Effect of Soy Proteins on the Growth of *Clostridium perfringens*¹

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Proteins that are used to fabricate imitation foods such as synthetic meats were evaluated for stimulative or inhibitory effects on the growth of *Clostridium perfringens*. Growth rate and extent were measured in thioglycolate medium without dextrose. This liquid medium contains Trypticase (BBL) which served as the protein control. For comparison, various soy proteins, synthetic meats, beef, turkey, sodium caseinate, and combinations of each were substituted for Trypticase. Meat loaf systems were also employed to determine the effects of protein additives to meat under actual meat loaf conditions. Growth of *C. perfringens* type A, strain S40, was measured in the respective media at 45°C at a pH of 7.0 and an Eh of below –300 mv. Viable populations were enumerated by agar plate techniques on Trypticase-sulfite-yeast-citrate-agar incubated anaerobically (90% N₂-10% CO₂) for 18 hr at 35°C. When compared to Trypticase, some soy proteins had stimulative effects on the growth of *C. perfringens*, whereas sodium caseinate and some soy proteins were inhibitory. In liquid medium in which meat or soy meat was the source of protein, there was a marked stimulation by beef, chicken, and soy beef. Soy chicken supported growth at a rate less than observed with Trypticase. Under actual meat loaf conditions, the addition of soy meat or protein additives to beef did not affect the growth of *C. perfringens*. The addition of protein additives to turkey meat loaves significantly enhanced the rate of growth of *C. perfringens*. The stimulative effects of some soy proteins are significant in relation to control of foodborne disease.

*Clostridium perfringens* foodborne disease continues to be a major concern to the food industry (2), especially that portion associated with cooked meat products which may be handled Improperly and will support growth of *C. perfringens*. Increases in commercial production of synthetic meats and increases in protein supplementation of natural meat systems directed our interest into this area. There is little current literature to determine what significance this addition of proteins has on the potential growth of *C. perfringens*. Our objective has been to determine whether protein fractions from sources such as soy protein enhance, retard, or do not affect the growth of *C. perfringens*. Protein fractions that are used to fabricate imitation foods such as synthetic meats and to supplement standard meat formulations were evaluated. A preliminary report of these findings has been presented (F. F. Busta, D. J. Schroder, and M. W. Ewers, Bacteriol. Proc., p. 2, 1970).

MATERIALS AND METHODS

*C. perfringens* strain S40 was obtained from H. E. Hall (National Center for Urban and Industrial Health, Cincinnati, Ohio). Stock cultures were maintained in Cooked Meat Medium (BBL) at room temperature. Inoculation cultures were grown at 45°C for 18 hr in Thioglycollate Medium without added dextrose (BBL). The culture inoculum was centrifuged at 4,080 × g for 10 min and decanted. The pellet was dispersed in 200 ml of sterile 6.25 × 10⁻⁴ M phosphate buffer (pH 7.0) and centrifuged. The pellet was dispersed in buffer, and the procedure was repeated twice. The final pellet was resuspended in 20 ml of phosphate buffer and serially diluted to obtain the proper inoculum. Microscopic examination indicated negligible clumping. All procedures were carried out under aseptic conditions.

The generation time was calculated by the formula

\[ G_t = \frac{t}{n} = \frac{t}{3.3 \log_6 B/B} \]

where the generation time \( G_t \) is equal to the time, \( t \) (the time elapsed between measurement of \( B \), the initial population, and \( b \), the final population), divided by the number of generations, \( n \) (number of generations being equal to 3.3 \( \log_6 B/B \)). (3). Both \( B \) and \( b \) were measured during the logarithmic phase of growth.

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Apparatus. The growth vessels consisted of 500-ml beakers (Pyrex Berzelius tall form without spout) fitted with a no. 14 rubber stopper carrying a combination pH electrode, $E_b$ electrode, and a syringe for obtaining samples. The $pH$ was measured with a combination-triple purpose glass electrode (Corning 476051). $E_b$ was measured with a platinum inlay electrode (Corning 46060) and the reference calomel of the combination glass electrode. The $pH$ and $E_b$ were measured with an expanded scale research Corning $pH$ meter (model 12). The medium was stirred by a Corning LM-S stirrer (Corning Glass Works, Medford, Mass.).

The stirring assembly and the inoculation needle were autoclaved in the medium at 121 C for 15 min. The platinum and glass electrodes were sterilized by immersion in a 0.05% sodium hypochlorite solution for 20 min followed by rinsing in sterile distilled water before inserting in the growth medium.

Studies showing the effect of added protein to meat loaf were carried out by the methods previously outlined (D. J. Schroder and F. F. Busta, J. Milk Food Technol., in press).

Growth media. Various test proteins were substituted for Trypticase (BBL) in the thioglycolate medium without added dextrose (Table 1) and autoclaved at 121 C for 15 min. The soy protein and sodium caseinate protein concentrations were adjusted to contain the same protein concentration (w/w) as the Trypticase control. Manufacturers' specifications were used for protein concentrations. This medium was selected as the growth medium because no sugar was present and the sole protein or peptide source was Trypticase. The meat loaf medium has been previously described (Schroder and Busta, J. Milk Food Technol., in press). One part protein additive was added to five parts meat unless stated otherwise.

The growth media were steamed before inoculation to ensure a low oxidation-reduction potential. The $pH$ of each medium was adjusted to 7.0 ± 0.1. The growth vessels were placed in a constant-temperature water bath maintained at 45 C. The $E_b$ and $pH$ were monitored throughout the incubation. Samples were taken at appropriate times to determine the extent of the lag, the log, and final stationary growth phase. These were serially diluted in 0.1% peptone dilution blanks, and the viable cell populations were estimated by plate count in triplicate with freshly prepared Trypticase-sulfite-yeast-citrate-agar. This medium contained Trypticase (BBL), 15.0 g; yeast extract (BBL), 10.0 g; sodium sulfite (Allied Chemical), 0.5 g; iron citrate (Mallinckrodt), 0.5 g; agar (Difco), 13.9 g; water (deionized), 1,000.0 ml; final pH 7.0 ± 0.1. This is similar to SPS agar (1) with the selective agents deleted. The agar plates were overlaid and incubated anaerobically at 35 C for 18 hr in an atmosphere of 90% N₂ and 10% CO₂.

The t test was employed to test the significance of differences between growth in the presence of the soybean proteins, sodium caseinate, beef, turkey, or combinations and growth with the standard Trypticase or meat control (4).

RESULTS

The reproducibility in population, $E_b$, and $pH$ in three trials with C. perfringens S40 in thioglycolate medium is demonstrated in Fig. 1. Initiating growth at several levels had little effect on the apparent growth rate, maximum population, or generation time. The relative $pH$ and $E_b$ values for each of the three trials are represented in the lower portion of the figure. The $pH$ remained between 7.5 and 7.0, and the $E_b$ generally registered below −300 mv down to −500 mv.

The influence of eight soy protein supplements and one sodium caseinate protein supplement on the growth of C. perfringens was tested. Generation times of two trials are presented in Table 2. The two right columns of the table relate the ratio of generation time in the protein under test to that observed in Trypticase during the same trial. Isolated soy protein batch 1, whipping protein, and soy glycamin resulted in significantly shorter generation times in comparison to Trypticase (t test, 5% level). The soy flour brand B, soy protein concentrate, textured soy flour, and isolated soy protein batch 2 sources resulted in generation times similar to Trypticase. Sodium caseinate and soy flour brand D were significantly inhibitory to the growth of C. perfringens (5% level).

Data presented in Fig. 2 show that, when isolated soy protein batch 1 was substituted for Trypticase, the generation time of C. perfringens S40 was reduced by more than one-third and the maximum extent of growth was increased 10-fold.

Table 3 relates data obtained in eight trials comparing isolated soy protein batches 1 and 2 to Trypticase as the protein or peptide source in

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**Table 1. Growth medium for Clostridium perfringens (final pH 7.0)**

| Constituent                          | Amt (g) |
|-------------------------------------|---------|
| Trypticase or substitution by       |         |
| proteins(*) (on a per cent protein  |         |
| basis) A, B, C, D, E, J, K, L       | 20.0    |
| Sodium chloride                     | 2.5     |
| Dipotassium phosphate               | 1.5     |
| Sodium thioglycollate               | 0.6     |
| L-Cystine                           | 0.4     |
| Sodium sulfite                      | 0.2     |
| Agar                                | 0.5     |
| Water                               | 1,000.0 |

* Proteins: A, soy protein concentrate; B, soy flour brand X; C, textured soy flour; D, soy flour brand Y; E, isolated soy protein; J, soy whipping protein; K, soy glycamin; and L, sodium caseinate.

* Measured in milliliters.
FIG. 1. Population, $E_h$ (in millivolts), and pH changes observed during incubation of Clostridium perfringens S40 in thioglycolate medium without glucose at 45°C.

Table 2. Growth rates of Clostridium perfringens in liquid media with various protein sources (Trypticase control)

| Sample                        | Generation time$^a$ (min) | Protein/Trypticase |
|-------------------------------|---------------------------|--------------------|
|                               | I     | II    | I     | II    |
| A (soy protein concentrate)   | 23.6  | 20.0  | 1.06  | 1.06  |
| B (soy flour brand B)         | 20.0  | 21.3  | 0.92  | 0.96  |
| C (textured soy flour)        | 23.9  | 21.4  | 1.07  | 0.99  |
| D (soy flour brand D)         | 22.3  | 23.1  | 1.30  | 1.16  |
| E (isolated soy protein batch 1) | 12.7  | 11.3  | 0.61  | 0.62  |
| F (isolated soy protein batch 2) | 18.5  | 19.4  | 0.97  | 1.01  |
| J (whipping protein) (enzyme modification) | 16.7  | 16.7  | 0.71  | 0.74  |
| K (soy glycycin)              | 16.4  | 19.2  | 0.82  | 0.86  |
| L (sodium caseinate)          | 42.0  | 53.7  | 2.40  | 2.50  |

$^a$ Generation time for Trypticase appears in parentheses.

$^b$ Significant difference at the 5% level from the Trypticase standard (t test).

In the case of batch 1, maximum populations of approximately $10^8$ were obtained in each of the four trials. In batch 1, all four trials of the isolated soy protein resulted in a higher population. The ratios of generation times in isolated soy protein to Trypticase ranged from 0.61 to 0.85 in the four trials. With batch 2 of the isolated soy protein, the growth was essentially the same in either medium.

Data in Table 4 show the influence of substituting beef, soy beef, or combinations of beef plus soy beef or beef plus textured soy flour for the Trypticase protein source in liquid media. Data on growth in turkey, soy chicken, or combinations of turkey plus soy chicken or turkey plus textured soy flour in liquid media are also shown in Table 4.

With Trypticase as the control, the use of beef or beef plus soy beef, soy beef alone, or beef plus textured soy flour as the sole source of protein
produced significantly shorter generation times. The use of turkey, soy chicken, and combinations of turkey and soy chicken or turkey plus textured soy flour produced generation times that were not significantly different from those observed with Trypticase.

Data used in Table 4 were reevaluated as shown in Table 5. These data show that soy beef or textured soy flour plus beef did not significantly influence growth of *C. perfringens* when compared to beef alone as the protein source.

Generation times observed with soy chicken or textured soy flour plus turkey were not significantly different from those observed in turkey alone (Table 5) when these were used as sole protein sources in liquid media. Note that growth rates in beef or soy beef were more rapid than in turkey or soy chicken.

Table 6 summarizes data on growth of *C. perfringens* under actual meat loaf conditions. The effects of the addition of soy beef, soy protein, and sodium caseinate to beef are shown as they relate to the beef control. Soy beef was used alone as a meat loaf but did not support growth to any extent. These data show no significant effect on growth by the additives to beef.

The data also show that there was a significant difference between beef and turkey and between beef and turkey plus Trypticase, i.e., growth in turkey and turkey plus Trypticase was slower than growth in the beef control. The addition of sodium caseinate and soy glycinin to turkey increased the growth rate of *C. perfringens* to the level observed in beef.

Under meat loaf conditions with turkey as the control, the addition of one part soy chicken to five parts turkey had no significant effect on the growth of *C. perfringens* (Table 6). The addition of a 1:1 mixture of soy chicken and 1:5 additions of isolated soy protein, sodium caseinate, Trypticase, or soy glycinin to turkey all significantly increased the growth rate in the turkey meat loaves. Soy chicken alone supported little or no growth. The $E_b$ remained at $+100$ to $+200$ mv, and there was slow death or no growth until about the 6th hr of incubation. Some growth did take place when the $E_b$ was reduced to approximately $-30$ to $-50$ mv. This is in contrast to the meat systems which quickly dropped the $E_b$ to $-300$ mv and reached $-500$ mv after 7 hr of incubation.

**DISCUSSION**

Stimulation of the growth of *C. perfringens* was observed with some soybean protein products when compared to Trypticase. These include an isolated soy protein. A basic difference between the products that cause stimulation and those that do not is the amount of refinement by the removal of a major portion of the nonprotein components. Manufacturers' literature states that isolated soy
proteins are over 92% protein and the whipping protein and soy glycinin are over 80% protein. The soy products that did not appear to stimulate growth ranged from 50 to 55% protein, with the exception of the soy protein concentrate which is approximately 70% protein. The processes involved in the concentration or isolation of the soy protein apparently improve it as a growth medium for *C. perfringens*. This could be due to the removal of some growth inhibitors or to some protein modification which could make the protein more available to the organism or to some change in availability of certain amino acids. The whipping protein has undergone some enzymatic modification which may effect some breakdown of the protein and facilitate easier uptake by the organism. Another factor may be the further concentration and isolation of the soy protein which in turn may affect the solubility of the soy protein and thus its availability to the organism.

This study has shown that, in the case of the isolated soy protein, some batches may have stimulative activity whereas others may not. This suggests that inadvertent changes in the process may modify the end product and cause a change in solubility or in some modification of the soy protein which may affect the growth of *C. perfringens*. Another possibility is that some factor may be added to the product to increase its functional properties or to prevent some undesired reactions.

Similarly, the converse may be applied to the soy flour brand D which caused some inhibition

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**Table 3. Growth of Clostridium perfringens in liquid media containing isolated soy protein or Trypticase**

| Determination | Log population/ml | Generation time G (min) | Ratio of isolated soy protein G/Trypticase G |
|---------------|-------------------|-------------------------|--------------------------------------------|
|               | Isolated soy protein | Trypticase | Isolated soy protein | Trypticase | Isolated soy protein | Trypticase |
| **Batch 1**   |                   |             |                      |                      |                      |
| Replicate 1   |                   |             |                      |                      |                      |
| Initial       | 1.00              | 0.95        | 12.7                 | 20.8                 | 0.61                 |
| Maximum       | 7.99              | 7.67        |                       |                      |                      |
| Replicate 2   |                   |             |                      |                      |                      |
| Initial       | 1.95              | 1.90        | 11.3                 | 18.2                 | 0.62                 |
| Maximum       | 8.30              | 7.34        |                       |                      |                      |
| Replicate 3   |                   |             |                      |                      |                      |
| Initial       | 3.20              | 3.63        | 17.3                 | 23.0                 | 0.75                 |
| Maximum       | 8.34              | 7.88        |                       |                      |                      |
| Replicate 4   |                   |             |                      |                      |                      |
| Initial       | 3.28              | 2.32        | 22.7                 | 26.8                 | 0.85                 |
| Maximum       | 7.93              | 7.60        |                       |                      |                      |
| **Batch 2**   |                   |             |                      |                      |                      |
| Replicate 1   |                   |             |                      |                      |                      |
| Initial       | 3.11              | 3.18        | 18.5                 | 19.2                 | 0.97                 |
| Maximum       | 7.75              | 7.30        |                       |                      |                      |
| Replicate 2   |                   |             |                      |                      |                      |
| Initial       | 2.94              | 3.08        | 19.4                 | 19.2                 | 1.01                 |
| Maximum       | 7.70              | 7.76        |                       |                      |                      |
| Replicate 3   |                   |             |                      |                      |                      |
| Initial       | 1.53              | 1.94        | 19.9                 | 19.8                 | 1.00                 |
| Maximum       | 7.30              | 7.18        |                       |                      |                      |
| Replicate 4   |                   |             |                      |                      |                      |
| Initial       | 2.27              | 2.21        | 19.9                 | 20.6                 | 0.97                 |
| Maximum       | 7.34              | 7.34        |                       |                      |                      |

**Table 4. Growth rates of Clostridium perfringens in liquid media containing meat, soy meat, and meat plus soy protein (Trypticase control)**

| Sample                  | Generation timea | Meat or soy meat/Trypticase |
|-------------------------|------------------|----------------------------|
|                         | I (min)          | II (min)                   | I (min) | II (min) |
| Beef 1                  | 14.3 (18.4)      | 13.4 (19.5)b               | 0.78    | 0.69     |
| Beef 2                  | 13.8 (18.3)b     | 17.3 (18.9)b               | 0.75    | 0.70     |
| Soy beef 1              | 16.5 (18.4)      | 13.6 (19.5)b               | 0.90    | 0.70     |
| Soy beef 2              | 18.3 (22.4)b     | 15.1 (18.9)b               | 0.82    | 0.80     |
| Beef and soy beef 1     | 13.0 (18.4)b     | 13.3 (19.5)b               | 0.71    | 0.68     |
| Beef and soy beef 2     | 13.4 (18.3)b     | 13.7 (18.9)b               | 0.73    | 0.72     |
| Beef and textured soy flour | 13.6 (21.8)b | 15.7 (18.9)b               | 0.62    | 0.83     |
| Turkey 1                | 19.5 (23.2)      | 13.1 (14.7)                | 0.84    | 0.89     |
| Soy chicken 1           | 18.6 (23.2)      | 26.0 (23.2)                | 0.80    | 1.12     |
| Soy chicken 2           | 23.8 (19.4)      | 17.5 (14.7)                | 1.22    | 1.19     |
| Turkey and soy chicken 1| 17.2 (19.2)      | 14.3 (14.7)                | 0.74    | 0.97     |
| Turkey and soy chicken 2| 18.4 (23.2)      | 17.7 (19.4)                | 0.79    | 0.96     |
| Turkey and textured soy flour | 20.7 (23.2) | 13.4 (14.7)                | 0.89    | 0.91     |

a Generation time for Trypticase appears in parentheses.

b Significant difference at the 5% level from the Trypticase standard (t test).
of the growth of *C. perfringens*. Sodium caseinate showed a major inhibition on the growth of the organism. Again protein availability and the presence of some inhibitory factors may play a part in its effects on growth.

The rate of growth of *C. perfringens* in liquid medium in which beef or soy was the sole protein source was more rapid than in Trypticase. This may be due to readily available growth factors in beef. Under meat loaf conditions, the rate of growth was rapid in beef and the additions of various amounts of soy, soy glycinin, isolated soy protein, and sodium caseinate had little effect on the growth rate. This indicates that beef

### Table 5. Growth rates of *Clostridium perfringens* in liquid media containing meat, soy meat, or soy protein (meat control)

| Sample | Generation time | Soy meat/meat |
|--------|-----------------|---------------|
|        | I (min) | II (min) | I  | II  |
| Beef as control<sup>a</sup> | | | | |
| Soybeef 1 | 16.5 (14.3) | 13.6 (13.4) | 1.15 | 1.01 |
| Soybeef 2 | 22.4 (13.8) | 15.1 (17.3) | 1.62 | 0.87 |
| Beef and soybeef 1 | 13.0 (14.3) | 13.3 (13.4) | 0.91 | 0.99 |
| Beef and soybeef 2 | 13.4 (13.8) | 13.7 (17.3) | 0.97 | 0.79 |
| Beef and textured soy flour | 13.6 (9.90) | 15.7 (17.3) | 1.37 | 0.91 |
| Turkey as control<sup>b</sup> | | | | |
| Soy chicken 1 | 18.6 (19.5) | 26.0 (14.2) | 0.95 | 1.83 |
| Soy chicken 2 | 23.8 (21.0) | 17.5 (13.1) | 1.13 | 1.34 |
| Turkey and soy chicken 1 | 18.4 (19.5) | 17.2 (14.2) | 0.94 | 1.21 |
| Turkey and soy chicken 2 | 17.7 (21.0) | 14.3 (13.1) | 0.84 | 1.09 |
| Turkey and textured soy flour | 20.7 (19.5) | 13.4 (13.1) | 1.06 | 1.02 |

<sup>a</sup> Generation time for beef appears in parentheses.

<sup>b</sup> Generation time for turkey appears in parentheses.

### Table 6. Growth rates of *Clostridium perfringens* in meat loaves containing meat, soy meat, and soy protein (meat control)

| Sample | Generation time | Soy meat/meat |
|--------|-----------------|---------------|
|        | I (min) | II (min) | I  | II  |
| Beef as control<sup>a</sup> | | | | |
| Beef-soy beef (5:1) | 11.4 (11.8) | 14.5 (15.2) | 0.97 | 0.95 |
| Beef-soy beef (1:1) | 14.5 (11.4) | 13.8 (15.2) | 1.27 | 0.91 |
| Beef and soy glycinin | 13.0 (11.8) | 13.1 (12.6) | 1.10 | 1.04 |
| Beef and promine | 14.9 (15.2) | 12.6 (12.6) | 0.98 | 1.00 |
| Beef and sodium caseinate | 13.1 (11.8) | 13.1 (15.2) | 1.11 | 0.86 |
| Soy bean alone | No growth | | | |
| Turkey | 16.6 (11.6)<sup>b</sup> | 12.4 (11.0)<sup>b</sup> | 1.43 | 1.13 |
| Turkey and Trypticase | 13.6 (11.6)<sup>b</sup> | 15.2 (11.0)<sup>b</sup> | 1.17 | 1.38 |
| Turkey and sodium caseinate | 14.0 (11.6) | 9.6 (11.0) | 1.21 | 0.87 |
| Turkey and soy glycinin | 10.3 (12.6) | 12.9 (12.6) | 0.82 | 1.02 |
| Turkey as control<sup>c</sup> | | | | |
| Turkey-soy chicken (5:1) | 14.2 (16.5) | 16.1 (17.6) | 0.86 | 0.91 |
| Turkey-soy chicken (1:1) | 14.9 (16.5)<sup>d</sup> | 14.0 (17.6)<sup>d</sup> | 0.90 | 0.80 |
| Turkey and promine | 12.5 (16.5)<sup>d</sup> | 11.1 (17.6)<sup>d</sup> | 0.76 | 0.63 |
| Turkey and sodium caseinate | 14.0 (16.5)<sup>d</sup> | 13.2 (17.6)<sup>d</sup> | 0.85 | 0.75 |
| Turkey and Trypticase | 13.6 (16.6)<sup>d</sup> | 19.5 (12.4)<sup>d</sup> | 0.82 | 0.77 |
| Turkey and soy glycinin | 10.3 (14.2)<sup>d</sup> | 12.9 (14.2)<sup>d</sup> | 0.72 | 0.91 |
| Soy chicken alone | No growth | | | |

<sup>a</sup> Generation time for beef appears in parentheses.

<sup>b</sup> Significant difference at the 5% level from the beef standard (*t* test).

<sup>c</sup> Generation time for turkey appears in parentheses.

<sup>d</sup> Significant difference at the 5% level from the turkey standard (*t* test).
is an excellent medium for the growth of *C. perfringens* and added protein sources have little effect. Soy beef alone as a meat loaf supported little or no growth.

Under meat loaf conditions, as in the liquid medium, the growth of *C. perfringens* was slower in turkey than it was in beef. The additions of soy chicken, isolated soy protein, sodium caseinate, Trypticase, and soy glycinin significantly stimulated the rate of growth of this organism in turkey meat loaves. The additions of sodium caseinate or soy glycinin to turkey brought the rate of growth to a level equivalent to that of beef.

As with meat loaves containing soy chicken alone, soy beef alone did not support any substantial growth of *C. perfringens*. *Eh* values in these meat loaves remained high throughout the 6-hr incubation period. These observations with soy meat loaves may be due to the physical nature of the meat loaf (i.e., somewhat more porous than regular meat loaves) or due to some other inadequacy of the soy meats.

In summary, these results show that certain soybean proteins were stimulative or inhibitory to growth of *C. perfringens*. Natural meat systems are highly stimulatory. Under actual meat loaf conditions, beef appears to be an excellent growth medium and the addition of soy meat, soybean, or other protein additives does not appear to affect its potential for fast growth of this organism. These observations are consistent with earlier findings (Schroder and Busta, J. Milk Food Technol., *in press*). This is not the case for turkey, in which protein additives did significantly enhance the growth of this organism. The stimulation may be due to the addition of certain factors which enhance the growth of *C. perfringens*, i.e., factors which are present in beef or soy protein and not in turkey.

The presence of stimulative or inhibitory factors in some soybean products or other proteins could be of public health concern and warrant their identification. Food processors fabricating synthetic meats or supplementing natural meats with protein preparations should be aware of their potential influence on growth of *C. perfringens*.

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