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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 $\log_{10}$ CFU/g. Inactivation of *E. coli* bacteria on CM and WPC80 extruded at 35 and 55°C averaged 4.8 log, 6.9 log and 1.8 log, 4.3 log, 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 extrudate 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 $\log_{10}$ 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 twin 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:

SME = RPM (run)/RPM (rated) X Newton meter (Nm) X Motor power rated/Feed rate

The temperature profiles for this study were set to maintain all 9, zones at the same temperature (Table 1). The temperatures were 35, 55, 75, and 95°C for both CM and WPC80. The product flow rates were higher, on average, for CM 119.3 to 123.9 g/min and 54.1 to 79.2 g/min for WPC80. The feed rates were slightly affected by the difference in bulk density 1.48 g/cm^3 for CM, and 1.43 g/cm^3 for WPC80, respectively. The screw elements were selected to provide low shear at 300 rpm; melt temperature and pressure were measured at die [5]. All products were collected in sterile bags and were immediately analyzed for colony forming units (CFU's) or stored for future bacterial analysis.

Moisture & water activity analysis: The Moisture content of the extruded corn meal and whey protein concentrates was determined by AACC method no. 925.09 [21], using a vacuum oven (Model No. 29, Precision, Winchester, VA).

Microbial analysis: To determine the initial number of background bacteria and the inoculated *E. coli* populations, 25g of treated and non-treated CM and WPC 80 products were weighed out and placed in a Stomacher bag (Dynatech Laboratories, Alexandria, VA) along with 75ml of 0.1% peptone-water, the contents were thoroughly mixed for 30s in a Stomacher (model 400, Dynatech Laboratories) at medium speed. Decimal dilutions of the samples were made with 0.1% peptone water, and aliquots (0.1ml) were plated in duplicate on tryptic soy agar (TSA, BBL/Difco, Sparks, MD) and sorbitol McConky agar (SMA, BBL/Difco, Sparks, MD). All plated samples were incubated at 37°C for 48h to determine the colony forming units (CFU/g).

Bacterial inactivation: Bacterial inactivation or log kill in extruded corn and whey protein concentrate was calculated this formula:

Log (No/N),

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

Statistical analyses: All experiments were done in triplicate with duplicate samples analyzed at each sampling time. Data were subjected to the Statistical Analysis System (SAS; SAS Institute, Cary, NC) for analysis of variance (ANOVA) and using the Bonferroni LSD method [22] to determine if there were significant differences ($p<0.05$) between mean values of number of cells recovered after each treatment.

Results and Discussion

The background populations of bacterial contamination determined from CM and WPC80 at each day of the study were below detection levels <10CFU/g. After inoculation of CM and WPC80 with *E. coli* bacteria, the bacterial populations determined in CM and WPC80 was approximately 8.8 $\log_{10}$ 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 $\log_{10}$ CFU/g, respectively. This information suggests that treatment at 35°C killed

		ZONE Temperature (°C)							
Product	Target Melt Temperature (± 2°C)	1 - 3	4	5	6	7	8	9	Feed Rate g/min
Corn Meal	35°C	0	0	0	2	2	35	35	122.2 ± 0.4
	55°C	0	20	25	30	35	45	50	122.1 ± 0.2
	75°C	0	35	45	50	60	65	70	123.9 ± 3.0
	95°C	0	40	55	65	78	88	92	119.3 ± 3.2
WPC-80	35°C	0	0	0	2	2	35	35	79.3 ± 3.8
	55°C	0	20	25	30	35	45	50	93.3 ± 6.4
	75°C	0	35	45	50	60	65	70	69.0 ± 2.3
	95°C	0	40	55	65	78	88	92	75.15 ± 11.2

Table 1: Extrusion processing conditions for corn meal and whey protein concentrate (80% protein) (WPC80).

approximately 4.8 and 1.8 $\log_{10}$ 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 *E. coli* populations in 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 R^2=0.850 (Figure 1A) and R^2=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	1.8 ± 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.

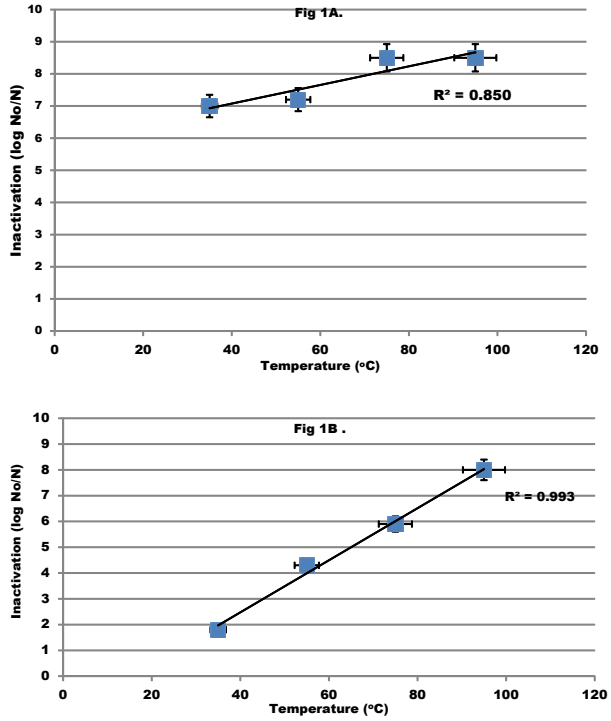

Figure 1: Correlation between inactivation of Escherichia coli K-12 (ATCC 23716) and treatment temperature during twin screw extrusion processing of corn meal (A) and whey protein concentrate (B). An average initial bacterial population in corn meal sample was approximately 8.8 log CFU/g.

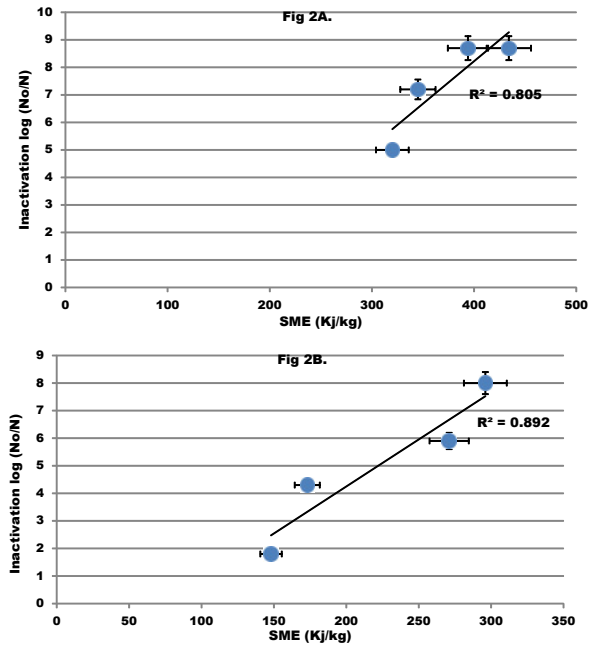

Figure 2: Correlation between inactivation of Escherichia coli K-12 (ATCC 23716) and pressure generated during twin screw extrusion processing of corn meal (A) and whey protein concentrate (B) at different temperatures. An average initial bacterial population in whey protein concentrate was approximately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

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

Values are means + STD of three experiments with duplicate determinations

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

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 R^2=0.805 (Figure 2A) and R^2=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

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 includes 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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