

# Food Fermentation Technology

Part 3

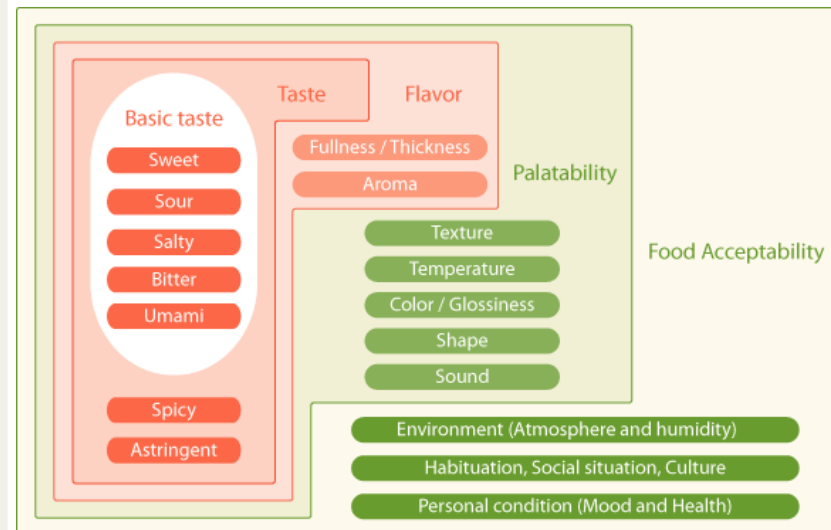
**Wilbert Sybesma**

**EPFL Course ENG-436**

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# Umami - History

- The discovery of umami - the fifth taste by a Japanese scientist: Dr. Kikunae Ikeda of Tokyo Imperial University
- Dashi stock made from kombu (kelp – seaweeds /algae) has long been an indispensable part of Japanese cuisine. It has also long been known that the active ingredients contained within kombu hold the key to its delicious taste.
- In 1908, Ikeda succeeded in extracting glutamate from kombu. He discovered that glutamate was the main active ingredient in kombu. He used the term 'umami' to describe its taste.
- He was sure that this taste was common with other savory foods, including those used in Western meals such as tomatoes and meat. Indeed it was discovered that these foods also contained umami.



<http://www.umamiinfo.com/2011/02/umami-culture-around-the-world.php>



Wikipedia

- Umami food or seasoning made from fermented beans and / or grains. It is normally available in either paste or liquid form.
- Umami seasoning made from fermented fish, prawns and / or other seafood. Available in either paste or liquid form.
- Umami food made from other ingredients.

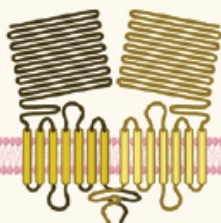
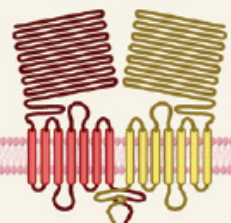
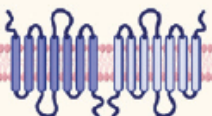
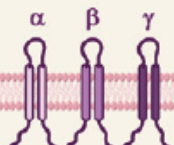
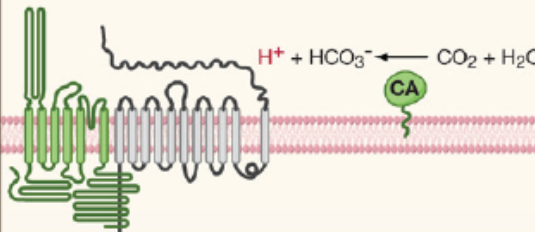
Brackets indicate ingredient(s) which deliver Umami.

## A large variety of Traditional fermented products



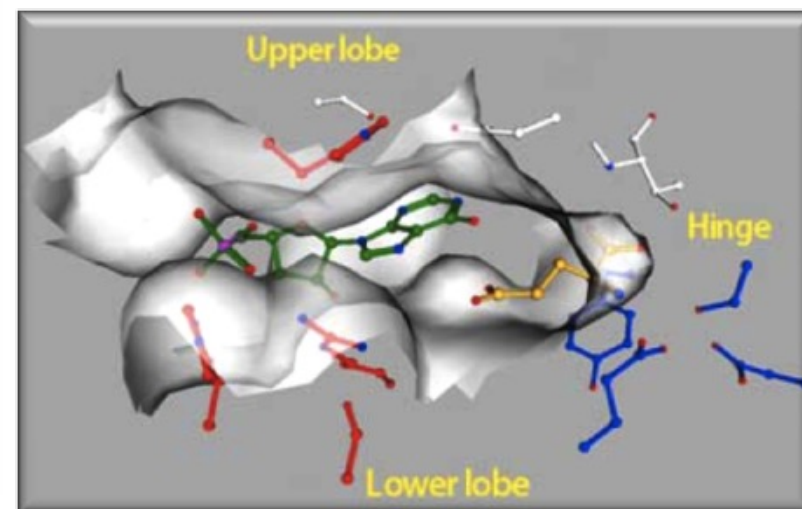
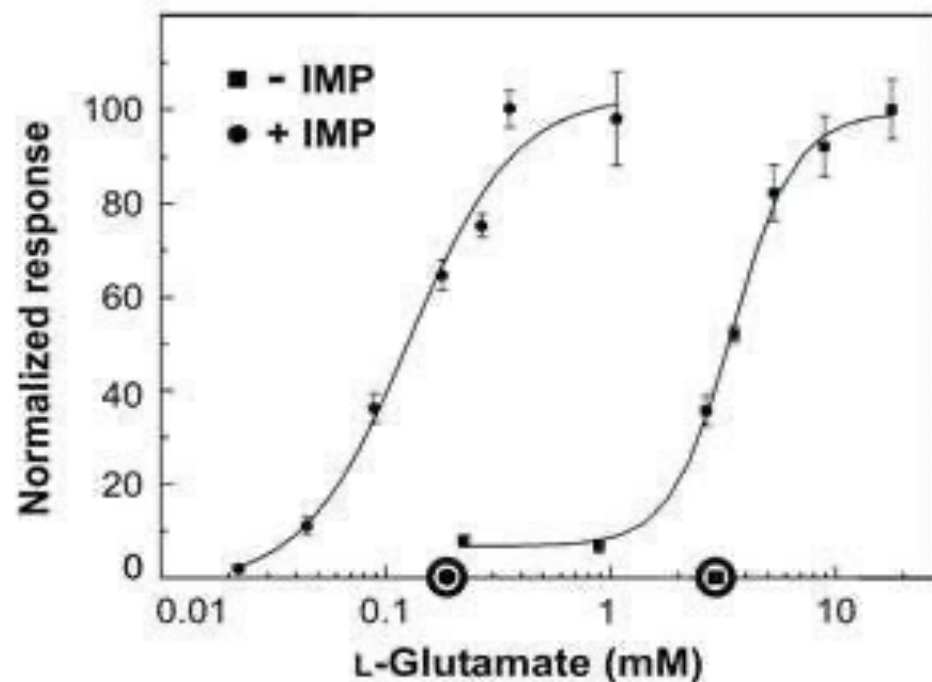
# Taste receptors can be used for discovery...

...they transduce signals for pleasant and aversive taste

Mammalian taste receptors and cells				
Umami	Sweet	Bitter	Sodium	Sour and carbonation cells
				
<b>T1R1+T1R3</b> <b>L-glutamate</b> L-amino acids glycine L-AP4  <b>Nucleotide enhancers</b> IMP, GMP, AMP	<b>T1R2+T1R3</b> <b>Sugars</b> Sucrose, fructose, glucose  <b>Artificial sweeteners</b> saccharin, acesulfame K, aspartame, cyclamate  <b>D-amino acids</b> D-alanine, D-serine, D-phenylalanine  <b>Glycine</b>  <b>Sweet proteins</b> Monellin, thaumatin	<b>~30 T2Rs</b> <b>Cycloheximide</b> (mT2R5)  <b>Denatonium</b> (mT2R8, hT2R4)  <b>Salicin</b> (hT2R16)  <b>PTC</b> (hT2R38)  <b>Saccharin</b> (hT2R43, hT2R44)  <b>Quinine</b> <b>strychnine</b> <b>atropine</b>	<b>ENaC</b> <b>Low NaCl</b> <b>Sodium salts</b>	<b>PKD2L1</b> <b>Acids</b> Citric acid Tartaric acid HCl  <b>CA IV</b> <b>Carbonated drinks</b>

# Umami taste: Synergy between MSG & nucleotide

- Umami reflects « taste of meat »
- Receptors can be used for screening





# Umami taste: Synergy between MSG & nucleotide

## Amino acids - a crucial element in flavor

The umami taste was initially found to appear with the presence of glutamate - a type of amino acid. After this discovery, research investigating the connection between amino acids - a structural element of protein - and the taste of food continued, and it was eventually discovered that each of the twenty kinds of amino acids possesses its own unique taste. The combination of these various tastes is an important element in determining the flavor of foods.

### Umami/Sour

- Aspartate
- Glutamate

### Bitter

- Phenylalanine
- Arginine
- Isoleucine
- Valine
- Tyrosine
- Leucine
- Methionine
- Histidine

### Sweet

- Glycine
- Threonine
- Serine
- Alanine
- Proline
- Glutamine

## Examples

### The Taste of Crab

Arginine itself is a bitter amino acid, but gives crab a seafood like taste.

The umami-rich glutamate also plays a role in giving crab its distinctive taste.

#### Essential composition

Amino acids	Nucleotides	Minerals
• Glycine	• Adenylate	• Sodium
• Alanine	• Guanylate	• Potassium
• Arginine		
• Glutamate		

### The Taste of Sea Urchin

It is methionine which plays the chief role in determining the flavor of sea urchin. By itself, this amino acid is very bitter, but it gives sea urchin its unique flavor. If methionine is removed from sea urchin extract, the overall taste becomes very similar to that of prawn or crab.

#### Essential composition

Amino acids	Nucleotides
• Glycine	• Guanylate
• Valine	• Inosinate
• Alanine	
• Methionine	
• Glutamate	

	GLUTAMATE		INOSINATE	
Japanese	Kombu/ Kelp	+	Dried bonito flakes	
Western	Onion	+	Leg of veal	
Chinese	Chinese cabbage Chinese leek	+	Chicken bones	

Synergistic effect of umami

<http://www.umamiinfo.com/2011/02/the-composition-of-umami.php>

# Soy Sauce Fermentation

- Soy sauce originated in China 2,800 years ago and spread to East and Southeast Asia.
- Like many salty condiments, soy sauce was originally a way to stretch salt, an expensive commodity. Chinese soy sauce, jiàngyóu, originally included fermented fish with soybeans used as filler. Soybeans became main ingredient for fermented fish sauce.
- Soy sauce has a distinct basic taste called umami, ("delicious taste") in Japanese, due to naturally occurring free glutamate. Umami was identified as a basic taste in 1908
- Soy sauce is a salty/brown liquid, obtained by a lengthy fermentation of soybean/wheat
- First, soybeans/wheat are blended with starter culture (*Aspergillus oryzae*). The mix is fermented a few days. Enzymes produced by the mold break down the protein. This culture (Koji), is transferred to fermentation tanks, where salt and water is added. This mash, called moroni, undergoes a further fermentation of 6 months.
- After this period, the mash is pressed to extract the shoyu.

<http://www.tempeh.info/fermentation/soy-fermentation.php>

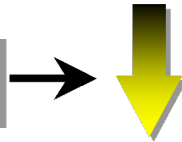
wikipedia

# Solid state fermentation of soybean and cereals

## PLANT RAW MATERIAL

e.g. soy, wheat, etc.

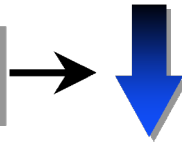
Moulds  
*A. oryzae*



Koji fermentation  
42 h

**Koji**

Water, Salt  
Yeasts



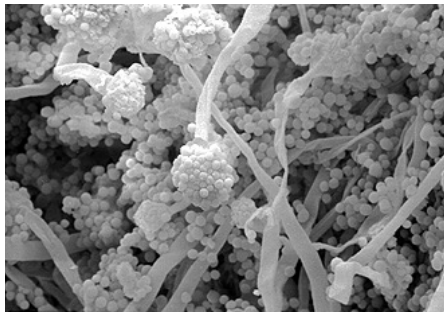
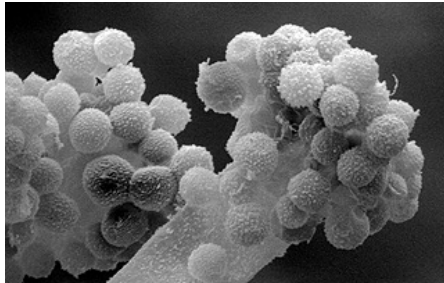
Yeast fermentation  
2-4 weeks

**Moromi**



Separation liquid-solid, sedimentation  
and pasteurisation

**RAW SAUCE**



*Aspergillus oryzae*

## History

- 2000-year tradition since the Han dynasty in China
- Spread to other Asian countries
- China: 70% Soybean/30% wheat
- Japan: 50% Soybean/50% wheat
- Indonesia: Black soybean
- “Koji” means “bloom of mold”
- “Moromi” means “mash”



**Koji**



# Soy Fermentation

**Table 1** Names, General Description, and Utilization of Fermented Soy Foods

Soy foods	Native names			General description	Uses
	Chinese <sup>a</sup>	Japanese	Others <sup>b</sup>		
Soy sauce	Jiang you (Chiang yu)	Shoyu	Kecap (In., Ma.) Tayo (Ph.) Kang jiang (Ko.)	Whole soybeans (or defatted soy flakes) and wheat fermented with <i>Aspergillus</i> , <i>Pediococcus</i> , <i>Torulopsis</i> , and <i>Zygosaccharomyces</i> . Dark brown liquid, salty and meaty.	All-purpose seasoning for dishes or soups
Soy paste	Dou jiang, Jiang (Chiang)	Miso	Tauco (In.,Ma.) Tao si (Ph.)	Whole soybeans with wheat flour, rice, or barley, fermented with <i>Aspergillus</i> , <i>Pediococcus</i> , <i>Zygosaccharomyces</i> , <i>Torulopsis</i> & <i>Streptococcus</i> . Light yellow to dark paste, salty & meaty.	All-purpose seasoning for dishes or soups
Tempeh	Tian bei	Tempe	Tempeh Kedelai (In.) Tempeh (Ma.)	Cooked and denulled soybeans fermented with <i>Rhizopus</i> . Soft beans bound by white mycelia, cakelike, nutty flavor.	Fried or cooked as part of meal, snack, or in soups
Natto	Na dou, Shui dou chi	Natto		Cooked whole soybeans fermented with <i>Bacillus natto</i> . Soft beans covered by viscous, sticky polymer, distinct aroma.	Seasoned and eaten with cooked rice
Soy nuggets	Dou chi (Toushih)	Hamanatto	Tao si (Ph.)	Whole soybeans fermented with <i>Aspergillus</i> . Soft beans with black color, salty and meaty taste	Cooked with vegetable and meat or served as seasoning
Sufu, Chinese cheese	Dou furu, Furu (Tou fu ju))			Tofu fermented with <i>Actinomucor</i> or <i>Mucor</i> . Creamy, cheeselike, salty, distinct aroma.	A condiment, served with or without further cooking
Sour soymilk	Suan dou nai			Lactic fermentation of soymilk	Frozen dessert
Soy yogurt					Yogurt replacement

<sup>a</sup> Mandarin Chinese (or Cantonese).

<sup>b</sup> In. = Indonesian, Ko. = Korean, Ma. = Malaysian, and Ph. = Filipino.

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)

# Soy Sauce Process (1)

## Ingredients and the Fermentation Process

Naturally brewed soy sauce is made using only four basic ingredients: soybeans, wheat, salt and water. Careful selection is required for these simple ingredients, as they directly influence the flavor and aroma of the soy sauce.

### ■ The Role of Soybeans and the Fermentation Process

The unique characteristics of soy sauce originate primarily from the proteins contained in soybeans. Soybeans are first soaked in water for an extended period, and then steamed at high temperatures.



### ■ The Role of Wheat and the Fermentation Process

Hydrocarbons contained in wheat are the component that gives soy sauce its fine aroma; the wheat also adds sweetness to the soy sauce. Wheat is roasted at high temperatures, then crushed by rollers to facilitate fermentation.



### ■ The Roles of Salt and Water and the Fermentation Process

Salt is dissolved in water. This salt-and-water solution controls the propagation of bacteria during the brewing process and acts as a preservative.

## Production of Koji, Mixing and Aging

### ■ Kikkoman's Aspergillus

Since its foundation, Kikkoman has been using its original Kikkoman Aspergillus, a type of fungus, to propagate koji mold. Koji mold is one of the most important elements in making soy sauce, and plays an essential role in fermenting the ingredients: this activity is the key to the taste of soy sauce.



### ■ Production of Shoyu Koji



Kikkoman's Aspergillus is mixed with processed soybeans and wheat, and then moved to a facility that provides the optimal environment for propagating koji mold. This three-day process results in the production of shoyu koji—the essential base of soy sauce.

### ■ Mixing

The mixing process starts from here: the shoyu koji is moved to a tank and mixed with the salt-and-water solution. This mixture is called moromi, a kind of mash, which is then fermented and aged in the tank.



### ■ Fermentation and Aging of Moromi



Moromi is aged for several months. Various actions take place in the tank, including lactic acid fermentation, alcoholic fermentation and organic acid fermentation, all of which impart to the moromi the rich flavor, aroma and color that are unique to soy sauce.

<http://www.kikkoman.com/soysaucemuseum/making/02.shtml>

# Soy Sauce Process (2)

## Pressing and Refining

### ■Pressing Soy Sauce from Moromi

Soy sauce is pressed from aged moromi. During pressing, moromi is poured into special equipment wherein the mash is strained through layers of fabric, with each layer folded into three sub-layers. After allowing the soy sauce to flow out of the moromi under the force of gravity, the moromi is then mechanically pressed slowly and steadily for about ten hours. It takes a considerable period of time to gradually press the mash in order to produce beautifully clear soy sauce.



### ■Pasteurization to Adjust Color, Flavor and Aroma

Soy sauce pressed from moromi is called "raw soy sauce." The Kikkoman plant is filled with a sweet scent resembling fresh fruit: this is the aroma of raw soy sauce. Raw soy sauce is left in a clarifier tank for three or four days to separate into its various components, with oil floating to the surface and sediment settling on the bottom. The clarified soy sauce is then run through a steam pipe to heat it: the main purpose is to pasteurize the soy sauce, but this process also halts the activity of the enzymes in order to stabilize the quality of the soy sauce. It also serves to adjust color, flavor and aroma.



### ■Reuse

During the pressing and clarifying processes, cake and oil are generated as by-products. Both of these resources are reused: the cake for livestock feed, the oil as fuel for machinery operations.

## Bottling, Inspection and Shipment

### ■Bottling



Pasteurized soy sauce is bottled automatically.

### ■Quality Inspection Conducted for Each Process

Kikkoman pays minute attention to quality control at every stage of soy sauce production. Quality inspections are carried out during every process to ensure that the highest standards are maintained. Inspectors analyze the ingredients and check the color, flavor and aroma of the soy sauce. Kikkoman's stringent control system upholds the finest, most consistent quality.



### ■Shipment

Only soy sauce that has passed all necessary inspections is released into the market. Thanks to this meticulous process, Kikkoman Soy Sauce—containing only natural ingredients—is delivered fresh to your table.



<http://www.kikkoman.com/soysaucemuseum/making/02.shtml>

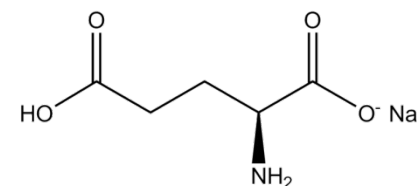
# *Corynebacterium glutamicum*

## A «work-horse for amino acid production»

- **Strain:** *Corynebacterium glutamicum* is a key fermentative bacteria in production of monosodium glutamate (MSG). Discovered 1957 in Japan as natural producer of glutamic acid, *C. glutamicum* is a gram-positive, facultatively anaerobic bacteria
- **Yield:** The strain was improved. Soil bacteria that produced large amounts of glutamic acid. Wild-type strains of this bacterium produced about 10 g/L glutamic acid, and with genetic engineering more than 100g/L.
- **Applications:** MSG, an umami taste enhancer, has been widely used in the food industry. *C. glutamicum* was grown in a medium of sugars, molasses or starch as substrate. Glutamic acid that they produce is filtered from the medium and neutralized to make MSG. After additional purification, crystallization, and drying, a white crystalline powder of MSG is obtained.
- **Suppliers:** E.g. Ajinomoto, Kirin (Japan), CJ, Daesang (Korea), Meihua , Fu Feng (PRC)



[http://farm1.static.flickr.com/190/510676658\\_425afb1323.jpg](http://farm1.static.flickr.com/190/510676658_425afb1323.jpg)

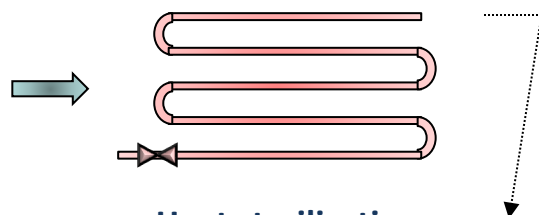




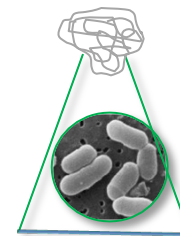
# MSG Production



**Sugar extraction  
(cane) or starch  
saccharification  
(tapioca)**



**Heat sterilisation**



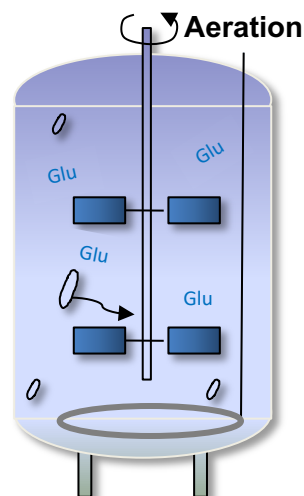
**Starter preparation  
*Corynebacterium glutamicum***



**Medium**



**Inoculum**



**Fermentation**  
excretion of glutamate  
out of the cells



**Separation,  
concentration**



**Precipitation  
with acid**



**Crystallization**



**Drying**



**MSG  
Crystals**





# Miso Fermentation

- *Aspergillus oryzae* (filamentous fungus) used in Chinese and Japanese cuisine to ferment soybean. Also used to saccharify rice and potatoes for alcoholic beverages (huangjiu, sake, shōchū) & rice vinegars. Domestication of *A. oryzae* occurred at least two thousand years ago.
- Natural miso can contain beneficial micro-organisms such as *Tetragenococcus halophilus* (LAB)
- Japanese drink miso soup in the morning. Believed to induce a health promoting effect for the consumer.
- Lighter/sweeter miso: Fermented/aged 1-2 months.  
Darker miso: Aged up to 2 years. Strong/salty taste

<http://www.tempeh.info/fermentation/soy-fermentation.php>



wikipedia



# Tempeh Fermentation

- Traditional fermented soy (Indonesia). Binds soybean into a cake, like a vegetarian burger patty
- Whole soybeans softened by soaking, dehulled, cooked. Speciality tempehs made from other/mixtures of beans
- A mild acidulent, e.g. vinegar may be added to lower pH to create an environment to favor growth of tempeh mold.
- Inoculation using spores of the fungus *Rhizopus oligosporus*. Beans are spread in a layer and fermented (24-36 h, 30 °C).
- A mild ammonia smell may accompany good tempeh as it ferments, but it should not be overpowering.

<http://www.tempeh.info/fermentation/soy-fermentation.php>



wikipedia



# Natto Fermentation

- Traditional Japanese food for >1000 years. Soybeans washed/soaked 12-20 h to increase size, then steamed 6 h
- Inoculum at 80°C to avoid contamination (*Bacillus natto* spores are heat resistant). Fermented 40 °C up to 24 h
- Aged at 5 °C, 1 week to develop stringiness. The bacilli develop spores. Peptidases break down the soybean protein. Bean softening due to pectic and proteolytic enzymes
- Odour of ammonia by fermentation of amino acids. Viscous/sticky polymers (polyglutamic acid) makes natto slimy
- *Bacillus subtilis natto* produces enzymes, vitamins, amino acids & other nutrients unique to natto

<http://www.tempeh.info/fermentation/soy-fermentation.php>



wikipedia



# Kimchi (1)

- Kimchi (gimchi, kimchee, kim chee): traditional fermented Korean dish from vegetables/seasonings.
- Hundreds variants of kimchi, made with vegetables (cabbage, radish, green onion, cucumber). Most popular: Chinese cabbage.
- High level of lactic acid bacteria ( $10^7 - 10^9$  cfu/ml), organic acids, various nutrients and functional components, that result from the fermentation
- Proposed benefits: Increase appetite, control body weight, prevent colon cancer, probiotics, decrease cholesterol, antioxidants (anti-aging, skin), increase immune function

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»



Wikipedia



# Kimchi (2)

- Anaerobic to minimize growth of aerobic microorganisms and stimulate LAB growth.
- Fermentation initiated by *Leuconostic mesenteroides*, producing lactic acid, acetic acid, CO<sub>2</sub>, and ethanol.
- As pH drops to 4.6-4.9 because of organic acids, *Leuconostic mesenteroides* is relatively inhibited.
- Fermentation continues with more acid tolerant species; *Pediococcus cerevisia*, *L. brevis*, *L. fermentum*, *L. plantarum*
- Yeast in end stage can produce tissue softening enzymes (pectinases can downgrade the kimchi quality).

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»



Wikipedia



<http://eng.cjcheiljedang.com>



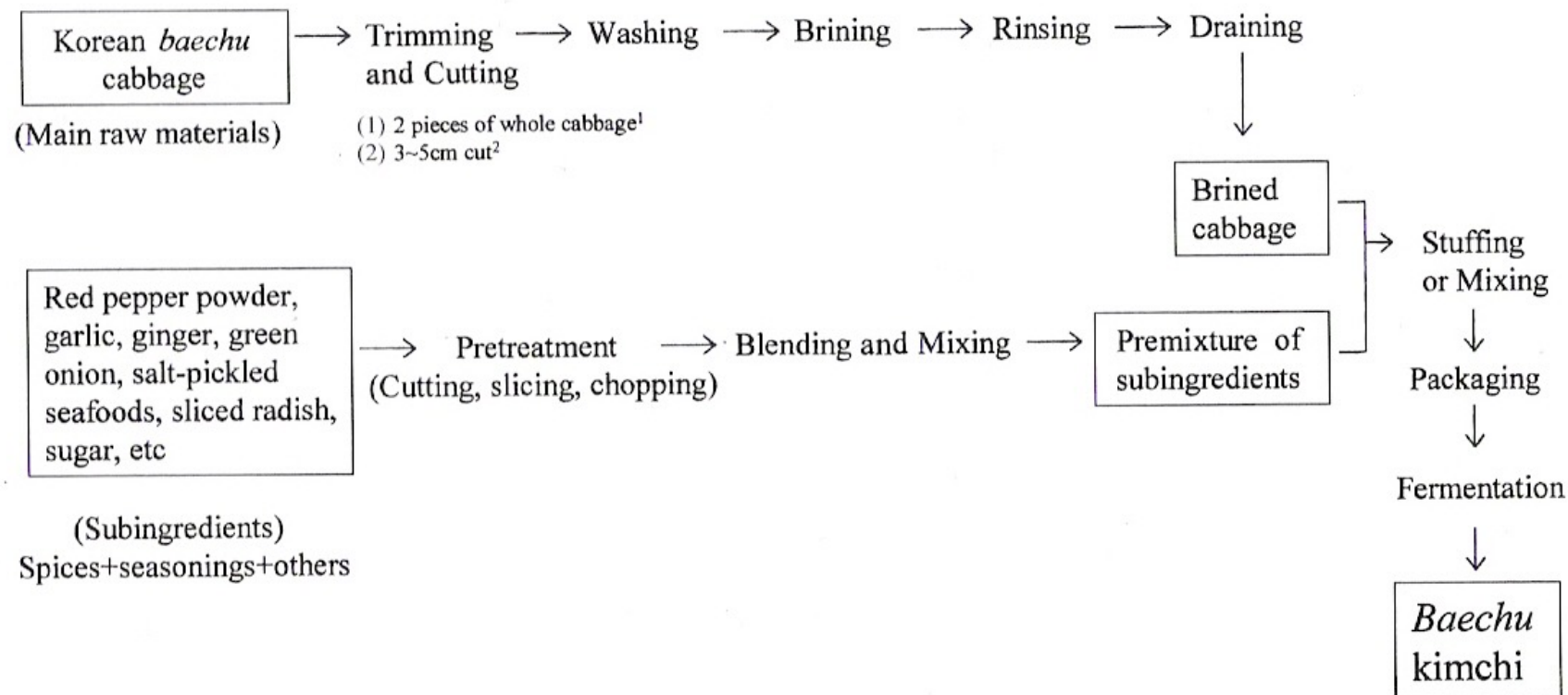
# Kimchi - Raw Materials

**Table 2** Raw Ingredients Used for the Preparation of Kimchi

Groups	Raw ingredients
Main raw vegetables	Baechu (Korean baechu cabbage), radish, pony-tail (chonggak) radish, young oriental radish, cucumber, green onion, lettuce, Western cabbage, leek, green pepper, etc.
Subingredients	
Spices	Red pepper, green onion, garlic, ginger, mustard, black pepper, onion, cinnamon, etc.
Seasoning	
Salt	Dry salt or brine solution
Salt-pickled seafood	Anchovy, small shrimp, clam, hairtail, yellow corbina, etc.
Other seasoning	Sesame seed, soybean sauce, monosodium glutamate, corn syrup, etc.
Other materials	
Vegetables	Watercress, carrot, crown daisy, parsley, mustard leaves, etc.
Fruits and nuts	Pear, apple, jujube, melon, ginkgo nut, pine nut, etc.
Cereals	Rice, barley, wheat flour, starch, etc.
Seafoods and meats	Shrimp, Alaska pollack, squid, yellow corbina, hairtail, oyster, beef, pork, etc.
Miscellaneous	Mushrooms, etc.

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# Kimchi - Flow chart



**Figure 1** Flow chart for processing of baechu kimchi. (1) Tongbaechu kimchi, (2) Matbaechu kimchi.

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»

# Kimchi - Bacteria isolated after fermentation

**Table 4** Frequency (%) of Gram-Positive Bacteria Isolated from Kimchi Fermented at 5°C, 15°C, and 25°C

Genus	Species	Subspecies	5°C	15°C	25°C
<i>Leuconostoc</i>	<i>mesenteroides</i>	<i>mesenteroides</i>	31.5	12	6.3
	<i>mesenteroides</i>	<i>cremoris</i>	0	4	4.8
	<i>mesenteroides</i>	<i>dextranicum</i>	10.1	0	1.6
	<i>paramesenteroides</i>		23.6	3	0.8
	<i>lactis</i>		0	1	0
<i>Streptococcus</i>	<i>lactis</i>		4.5	0	1.6
	<i>iniac</i>		0	0	0.8
	<i>agalactiae</i>		0	0	0.8
	<i>raffinolactis</i>		0	0	11.1
<i>Pediococcus</i>	<i>pentosaccus</i>		0	0	4.0
	<i>inopinatus</i>		2.2	6	0
	<i>acidilactici</i>		0	1	0
<i>Lactobacillus</i>	<i>plantarum</i>		0	15	36.5
	<i>maltaromicus</i>		12.3	8	5.6
	<i>homochiochii</i>		0	7	4.8
	<i>brevis</i>		0	0	3.2
	<i>curvatus</i>		0	0	2.4
	<i>minor</i>		10.1	3	0.8
	<i>sake</i>		4.5	9	0.8
	<i>confuses</i>		0	0	0.8
	<i>hilgardii</i>		0	0	0.8
	<i>fructosus</i>		0	15	0.8
	<i>farciminis</i>		0	3	1.6
	<i>coryniformis</i>	<i>coryniformis</i>	0	0	0.8
	<i>casei</i>	<i>rhamnosus</i>	0	0	0.8
	<i>divergens</i>		1.1	0	0
	<i>alimentarius</i>		0	4	0
	<i>bavaricus</i>		0	2	0
	<i>yamanashiensis</i>		0	4	0
	<i>amylophilus</i>		0	1	0
<i>Bacillus</i>	<i>cereus group</i> <sup>a</sup>		0	0	4.8
	<i>circulans</i>		0	0	4.0

<sup>a</sup> *B. cereus*, *B. anthracis*, *B. mycoides*, *B. thuringiensis*.

Source: Ref. 21.

No need to remember details

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»

# Pickling

- Preserve by anaerobic fermentation in brine (salt in water) to produce lactic acid, or marinate/store in vinegar
- pH <4.6, which is sufficient to kill most bacteria. Pickling preserve foods for months. Anti-microbial herbs/spices (mustard seed, garlic, cinnamon, cloves) are often added
- Low Salt & Temp: *Leuconostoc mesenteroides* dominates, producing a mix of acids, alcohol, and aroma compounds.
- Higher Temp: *L. plantarum* dominates, producing lactic acid. Often start with *Leuconostoc*, then LAB at lower pH
- Other homo-fermentative strain: *Pediococcus cereviseae*

Wikipedia



Wikipedia



# Sauerkraut

- Fermentation by LAB introduced naturally; air-borne bacteria on raw cabbage leaves
- Yeasts may yield soft sauerkraut/poor flavor if fermentation temperature is too high
- The fermentation process has three phases.
  1. Anaerobic bacteria (*Klebsiella*, *Enterobacter*), producing acidic environment that favors later bacteria.
  2. The acid levels too high for many bacteria. *Leuconostoc mesenteroides* and other *Leuconostoc* take dominance.
  3. *Lactobacillus* species, including *L. brevis* & *L. plantarum*, ferment remaining sugars, further lowering the pH.
- Properly cured sauerkraut sufficiently acidic to prevent *Clostridium botulinum* (botulism toxins). pH 3.3 – 3.8



Wikipedia



<http://www.about.ch/culture/food/sauerkraut.html>



# Sourdough Bread

- Starter: stable symbiotic bacteria & yeast present in flour
- Yeasts, e.g. *Candida milleri* or *Saccharomyces exiguus* populate symbiotically with e.g. *L. sanfranciscensis*
- Fresh culture begins with flour/water. Naturally occurring amylase breaks down starch. Maltase generates glucose/fructose that yeast can metabolize
- The LAB feed on the metabolism products of yeast
- Balanced, symbiotic culture after repeated feedings. 20-25% of sourdough starter is mixed into the dough
- Benefits: Dough leavening, flavor, increased nutritional value, enhance mineral bioavailability, extend shelf-life



<http://sourdough.com/forum/bethesda-bakers-3-starter-maintenance>



<http://sourdough.com/forum/bao-rise-fall-and-rise>

# Tea Fermentation

- *Camellia sinensis* leaves oxidize, if not dried. Leaves turn darker as chlorophyll breaks down. Tannins are released
- Enzymatic oxidation process, known as “fermentation”, is caused by plant's enzymes and causes the tea to darken.
- Darkening stopped by heating; deactivates enzymes.  
Black teas: oxidization stops simultaneously with drying
- Tea is classified based on techniques and processes.
  - White tea: Wilted (dried) and unoxidized
  - Yellow tea: Unwilted and unoxidized, but allowed to yellow
  - Green tea: Unwilted and unoxidized
  - Oolong: Wilted, bruised, and partially oxidized
  - Black tea: Wilted, sometimes crushed, and fully oxidized
  - Post-fermented tea: Green tea allowed to ferment



<http://www.dethlefsen-balk.de/ENU/10795/ErnteVerarbeitung.html>



[http://hojotea.com/item\\_e/b06e.htm](http://hojotea.com/item_e/b06e.htm)

# Coffee Fermentation

- Coffee is fermented to ease removal of a mucilage layer from the seed/inner integument to which it adheres
- Wet processing: pulp removed by breaking down cellulose by fermenting beans and washing with water
- Fermentation monitored to prevent undesirable flavors
- Fermentation takes 24-36 h, depending on temperature, thickness of mucilage layer and enzyme concentration
- It is a mixed yeast/bacterial fermentation:
  - Yeasts: *Saccharomyces apiculatus*, *Hanseniaspora uvarum*, *Pichia kluyveri*, *Kluyveromyces marxianus*
  - Bacteria: lactic acid bacteria, *Enterobacteriaceae*, *Bacillus*



<http://www.coffeeresearch.org/agriculture/processing.htm>



<http://recipes.howstuffworks.com/coffee5.htm>

# Kopi Luwak coffee

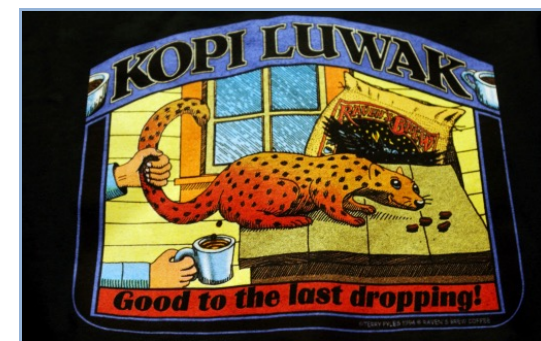
- Kopi luwak (civet coffee): one of most expensive and low-production coffee (Price up to 1000 US \$/kg)
- Beans of coffee berries which have been eaten by Asian Palm Civet (*Paradoxurus hermaphroditus*), then passed through its digestive tract
- Eats berries for the fleshy pulp. Proteolytic enzymes in stomach, generating peptides & amino acids
- Passing through a civet's intestines the beans are then defecated, keeping their shape
- After gathering, washing, sun drying, light roasting and brewing: aromatic coffee with low bitterness



<http://cafe.cafes-pfaff.com/p-853-indonesie-kopi-luwak-cafe-moulu.html>



<http://poshalert.blogspot.com/2010/07/kopi-luwak-civet-coffee.html>



<http://www.roughguides.com/website/Travel/SpotLight/ViewSpotLight.aspx?spotLightID=535>



# Cocoa Fermentation



## Microbial fermentation in the pulp

**Anaerobic:** Pulp sugars converted to ethanol and CO<sub>2</sub> by yeast

Metabolism of citric acid by yeast (slow pH rise)

Pectin degradation - "sweating" - allows aeration

Inhibition of yeast - end of anaerobic phase

**Aerobic:** Acetic acid bacteria - oxidation of ethanol to acetic acid

Temperature rises to 45-50 °C

Acetic acid and heat induces bean death and loss of cell compartmentation

### Enzyme reactions:

Proteases: Storage proteins to peptides and amino acids

Invertase: Sucrose to glucose and fructose

Glycosidases: Anthocyanidine pigment degradation

Polyphenol oxidase: Oxidation of polyphenols (epicatechin, catechin)

Acetic acid  
Heat  
Water

Pulp

Bean  
(cotyledon)

**Accumulation of flavour precursors**

**Reduction of astringency**

**Formation of brown insoluble pigment**





# Dawadawa

- Diet among low-income groups in tropical Africa is often a mixture of cereals or starchy foods and grain legumes. Sometime other protein-rich seeds are included. Legumes and protein-rich seeds are added as supplement to high protein foods of animal origin, which are too expensive for majority.
- Dawadawa produced by fermentation (3-4 days) of soybean or dried African locust bean *Parkia biglobosa*
- Micro-organisms associated with fermentation of “dawadawa”, were isolated and identified: Three species of bacteria (*Bacillus subtilis*, *Leuconostoc mesenteroides*, *L. dextranicus*) were predominant and most actively involved organisms.



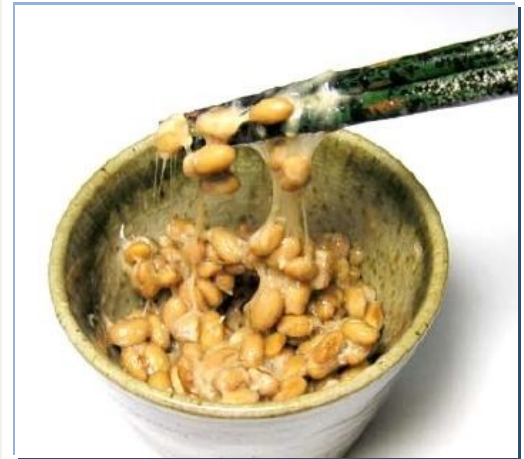
<http://opiom.net/joomla.php?q=african-locust-beans&page=6>



<http://opiom.net/joomla.php?q=locust-beans-iru&page=4>

# *Bacillus subtilis*

- *Bacillus subtilis*, known as hay/grass bacillus, is Gram-positive, catalase-positive soil bacterium
- Catalase is found in nearly all organisms exposed to oxygen:  $2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$
- In the genus *Bacillus*, *B. subtilis*, has ability to form a tough, protective endospore, allowing to tolerate extreme environmental conditions
- Enzymes of *B. subtilis* & *B. licheniformis* are used in food processing and laundry detergents
- *B. subtilis* produces the proteolytic enzyme subtilisin (sold as alcalase by Novozymes).



<http://www.scienceknowledge.org/2010/05/23/the-genome-of-bacillus-subtilis-natto-sequencing/>



<http://www.scienceknowledge.org/2010/05/23/the-genome-of-bacillus-subtilis-natto-sequencing/>

# Malting

- Malt: The grains germinate by soaking in water, and are then halted from germinating further by drying
- Malting develops the enzymes required to modify the grain's starches into sugars such as glucose, fructose & maltose. It also develops other enzymes, such as proteases, which break down the proteins in the grain into forms which can be used by e.g. yeast.
- Malted grain is used to make beer, whisky, malted shakes, malt vinegar, confections such as Maltesers & Whoppers, beverages such as Ovomaltine & Milo.
- Germination can generate flavor precursors, flavors, vitamins and reduction of anti-nutritional factors



[http://en.wikipedia.org/wiki/File:Sjb\\_whokey\\_malt.jpgx](http://en.wikipedia.org/wiki/File:Sjb_whokey_malt.jpgx)



# Tofu and Stinky Tofu

- Coagulating soy milk and pressing the curds.
- Coagulation of the protein and oil (emulsion) suspended in the boiled soy milk is the most important step in the production of tofu. This process is accomplished with the aid of coagulants (salts and acids). Enzymes are not yet used commercially but shows potential for firm and "silken" tofu.
- The traditional method for stinky tofu is to prepare a brine from fermented milk, vegetables, fish, meat; the brine can also include dried shrimp, amaranth greens, mustard greens, bamboo shoots, and Chinese herbs
- The mixed culture contains *Bacillus*, *Streptococcus*, *Enterococcus* and *Lactobacillus* species.
- When *Bacillus spaericus* is cultured in meat & fish brine, ammonia increases from 100 mg/l to 3400 mg/l in 10 weeks.
- The pH drops first from 6.5 to 4.7, then increasing to 7.5
- The protein of tofu is hydrolyzed by proteases.

<http://www.tempeh.info/fermentation/soy-fermentation.php>



Wikipedia





# Lactobacillus isolated from Sourdough

**Table 5** Groups of Lactobacillus Isolated from Sourdoughs

Characteristics	Obligately homofermentative	Facultatively heterofermentative	Obligately heterofermentative
Growth at 15°C	—	+	+ / —
Growth at 45°C	+	—	+ / —
Pentose fermentation	—	+	+
CO <sub>2</sub> from glucose	—	—	+
CO <sub>2</sub> from gluconate	—	+ <sup>a</sup>	+ <sup>a</sup>
FDP <sup>b</sup> aldolase present	+	+	—
Phosphoketolase present	—	+ <sup>c</sup>	+
<i>Lactobacillus</i>	<i>L. acidophilus</i> <i>L. amylovorus</i> <i>L. delbrueckii</i> spp. <i>bulgaricus</i> <i>L. delbrueckii</i> spp. <i>delbrueckii</i> <i>L. farciminis</i> <i>L. helveticus</i> <i>L. leichmanni</i> <i>L. mindensis</i>	<i>L. alimentarius</i> <i>L. casei</i> <i>L. curvatus</i>  <i>L. paralimentarius</i>  <i>L. plantarum</i> <i>L. rhamnosus</i>	<i>L. brevis</i> <i>L. buchneri</i> <i>L. fermentum</i>  <i>L. fructivorans</i>  <i>L. frumenti</i> <i>L. hilgardii</i> <i>L. panis</i> <i>L. pontis</i> <i>L. reuteri</i> <i>L. sanfranciscensis</i> <sup>d</sup> <i>L. viridescens</i>

<sup>a</sup> When fermented.

<sup>b</sup> FDP fructose-1,6-diphosphate.

<sup>c</sup> Inducible by pentoses

<sup>d</sup> Former names of some of the bacteria: *L. brevis* spp. *lindneri* and *L. sanfrancisco*.

Source: Ref. 46.

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»



# Yeasts isolated from Sourdough

**Table 6** Yeasts Isolated from Sourdoughs and Their Synonyms

Perfect fungi	Imperfect fungi	Synonyms
<i>Saccharomyces cerevisiae</i> <i>S. exiguus</i>	<i>Candida holmii</i>	<i>Torulopsis holmii</i> <i>Torula holmii</i> <i>S. rosei</i>
<i>S. delbrueckii</i> <i>S. uvarum</i>	<i>C. milleri</i>	<i>Torulopsis holmii</i> <i>Torulaspora delbrueckii</i> <i>S. inusitatus</i>
<i>Issatchenkia orientalis</i>	<i>C. krusei</i>	<i>S. krusei</i> <i>Endomyces krusei</i>
<i>Pichia anomala</i> <i>P. membrifaciens</i>	<i>C. peliculosa</i> <i>C. valida</i>	<i>Hansenula anomala</i>
<i>P. norvegensis</i> <i>P. polymorpha</i> <i>P. satoi</i> <i>Endomycopsis fibuligera</i>		<i>S. fibuliger</i>

*C.*, *Candida* *P.*, *Pichia*; *S.* *Saccharomyces*

Source: Refs. 128 and 129.

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»