Fermentation Microorganisms and Flavor Changes in Fermented Foods

R.F. MCFEETERS

ABSTRACT: Food fermentation processes often result in profound changes in flavor relative to the starting ingredients. However, fermenting foods are typically very complex ecosystems with active enzyme systems from the ingredient materials interacting with the metabolic activities of the fermentation organisms. Factors such as added salt, particle sizes, temperature, and oxygen levels will also have important effects on the chemistry that occurs during fermentation. This is a brief review of recent research on flavor changes in food fermentations. The emphasis will be on the role of lactic acid bacteria in changing the compounds that help determine the character of fermented foods from plant-based substrates.

Introduction

Lactic acid bacteria influence the flavor of fermented foods in a variety of ways. In many cases, the most obvious change in a lactic acid fermentation is the production of acid and lowering pH that results in an increase in sourness. Since most of the acid produced in fermentations will be produced by the metabolism of sugars, sweetness will likely decrease as sourness increases. The production of volatile flavor components tends to be the first mechanism considered for the development of flavor specific to a particular fermented food. In addition to this direct mechanism, however, there are less direct ways in which fermentation microorganisms affect flavor. Lowering the pH in lactic acid fermentations may reduce the activity or completely inactivate enzymes in the plant that generate either flavor components or flavor precursor compounds. Finally, the fermentation microorganisms may directly metabolize precursor flavor compounds or flavor components themselves. Some examples of these different flavor modification mechanisms will be given.

Prevention of flavor formation

Purge-and-trap analysis of the volatile components found in cucumber slurries before and after cucumbers were fermented in a 2% reduced-salt brine. Comparison of volatile components before and after fermentation led to the conclusion that the major effect of the fermentation on flavor volatiles was to prevent enzymatic formation of *E*,*Z*-2,6-nonadienal and 2-nonenal by enzymes present in cucumbers (Zhou and McFeeters 1998). These aldehydes are the major compounds responsible for fresh cucumber flavor (Schieberle and others 1990). However, a few days into cucumber fermentation, the pH drops low enough to inactivate the enzymes which form these compounds when cucumber tissue is disrupted. Among the volatile components identified in the fermented cucumbers, only benzaldehyde, ethyl benzene, and *o*-xylene were not observed in fresh cucumber slurries (Table 1). The lack of the flavor impact of volatile aldehydes is certainly the major effect of the fermentation on flavor.

More recently, Marsili and Miller (2000) found a low volatility flavor impact compound in fermented pickled cucumber brines. Addition of saturating salt to brine samples heated to 50 °C, sampling with an SPME (solid-phase microextraction) fiber and followed by GC-olfactometry led to recognition of a compound with an odor close to that of the fermentation brine. The compound with a fermentation brine odor was identified as *trans*-4-hexenoic acid. They also tentatively identified the presence of *cis*-4-hexenoic acid. In a reconstitution experiment, a solution that contained 25 ppm *trans*-4-hexenoic acid, 10 ppm phenyl ethyl alcohol, 0.65% lactic acid, 0.05% acetic acid, and 8% NaCl had an odor very similar to that of brine from fermented cucumbers. The concentrations of the lactic acid, acetic acid, and NaCl are reasonable for commercial brines after the completion of fermentation. The addition of phenyl ethyl alcohol gave only a small improvement in the odor match. Thus, the *trans*-4-hexenoic acid was the key component in the simulated brine solution. Unfortunately, the origin of *trans*-4-hexenoic acid in fermentation brines is not known.

Author McFeeters is with the U.S. Dept. of Agriculture, Agricultural Research Service, and the North Carolina Agricultural Research Service, Dept. of Food Science, NC State Univ., Raleigh, NC 27695-7624 (E-mail: rfm@unity.ncsu.edu).

Volatile odor changes caused by lactic acid fermentation

In contrast to the cucumber fermentation, where most volatile components did not change substantially, Czerny and Schieberle (2002) observed a number of changes in odorants when whole meal wheat flour was fermented by a commercial sourdough starter culture. Using aroma extract dilution analysis, they identified 14 compounds that had substantial odor intensity in fermented sourdough. All of these odorants were present in dough before and after fermentation, so the fermentation did not result in formation of compounds not present in the flour, nor did fermentation completely remove odor components. However, most of the compounds changed during fermentation, with changes ranging from 7-fold decreases to 9-fold increases in concentration (Table 2). Unsaturated aldehydes present in the flour decreased in the fermentation. As would be expected for heterofermentative lactic acid bacteria, there was a major increase in acetic acid. In addition, components such as 2- and 3-methylbutanal and 2- and 3-methylbutanoic acid, which form as a result of amino acid degradation, also increased.

Flavor precursor formation caused by lactic acid fermentation

When sourdough bread is baked, one of the characteristic odor compounds generated in the baking process is 2-acetyl pyrroline, which has a roasted, popcorn-like odor, with an odor threshold of only 20 ppt (Schieberle 1995). The precursor of 2-acetyl pyrroline during baking is ornithine. Ornithine is synthesized from free arginine by heterofermentative lactic acid bacteria. De Angelis and others (2002) have shown that *Lactobacillus sanfranciscensis* has the enzymes arginine deiminase and ornithine transcarbamoylase, which can convert arginine to ornithine. However, *L. sanfranciscen-*

Table 1—Peak area ratios of volatile components in fermented cucumbers relative to fresh cucumbers (Zhou and McFeeters 1998).

Compound[1]	Fermented/ fresh ratio	Compound	Fermented/ fresh ratio
(*E*,*Z*)-2,6-nonadienal	0.007	2-methyl butanol	1.00
2-undecanone	0.31	ethyl butyrate	1.03
3,7-dimethyl-(*E*,*E*,*E*)-1,3,6-octatriene	0.38	3-methyl butanol	1.06
hexanal	0.43	dimethyl disulfite	1.09
2-methoxy-3-(1-methylethyl)pyrazine	0.57	eucalyptol	1.15
hexyl acetate	0.62	propyl acetate	1.25
octanol	0.76	tetrahydro-4-methyl-2-(2- methyl-1-propenyl), 2H-pyran	1.26
2-heptanol	0.78	tetradecane	1.28
1-(2,6,6-trimethyl-(*E*,*E*)-1,3- cyclohexadien-1-yl)-2-buten-1-one	0.79	dimethyl trisulfide	1.31
decanal	0.80	2-methyl-1-butanol acetate	1.44
octanal	0.87	methyl 3,7-dimethyl- (*E*,*E*)-2,6-octadienate	1.53
2-nonanone	0.87	geraniol	1.54
nonanal	0.87	alpha-caryophyllene	1.89
hexadecane	0.88	linalool	9.79
2-ethyl-1-hexanol	0.90	ethyl benzene	*
hexanol	0.94	*o*-xylene	*
(E)-2-heptenal	0.95	benzaldehyde	*
2-pentanol	1.00		

[1]Compound was only observed in fermented cucumbers

sis only increased the ornithine concentrations during dough fermentation when additional arginine was added. Thiele and Vogel (2002) reported that ornithine accumulated during sourdough fermentation only when *Lactobacillus pontis* was used as the starter culture. No increase in ornithine was found when the starter was *L. sanfranciscensis*. This result indicates the difficulty that can be encountered between showing that a fermentative organism can carry out a reaction and showing that, in the complex conditions existing in a food fermentation, the expected transformation will, in fact, occur.

While 2-acetyl-pyrroline is apparently formed from ornithine by chemical reactions in bread baking, it may be formed by lactic acid bacteria in wine. It is one of the compounds associated with the highly objectionable 'mousy' off-flavor in wine. Costello and Henschke (2002) showed that *Lactobacillus hilgardii*, a lactic acid bacterium that occurs in wine, could produce the compound from ornithine in a defined medium with a high cell density. Formation of 2-acetyl-pyrroline also required fructose as a fermentable sugar, ethanol, and iron (Fe^{+2}) in the medium.

Table 2—Comparison of the amounts of 14 odorants in whole meal flour, the sourdough starter, and the fermented sourdough (Czerny and Schieberle 2002)

Compound	Amount (µg) Flour	Starter	Amount in sourdough (µg) Calc.	Meas.
3-methylbutanal	31	20	51	158
2-methylbutanal	15	5.4	20	33
2- and 3-methylbutanoic acid	126	12	138	361
3-(methylthio)propanal	25	2.2	27	14
hexanal	2240	14	2254	2070
(*Z*)-4-heptenal	4	0.1	4.1	0.8
(*E*)-2-nonenal	52	1	53	20
(*E*,*Z*)-2,6-nonadienal	13	0.2	13	3.8
(*E*,*Z*)-2,4-decadienal	362	0.5	363	52
(*E*,*E*)-2,4-decadienal	338	1	339	48
acetic acid	43600	5400	49000	427000
butanoic acid	1400	110	1510	4690
pentanoic acid	2320	120	2440	4800
vanillin	582	10	592	288

[1]Amount in 200 g of flour.
[2]Amount in 20 g of sourdough starter.
[3]Calculated amount present before fermentation of a mix of 200 g of flour, 20 g of starter culture, and water (130 mL).
[4]Amount determined in 350 g of fermented sourdough.

Metabolism of phytochemicals in sauerkraut

Sauerkraut undergoes a sequential fermentation that is initiated by heterofermentative lactic acid bacteria and completed by homofermentative bacteria. Sulfur compounds derived from S-methyl cysteine sulfoxide and glucosinolates in the raw cabbage must have a major effect on the flavor developed during fermentation. S-methyl cysteine sulfoxide is degraded by cysteine sulfoxide lyases that are present in Brassica vegetables to produce methyl methane thiosulfinate. This compound can then be degraded further by chemical reactions to produce methanethiol, dimethyl disulfide, and dimethyltrisulfide (Hsi and Lindsay 1994). In both commercial sauerkraut fermentations (Daxenbichler and others 1980) and reduced-salt fermentations (Viander and others 2003) the glucosinolates disappear during fermentation and produce degradation products in the sauerkraut. Myrosinase, an enzyme which hydrolyzes glucosinolates, is present in plant tissues, but is not found in lactic acid bacteria. The hydrolysis products may then undergo further nonenzymatic chemical reactions. There is no evidence that fermentation microorganisms are directly involved in the metabolism of degradation products derived from either S-methyl cysteine sulfoxide or glucosinolates. The fermentation process is certainly necessary for the development of typical sauerkraut flavor because acidification of cabbage does not result in a product with sauerkraut odor or flavor (Lonergan and Lindsay 1979). However, when sulfur compounds were analyzed in commercial sauerkraut products in the United States and compared to sensory analysis of the sauerkraut flavor, it was not possible to relate differences in specific sulfur compounds to differences in flavor (Trail and others 1996). Currently, it is not at all clear how the flavor of sauerkraut is generated by the fermentation process.

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