Functional Characteristics of Extruded Blends of Whey Protein Concentrate and Corn Starch

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ABSTRACT

The aim of this work was to study the effects of extrusion barrel temperature (70–180°C), feed moisture (18–30%), pH (3–8), different proportions of corn starch (75–95%), and whey protein concentrate (WPC, 80% protein concentration) (25–5%) on the preparation of functional blends. Expansion index (EI), bulk density (BD), compression force (CF), color, water absorption index (WAI), water solubility index (WSI), gel strength (GS), syneresis of the gel, and in vitro digestibility were evaluated. Barrel temperature and the proportion of WPC had significant effects on BD; at higher temperatures, BD was lower. Feed moisture and pH had significant effects on EI; with lower moisture and higher pH, the EI increased. An interaction of barrel temperature and feed moisture had an effect on WAI; at lower moisture content, the temperature effect was nonexistent, whereas at higher temperatures and feed moisture content, the WAI increased. The pH level had a significant effect on WSI, showing high WSI when lower pH levels were used. Color analysis showed that higher protein content and pH generated higher ΔE values; low feed moisture and low pH resulted in gel syneresis. Higher in vitro digestibility was obtained when a higher WPC proportion and pH were used. Extruded WPC-CS blends under alkaline and acidic conditions were affected by the preparation of diverse formulations that potentially can be used in foods to improve some functional and protein content.

Whey proteins have a high protein efficiency ratio and are widely accepted and used in many products due to their beneficial nutritional and functional properties. They are an option for food fortification; however, they are expensive (Martinez-Serna and Villota 1992; Onwulata et al 1998, 2006). They are used in foods to improve texture, flavor, and color, and to increase protein content. However, fortification with whey proteins to enhance nutritional quality has been limited due to reported adverse effects when protein supplementation is significant (>10%). To further increase the amount of whey and milk proteins, these proteins have been extruded in blends with starch, which reduces cost in preparation of expanded snacks (Martinez-Serna and Villota 1992; Onwulata et al 2001a,b; Fernandez-Gutierrez et al 2004); texturized proteins (Onwulata et al 2006); and third-generation snacks (Limón-Valenzuela et al 2006). During extrusion, control and management of the interactions between starch and protein is critical; the folding of globular proteins changes to improve interaction with other ingredients and create new product formulas (Onwulata et al 2006). Also, amylose-extruded products may be used as a binder to hold proteins because of their linear structure and their ability to form hydrogen bonds in extruded products (Matthey and Hanna 1997). Starch and proteins processed as individual components offer a wide range of functional properties. The extrusion process can induce structural and functional changes in both polymers and starch-protein interactions that contribute to the protein three-dimensional network stability after extrusion, improving the functional and nutritional properties of the new biopolymer to be used in diverse food systems (Li and Lee 1996; Gezimati et al 1997; Gropper et al 2002). However, the variability in whey composition and differences in extracting processes, the lack of knowledge about interactions with other components such as carbohydrates during extrusion, as well as the influence on texture formation, had limited their use (Matthey and Hanna 1997). Thus, the aim of this research was to prepare functional blends of whey protein concentrate and corn starch (WPC-CS) and evaluate the effect of some extrusion variables in alkaline and acidic conditions.

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MATERIALS AND METHODS

Materials

Corn starch (CS) and whey protein concentrate 80 (WPC 80) were purchased from IMSA, S.A. (Mexico, DF) and America Alimentos, S.A. de C.V., (Zapopan, Jal, Mexico), respectively.

Chemical Composition

Official methods (AOAC 1999) were used to analyze total protein (No. 988.05) and lipid (No. 920.35) contents. The moisture content was determined using Approved Method 44-19 (AACC International 2000).

The WPC-CS blends were prepared by mixing WPC 80 (25–5%) with CS (75–95%). The pH was adjusted by adding NaOH or HCl (concentration of 0.1–1.0*N*) and moisture concentration was adjusted to the levels indicated in Table I. The samples were stored in polyethylene bags at 4°C for subsequent extrusion processing. The extrusion process was conducted using a single-screw extruder, designed and manufactured by Cinvestav-IPN, Mexico. The screw compression ratio was 1:1 with a 5.0-mm die nozzle. The barrel was equipped with electrical cartridge heaters and three independently controlled heating and cooling zones. Barrel temperature in the final zone (zone 3) varied from 70 to 180°C according to the experimental design (Table I). The feed rate (73 g/min) and the screw speed (43 rpm) were constant throughout the experiment.

The extrusion variables were barrel temperature (70–180°C), feed moisture (18–30%), pH (3–8), and the ratio of WPC and corn starch (CS). The feed rate of WPC-CS blends varied from 1.74 to 7.06 kg/hr and the feed rate depended on the moisture concentration in the blends. Extruded blends were dried to the desired moisture (9.5–10.5%) in a convection oven (40°C) for 18 hr. Depending on the analysis, the final extruded treatments were either used whole or were milled using a hammer mill (model 200, Pulvex, Mexico) with a 250-µm sieve and packed into polyethylene bags for storage and further analysis. The second order of experimental design without repetitions was used for the extrusion measurements of WPC-CS.

Expansion Index

Dried extruded blends (not milled) were cut into 5-cm pieces and then the expansion index (EI) was determined according to the method described by Jin et al (1994). The expansion index (average) for each extruded product was derived from 20 measurements.

Bulk Density

The bulk density (BD) was determined using the method of Gujska and Khan (1991). The average diameter and length were measured and the apparent volume (V, m^3) was computed as

$$V = \left(\frac{\pi \cdot d^2 \cdot l}{4} \right)$$

where d (mm) is the average diameter and l (mm) is the length of the extruded product. BD values were derived from 20 analyses of each product.

Penetration Force

Penetration force (PF) tests were performed using a texture analyzer (TA-XT2, Stable Micro Systems, Texture Technologies Corp., Scarsdale, NY). Ten randomly chosen extruded pieces adjusted to 8% moisture and cut into 2.5-cm lengths were placed in an Ottowa compression piston cell. The maximum penetration force (in Newtons) necessary to break extruded products was recorded. Twenty measurements were taken for each treatment.

Color

The color was measured in extruded treatments milled to 250 μm. Color was recorded using a Mini Scan Hunter Lab instrument (CE96, Hunter Associates Laboratory, Reston, VA) following the method of Jin et al (1994). The color difference for each sample was calculated using the equation

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

Each assay was the average of 20 samples. The blank values were $L = 92.26$, $a = -0.81$, $b = 0.62$.

Water Absorption and Water Solubility Indexes

Water absorption (WAI) and water solubility (WSI) indexes were kept at two temperatures (30 and 75°C). These temperatures indicated the behavior of the blends below and above the typical starch gelatinization temperature. These values were registered according to the method of Anderson et al (1969). Three repetitions were made for each analysis.

Gel Strength and Syneresis

Samples of gel were prepared according to the method of Aguilera and Rojas (1996). These authors reported that gel properties varied according to the pH level. Samples were adjusted to different values of pH 4.5 (GF4.5) and pH 7.0 (GF7.0). The pH was adjusted by adding HCl (0.1–1.0*N*) or NaOH, and a third sample was maintained at the original pH after extrusion (GFE). A universal texture analyzer (TA-XT2, Texture Technologies Corp., Scarsdale, NY, USA/Stable Micro System, Godalming, Surrey, UK) was used in the compression puncture mode to record the force required to penetrate the gel (Tjahjadi and Breene 1984). Three repetitions were made for each analysis.

In Vitro Digestibility

In vitro digestibility of protein was measured according to the method of Hsu et al (1977). A multienzyme system consisting of trypsin, chymotrypsin, and peptidase was used. The multienzyme solution was freshly prepared before each series of tests and its activity was determined using casein of known in vitro digestibility. Two repetitions were made for each analysis.

Experimental Design and Data Analysis

A factorial design of 16 assays was used in such a way as to allow the estimation of main, double interaction, and quadratic effects. Restrictions in the randomization order of the assays were taken into account in the statistical analysis. All generated data were analyzed using regression models and Tukey test (Box et al 1978). The experimental design and its independent variables are shown in Table I. The statistical analysis was conducted using the Statistical Analysis System (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Chemical Composition

Chemical composition (wb) of the CS and WPC 80 was protein (N × 6.25) 0.17 and 73.77, respectively, and lipid 0.70 and 0.72, respectively. Reported values were the average of two repetitions.

Expansion Index and Bulk Density

Statistical analysis showed that feed moisture ($P = 0.0125$) and pH level ($P = 0.038$) had the greatest effect in these evaluated responses, and that the protein factor at high barrel temperatures was significant ($P = 0.0384$). All the blends extruded at 180°C barrel temperatures showed the lowest values for EI ($P < 0.0001$), and the rest of the extruded blends had similar values for EI and BD. Samples extruded at low feed moisture (18%) and barrel temperature (70°C), alkaline pH 8, and with 5% WPC added in the blends, showed maximum EI values slightly higher than the other samples extruded at barrel temperatures <180°C. Probably high barrel temperatures increased the amount of the fragmented

TABLE I
Experimental Design for Extrusion and Response Variables of Expanded Pellets of Extruded Blends[a]

	Factors				Response Variables		
Assay	P (%)	FM (%)	pH	BT (°C)	EI	BD	PF
1	25	30	8	70	1.3	1,000	30.4
2	25	18	3	70	1.4	1,200	154.5
3	5	30	3	70	1.4	1,000	62.0
4	15	23	5.5	70	1.3	1,000	69.0
5	5	18	3	70	1.4	1,100	68.7
6	25	30	3	70	1.4	900	20.6
7	5	18	8	70	1.5	1,400	231.9
8	25	18	8	70	1.4	1,200	104.5
9	15	30	3	125	1.3	1,000	82.2
10	5	18	5.5	125	1.4	1,100	165.6
11	25	23	8	125	1.3	1,000	107.1
12	15	18	8	180	0.6	1,600	59.7
13	25	18	3	180	0.7	1,000	35.6
14	5	30	8	180	0.6	1,100	102.2
15	5	23	3	180	0.5	1,200	79.3
16	25	30	5.5	180	0.7	1,000	70.0

[a] P, protein content in the blend; FM, feed moisture; BT, barrel temperature; EI, expansion index; BD, bulk density ($kg \cdot m^{-3}$); PF, penetration force (Newtons).

starch and the protein denaturated and lost solubility, becoming insoluble and aggregate and thus decreasing EI. Passing a threshold temperature, which depends both on the type of starch and the moisture content, expansion decreases with temperature. This is most likely due to excessive softening and potential structural degradation of the starch melt, which becomes unable to withstand high vapor pressure and therefore collapses (Kokini et al 1992). Previous findings showed that maximum expansion was found with whey and milk protein content <10%, feed moisture >25%, pH 6–7, and barrel temperature (125–130°C) (Matthey and Hanna 1997; Onwulata et al 1998, 2001a; Fernandez-Gutierrez et al 2004). Probably, as referred to by Cha et al (2001), although higher temperature leads to greater vapor pressure and increased driving force for expansion, it may also lead to a decrease in melt viscosity and, consequently, a greater tendency for the extrudates to collapse. In addition, it is possible to increase gelation, microcoagulation, formation of starch-protein bonds, and digestibility. According to Faubion and Hoseney (1982), the effect of proteins on expansion depends on the type and concentration. Proteins affect expansion due to their ability to affect water distribution in the matrix and their macromolecular structure and conformation, which affect the extensional properties of extruded melts. They also contribute to the formation of widespread networking through covalent and nonbonding interactions that take place during extrusion (Madeka and Kokini 1992; Li and Lee 1996). Camire et al (1990) and Seker (2005) reported that during extrusion a protein-starch interaction could be a possible, decreasing the free expansion of amylopectin chains and inhibiting the release of water content, thus decreasing expansion and increasing density more easily. In general, the BD values were slightly modified (900–1,600 kg/m^3). These results are consistent with the findings reported by Onwulata et al (2001a), who reported that the addition of WPC, whey isolate, or casein did not change the treatment density significantly. In this research, extruded treatments with high BD acquired a dense and rigid structure, probably because in the evaluated extrusion conditions, the starch was not fully gelatinized and plasticized, thus decreasing EI. Zaleska et al (2001) reported that potato starch-casein complexes induced by mixing in aqueous solutions and electrosynthesis formed some covalent bonds at lower pH with the involvement of carboxylic groups of protein and hydroxyl groups of starch. Furthermore, these researchers added that kinetic measurements and analysis of thermogravimetric thermograms suggested that there was a preference for the formation of the starch-casein 1:1 complex. In baths containing a small excess of protein, the 1:1 complex coprecipitated with casein.

Penetration Force

The analysis of PF showed the lowest values for the blends extruded with 25% of WPC at high feed moisture (30%), low barrel temperature (70°C), and pH 3 or 8. Probably extreme pH values contribute to the increase in the severity of the process, resulting in protein denaturation and increasing starch degradation, developing more fragile, breakable treatments, and resulting in lower PF values than the other extruded blends. On the other hand, the blends extruded at 70 and 125°C barrel temperature with low protein content, low feed moisture, and neutral or alkaline pH increased PF values (P = 0.0127), as did the blends processed at 180°C with low protein content (P = 0.0490). Probably the low feed moisture and high proportion of starch in the blends reduced starch conversion/gelatinization, ultimately resulting in decreased expansion. As a result, less water was available for hydration; the starch granules were insufficiently plasticized by water and thus did not melt during extrusion, which led to increased PF values. Onwulata et al (2001b) reported that with the addition of WPC to corn starch during extrusion, the moisture differential will affect not only the melt transition temperature of the starch but the uniformity of the cooking, with the potential for the development of hard, partially cooked particles in the final treatment. In addition, under those conditions, the extruded blends probably formed a gel stabilized by hydrophobic interactions and disulfide bonds as indicated by McSwiney et al (1994) and Monahan et al (1995). Fernandez-Gutierrez et al (2004) found that the PF of extruded casein-starch blends was strongly dependent on moisture content and casein proportion in the blend. The higher CF values were found at starch concentrations of ≈50 and 25% moisture content. For concentrations higher or lower than these, the extruded products obtained were softer and consequently had lower CF values.

Fernandez-Gutierrez et al (2004) attributed this behavior to the formation of hydrogen bridge bonds and van der Waals interactions that were not present under the initial conditions. Furthermore, the addition of three different milk proteins (casein, WPC, or WPI at 25%) increased the breaking strength of corn treatments extruded at low feed moisture (Onwulata et al 2001a). In addition, whey products extruded alone at a concentration of 25% reduced expansion and water absorption and increased density bulk.

Onwulata et al (1998) reported that the addition of WPC (25%) to corn, rice, and potato flour had minimal effects on the texture of extruded products, although by reducing moisture and adding reverse screw elements, EI and breaking strength increased.

WAI and WSI

Table II shows the data of WAI30, WAI75, WSI30, and WSI75. Statistical analysis of WAI30 showed that the barrel temperature (P = 0.0007) factor and its interaction with feed moisture (P = 0.0010) were significant. However, feed moisture did not show a significant effect (P = 0.6455). At low feed moisture, the effect of barrel temperature was not significant, while at high levels of both factors, the WAI30 values increased. Thus, the blends extruded at 180°C barrel temperatures, low and high feed moisture, and extreme pH values had the highest WAI30 values, regardless of the protein content in the blends. In the WAI75 sample, barrel temperature (P = 0.0101) and pH (P = 0.0245) were significant factors without interaction between them, although high levels of both factors resulted in an increase for WAI75. CS WAI values were 2.128 and 7.736, whereas WPC values were 6.320 and 5.220 at 30 and 75°C, respectively. Extruded blends increased WAI values from 1.5 to 3.5× more than CS and, in some cases, higher than WPC. On the other hand, the WAI of all the extruded blends registered at 75°C increased from approximately 3 to 5×, similar to CS, whereas the WAI of WPC was lower than all of the extruded blends. Blends extruded at 180°C with intermediate or high feed moisture and low or high protein content had the highest WAI75 values regardless of pH values in the blends. The high hydration capacity of the blends probably depends mainly on the inter- and intra-molecular bonds between WPC and amylose and amylopectin induced by high barrel temperatures, as well as on the changes that the starch undergoes during extrusion. Similarly Onwulata et al (2001a) reported that corn products formulated with three different milk proteins (casein, WPC, and WPI added at 25%) increased WAI with WPC, decreased WAI with WPI, while no variation was found with the addition of casein. These functional characteristics of the extruded WPC-CS make possible its application as a thickening agent in the food industry and its improved nutritional value (Hudson and Daubert 2002). Similar behavior in WAI of extruded cornmeal-WPC was reported by Kim and Maga (1987). These researchers found an increase in WAI when barrel temperature and feed moisture were increased, and a decrease when WPC was increased in the blends. In addition, increasing casein in extruded blends of casein-wheat starch increased WAI and decreased WSI (Fernandez-Gutierrez et al 2004). In the WSI30, the significant factor was pH (P = 0.0358); at low pH values, the WAI30 values were high. The statistical analysis of WSI75 did not show significant effects, except for samples extruded with low feed moisture and low pH values, which showed the highest values. Probably under these conditions, the hydrolyzed and fragmented starch was able to interact with the protein, which prevents the denaturation of the protein and renders the blend more soluble (Fernandez-Gutierrez et al 2004).

According to Onwulata et al (2003), the degree of extrusion-induced insolubility (denaturation) or texturization determined by lack of solubility at pH 7 for WPI increased from 30 to 60, 85, and 95% at 35, 50, 75, and 100°C, respectively. Extruded blends in acidic conditions showed the highest WSI values, increased by low feed moisture, whereas at alkaline conditions, the WAI of the extruded blends was high and the WSI was low. The protein factor was not significant, although probably when associated with pH, the synergetic effect of both factors denaturalized the protein and modified the WAI30. The level of denaturation and subsequent insolubility depends on heating temperature and time, and the pH of the whey depends on heating (Ennis and Mulvihill 2000). The addition of WPC and WPI to extruded corn starch products decreased solubility patterns significantly, while the addition of casein increased these values (Onwulata et al 2001a). Also Fernandez-Gutierrez et al (2004) found that increasing the barrel temperature of casein-starch blends from 126 to 194°C increased the WSI. Onwulata et al (2006) reported similar results during extrusion of WPI under acidic and alkaline conditions. These researchers reported that extruded WPI significantly increased solubility in relation to the control (raw WPI), although the highest values for solubility were for the samples extruded under acidic conditions. In addition, Onwulata et al (2006) found that the extrusion process was more severe in alkaline conditions than in acidic conditions, as revealed by the high water index values found in alkaline conditions. These differences are probably due to the starch's protective effect against protein denaturation.

Gel Firmness

Table II shows the gel strength data at three different pH values. The statistical analysis of GF7.0 showed significant effects in protein content (P = 0.002), feed moisture (P = 0.0005), and pH (P = 0.0172). Thus, decreasing protein content and increasing feed moisture and pH increased GF7.0 values. At this pH level, extensive unfolding facilitates sufficient interactions to form a gel (Monahan et al 1995). Opaque gels formed by heating solutions of whey protein isolate at >75°C at pH close to the isoelectric point are believed to be stabilized by hydrophobic interactions and disulfide bonds (McSwiney et al 1994). Whey proteins at pH 11 (high) formed gel at room temperature. On the other hand, only the protein factor was significant (P = 0.0036) for GFE. A decrease in protein content in the blends results in an increase in GFE, according to the findings of Aguilera and Rojas (1996). These researchers reported that the heat-induced gels had improved mechanical properties when a small proportion of whey protein isolate (10–20%) is substituted by cassava starch. The values of the four analyzed factors affected GF4.5. Protein and feed moisture were highly significant ($P < 0.0001$), as was the interaction between pH and barrel temperature (P = 0.0179). Thus, at low protein content and high feed moisture, GF4.5 values increased; however, at high barrel temperatures, the pH effect was not significant, although low values of both factors decreased GF4.5 values. The blend extruded at low feed moisture (18%) and protein (5%) under acidic conditions (pH 3) was completely hydrolyzed and did not form a gel and thus was not considered for the statistical analysis. The highest values of GF were for the samples extruded with low protein content (5%) and high feed moisture (18 and 30%), except the sample extruded with 15% protein. The lowest values of GF were for the samples extruded under acidic conditions and intermediate feed moisture (18%). While aggregation takes place mainly through thiol/disulphide exchange reaction in a neutral or alkaline pH, noncovalently linked aggregates are predominantly formed in an acidic environment (Spiegel and Huss 2002). In this research, varying extrusion conditions from 70 to 180°C barrel temperature, acidic (pH 3) or alkaline (pH 8) and 5 or 15% WPC concentration in blends allowed the formation of gels. Muhrbeck and Elliasson (1991) showed that in the interaction between starch and BSA (bovine serum albumine), starch cannot form a continuous network but will act as a filler, reinforcing the BSA matrix.

Also, casein and starch formed complex structures by continuous networks of protein with hydrated starch fractions, formed mainly by amylopectin, while amylose was included in the continuous network.

Gel Syneresis

Table II shows the syneresis values of the extruded blends at three different pH values. The statistical analysis did not show significant effects. Most of the assays did not show syneresis and, in some samples, these values were minimal. Mixed gels of cassava starch-WPI show synergistic behavior, which has been attributed to the hydration and swelling of starch granules and the formation of a continuous phase WPI network around them (Aguilera and Baffico 1997). Samples extruded at low barrel temperatures under acidic conditions showed the highest syneresis values and low gel strength. Probably in those extrusion conditions, the protein was denaturated and the starch hydrolyzed without any possibility of complex formation as referred by Pomeranz (1985). This researcher

TABLE II
Functional Characteristics of Extruded Blends of Whey Protein Concentrate and Corn Starch[a]

	WAI		WSI (°C)		Gel Force			Syneresis (%)		
Assay[b]	30 (°C)	75 (°C)	30 (°C)	75 (°C)	GF4.5	GFE	GF7.0	GF4.5	GFE	GF7.0
1	3.5	6.8	6.8	11.7	18.0	16.2	19.0	0	0	3
2	4.8	6.9	24.4	27.8	5.8	7.6	7.7	27	26	22
3	3.8	6.3	7.8	8.3	24.0	28.1	22.5	0	0	0
4	3.7	6.9	11.3	11.1	19.6	17.5	18.6	0	0	0
5	3.7	5.8	22.8	63.2	0.0	0.0	0.0	57	75	66
6	3.3	6.5	14.0	19.7	13.9	8.5	14.2	0	0	0
7	4.9	7.2	13.7	20.6	23.2	24.3	23.0	0	0	0
8	5.2	7.9	12.5	20.6	13.5	11.8	14.8	3	2	1
9	4.2	7.2	25.0	15.2	21.4	20.3	22.7	3	0	0
10	4.9	8.2	9.4	14.2	14.1	15.1	15.0	0	0	1
11	4.7	7.3	12.6	15.7	14.1	14.1	14.6	0	1	0
12	4.8	8.5	13.1	18.0	14.9	15.5	12.7	1	1	0
13	4.4	8.0	16.8	22.0	7.8	6.9	8.4	5	2	2
14	7.1	9.7	7.6	12.6	23.4	19.0	22.5	0	0	0
15	5.3	7.3	12.1	17.1	17.3	16.0	18.9	0	0	0
16	5.6	6.5	8.6	13.4	16.4	16.7	15.9	0	0	0

[a] WAI, water absorption index; WSI, water solubility index; GF4.5 and GF7.0, gel strength at pH 4.5 and 7.0; GFE, original pH after extrusion.
[b] Levels shown in Table I.

reported that proteins form complexes with starch granules over the surface of the granules, thus preventing the release of the exuded liquid from the granules. Furthermore, Piyasena and Chambers (2003) reported that more whey was released at low a pH than at a high pH of renneted curd added to whey protein dispersions (WPD). Thus, relatively more moisture was retained in curd obtained from milk with WPD at a high pH.

Extruded WPI at pH 13.2 favored the formation of aggregates (Onwulata et al 2006). According to these researchers, the network structure in a heat-induced globular gel depends strongly on the balance between the attractive and repulsive forces during aggregation. If the pH is far from the isoelectric point, and the ionic strength is sufficiently low, then intermolecular electrostatic repulsion is dominant. In addition, heat induces gelation of globular proteins at low pH (2 to 2.5) and at the molecular level rod-like structures are formed (Onwulata et al 2003), whereas aggregation of globular proteins occurs at pH 7. Starch derivative, starch, and whey proteins (WP) are commonly added to milk-based formulations (cheese, custards, and yogurts) to improve viscosity, texture, minimize syneresis, and increase stability throughout storage life (Punidadas et al 2000; Schmidt et al 2001; Malinski et al 2003).

Color

The effect of extrusion on color values of CS-WPC extrudates is shown in Table III. CS had the highest *L* value and WPC had the highest *b* value. The statistical analysis showed that the significant factors in the extruded treatments were protein content (P = 0.0207) and pH (P = 0.0010) in extruded blends. Thus, samples extruded at low protein content and low pH had the highest *L* values. On the other hand, barrel temperatures and feed moisture did not show significant effects on *L* values. Thus, increasing the WPC in the blends decreased luminosity (*L* values) and increased *b* values. In general the *b* color parameter of the blends showed significant effects from protein content ($P < 0.0001$) and pH (P = 0.0026). Feed moisture did not show significant effects (P = 0.8883), although it showed significant effects in its interaction with pH (P = 0.0096). On the color scale, the *b* coordinate is a measure of yellowness; *b* values increased when protein content and pH were increased. At low feed moisture, *b* values increased as pH values were increased. High levels of protein (P = 0.0026) and pH (0.0196) increased ΔE values. The highest ΔE values were for blends extruded at alkaline pH. Also, Onwulata et al (2006) reported a significant increase in *b* color values and a decrease in ΔE values of WPI extruded under acidic and alkaline conditions related to raw WPI. However, the *b* values found in this research were higher and the ΔE values were lower, which was attributed to the high *L* values of CS. Similar to the findings of Onwulata et al (2006), the high *b* values of extruded blends under alkaline conditions were probably due to the effect of ammonium sulfide which was olfactorally perceptible. Substituting WPI in expanded corn meal increased lightness even at high temperature (>140°C) where browning was expected (Onwulata et al 2003). Changes in color intensity are caused by the Maillard reaction between reducing sugars (dextrinized starch) and amino groups from casein (Wen et al 1990). However, high casein concentration, at high or low feed moisture contents, facilitates reaction with the reducing sugars, thus intensifying the change in color when the barrel temperature is increased (Hsieh et al 1990). The color intensity of extruded casein-starch blends is also increased by high starch concentrations due to the presence of reduced sugars because of starch dextrinization, especially at higher barrel temperatures from 126 to 194°C (Fernandez-Gutierrez et al 2004).

In Vitro Digestibility

Statistical analysis showed that the significant effects for this response variable were protein content (P = 0.0020) and pH (P = 0.0084). High levels of these parameters results in high in vitro digestibility values; barrel temperature and feed moisture were not significant (Table III). The blend extruded with 25% protein content and alkaline pH 8 had the highest value of in vitro digestibility compared with the rest of the assays. On the other hand, the assays processed with low protein and low pH had the lowest digestibility values. The extrusion process can improve protein digestibility by denaturation, thus increasing its susceptibility to enzymatic hydrolysis. However, some interactions can decrease digestibility due to nonenzymatic browning reactions and the formation of cross-linking reactions (Camire et al 1990; Arêas 1992; Ledward and Tester 1994; Camire 2000, 2001; Moraru and Kokini 2003). SDS-PAGE electrophoretic analysis of WPI and various heat-treated samples showed that, even at the highest barrel temperatures, the extrusion process does not affect the overall protein percentage (Onwulata et al 2003). Furthermore, these researchers reported that although the amount of denatured protein increased with increasing temperature, denaturation had a minimal overall effect on protein digestibility. Therefore, the interesting result is the increased protein denaturation without a significant loss of diges-

TABLE III
Color of Extruded Blends and In Vitro Digestibility of Raw and Extruded Blends of Whey Protein Concentrate and Corn Starch

	Color[b]			In Vitro Digestibility	
Assay[a]	*L*	*b*	ΔE	Raw Blends	Extruded Blends
1	84.4	12.4	11.6	85.9	87.7
2	85.2	11.8	10.9	85.9	85.6
3	87.8	10.8	9.8	82.8	83.3
4	87.6	12.3	11.3	85.7	86.1
5	79.7	8.5	10.4	82.8	82.0
6	85.8	11.9	11.1	85.9	85.1
7	84.7	10.8	10.0	82.8	85.4
8	83.2	15.7	15.2	85.9	85.2
9	87.3	10.6	9.7	85.7	84.9
10	88.0	10.8	9.8	82.8	84.1
11	84.5	13.1	12.3	85.9	85.8
12	85.9	12.6	11.7	85.7	85.8
13	87.2	11.3	10.3	85.9	85.1
14	83.9	10.2	9.7	82.8	nd
15	89.0	9.5	8.7	82.8	83.1
16	85.8	12.7	11.8	85.9	86.3
Corn starch	94.2	4.9	8.4	–	–
Whey protein concentrate	79.9	15.8	16.5	87.5	–
Casein	–	–	–	89.5	–

[a] Levels shown in Table I.
[b] *L*, lightness; *b*, yellowness; Δ*E*, color difference.

tibility due to extrusion at <100°C. The values of digestibility of extruded WPI reported by these researchers varied from 84.5 to 89.6%, slightly higher than the values found in this research. This was probably due to the low barrel temperatures (<100°C) and high feed moisture (30%) used.

CONCLUSIONS

The global effect of the four factors analyzed during the extrusion process influenced the structural and functional properties of extruded blends. High barrel temperatures and protein content decreased the EI of the blends. Blends extruded with high protein content and pH increased the yellow color of the blends. The WAI and WSI varied according to the processing conditions, although the effects of the acidic conditions during extrusion were more severe in these response variables. The strongest gels, and those with decreased gel syneresis, were those with low protein content and high feed moisture. Protein content and pH were the most important variables influencing the in vitro digestibility, although in general, most of the blends had improved values. Our findings reveal the importance of substrate pH in combination with the extrusion conditions to prepare diverse extruded blends of starch-WPC that could be utilized by producers of milk-based products to optimize yield and composition and improve nutritional value

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