SALTINE CRACKER FLAVOR. I. CHANGES IN ORGANIC ACIDS AND SOLUBLE NITROGEN CONSTITUENTS OF CRACKER SPONGE AND DOUGH[1]

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ABSTRACT

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Total soluble nitrogen, peptides, primary amines, and ammonia formed in cracker doughs as a result of lengthy fermentation were measured. All components increased significantly with 20 hr sponge fermentation. Proteolytic and hydrogen ion activities also increased steadily. Lactic acid was predominantly produced, followed by acetic and smaller amounts of propionic, butyric, valeric, and isovaleric acids.

Saltine crackers are produced with a procedure that requires 25 hr fermentation. Johnson and Bailey (1), in a fundamental study of crackers involving investigation of physical and chemical changes in dough during fermentation, questioned the necessity of prolonged sponge fermentation that degrades gluten proteins and saturates dough with carbon dioxide. As appreciable dry matter is lost during fermentation, they suggested that other, less wasteful, means might cause the same changes that lengthy fermentation does, and proposed that further research be directed toward a procedure to degrade flour proteins with suitable proteolytic enzymes. To a limited extent, proteolytic enzymes already are used to improve cracker quality and flavor. Literature on cause and control of flavor of crackers is scant.

Many others have attempted to deal with it, but few references in the literature treat the problem in a scientific manner. The investigation reported here was to study chemical changes related to flavor precursor components (organic and amino acids) during 25 hr fermentation.

MATERIALS AND METHODS

For cracker sponge-and-dough preparation, this commercial formula was used:

Ingredients	*Sponge* *lb*	*Dough* *lb*
Soft red winter wheat flour	65.0	35.0 (25 wheat flour, 10 rye)
Water	22.0	...
Yeast	0.40	...
Protease[a]	One tablet/100 lb flour [62,000 hemoglobin (Hb units)]	...
Lard	11.0	...
Salt	...	1.80
Malt (nondiastatic)	...	1.40
Sodium bicarbonate (soda)	...	0.45

[a]Fungal protease tablets, Pennwalt Food Industries, Dallas, TX.

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A soft red winter wheat flour containing 8.7% protein was used for crackers. The sponge was mixed 3 min at low speed and fermented 20 hr at 27° C and 90% rh. The sponge was mixed with the remaining ingredients 5 min at low speed and the dough fermented 5 hr at 27° C and 90% rh. The fermentation troughs used in this experiment were biologically active having a thin film of dough leftovers from the previous batches on the sides of the troughs. Doughs were taken for analysis after 5, 10, 15, 20, and 25 hr fermentation.

Amino Acid Analysis

A 100-g portion of sponge or dough was extracted with 70% ethanol, centrifuged, deproteinized with 1% picric acid, and recentrifuged. Excess picric acid was removed by passing the extract through an anion-exchange resin (Dowex 2-X, chloride form). The effluent was evaporated to 10 ml in a rotary evaporator. The pH was brought to 7–8 with 1*N* NaOH and the sample was let stand 4 hr at room temperature to convert cysteine to cystine. The pH was adjusted to 2.2 with 1*N* HCl and was diluted to 50 ml with a citrate buffer. Total soluble nitrogen of all prepared samples was determined (3). Amino acids were analyzed with a Beckman amino acid autoanalyzer (Model 120B). A known standard mixture was used in calculations and results were expressed in moles and mg amino acid/100 g dough (dry basis).

Primary Amino Groups

Primary amino groups were determined using 1 ml of the deproteinized extract and the procedure of Satake *et al.* (4). A standard curve was established for the reaction with glycine, and results were expressed in micromoles (μmol) of primary amino groups equivalent to μmol of glycine.

Peptides

Peptides were determined quantitatively by the biuret reaction (5). A standard curve was established for the reaction with glycylglycylglycine and results were expressed in μmol of primary amino groups equivalent to μmol of glycylglycylglycine.

Proteolytic Activity

The modified Ayre-Anderson procedure (6) was used to determine proteolytic activity of cracker sponge and dough. Samples were lyophilized at 60° C, ground finely, and stored until analyzed.

Short-Chain (C_2–C_5) Free Fatty Acids and Their Salts

Cracker sponge or dough (500 g) was extracted four times: twice with acetone, centrifuged, then with aqueous acetone (80 and 60% v/v, respectively), and recentrifuged. Supernatants were collected and combined. Volume was about 3–4 liters. The extracts were distilled at 56° C under slightly reduced pressure. Distillates were collected in two cold traps of ice cold water and Dry Ice-ethanol. Ten milliliters of 2*N* NaOH was placed into the first trap to convert the volatile organic acids to their sodium salts. After about 90% of the extract was distilled, the pH was adjusted to 2.0 by 1*N* H_2SO_4 and 200 ml of distilled water added. Distillation continued to almost dryness. Distillates of the two traps were combined (pH >7.0) and concentrated at 45° C on a rotary evaporator

to 40–50 ml. To retain the organic acids, this concentrate was passed through basic anion-exchange resins (Dowex-1 chloride form). Organic acid was recovered with 1*N* HCl. Effluent was neutralized to pH 7–8 with 2*N* NaOH and evaporated to the least possible volume without precipitation.

The efficiency of extracting organic acids by acetone was established by using labeled sodium butyrate (New England Nuclear, 575 Albany St., Boston, MA). A solution of 2.5 ml ethanol containing 14.8 mg labeled sodium butyrate with activity equivalent to 0.25 milli-Curie (mCi) (1 mCi gives 5×10^9 counts/min, cpm) was diluted (0.2 ml to 100 ml). The quenching effect of acetone was studied by adding 0.1, 0.2, 0.4, 0.6, 0.8 ml of acetone to 0.5 ml of the diluted labeled sodium butyrate. Counts per minute were measured with a Beckman LS 200-B Scintillator. The results showed that acetone significantly quenched labeled sodium butyrate. In subsequent measurements of extraction efficiency, the extracts were diluted 20 times with distilled water before counting, to eliminate as much of the quenching effect as possible. The relation between cpm and labeled sodium butyrate concentration was linear.

Fifty milliliters of diluted, labeled sodium butyrate was thoroughly mixed with 150 g flour and 20 g shortening and then extracted in a Waring Blendor twice with acetone. After each mixing, the suspension was allowed to precipitate, was decanted, and the volumes of supernatants were measured separately and designated as V_1 and V_2. The residue was then extracted by 80% aqueous acetone, centrifuged, followed by a fourth extraction with 60% aqueous acetone and recentrifuging. Extract volumes were designated V_3 and V_4, respectively. Counts per minute were measured in a sample of each extraction and the extraction percentage was calculated. First, second, third, and fourth extractions were 24.83, 23.98, 45.36, and 3.23%, respectively, for a total of 97.4% of the added labeled sodium butyrate.

Organic acids were separated with a Barber-Coleman gas chromatograph equipped with a flame-ionization detector. The column was a U-shaped glass column (3 ft × 3 mm) packed with 60–80-mesh Carbopack A sp 1000. Extracts of cracker doughs were acidified with 1*N* H_2SO_4 to pH 2, and 6 to 8 μl were injected into the column. Carrier gas (20 ml/min) was used with an injector temperature of 180°C and detector at 300°C. A temperature programmer was used, starting

TABLE I
Effect of Fermentation on Total Soluble Nitrogen, Peptides, and Primary Amine on Cracker Sponge and Dough

	Fermentation Time, hr					
	0[a]	5[a]	10[a]	15[a]	20[a]	25[b]
Total soluble nitrogen[c]	17	14	31	46	51	60
Peptides[d]	75	553	735	435	623	637
Primary amines[d]	206	275	177	309	505	1001

[a]Sponge.
[b]Dough.
[c]mg/100 g dough, dry basis.
[d]μmol/100 g dough, dry basis.

TABLE II
Effect of Fermentation Time on Free Amino Acid Content of Saltine Cracker Sponge and Dough

Amino Acid	Fermentation Time, hr						
	0[a]	5[a]	10[a]	15[a]	20[a]	25[b]	Change after 25 hr
	μmol/100 g, dry basis						%
Monoamino-monocarboxylic acid							
Glycine	7.97	21.99	14.54	20.74	25.31	65.07	+ 715
Alanine	27.29	59.73	11.82	60.02	93.73	126.40	+ 363
Valine	14.46	11.44	3.71	11.40	22.14	55.35	+ 285
Leucine	8.41	17.68	trace	16.23	34.31	54.10	+ 545
Isoleucine	8.41	6.24	1.44	5.39	12.21	35.53	+ 325
Dicarboxylic acid							
Aspartic acid	16.22	7.28	3.86	7.67	7.63	99.00	+ 510
Glutamic acid	7.81	26.74	15.00	27.37	38.26	121.16	+1450
Secondary amino acid							
Proline	8.85	41.01	15.83	42.18	49.62	81.99	+ 825

Basic amino acid							
Lysine	10.26	15.60	10.00	23.89	29.96	58.59	+ 471
Arginine	10.91	16.64	11.67	15.52	19.85	28.68	+ 163
Histidine	1.18	2.90	1.74	2.72	3.16	12.96	+1017
Ammonia	106.41	128.97	88.87	154.82	95.43	207.60	+ 88
Aromatic amino acid							
Tyrosine	2.51	6.09	trace	4.57	9.16	15.96	+ 542
Phenylalanine	5.61	8.47	trace	6.27	15.27	26.68	+ 374
Sulfur-containing acid							
Cystine	2.80	3.27	1.74	1.15	6.39	16.78	+ 500
Methionine	1.92	3.12	trace	3.63	6.87	12.47	+ 545
Hydroxy amino acid							
Serine	26.11	25.70	13.51	23.74	32.06	152.82	+ 486
Threonine	5.01	8.32	5.07	7.44	7.63	39.12	+ 677

[a]Sponge.
[b]Dough.

at 115°C for 6 min and going up 5°C/min to 140°C. Aqueous acid standards were chromatographed to establish relative retention times and peak areas for each acid containing 0.1% acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. Results were expressed as mg and μmol organic acid/100 g cracker dough (dry basis).

Lactic Acid

A 10-g sample of sponge or dough was mixed with about 40 ml distilled water in an Osterizer for 10 min. To the mixture, 6 ml 1*N* H_2SO_4 and 5 ml 20% phosphotungstic acid were added and diluted to 100 ml. After being shaken and filtered, 50 ml aliquot was extracted with ether in a liquid extractor (8). Barker and Summerson's colorimetric method (9) was used to determine lactic acid with hydroxydiphenyl for final color development. A standard curve was established with calcium lactate. Results were expressed in mg lactic acid/100 g dough (dry basis).

RESULTS AND DISCUSSION

Changes in Concentration of Peptides, Primary Amino Groups, and Free Amino Acids during Fermentation

Effects of fermentation on total soluble nitrogen peptides and amino groups of cracker sponges and dough are shown in Table I. Fermentation caused a steady increase in concentration of all the compounds or groups. The fermentation process, mediated by yeast and bacterial enzymes, seemed to induce a dynamic change in quantity as well as type of primary amino groups in sponge and dough. The primary amino content increased only slightly during the first 5 hr of fermentation, probably because of little yeast and bacteria activity (lag phase). Proteolytic activity was relatively high, then decreased after the 10th hr (high yeast and bacterial activity, log phase), followed by a sharp increase. Such dynamic changes in primary amino groups may increase the reactivity of primary amino groups capable of reacting with reducing sugars during baking. Increased ethanol-soluble nitrogen suggests large changes in physical dough properties mainly from proteolytic activity.

Changes in Free Amino Acids during Fermentation

Data in Table II show total free amino acid content increased during the first 5 hr of fermentation, which results from low yeast and bacterial metabolism. However, total amino acid decreased markedly after 10 hr of fermentation, which suggested extremely high metabolic activity of yeast and bacteria and supports the findings of Morimoto (10) and Micka (11). During the last 10 hr of fermentation, the amino acid content again increased; consequently, microorganism activity steadily decreased. After 25 hr fermentation, total free amino acids, particularly glutamic acid, increased markedly. Ammonia increased least during fermentation (88%), which suggested that ammonia was the most suitable nitrogen metabolite for microorganisms during fermentation.

Protease Activities

One characteristic of the saltine cracker formula is an extremely high level of fungal protease (62,000 Hb units/100 lb flour), to condition the dough and to

allow proper baking development of the crackers. Proteases released by microorganisms are less significant than those provided by fungal protease supplement. Proteolytic activity in the sponge increases as pH is lowered.

Morimoto (10), who measured proteolytic activities of soda-cracker sponge and dough reported that the activities did not change significantly during fermentation. By the method he used, the role of hydrogen-ion activity was neglected. With buffer solution, the pH of the sample was adjusted to the corresponding pH of sponge or dough that had been sampled. The results (Table III) show that, as pH of the sponge decreased, protease activity increased. Protease activity increased almost ten times after 20 hr of fermentation. Adding soda drastically decreased protease activity equal to 5 hr sponge fermentation. The protease activity then increased slightly during 5 hr of dough fermentation.

The results explain variations in total amino acid contents. During the first 5 hr of fermentation, yeast and bacteria are less metabolically active (lag phase). With a steady increase in proteolytic activity, the free amino acid content of the sponge increased. After 10 hr, yeast and bacteria, as reported by Morimoto (10) and Micka (11), are at their peak of metabolic activity and free amino acid content of sponge is drastically decreased. As the sponge fermentation continues, metabolic activities of yeast and bacteria remain essentially constant (stationary

TABLE III
Effect of Fermentation Time on Protease and Hydrogen Ion Activities

	Fermentation Time, hr						
	0[a]	5[a]	10[a]	15[a]	20[a]	20[b]	25[b]
Protease activity, Hb units/100 g	126	222	540	686	1242	209	242
pH	5.6	5.4	4.6	4.1	4.0	6.9	6.7

[a]Sponge.
[b]Dough.

TABLE IV
Effect of Fermentation on Organic Acids or Saltine Cracker Sponge and Dough

Fatty Acids	Fermentation Time, hr					
	0[a]	5[a]	10[a]	15[a]	20[a]	25[b]
	μmol/100 g, dry basis					
Acetic acid	35.2	69.8	147.2	160.2	177.7	185.2
Propionic acid	14.2	22.3	28.4	31.2	35.4	38.4
Isobutyric acid	10.3	19.8	25.7	29.8	35.2	42.2
n-Butyric acid	10.6	11.4	15.3	15.8	16.3	19.4
Isovaleric acid	7.3	11.9	19.8	22.2	23.5	27.2
n-Valeric acid	8.9	12.0	16.3	17.6	18.2	20.2
Lactic acid	750	970	2260	4350	4820	4890

[a]Sponge.
[b]Dough.

phase), protease activity increases greatly, and total free amino acids increase. After soda was added, microorganism metabolism was retarded (low production of acids) but protease activity was still relatively high so total free amino acid content of cracker sponge and dough reached maximum after 25 hr fermentation.

Short-Chain Fatty Acids and Their Salts

Amounts of free fatty acids (C_2–C_5) from soda cracker sponge and dough were identified and quantitatively measured by comparing their retention time and areas under peaks with those of a standard solution. Results (Table IV) indicate increases of all free fatty acids and their salts. However, the greatest rate of increase was between the 5th and 10th hr of fermentation—supposedly the time of high microorganism activities.

Lactic Acid

Amounts of lactic acid and its salts in cracker sponge and dough are also shown in Table IV. As fermentation proceeded, lactic acid production increased due to continuous bacteria and yeast activity. Although free fatty acids and lactic acid in cracker sponge and dough could originate from both fermentation and lipase activity, the main route probably is from α-keto acids supplied by glycolysis and transamination of amino acids. Free fatty acids can be formed by oxidation of aldehydes resulting from decarboxylation of keto acids. Then the content of free amino acids and sugars available in dough systems would affect acid formation.

CONCLUSION

Extending fermentation time of cracker sponge and dough markedly increased soluble nitrogen, primary amine groups, and free amino acids, all of which are related to the final flavor of saltine cracker. Highest metabolic activity was after 10 hr of fermentation.

Similar compositions of fatty acids at different fermentation stages of cracker dough were evident. The ways yeast and bacteria influence fatty acid composition of a fermented dough have not been clarified in detail. However, one may assume that fatty acids in yeast and bacteria cells can readily pass into the surrounding media.

The swelling of gluten protein and dough development in cracker sponge and dough may be caused by free fatty acids developing. We think that organic acids, including predominant lactic and acetic acids, which have high ionization constants, are the major substances involved in gluten development.

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