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Retention of Essential Amino Acids during Extrusion of Protein and Reducing Sugars

Shivendra Singh,[†] Lara Wakeling,[†] and Shirani Gamlath*,[§]

School of Science and Engineering, University of Ballarat, Victoria 3353, Australia, and School of Exercise and Nutrition, Faculty of Health, Medicine, Nursing and Behavioural Sciences, Deakin University, Burwood, Victoria 3125, Australia

This research investigates the retention of essential amino acid profiles of products during the extrusion of proteins and reducing sugars. Animal proteins (egg and milk protein at 10 and 30% levels) and reducing sugars (fructose and galactose at 0, 2, and 8% levels), with pregelatinized wheat flour, were extruded at 110 and 125 °C product temperatures and feed moistures of 19 and 23.5% for egg protein and 13.75 and 16% for milk protein. The nutritional property analyzed was essential amino acid retention, and sugar retention was also considered to understand the relationship of sugars with retention of amino acids. Lysine showed the lowest retention (up to 40%) of all the essential amino acids. Retention of other essential amino acids varied from 80 to 100% in most situations. Apart from lysine, tryptophan, threonine, and methionine were found to be significantly changed ($P < 0.05$) with processing conditions. Increased protein and sugar levels resulted in a significant degradation of lysine. Greater lysine retention was found at a lower temperature and higher feed moisture. Results of sugar retention also showed similar patterns. The products made from fructose had greater lysine retention than products made from galactose with any type of protein. The outcomes of this research suggested that the combination of milk protein and fructose at a lower temperature and higher feed moisture is most favorable for developing high-protein extruded products.

KEYWORDS: Extrusion; egg protein; milk protein; fructose; galactose; essential amino acid; Maillard reaction; lysine retention

INTRODUCTION

Parallel to the increased applications of extrusion cooking, interest has grown in the nutritionally relevant effects of this process. Prevention or reduction of nutrient destruction, together with improvement in starch or protein digestibility, is clearly of importance in most extrusion applications (*1*). Nutritional concern associated with extrusion cooking is highest when extrusion is used specifically to produce nutritionally balanced or enriched foods (weaning foods, dietetic foods, meat replacers). The extent of modifications in nutritional properties of the extruded product is largely dependent upon the feed mixture composition and extrusion parameters (*2–4*).

For the development of nutritious snack products, balanced nutritional composition in the final product is essential. To attain greater nutritional value, selection of raw materials (protein, sugars, and other components) is critical. The major proteins used in extruded foods are oilseed proteins, such as soy flours or concentrates, and cereal proteins, mainly from wheat (*5*). Interest in using other proteins (egg albumen, milk protein, whey protein, and sodium caseinate) is growing (*6, 7*). Egg and milk proteins are commonly referred to as the "perfect proteins"; these are the common reference to which other proteins are compared. Protein digestibility corrected amino acid score (PDCAA) and biological value are commonly used to measure protein quality, with egg and milk proteins scoring 1.0 for the PDCAA and 100 for biological value, which are the highest measures for both (*8*). Additionally, egg and milk proteins contain all essential amino acids needed by the body for optimum growth, but little published data are available on the extrusion of egg and milk proteins. Accordingly, there is a need to undertake research in this area to determine the effect of extrusion on egg and milk proteins.

Generally, sugar is used to produce flavor and browning characteristics and plays an important role in controlling texture and mouthfeel in extruded snack products (*9*). Sucrose and fructose are commonly used in the production of extruded products (*9–12*), but there is no published information concerning the extrusion of galactose. Thus, fructose and galactose are the sugars of interest for research.

During the extrusion process, the interaction of protein and reducing sugars, known as the Maillard reaction, causes the deterioration of the nutritional quality of extruded products. The Maillard reaction occurs between free amino groups of protein

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* Author to whom correspondence should be addressed (telephone 61 3 92517267; fax +61 3 9244 6017; e-mail shirani.gamlath@deakin.edu.au).

[†] University of Ballarat.

[§] Deakin University.

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and carbonyl groups of reducing sugars. Although the Maillard reaction is beneficial in terms of color and flavor development in food products, it negatively affects protein nutritional quality. Loss of essential amino acids resulting from the Maillard reaction during the extrusion process is the main concern in developing a nutritionally high-quality product (*13–17*). The extent of Maillard reaction depends on the presence of reducing sugars and their reactivity (*18*). Use of less reactive sugars may be helpful to control the Maillard reaction to some extent, leading to greater retention of amino acids in extruded products. Therefore, a less reactive sugar, fructose, and a highly reactive sugar, galactose, were chosen to investigate the influence of reactivity of sugars on the quality of extruded products. In addition, the glycemic index (GI), which is a quantitative assessment of foods based on postprandial blood glucose response, is currently one of the important aspects in the development of nutritional products (*19*). Several health benefits (prevention or management of several chronic Western diseases including diabetes, coronary heart disease, and possibly certain cancers) exist for reducing the rate of carbohydrate absorption by means of a low GI diet. It is known that the glycemic indices of fructose (*23*) and galactose (*28*) are very low (*20*). Overall, fructose and galactose as reducing sugars and egg and milk proteins as a source of protein have been selected for this project.

To identify a suitable base material, several preliminary studies had been conducted with different materials (rice flour, wheat flour, and tapioca starch). These studies revealed that the processing capabilities of this extruder were not sufficient for proper gelatinization and expansion of extrudates made from these base materials. In addition, it was not possible to achieve stable running conditions with the tested materials (after a short run at steady conditions, barrel jamming occurred as a result of high pressure). Thus, on the basis of the preliminary study, pregelatinized wheat flour was chosen as a base material for this study.

Extrusion conditions such as high barrel temperatures and low moisture promote the Maillard reaction, which is one of the major causes of nutritional changes during the process. In addition, it is well-known that among different process variables, feed moisture and temperature have important effects on the nutritional quality of extruded products. Thus, feed moisture and temperature were the process variables chosen to determine the influence of extrusion conditions (feed moisture and temperature) on the nutritional characteristics of extruded products.

Therefore, the objective of this research was to investigate the effects of different types and levels of egg and milk proteins, reducing sugars (fructose and galactose), and process variables (feed moisture and temperature) on the essential amino acid profile of extruded products. To understand the relationship of sugars with retention of amino acids, sugar retention was also examined.

MATERIALS AND METHODS

Materials. Pregelatinized wheat flour, egg and milk proteins, and fructose and galactose were used as the raw materials in different combinations. Pregelatinized wheat flour, supplied by Penford Australia Limited, was used as a base material for all experiments. Milk protein isolate (ALAPRO 4900), supplied by Fonterra Ltd., New Zealand, and egg protein (high-whipping spray-dried egg whites), produced by GF Ovodry S.r.i and imported by Med-Chem Ingredients Pty Ltd. (Australia) were used as protein sources. Galactose (Fonterra Ltd.) and Cornsweet Crystalline Fructose (ADM Australia Pty Ltd.) were used as reducing sugars. The specifications for raw materials are presented in **Table 1**.

Table 1. Specifications of Raw Materials

specification	pregelatinized wheat flour	milk protein isolate	egg albumen
moisture (%)	10.0 (max)	4.0	8.0
protein (%, dry basis)	10.5	90.4	80.0 (min)
fat (%)		1.7	0.25 (max)
pH	5.0–7.0	7.1	7.0 ± 1.5
reducing sugars (%)		0.025 (max)	1.4 (lactose)
sodium (mg/100 g)		600	
calcium (mg/100 g)		2300	
ash (%)		7.1	
bulk density (g/mL)	0.3–0.4		0.45 min
sieve analysis (% pass through 250 μm)	30 (max)		100

Experimental Design and Preparation of Raw Materials. The experimental design consisted of two types of protein (egg and milk protein) and two types of reducing sugars (fructose and galactose) with two independent process variables (product temperature and feed moisture). Feedstocks containing two levels of each protein and three levels of each sugar were prepared as shown in **Figure 1**. Each feedstock was mixed in a rotary mixer for 10 min. The feedstocks were later extruded.

Extrusion of Samples. A Werner and Pflederer Continua 37 corotating twin-screw extruder (Stuttgart, Germany) with an L/D ratio of 27:1 and an outer screw diameter of 37.4 mm was used. The screw barrel consisted of three individual barrel sections (zones), which were held together by tie-rods. The first and second zones were maintained at 60 and 90 °C, respectively, and the third zone temperature was either 110 or 125 °C according to product temperature. The screw configuration (from feed section to die) used to process the extrudates consisted of two forward elements (30 mm each), a reverse element (10 mm), two forward elements (30 mm each), a reverse element (10 mm), three forward elements (30, 10, and 30 mm), a high-pitch element (20 mm), and a forward element (30 mm). A circular die (12 mm diameter) was used for all experiments.

A total of 20 kg of each mixture was processed through the extruder using the conditions outlined in **Figure 1**. Screw speed was kept between 200 and 250 rpm to achieve the particular product temperature. The input feed rate was constant for achieving particular feed moisture. The pressure behind the die varied from 0 to 70 bar. Temperatures of all three barrel sections, screw speed, product pressure, feed rate, and time were recorded for each trial. Extrudates were collected over a period of 3–5 min, during which time the main extrusion variables showed maximum stability. The extrudates were collected and allowed to cool to room temperature. The samples were packed into hermetically sealed polyethylene bags and stored at room temperature until analyses were performed.

Sugar Analyses. Sugar analyses were performed using the procedure of Wills et al. (*21*). The extrudate samples were assessed using a Varian HPLC, equipped with a model 9010 pump, a model 410 autosampler, and a differential refractive index detector (model 410, Waters, Milford, MA). The column used was a Carbohydrate Analysis column (Zorbax, Agilent Technologies, 250 × 4.6 mm), with a mobile phase of 75% v/v acetonitrile/water. External standard solutions of fructose, galactose, and glucose were prepared for identification (using retention times) and quantification purposes.

Amino Acid Analysis. For quantitative amino acid analysis, the samples were weighed out in duplicate (approximately 200–300 mg), and 20% HCl (10 mL) was added to the vials. For tryptophan analysis, the samples were weighed out in duplicate (approximately 100–200 mg), and 5 M sodium hydroxide (3 mL) was added. In both analyses, the samples were hydrolyzed for 24 h at 110 °C. After hydrolysis, an aliquot was taken and derivatized using a Waters AccQFluor reagent kit containing the borate buffer and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbonate (AQC) derivatizing agent. The amino acid derivatives were separated and quantified by reversed phase (C18) HPLC. The HPLC consisted of a Waters Alliance 2695 separation module, a Waters 474 fluorescence detector, and a Waters 2487 dual λ absorbance detector in series. The control and analysis software was Waters

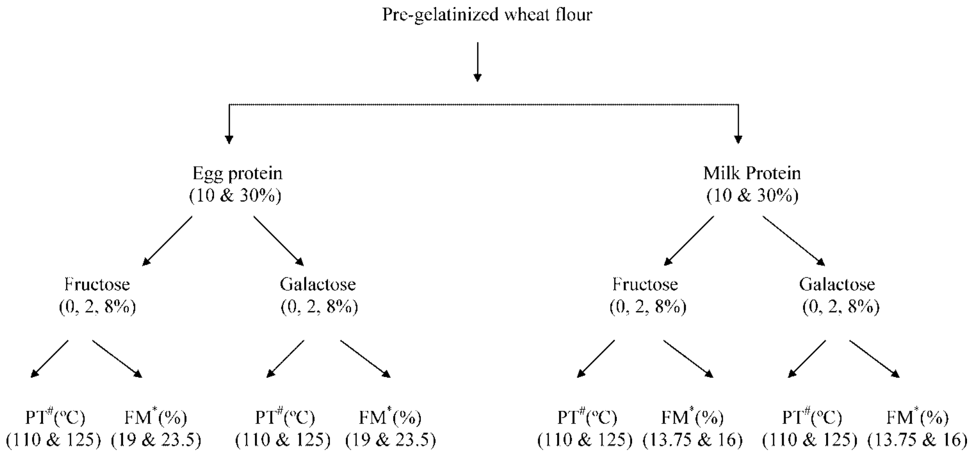

Figure 1. Experimental design for this research: #, product temperature; $*$, feed moisture.

Table 2. Essential Amino Acid Retention

	essential amino acid retention (%) with 8% sugar level at 125 °C[a]							
	egg protein[b]				milk protein[c]			
	10%		30%		10%		30%	
amino acid	F	G	F	G	F	G	F	G
tryptophan	97.88 a	96.27 a	96.92 a	95.85 a	83.53 b	82.66 b	83.26 b	81.28 b
leucine	91.35 a	89.99 a	91.00 a	88.82 a	84.41 b	82.16 c	83.48 c	81.73 c
phenyl-alanine	90.29 a	84.08 b	89.59 a	82.37 c	83.65 c	81.26 c	82.16 c	80.41 c
valine	90.56 a	89.71 a	90.24 a	88.37 a	84.48 b	82.42 b	83.22 b	82.30 b
threonine	97.96 a	89.94 b	91.98 b	88.78 b	84.86 c	83.42 d	84.03 c	82.92 d
isoleucine	90.63 a	89.17 a	90.06 a	88.13 a	83.23 b	80.93 b	82.52 b	80.18 b
methionine	89.75 a	83.13 b	87.67 c	80.51 d	82.93 d	80.25 d	80.36 d	80.39 d
lysine	71.72 a	49.29 b	68.34 c	45.70 d	82.42 e	41.46 f	79.58 g	40.60 h

[a] Means with different letters in each row are significantly different ($P < 0.05$). Reported values represent the mean of four values. F,f ructose; G, galactose. [b] Processed at 19% feed moisture. [c] Processed at 13.75% feed moisture.

Empower Pro Module (Waters Corp.). The amino acids analyzed included tryptophan, leucine, phenylalanine, valine, threonine, isoleucine, methionine, lysine, asparagine, serine, glutamine, glycine, histidine, arginine, alanine, proline, and tyrosine.

Statistical Analysis. A four-factorial (protein level, sugar type and level, product temperature, feed moisture) experimental design was adopted for both sources of protein (egg and milk proteins). The data obtained from the analyses were statistically analyzed using the statistical package of SPSS, version 12.0 with a probability of $P < 0.05$. The main effects and all two-way interactions (protein level, sugar type and level, product temperature, and feed moisture) were identified using Univariate Analysis of Variance. Pairwise comparisons were performed using the Bonferroni method (SPSS, version 12.0). The effects of feed compositions and processing conditions on the nutritional properties of the extruded product were studied.

RESULTS

Essential Amino Acids Retention. The results of retention of essential amino acids showed that lysine had the least retention (up to 40%) of all the essential amino acids, compared to 80–100% retention for the other essential amino acids in most situations. Apart from lysine, retentions of tryptophan (up to 81.28%), threonine (up to 82.92%), and methionine (up to 80.25%) were found to be significantly changed ($P < 0.05$) with processing conditions. Changes in leucine and phenylalanine retention with an increased level of galactose were also statistically significant ($P < 0.05$). There were no significant changes ($P > 0.05$) observed in the retention of valine and isoleucine with the different feed mixtures and processing conditions. Combination of egg protein and galactose seemed to have a more detrimental effect on amino acid retention than milk protein and fructose. Extrudates having the highest (8%) initial level of sugar and processed at low feed moisture and high temperature showed the greatest loss in essential amino acids. Thus, essential amino acid retention in the products made from different feed mixtures containing 8% sugar processed at 125 °C and 19 and 13.75% feed moisture for egg protein and milk protein, respectively, are presented in **Table 2**. No significant changes ($P > 0.05$) were found in the retention of essential amino acids, except lysine, in the extrudates made from milk protein. Therefore, it was decided to investigate the effects of the extrusion process on the retention of lysine in further detail. These investigations of the relationship between lysine retention, composition of feed mixture, and process variables are presented in detail.

Lysine Retention with Egg Protein. The retention of lysine was affected by types and levels of sugars, levels of egg protein, feed moisture, and temperature during the process. Products made from fructose showed significantly greater ($P < 0.001$) retention of lysine compared to those extruded with galactose at the same levels. Lysine retention significantly decreased ($P < 0.001$) as sugar (fructose and galactose) levels increased. The interaction effect (protein level–sugar type and level) significantly ($P < 0.05$) influenced the lysine retention in the extruded products. It was observed that lysine retention in the extrudates tended to be significantly lower ($P < 0.05$) for the samples having higher levels of egg protein for all sugar types and levels, but the differences were greater with galactose. Significantly ($P < 0.001$) greater lysine retention was observed as feed moisture increased and temperature decreased. The samples, containing fructose, extruded at 23.5% feed moisture and 110 °C product temperature showed the highest lysine retention.

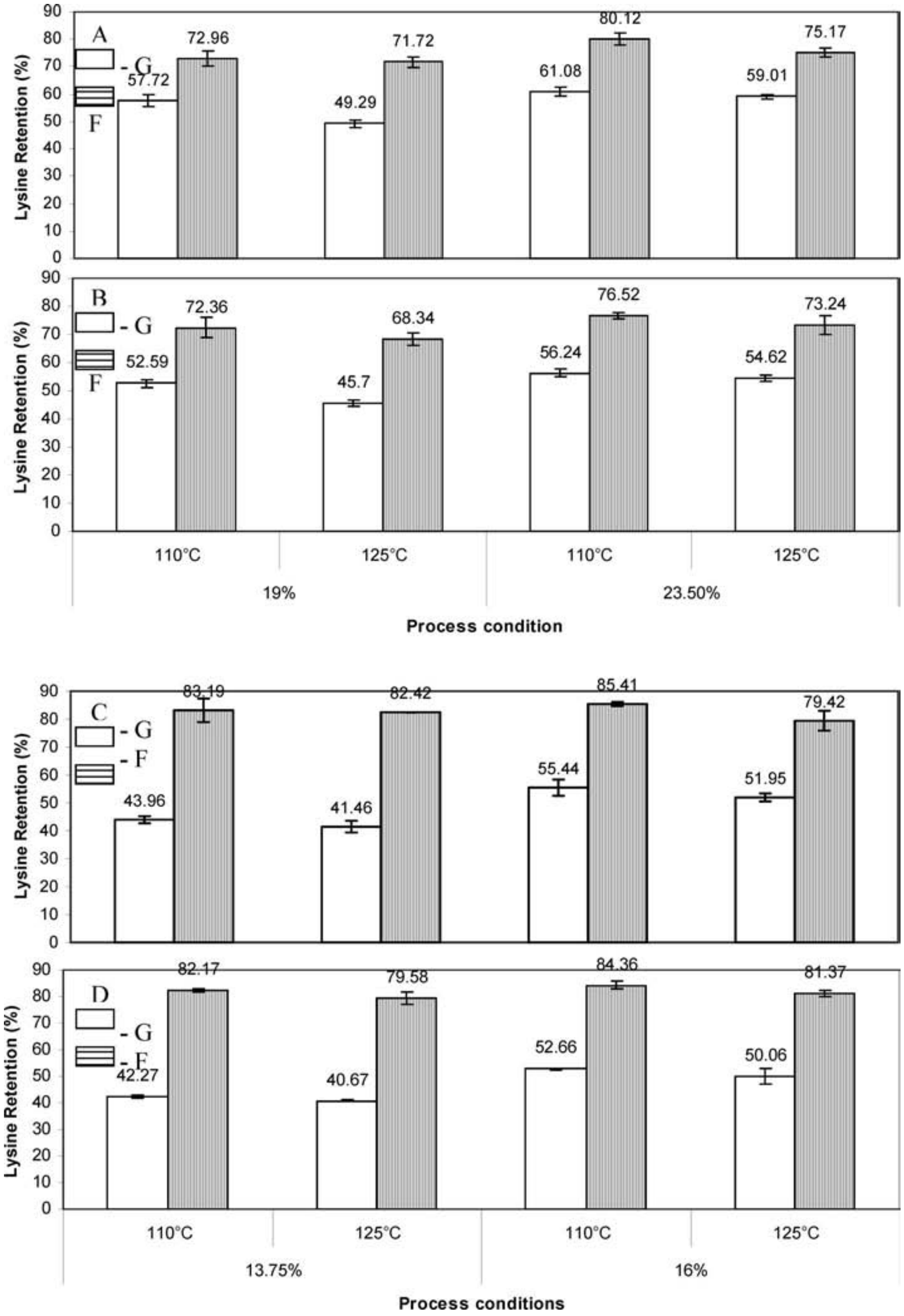

Figure 2. Lysine retention comparison [galactose (G) vs fructose (F)] at 8% initial sugar level with (**A**) 10% egg protein, (**B**) 30% egg protein, (**C**) 10% milk protein, and (**D**) 30% milk protein. Vertical bars represent standard deviation of four values.

Lysine Retention with Milk Protein. Lysine retention with 10 and 30% milk protein at different levels of fructose and galactose significantly changed with galactose levels, feed moisture, and temperature. The retention of lysine did not significantly change ($P > 0.05$) with different levels of fructose. The retention of lysine was significantly ($P < 0.001$) greater in the products with fructose compared to those with any level of galactose. The milk protein level did not significantly affect ($P > 0.05$) the amount of lysine retained in the extruded products. Significantly greater ($P < 0.05$) lysine retention was observed at the higher feed moisture with all sugar types and levels. The retention of lysine significantly ($P < 0.001$) decreased with increasing temperature. Overall, fructose as a sugar source and 16% feed moisture and 110 °C product temperature as processing conditions were more favorable to produce nutritious extruded products.

Lysine Retention Comparison at the 8% Sugar Level. The greatest degradation of lysine occurred at 8% fructose and galactose, so a comparison of lysine retention between the products made from fructose and galactose at the level of 8% with 10 and 30% of both egg protein and milk protein is presented in **Figure 2**. At 10% egg protein, products made from fructose had a greater lysine retention (71–80.5%) compared to 49–61% retention in the products with galactose (**Figure 2A**). A similar trend was observed for 30% egg protein (**Figure 2B**). However, losses at 30% egg protein were comparatively higher than at the 10% level. In the products containing 10% milk protein, only 41–55.5% lysine retention was found with galactose, whereas a much greater retention of lysine (79–85.5%) was found with fructose (**Figure 2C**). At a 30% level of milk protein, again a similar pattern was observed (**Figure 2D**).

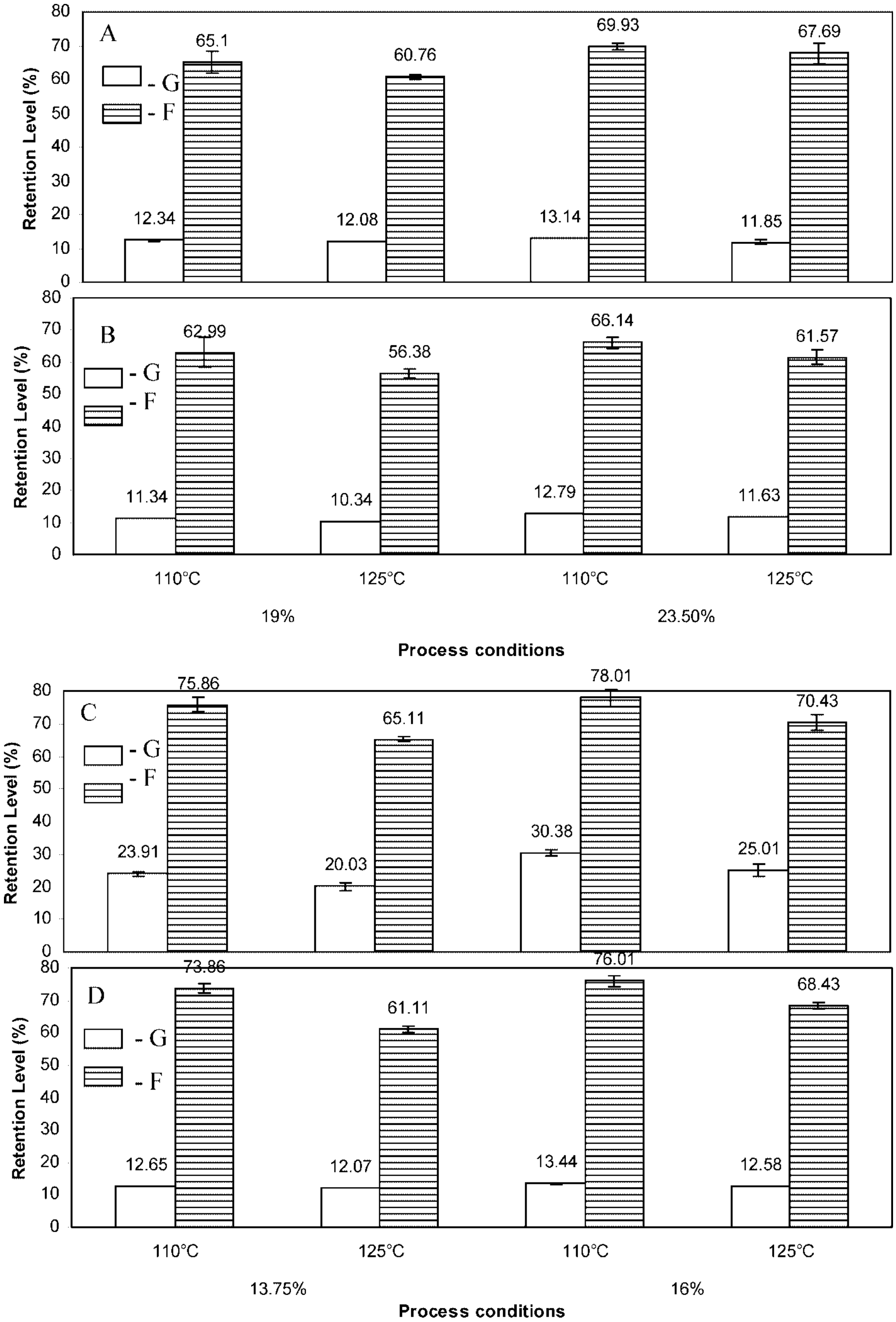

Figure 3. Sugar retention comparison [galactose (G) vs fructose (F)] at 8% initial sugar level with (**A**) 10% egg protein, (**B**) 30% egg protein, (**C**) 10% milk protein, and (**D**) 30% milk protein. Vertical bars in all figures represent standard deviation of four values.

Overall, it was found that products made from fructose had significantly greater ($P < 0.001$) lysine retention compared to those made from galactose under the same processing conditions.

Sugar Retention. The retention of sugars followed a trend similar to that observed with lysine retention. A greater retention of sugar (fructose and galactose) was observed at lower initial levels of the respective sugars, lower levels of egg and milk proteins, a higher feed moisture, and a lower temperature. Comparison of fructose and galactose retention at an initial level of 8% revealed that fructose had significantly higher retention values compared to galactose under all processing conditions at 10 and 30% of both egg and milk protein (**Figure 3**).

Color of the extruded material is another indication of the extent of the Maillard reaction. **Figures 4** and **5** support the argument that losses of these amino acids and sugars may be due to Maillard reaction (browning reaction), indicating darker color in samples with lower lysine and sugar retention. Samples with galactose showed greater browning compared to fructose with both proteins. Overall, fructose had a greater retention in the products compared to galactose under all processing conditions.

DISCUSSION

Investigations of the effects of reducing sugars (fructose and galactose) on the amino acid profile revealed that lysine showed the greatest loss of all the essential amino acids (**Table 2**). Only minor losses were found in other essential amino acids. The results revealed a decrease in the retention of threonine and methionine with an increase in temperature. A similar finding of minor loss (13%) of methionine was also reported during extrusion cooking

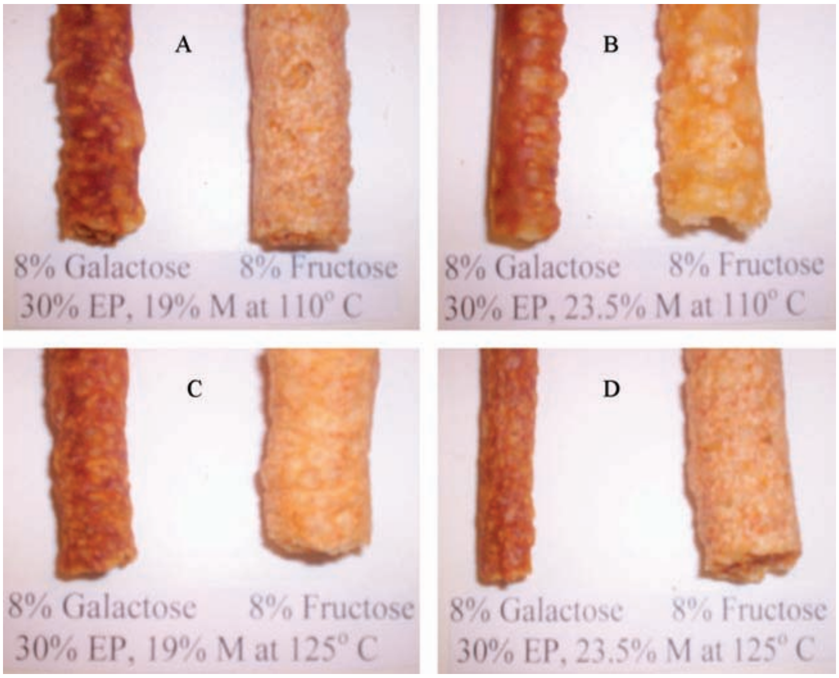

Figure 4. Browning comparison between 8% galactose and 8% fructose with 30% egg protein at (**A**) 19% M, 110 °C; (**B**) 23.5% M, 110 °C; (**C**) 19% M, 125 °C; and (**D**) 23.5% M, 125 °C. EP, egg protein; M, feed moisture.

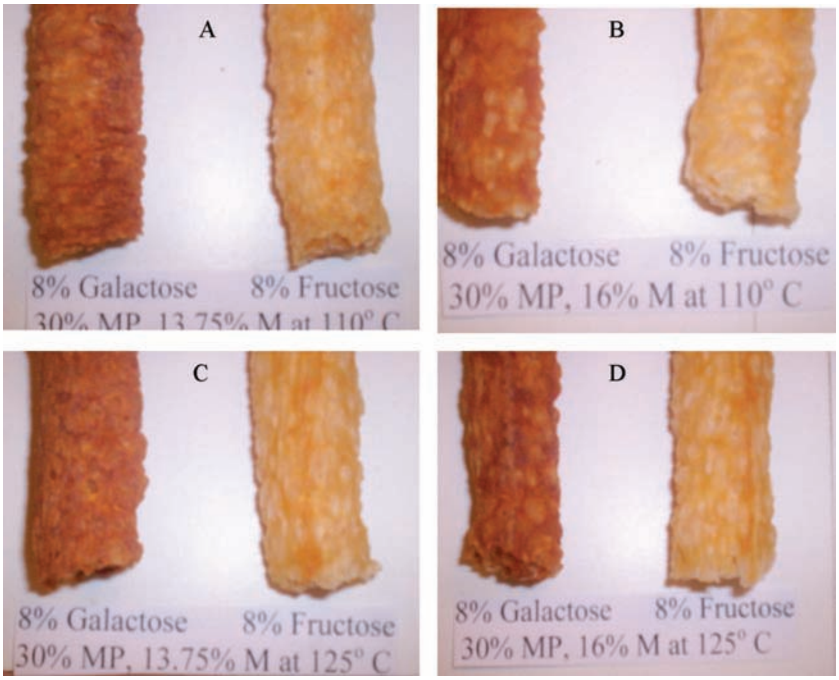

Figure 5. Browning comparison between 8% galactose and 8% fructose with 30% milk protein at (**A**) 13.75% M, 110 °C; (**B**) 16% M, 110 °C; (**C**) 13.75% M, 125 °C; and(**D**) 16% M, 125 °C. MP, milk protein; M, feed moisture.

of soybeans at a barrel temperature of 127–154 °C, a feed moisture of 14%, and a residence time of 20 s (*15*). Decreases in phenylalanine, tyrosine, serine, and isoleucine with increasing temperature were reported during extrusion of potato flakes at a barrel temperature of 70–160 °C, a feed moisture of 48% and a screw speed of 100 rpm (*22*). Minor losses of cysteine, aspartic acid, tyrosine, and arginine at lower moisture content (<14.5%) were also reported by Bjorck and Asp (*15*) and Iwe et al. (*23*), but there is no literature available for losses of leucine, tryptophan, and threonine. In this study, there were no significant changes in the retention of essential amino acids, except lysine, in the products made from milk protein.

The results for lysine retention confirmed that it decreased as sugar content, protein level, and temperature increased and feed moisture decreased. The reduction in lysine content is attributed to the occurrence of the Maillard reaction. Several investigations also confirmed similar results for the losses of lysine (*15*–*19*). The Maillard reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars and leads to a decrease in the availability of the amino acids involved. The rate of the Maillard reaction depends on the reactivity of the amine involved and the reducing sugar. Lysine appears to be the most reactive amino acid due to the fact that it has two available amino groups (*20*). This supports the fact that the greatest loss of all the essential amino acids was for lysine. It was evident from the results of this study that extrudates made from galactose showed a greater loss of lysine compared to those made from fructose, which suggests greater participation of galactose in the Maillard reaction, resulting in less retention of lysine in the products as compared to fructose. This was supported by the results of less retention of galactose in the products than of fructose (**Figure 3**). This may be the result of higher reactivity of galactose in the Maillard reaction compared to fructose. The higher reactivity of galactose can be explained by its higher amount present in the acyclic form (*24, 25*). The acyclic form of the sugar is the form in which the sugar reacts with the lysine residues in the Maillard reaction. This suggests that fructose is a better option than galactose for achieving higher nutritional properties in extruded products. By comparing levels of sugars and proteins, it was found that losses of lysine were greater at higher levels (8%) of fructose and galactose and 30% egg and milk proteins. This could be explained by a greater availability of carbonyl groups of fructose or galactose and the ε-amino group of lysine for reaction. Similar results of pronounced losses of lysine at higher levels of soy addition, which apparently has a higher lysine content, during the extrusion of soy–sweet potato mixtures were reported by Iwe et al. (*26*).

There were minor losses in lysine retention without the addition of sugars in the feed mixture, which may be due to hydrolysis of starch. Free sugars may be produced from starch hydrolysis during extrusion to react with lysine and other amino acids with free terminal amines ((*9, 13,*) (*27*)). A similar finding of losses of available lysine in the cereal mixture extruded without the addition of sugars was reported by Beaufrand et al. (*28*). The results revealed that products made from milk protein (especially with galactose) showed less retention of lysine compared to those made from egg protein. This may be attributed to a higher amount of lysine present in milk protein than egg protein and more severe conditions (low feed moisture) being used with milk protein extrusion.

The effect of extrusion conditions on the nutritional quality of extruded products is highly dependent on the moisture content and temperature. Protein nutritional value generally reflects the degree of heat treatment received by the product (*29*). In this study, greater lysine damage was reported under severe conditions (low feed moisture and high temperature). This might be due to the high energy inputs required for extrusion under these conditions, resulting in the high friction and, consequently, the high degree of molecular fragmentation and thus irreversible chemical modification of amino acids (*2*, *30*–*32*). The explanations for damaging effects at low water contents include an increase in local temperature through intense shear forces, specific mechanical effects (splitting of the glycosidic bonds of starch, decrease in the diffusion barrier existing at low water content), an enhancing effect of low moisture on the Maillard condensation, or a combination of these effects (*2*). The results indicated that a higher feed moisture content improved lysine retention. This beneficial effect of moisture on lysine retention could be a secondary effect because water, by functioning as a lubricant, reduces friction during the extrusion process (*3*). Overall, it could be inferred from the results of this study that high feed moisture and low temperature offer a positive effect on lysine retention.

In conclusion, lysine was found to be the most seriously affected amino acid during extrusion cooking of pregelatinized wheat flour with egg and milk proteins and fructose and galactose. Minor losses of leucine, phenylalanine, methionine, tryptophan, and threonine were found during the extrusion of egg protein, whereas with milk protein only lysine showed a significant reduction. Lysine retention significantly decreased with increasing levels of fructose and galactose and egg and milk proteins in the feed mixtures. Lower lysine retention was found at higher product temperature and lower feed moisture. Sugar analysis revealed that the retention of galactose was much lower compared to fructose in the extruded products, suggesting a higher interaction of galactose with amino acids in the Maillard reaction. Products containing fructose have a higher lysine retention compared to those containing galactose, which suggests that fructose is a better alternative than galactose for developing highly nutritious extruded products. To achieve greater retention of lysine and other essential amino acids, high feed moisture and low temperature were the more favorable conditions.

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