Behavior of *Escherichia coli* Bacteria in Whey Protein Concentrate and Corn Meal During Twin Screw Extrusion Processing at Different Temperatures

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Abstract

New studies on the development of low-temperature extruded value-added nutritional foods containing corn meal and whey protein isolate have been reported. However, information on the effect of extrusion treatment parameters on microbial safety of foods below 100°C is limited. In this study, we investigated the effect of extrusion treatments at 35°C, 55°C, 75°C, and 95°C on reduction of *E. coli* cell populations inoculated onto corn meal (CM) and whey protein concentrate (WPC80) at 8.8 log₆ CFU/g. Inactivation of *E. coli* bacteria on CM and WPC80 extruded at 35 and 55°C averaged 4.8 log₆, 6.9 log and 1.8 log₆, 4.3 log₆, respectively. Extrusion treatment at 75°C and above reduced *E. coli* (ATCC-25922) cells on CM to below detection (<20 CFU/g); but treatment at 95°C was needed to achieve a similar below detection (<20 CFU/g) for WPC80. The results of this study suggest that corn meal products extruded at 75°C or above, and whey protein isolate extruded at 95°C, will enhance the microbiological safety of the extrudates.

Keywords: Twin screw extruder; Inactivation; *Escherichia coli*; Corn; Whey protein

Introduction

Physical and chemical treatments are used in food processing to eliminate or at least reduce the presence of pathogenic and spoilage microorganisms in foods [1-3]. Functionalized healthy food ingredients have been developed using micro-texturing and micro structuring processes such as micro articulation, micro-shear, and extrusion texturization processes [4]. Food ingredients developed by this extrusion have improved texture with enhanced physical properties including ex-vivo or in-vivo functionality [5]. Texturization of proteins by extrusion processing occurs at temperatures ranging from 50 to 100°C at short residence times of approximately 2 min [6]. However, information of this processing treatment on the survival and viability loss of microbial organisms present on treated food items is limited.

Several studies on the use of extrusion processing to inactivate microbial populations in food ingredients have been reported [7-12]. Likimani et al. [13] proposed a methodology for determining the destruction of bacterial spores in a Brabender single-screw Plastic order extruder at different processing conditions. The authors used the average time at a temperature of 95°C to calculate the D value which was referred to as the extrusion D value or extrusion death rate. In that study, the authors also stated that destruction of spores in the extruder was a function of temperature and residence time at constant moisture levels (18%) for a corn-soybean (70%/30% [wt/wt]) mixture.

There are two major reactions that occur during extrusion cooking, (1) the extruded-starches are gelatinized and (2) proteins are denatured. For example, conversion of starch (gelatinization and melting) during extrusion cooking was reported to follow zero-order kinetics in limited water environments [14,15]. As the material flows in the extruder, the presence of thermal and shear forces causes the temperature of the starch granule to rise, causing it to melt and gelatinize when temperature reaches at or above 90°C for high moisture processing or for high shear, low moisture processing. Protein denaturation and starch gelatinization are the result of water content, pressure and shear in the evaporation process [16]. Starch gelatinization affects many extrude properties, such as water stability, digestibility, and expansion ratio. However, the extent of starch gelatinization by itself depends on starch type, particle size and the extrusion process parameters [17]. For example, for the waxy maize starch, granule destruction by mechanical forces can occur at much lower temperatures than those required for gelatinizing or melting the starch granule under conditions where no mechanical energy is applied [18]. Yam et al. [19] reported that shear energy alone was responsible for the conversion of corn meal at low temperatures. Starch gelatinization during extrusion is crucial because it affects feed digestibility, product expansion. Degradation of DNA into different fragments sizes in processed maize product by cooking or extrusion has been estimated using Quantitative Real-Time PCR [20]. Therefore, extrusion processing can affect enzymes and microorganisms present in contaminated corn meal or whey protein concentrate. There is limited information concerning the behavior of bacterial populations in corn meal and whey protein concentrate during extrusion treatments at 35°C to 95°C. The aim of our study was to investigate the effect of temperature on twin screw extrusion processing on the survival of inoculated *E. coli* populations in corn meal (CM) and whey protein concentrate (WPC80). Also the influence of pressure generated during extrusion processing on bacterial inactivation was investigated. Information derived from this study would point to appropriate HACCP protocols that would enhance the microbial safety of low-temperature extruded foods.

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Material and Methods

Materials

The standard coarse corn meal with a mean particle size of ~710 μm was used for this study and was purchased from (J.M. Swank Co., North Liberty, IA), and whey protein concentrate 80% protein, WPC80 (Davisco Foods International, Inc., Eden Prairie, MN).

Test strains and preparation of inocula

E. coli K-12 (ATCC 23716) from the U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center culture collection was used in this study. Cell culture was maintained on tryptic soy agar (TSA) at 4°C. Prior to use, the cells were inoculated by loop in tryptic soy broth (TSB; Remel, Inc., Lenexa, KS) and then incubated at 37°C for 16-18 h with shaking. A 1 ml cell aliquot was transferred to 4 different beakers, each containing 200 ml of TSB and incubated at 37°C for 24 h. The cell suspensions that developed overnight were centrifuged at 3,000 g for 10 min at 5°C. The cell pellets were washed with equal volumes (200 ml) of sterile phosphate-buffered saline (PBS, pH 7.2) solution. The cell pellets of all washed E. coli bacteria were re-suspended in 12 L of de-ionized sterile water to make a suspension of ~9.23 log₁₀ CFU/ml and this inoculum was referred to as E. coli water throughout this study.

Sample preparation and processing

The E. coli K-12 (ATCC 23716) water was used as the feed water during processing of corn meal (CM) and whey protein concentrate (WPC80) at room temperature (~23°C). The CM and WPC80 with the E. coli water was mixed as follows and is referred to as the “Feed”. The CM and WPC was conveyed into the extruder at a set speed of 600 RPM with a series 6300 digital feeder, type T-35 screw volumetric feeder (K-Tron Corp., Pitman, NJ), and E. coli water was added into the extruder at the rate of 1.86 L/h with an electromagnetic dosing pump (Milton Roy, Acton, MA). The ZSK-30, smooth barrel, twin, co-rotating screw extruder (Krupp, Werner Pfleiderer Co., Ramsey, NJ) with nine independently set temperature zones were used. The moisture content of the dry feed was 7% and the feed rate at 600 rpm was 7.452 kg/h. The operating torque for CM averaged 13.2 ± 3.1 Nm and 7.7 ± 2.3 Nm for WPC80, the rate of water addition was 31 ml/min and the specific mechanical energy (SME) calculated for CM averaged 375.5 Kj/kg and 222.00 Kj/kg for WPC80. The SEM was calculated using the following formula:

\[ \text{SME} = \text{RPM} \times \text{V} \times \text{P} \times \text{N} \]

Where No = count of bacteria before treatment, N = count of bacteria after treatment.

Bacterial inactivation:

The background populations of bacterial contamination determined from CM and WPC80 at each day of the study were below detection levels <10 CFU/g. After inoculation of CM and WPC80 with E. coli bacteria, the bacterial populations determined in CM and WPC80 was approximately 8.8 log₁₀ CFU/g and this number is considered to be the initial bacterial population before treatment. Extrusion treatment at temperature tested decreased E. coli population in CM and WPC80 (Table 2). For example, after treatment at 35°C, the number of surviving E. coli bacteria in CM and WPC80 averaged 4.0 and 6.9 log₁₀ CFU/g, respectively. This information suggests that treatment at 35°C killed bacteria in the samples.

Results and Discussion

The bacterial populations of bacterial contamination determined from CM and WPC80 at each day of the study were below detection levels <10 CFU/g. After inoculation of CM and WPC80 with E. coli bacteria, the bacterial populations determined in CM and WPC80 was approximately 8.8 log₁₀ CFU/g and this number is considered to be the initial bacterial population before treatment. Extrusion treatment at temperature tested decreased E. coli population in CM and WPC80 (Table 2). For example, after treatment at 35°C, the number of surviving E. coli bacteria in CM and WPC80 averaged 4.0 and 6.9 log₁₀ CFU/g, respectively.

Table 1: Extrusion processing conditions for corn meal and whey protein concentrate (80% protein) (WPC80).
approximately 4.8 and 1.8 log₁₀ CFU of the E. coli population in CM and WPC80, respectively (Table 2). The population of E. coli bacteria killed in CM treated at 55°C and above was significantly (<0.05) greater than treatment at 35°C. Treatment at 75°C or above, led increased bacterial inactivation and the populations of E. coli bacteria in the extruded CM samples were below detection level (<20 CFU/g) at temperatures above 75°C. For the WPC80, increased bacterial inactivation occurred at 55°C and at 95°C, the E. coli populations were below detection (<20 CFU/g). Extrusion processing at all temperatures reduced bacteria in the extruded CM and WPC80; however bacterial inactivation occurred at lower temperatures for CM than WPC80.

Correlation coefficients for bacterial inactivation and extrusion temperature for CM and WPC80 were R²=0.850 (Figure 1A) and R²=0.993 (Figure 1B), respectively. Extrusion at 95°C effectively resulted in complete microbial elimination in CM and WPC80.

| Product                  | Temperature (°C) | Log Kills (CFU) |
|--------------------------|------------------|-----------------|
| Corn meal                | 35               | 4.8 ± 0.2       |
|                          | 55               | 6.9 ± 0.1       |
|                          | 75               | < 20 CFU        |
|                          | 95               | < 20 CFU        |
| Whey protein concentrate | 35               | 4.6 ± 0.2       |
|                          | 55               | 4.3 ± 0.1       |
|                          | 75               | 5.9 ± 0.2       |
|                          | 95               | < 20 CFU        |

An average initial bacterial population in each sample was approximately 8.8 log CFU/g for corn meal and whey protein concentrate.

Values are means ± STD of three experiments with duplicate determinations

Table 2: The number of decimal reductions of inoculated populations of E. coli cells in corn meal and whey protein concentrate during twin screw extrusion processing at different temperatures.

The SME for CM was higher as was bacterial inactivation. A further investigation was carried out to understand the impact of SME on bacterial inactivation during the extrusion process. The correlation coefficient between the inactivation of bacteria and SME during extrusion processing of CM and WPC80 were R²=0.805 (Figure 2A) and R²=0.892 (Figure 2B), respectively. The results show that shear contributed to the bacterial inactivation in CM and WPC80 and that shear and temperature were positively correlated. On the average, the water activity (aw) in WPC80 was slightly lower than the numbers determined for CM at all temperatures used to process the ingredients (Table 3). The moisture content for CM treated at 35°C, 55°C and 75°C averaged 35.90%, and treatment at 95°C resulted to similar moisture content. Unlike the CM, increases in treatment temperature led to a decrease in the moisture content of WPC80. For example, WPC80 treated at 35°C had moisture content of 42.12 ± 0.12 and those treated at 75°C and above had moisture content of approximately 36%. This result indicates that higher treatment temperature (75°C and 95°C) is needed to reduce the moisture content of WPC80 to approximately 36%. For example, several studies have been conducted on the effect of moisture content on extrudate properties for starches and protein-based feed materials [23-30]. In all these studies, the authors found

Figure 1: Correlation between inactivation of Escherichia coli K-12 (ATCC 23716) and treatment temperature during twin screw extrusion processing of corn meal (A) and whey protein concentrate (B). An average initial bacterial population in corn meal sample was approximately 8.8 log CFU/g.

Figure 2: Correlation between inactivation of Escherichia coli K-12 (ATCC 23716) and pressure generated during twin screw extrusion processing of corn meal (A) and whey protein concentrate (B) at different temperatures. An average initial bacterial population in whey protein concentrate was approximately 8.8 log CFU/g.

Table 3: Influence of extrusion temperature on water activity and moisture content of corn and whey meal.

| Product     | Temperature (°C) | Water activity (aw) | Moisture content (%) |
|-------------|------------------|---------------------|----------------------|
| Corn        | 35               | 0.997 ± 0.001       | 35.95 ± 0.22         |
|             | 55               | 0.996 ± 0.003       | 35.90 ± 0.20         |
|             | 75               | 0.986 ± 0.003       | 35.85 ± 0.02         |
|             | 95               | 0.992 ± 0.001       | 35.49 ± 0.50         |
| Whey        | 35               | 0.986 ± 0.002       | 42.12 ± 0.06         |
|             | 55               | 0.982 ± 0.000       | 40.37 ± 0.16         |
|             | 75               | 0.982 ± 0.001       | 36.57 ± 0.24         |
|             | 95               | 0.981 ± 0.001       | 36.45 ± 0.15         |
a correlation between moisture loss and structural changes for both starches and protein-based products which agrees with these results for torque and SME for CM and WPC80. The results of this study showed differences in bacterial inactivation on CM and WPC80 treated at temperatures below 75°C. Previously, we reported that initial bacterial counts post processing in most cases include both surviving and injured cells [31-33]. The effect of storage temperatures on the recovery of injured populations is being investigated. The results obtained will help in our understanding of the behavior of E. coli bacteria on CM and WPC80 extrudates stored at 5°C and room temperature (22°C).

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