Nutritional Improvement of Corn Meal by Fermentation by
\textit{Bacillus licheniformis} and \textit{Enterobacter cloacae}^1

MARION L. FIELDS* and FU GEN YOA

Department of Food Science and Nutrition, University of Missouri-Columbia, Columbia, MO 65211

(Received for Publication November 23, 1988)

ABSTRACT

Fermentation of corn meal by \textit{Bacillus licheniformis} strain 6 significantly (P<0.05) increased the percent relative nutritive value (%RNV). Both \textit{B. licheniformis} strain 6 and \textit{Enterobacter cloacae} strain 18 significantly (P<0.05) increased the methionine and tryptophan content. Only \textit{E. cloacae} increased the lysine content. Both bacteria increased total folacin significantly (P<0.05). A pH of 7-8, a temperature of 35°C, and a fermentation time of 4-6 days resulted in higher yields of total folacin than other combinations of conditions. Mixed cultures of the two bacteria increased lysine, methionine, tryptophan, and total folacin (P<0.05) when compared to nonfermented corn meal.

People who consume corn as a major part of their diet may be deficient in certain amino acids such as lysine, tryptophan, and methionine. Vitamin such as folacin may also be deficient. Fermentation of corn meal offers a partial solution to the problem by selective fermentation where selected microorganisms may be used.

Murdock and Fields (15) studied the B-vitamins in natural lactic acid fermentation of corn meal. They found that after lactic acid fermentation, the total folacin content of corn meal doubled or tripled as compared to nonfermented corn meal. Industrial fermentations may improve the vitamin content even more than a natural lactic acid fermentation. Therefore, fermentation of corn meal may be one method of alleviating part of the folacin deficiency problem in developing countries.

Natural lactic acid fermentation of corn meal increased folacin content (10) and percent relative nutritive value (%RNV) (11,20). This may have been due to the naturally occurring microorganisms in the fermented corn meal. Only limited information has been reported on the production of folacin by specific microorganisms in fermented corn meal and their effect on other nutrients in the product. Since corn is deficient in lysine, methionine, and tryptophan, increasing their availability simultaneously with increasing the vitamin content is desirable. In preliminary studies, we found that \textit{B. licheniformis} strain 6 and \textit{E. cloacae} among 35 microorganisms isolated from fermented corn meal produced the highest levels of folacin and were, therefore, selected for further study (24). In these studies, folacin was measured by the microbiological method as described by Keagy (14).

The objectives of this research were to determine the influence of \textit{B. licheniformis} strain 6 and \textit{E. cloacae} strain 18 on %RNV and the availability of lysine, methionine, and tryptophan in corn meal, to determine the effect of pH, temperature, and fermentation time on production of total folacin, and lastly to produce total folacin under optimal conditions by \textit{B. licheniformis} strain 6 and \textit{E. cloacae} strain 18 in fermented corn meal.

MATERIALS AND METHODS

Preparation of nonfermented and fermented corn meal

Preparation of nonfermented and fermented corn meal followed the procedure of Chung and Fields (4). Corn was cleaned and ground through a 1-mm screen. A 1:4 slurry of the corn meal with tap water was made and steamed at 100°C for 15 min, and blended in a blender to break up clumps of starch to make a more homogenous slurry to use in the fermentations. The corn meal was dried at 52°C and reground again in a Wiley mill using a 1-mm screen.

Bacteria used in fermentations

\textit{B. licheniformis} strain 6 and \textit{E. cloacae} strain 18 were isolated from fermenting corn meal and identified by Dyer (7). These strains were selected for this study because they were the best producers of folacin and also producers of vitamin B$_{12}$, riboflavin, pantothenic acid (2), and vitamin B$_6$ (21).

Fermentation variables

Fermentation variables (lysine, methionine, and tryptophan content; %RNV; folacin content as influenced by pH, temperature, and time of fermentation of corn meal) were the same as used by Chung and Fields (4) for determining the production of riboflavin and vitamin B$_{12}$.

Determination of available lysine, tryptophan, and methionine

Samples for the determination of available lysine, trypto-
phosphorus, and methionine were prepared according to Ford (9). The microbiological method was used to analyze the availability of the amino acids, lysine, methionine, and tryptophan (5).

**Determination of %RNV**

A microbiological assay, using a ciliated protozoan *Tetrahymena pyriformis*, was used in measuring the %RNV (protein quality) of the nonfermented (control) and fermented corn meal (19).

**Vitamin determination**

The microbiological assay procedures for folacin were according to Keagy (14) and Difco (5). According to Keagy in the microbiological assay procedure, the term folacin refers to a microbiologically active folic acid when measured by the *Lactobacillus casei* procedure; values were calculated, therefore, as folacin and not folic acid. Folic conjugate in this research was chicken pancreas folic acid. Folic conjugate in this research was chicken pancreas.

**pH adjustments**

The pH of the 1:17 (w/v) slurry was adjusted by using 1 N HCl or 1 N NaOH and the corn meal was autoclaved at 121°C for 45 min. Samples were adjusted to pH 5, 6, 7, and 8. The fermented corn meal was dried at 52°C, reground again in a Wiley Mill using a 1-mm screen. The reground corn meal was stored at 4°C in the dark until needed.

**Effect of length of fermentation**

The influence of time on the production of folacin was determined each d for 6 d.

**Statistical analyses**

Analysis of variance (18) was used to determine if there were statistically significant differences among means for treatments and replications. When significant (P<0.05) differences were found, Duncan’s (6) new multiple range and multiple F tests were used to locate the means that differed. Standard deviations were also determined and are reported with the means.

### RESULTS AND DISCUSSION

**Relative nutritive value and available amino acids**

The mean and standard deviation of %RNV of fermented and nonfermented corn meal are presented in Table 1. The %RNV of corn meal fermented with only *B. licheniformis* strain 5 was significantly (P<0.05) higher than the other treatments (Table 1).

**TABLE 1. Means and standard deviations for percent relative nutritive value (%RNV) of nonfermented and fermented corn meal.**

<table>
<thead>
<tr>
<th>Corn meal</th>
<th>%RNV $^{1,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfermented (control)</td>
<td>84.6 ± 2.7$^b$</td>
</tr>
<tr>
<td>Fermented with <em>B. licheniformis</em> $^3$</td>
<td>92.2 ± 1.5$^c$</td>
</tr>
<tr>
<td>Fermented with <em>E. cloacae</em> $^4$</td>
<td>87.2 ± 1.3$^b$</td>
</tr>
<tr>
<td>Fermented with mixed culture $^5$</td>
<td>86.6 ± 1.4$^c$</td>
</tr>
</tbody>
</table>

$^1$N = 3. Where letters differ, means differ significantly (P<0.05) from each other.
$^2$Dry weight basis.
$^3$Fermented at pH 8.0, 35°C for 5 d.
$^4$Ratio cell number (1:1) of *B. licheniformis* and *E. cloacae* at pH 8.0, 35°C for 5 d.

bacteria involved in fermentation caused a difference in %RNV.

Available methionine and tryptophan of corn meal were increased (P<0.05) by fermentation with pure and mixed cultures of *B. licheniformis* strain 6 and *E. cloacae* strain 18 (Table 2). No significant difference was found in available lysine, between the control and corn meal fermented with *B. licheniformis* strain 6. However, available lysine of corn meal fermented with *E. cloacae* strain 18 and the mixed culture were significantly (P<0.05) increased (Table 2). Tongnual et al. (20) also found that available lysine, methionine, and tryptophan of corn meal and a corn-soybean mixture were significantly (P<0.05) increased by a natural lactic acid fermentation. The magnitude of the increase was 2 to 3 fold for lysine, methionine, and tryptophan. Similar results were obtained with other cereal grains (11,13,26). Hamad and Fields (11) reported that fermented wheat meal was significantly (P<0.01) increased in lysine over nonfermented wheat meal. Kazanas and Fields (13) found that a natural lactic acid fermentation of ground grain sorghum produced a significant (P<0.01) increase in available lysine/leucine, isoleucine, and methionine. Zamora and Fields (26) stated that the limiting amino acids

**TABLE 2. Means and standard deviations for available lysine, methionine, and tryptophan in nonfermented and fermented corn meal.**

<table>
<thead>
<tr>
<th>Amino acid (µg/100 g)</th>
<th>Lysine</th>
<th>Methionine</th>
<th>Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfermented (control)</td>
<td>66.4 ± 2.9$^c$</td>
<td>20.8 ± 2.3$^b$</td>
<td>10.4 ± 0.8$^b$</td>
</tr>
<tr>
<td>Fermented with <em>B. licheniformis</em> $^3$</td>
<td>53.5 ± 10.6$^a$</td>
<td>52.4 ± 15.5$^b$</td>
<td>20.3 ± 4.7$^b$</td>
</tr>
<tr>
<td>Fermented with <em>E. cloacae</em> $^4$</td>
<td>194.2 ± 8.3$^a$</td>
<td>118.1 ± 15.8$^a$</td>
<td>43.0 ± 2.9$^a$</td>
</tr>
<tr>
<td>Fermented with mixed culture $^5$</td>
<td>101.2 ± 18.2$^b$</td>
<td>80.3 ± 5.2$^b$</td>
<td>23.6 ± 1.7$^b$</td>
</tr>
</tbody>
</table>

$^1$N = 4. Where letters differ within a column, means differ significantly (P<0.05) from each other.
$^2$Dry weight basis.
$^3$Fermented at pH 8.0, 35°C for 5 d.
$^4$Ratio cell number (1:1) of *B. licheniformis* and *E. cloacae* at pH 8.0, 35°C for 5 d.
methionine, isoleucine, and tryptophan increased significantly (P<0.05) in chickpeas, and the methionine and isoleucine content increased significantly (P<0.05) in corn meal after fermentation. These data show that fermentation can improve the amino acid balance and thereby improve the nutritional value of corn meal.

Effect of initial pH on total folacin
The initial pH of the corn meal influenced the production of total folacin. The highest yields of total folacin were obtained at an initial pH 7 or 8 for both E. licheniformis strain 6 and E. cloacae (Table 3); therefore a pH of 7 or 8 should be used to stimulate total folacin production.

Effect of temperature on total folacin
The quantities of total folacin produced by B. licheniformis strain 6 and E. cloacae strain 18 were a pH of 7-8, a temperature of 35°C, and a fermentation time of 4-6 d. The optimal conditions for both strains of bacteria were a pH of 7-8, a temperature of 35°C, and a fermentation time of 4-6 d. Murdock and Fields (15) found no significant difference in production of total folacin when fermentation of corn meal with pure and mixed cultures of both microorganisms was carried out by the presence of a natural microflora. The lack of agreement in findings for the two studies may be due to the fact that pure cultures of B. licheniformis strain 6 and E. cloacae strain 18 were used in the present investigation.

Effect of length of fermentation
Data illustrating the effect of fermentation time on the production of total folacin by B. licheniformis strain 6 and E. cloacae strain 18 are presented in Table 5. Increasing the length of fermentation resulted in higher production of total folacin in 4 d of natural lactic acid fermentation of corn meal carried out by the natural microflora. The lack of agreement in findings for the two studies may be due to the fact that pure cultures of B. licheniformis strain 6 and E. cloacae strain 18 were used in the present investigation.

Production of total folacin under optimal conditions
The optimal conditions for both strains of bacteria were a pH of 7-8, a temperature of 35°C, and a fermentation time of 4-6 d resulted in high yields of total folacin. To maintain these parameters, pure culture must be used to maintain these conditions.

The quantities of total folacin produced by B. licheniformis strain 6 and E. cloacae strain 18 are listed in Table 6. The total folacin of corn meal fermented with pure and mixed cultures of both microorganisms was significantly (P<0.05) higher than in nonfermented corn meal. The total folacin in corn meal fermented with E. cloacae strain 18 was significantly (P<0.05) higher than in nonfermented corn meal. The total folacin present in nonfermented corn meal might stimulate or inhibit the production of total folacin by microorganisms during fermentation. However, according to the findings of Van Lanen and Tanner (22), the presence of a vitamin does not affect its synthesis. This helps to explain why a corn meal, which already contained sufficient vitamins for microbial growth, did not inhibit the synthesis of total folacin, thus allowing the vitamin to increase in concentration with time.

The lack of total folacin causes one of the most com-

---

**TABLE 3. Means and standard deviations for total folacin produced by B. licheniformis strain 6 and E. cloacae strain 18 in corn meal at different initial pH values.**

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Total folacin (µg/100 g)</th>
<th>B. licheniformis</th>
<th>E. cloacae</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>57.6 ± 4.35b</td>
<td>45.7 ± 11.26c</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>57.1 ± 5.11b</td>
<td>58.0 ± 9.24b</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>71.3 ± 6.24b</td>
<td>85.7 ± 17.46c</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>78.7 ± 13.81c</td>
<td>86.1 ± 17.88c</td>
<td></td>
</tr>
</tbody>
</table>

1N = 4. Where letters differ within a column, means differ significantly (P<0.05) from each other.
2Dry weight basis.
3Incubated at 35°C for 4 d.

**TABLE 4. Means and standard deviations for total folacin produced by B. licheniformis strain 6 and E. cloacae strain 18 in corn meal at different temperatures.**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>B. licheniformis</th>
<th>E. cloacae</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>58.6 ± 3.3b</td>
<td>75.0 ± 9.6a</td>
</tr>
<tr>
<td>35</td>
<td>61.6 ± 4.2a</td>
<td>91.4 ± 8.7a</td>
</tr>
<tr>
<td>40</td>
<td>59.9 ± 3.8b</td>
<td>94.1 ± 29.4a</td>
</tr>
<tr>
<td>45</td>
<td>53.8 ± 2.1b</td>
<td>92.4 ± 16.3a</td>
</tr>
</tbody>
</table>

1N = 4. Where letters differ within a column, means differ significantly (P<0.05) from each other.
2Dry weight basis.
3Incubated at pH 8 for 4 d.

**TABLE 5. Means and standard deviations for total folacin produced by B. licheniformis strain 6 and E. cloacae strain 18 in corn meal at different times.**

<table>
<thead>
<tr>
<th>Fermentation time (d)</th>
<th>Total folacin (µg/100 g)</th>
<th>B. licheniformis</th>
<th>E. cloacae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.0 ± 1.9b</td>
<td>24.5 ± 3.0a</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31.3 ± 1.8b</td>
<td>42.8 ± 3.6a</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36.8 ± 3.5b</td>
<td>47.6 ± 4.0b</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>43.4 ± 5.4bc</td>
<td>56.8 ± 3.7b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>47.6 ± 9.8b</td>
<td>63.6 ± 4.8b</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>54.3 ± 10.1b</td>
<td>61.5 ± 5.9b</td>
<td></td>
</tr>
</tbody>
</table>

1N = 4. Where letters differ within a column, means differ significantly (P<0.05) from each other.
2Dry weight basis.
3Incubated at pH 8 and 35°C.
TABLE 6. Means and standard deviations for total folacin produced under optimal conditions for fermentation of corn meal by \(B. \) licheniformis strain 6 and \(E. \) cloacae strain 18.

<table>
<thead>
<tr>
<th>Fermentation of</th>
<th>Total folacin ((\mu g/100 , g))</th>
<th>Total$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfermented</td>
<td>25.3 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>(B. ) licheniformis</td>
<td>65.6 ± 8.12</td>
<td></td>
</tr>
<tr>
<td>(E. ) cloacae</td>
<td>108.3 ± 14.97$^1$</td>
<td></td>
</tr>
<tr>
<td>Mixed culture</td>
<td>78.1 ± 9.52$^2$</td>
<td></td>
</tr>
</tbody>
</table>

$^1N = 4$. Where letters differ within a column, means differ significantly (P<0.05) from each other.

$^2$Dry weight basis, At 35°C, pH 8 for 5 d.

$^3$Conjugase treated (chicken pancreas).

mon deficiencies encountered in clinical practice of human beings. Megaloblastic anemia, caused by folacin deficiency is a major world health problem (12). The World Health Organization (23) reported that megaloblastic anemia was diagnosed in 2.5 to 5.0% of the pregnant women in developed countries and in developing countries, but total folacin deficiency occurred even in children, men, and non-pregnant women. Fermentation of corn meal just to produce folacin would be justified from a health point of view, but in addition we know that these two bacteria also produce vitamin B\(_{12}\) and riboflavin (7) and pantothenic acid (2).

The highest amount of total folacin found in this research was 108.3 \(\mu g/100 \, g\). This value exceeds the amount reported by Bogert, Briggs, and Calloway (1) for some of the foods that they listed as good sources of folacin. Their values for some of the foods were as follows (\(\mu g/100 \, g\)): beet greens, 60; filbert nuts, 65; kale, 70; mustard greens, 60; spinach, 75; and walnuts, 75.

But as important as vitamins are to nutrition, the data in this study show that fermentation by \(B. \) licheniformis strain 6 produced a better amino acid balance, and both bacteria produced significant increases in lysine, methionine, and tryptophan deficient amino acids of corn.

Research on the practical uses of fermented corn meal and other cereal meals to increase the nutrition of man and animals needs further work especially on the possible use of large commercial equipment. One can say, however, that when the nutrition of a food item is increased, nutritional benefits to consumers occur. This research reported here is part of a larger study aimed at deriving the most benefits from the least number of microorganisms. The multiple nutritional improvements brought about by these bacteria make them important in the upgrading of corn meal by fermentation.

REFERENCES


16. Nanson, N. J., and M. L. Fields. 1982. Effect of \(Lactobacillus\) \(fermentum\), \(Bacillus\) \( sublicus\), \(Bacillus\) \(cereus\), and \(Pseudomonas\) \(maltophilia\) singly and in combination on the relative nutritive values of fermented corn meal. J. Food Sci. 47:1294-1295.


JOURNAL OF FOOD PROTECTION. VOL. 53. MARCH 1990