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High performance liquid chromatographic (HPLC) determination of available lysine in milk protein-maize composite extrudates and its stability during storage

T. R. Thirumuruga Ponbhagavathi*, Ashish Kumar Singh, P. Narender Raju and Neelam Upadhyay

Dairy Technology Division, ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India

E-mail: ponbhagavathi2015@gmail.com

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Effect of three processing variables: Milk protein sources (Rennet Casein and Whey Protein Concentrate-70 (WPC-70)), feed composition (4, 6 and 8% of milk protein source in addition to maize), feed moisture content (12,14,16%) on available lysine using HPLC was studied following extrusion processing of mixtures of milk protein and maize composite flour at extrusion temperature 100°C with 340 rpm and optimized product was studied for storage stability at two different temperatures 25°C and 37°C. Results showed that rennet casein addition at 8% with 12% feed moisture content resulted in higher available lysine of 8.84 g/kg of sample whereas the WPC-70 addition at 8% with 14% feed moisture content had significantly ($p < 0.05$) lower available lysine content of 5.48 g/kg of sample due to higher denaturation at 100°C. The available lysine content of the product on the initial day was found to be 8.36 (g/Kg of Sample) which reduced after one month of storage to 4.28 g/Kg of Sample and 2.97 g/Kg of Sample at 25°C and 37°C, respectively. Optimum extrusion parameters and storage conditions resulting in maximum available lysine was estimated.

Keywords: Maillard reaction, available lysine, milk protein-maize composite extrudates, 1-fluro-2,4-dinitrobenzene (FDNB), HPLC.

Introduction

Extrusion cooking is a high temperature – short time process, which involves cooking, forming and drying the product in an integrated process is one of the most important processing techniques used in the development of food products[1]. During an extrusion process the wet raw material is subjected to high temperature, pressure, and intense shear forces that cause intensive changes in raw material components, which favors the development of browning reaction leading to the formation of maillard end products[2]. Although extrusion processing results in increased protein digestibility, overheating causes degradation and reduces the bioavailability of protein and essential amino acids like lysine due to exposure to high temperatures[3].

The Maillard reaction is maximal at water activity values in the range 0.5–0.7[4]. During extrusion process, intermediate moisture systems are produced and lysine losses increase considerably. Several reports had attempted to relate Maillard reaction and discoloration or browning to loss of lysine. The major nutritional change due to extrusion processing in soya-sweet potato mixtures was the loss of lysine, probably via reaction with reducing sugars[5]. Nutritional quality of corn snacks enriched with nanofiltered whey powder was studied for available lysine content at different feed moisture content[6].

Lysine content of cereal products is generally low and is one of the most sensitive and limiting amino acids in the human diet[7]. Milk protein is relatively high in protein and lysine and its production has considerably increased in recent years. Hence, combination of milk protein-maize in a food system can fulfill the nutrient quality of cereals. Extrusion of milk protein-maize system might favor Maillard reaction and lysine loss due to the presence of both reducing sugars and ε-amino group of lysine. The length and conditions of storage can also influence the progress of the Maillard reaction that occurs during their processing[8].

The 1-fluro-2,4-dinitrobenzene (FDNB) procedure has been considered as a standard reference method for monitoring reactive lysine[9]. The N-ε-[2,4-dinitrophenyl]-L-lysine (ε-DNP-Lysine) formed after FDNB derivatization is measured

using chromatographic methods. The aim of the study was to determine the effect of the addition of milk proteins on available lysine content in milk protein-maize extrudates from the raw material of different moisture content and their stability during storage at different temperature.

Results and discussion

Available lysine content of extrudates:

Lysine measurement has received much attention because lysine is the limiting essential amino acid in many foods as well as it is one of the very reactive amino acids, and its availability is often monitored as an indicator of the severity of processing on the nutritional quality of protein foods[10]. The ε-amino group in lysine facilitates its participation in degradational reactions affecting the bioavailability and nutrition value of protein rich food products during heat processing and storage[11]. Maillard reaction occurs in many foods, including dairy products during extrusion processing. In an early step, the free amino groups of the proteins react non-enzymatically with reducing sugars and in the advanced step of Maillard reaction, proteins are modified into colored, fluorescent and cross-linked molecules. The Maillard reaction plays an important role in the production of undesirable organoleptic characteristics (flavor and color)[12] and in the decrease of nutritional quality, especially loss of lysine[13].

The available lysine content of extrudates are presented in Fig. 1. The description of samples are Control - 100% maize extrudates ; C0812- Rennet Casein 8% + maize +12% feed moisture; C0612- Rennet Casein 6% + maize +12% feed moisture; W0814 - WPC-70 + maize + 14% feed moisture; W0412 - WPC-70 + maize + 12% feed moisture; C0814 - Rennet Casein 8% + maize + 14% feed moisture. There was improvement in available lysine content with incorporation of milk protein in formulation. At 100°C, the losses were greater in whey proteins because of the denaturation of whey proteins, which may favor the Maillard reaction[6].

The control sample was found to have significantly ($p < 0.05$) lower available lysine content of 1.83 g/kg of sample.

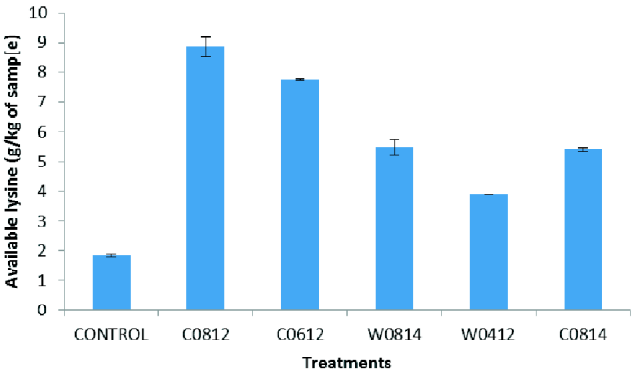

Fig. 1. Available lysine content of milk protein-maize based extrudates.

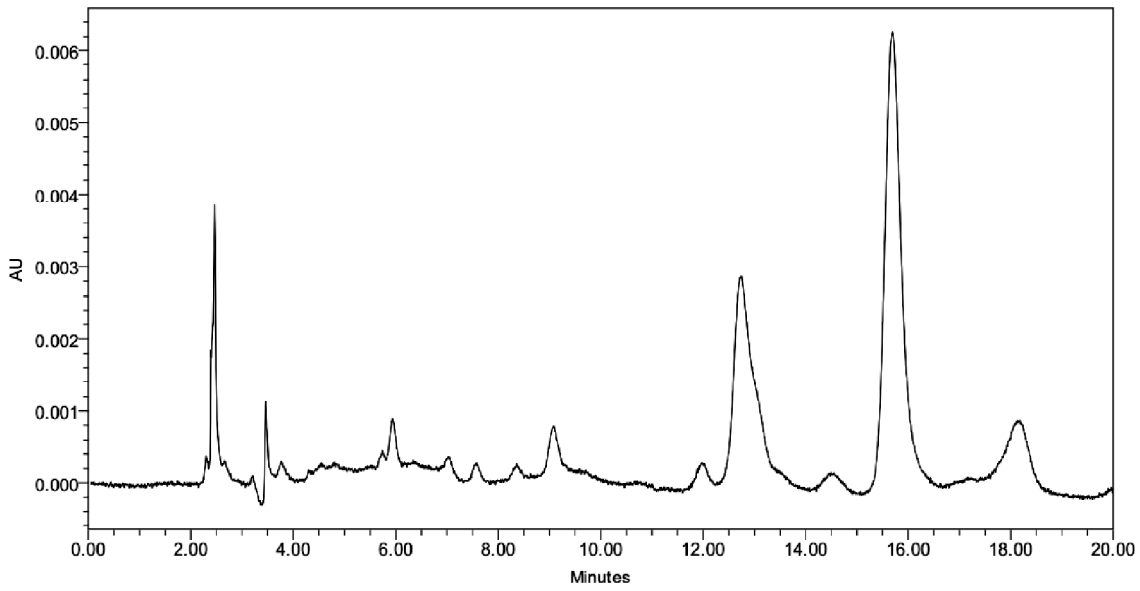

Fig. 2. Chromatogram of C0812 with area 197855 at retention time 15 min.

Similar trend of results were found in cereals that the available lysine were in the range of 1.71–1.78 g/kg of sample[13]. Both protein content and feed moisture influenced the available lysine content significantly. Increasing the protein content at lower feed moisture resulted significantly higher available lysine content than extruding at higher moisture content. In our study C0812 resulted in higher available lysine of 8.84 g/kg than C0814 of 5.40 g/kg. This result was supported by available lysine content of peanut meal extrudates[10]. Among the milk protein incorporated extrudates maximum available lysine was found for C0812, whereas W0412 had lowest values for the same. The extrudates resulted in higher available lysine content was studied for the stability at different temperature storage condition.

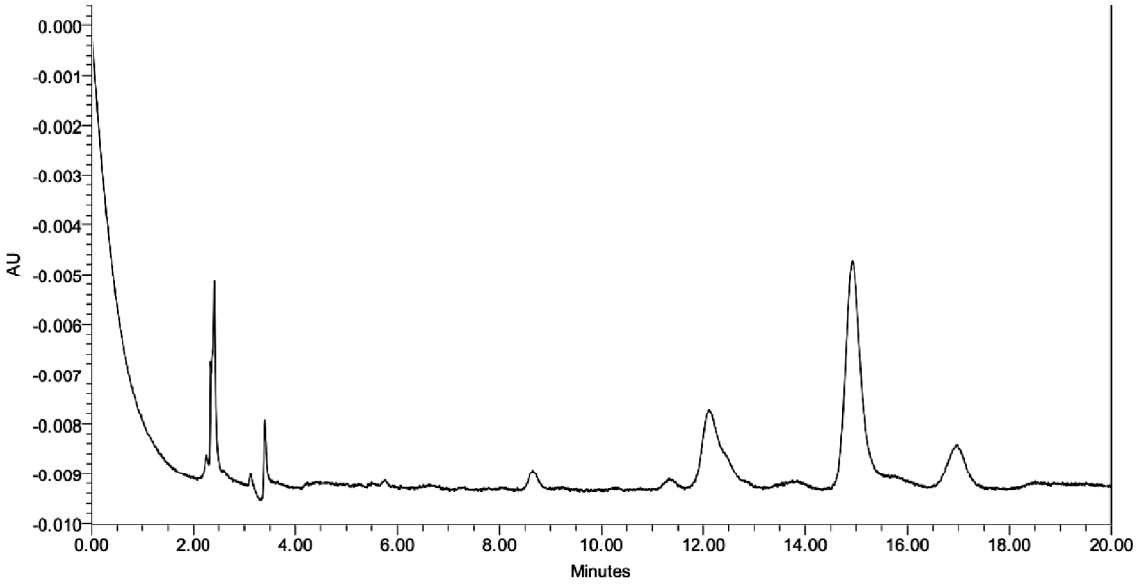

Fig. 3. Chromatogram of C0814 with area 123562 at retention time 15 min.

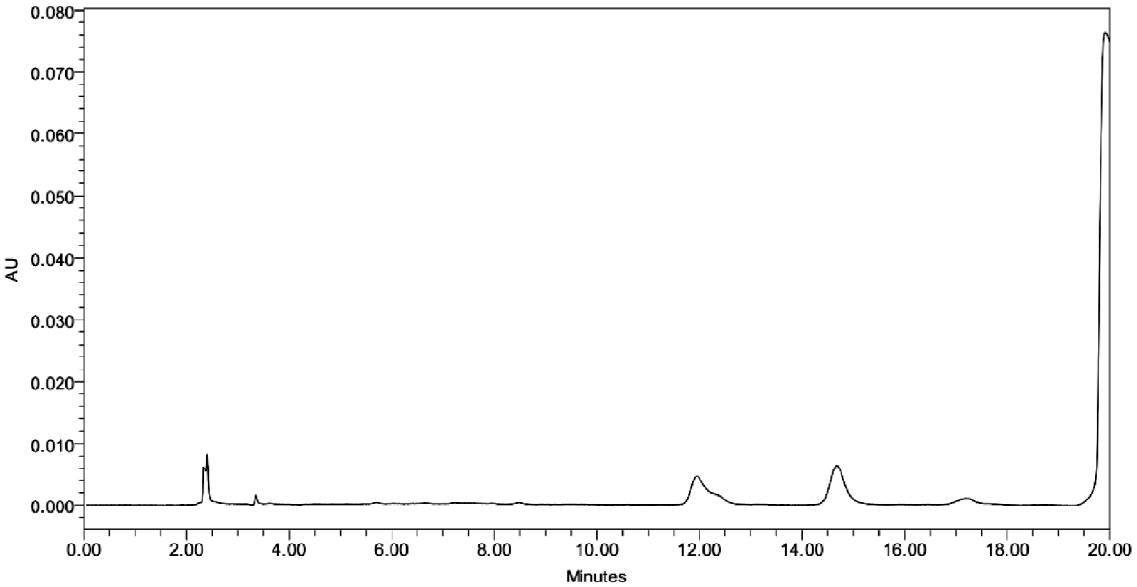

Fig. 4. Chromatogram of W0814 with area 118104 at retention time 15 min.

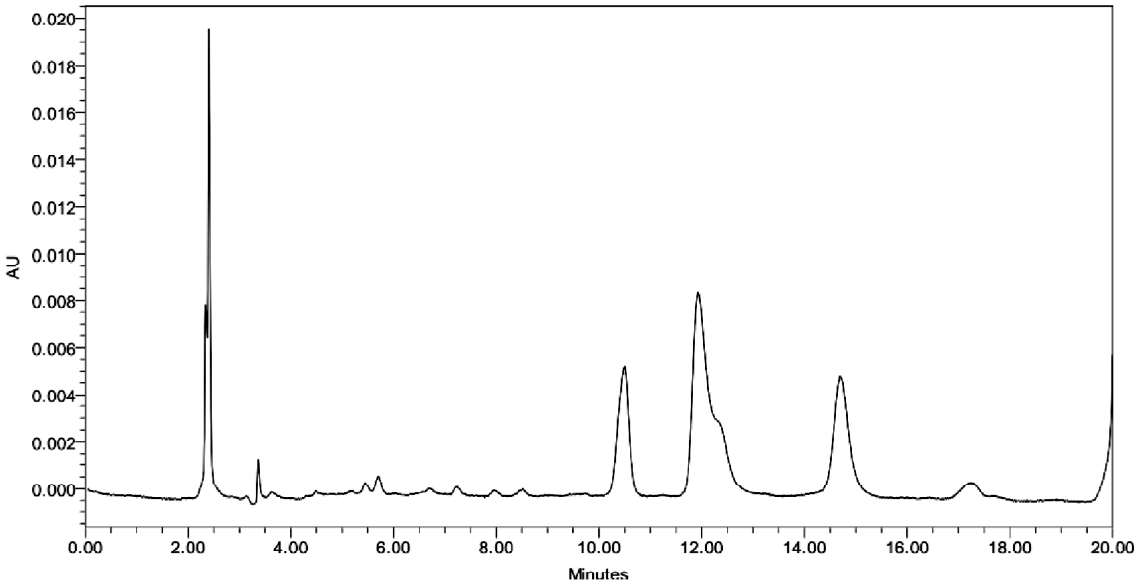

Fig. 5. Chromatogram of W0412 with area 94349 at retention time 15 min.

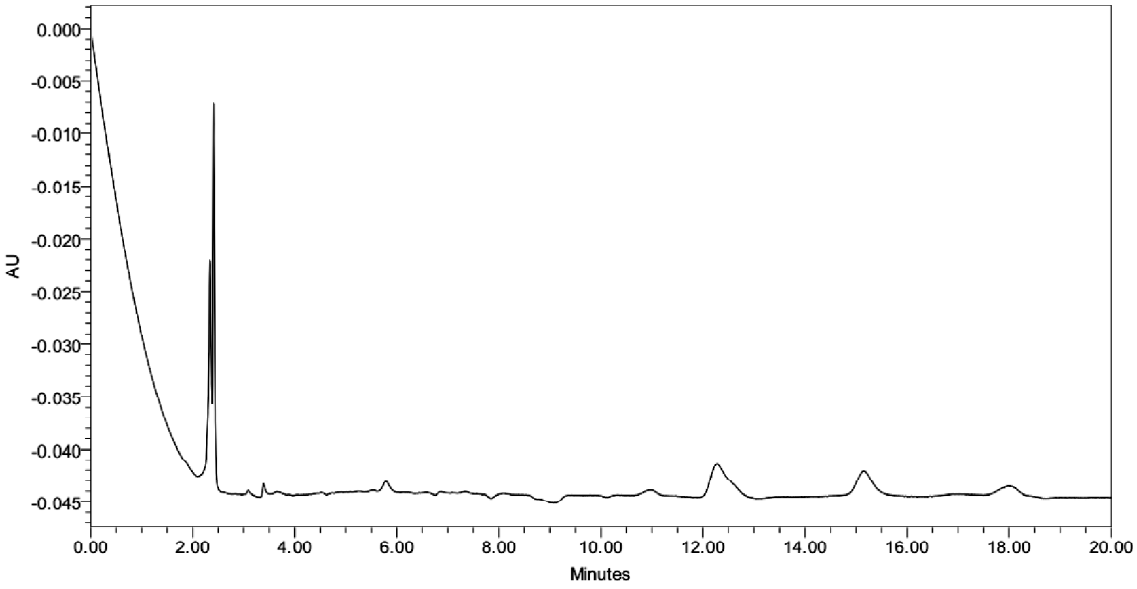

Fig. 6. Chromatogram of C0814 with area 123562 at retention time 15 min.

Changes in available lysine content of extrudates during storage:

Extrusion processing generates heat friction which promotes interaction among the sugars and free amino acids or peptides/proteins leading to Maillard browning reaction[14]. Lysine is one among the amino acids mostly take part in Maillard reaction. The ε-amino group of protein-bound lysine can react with glucose, maltose or lactose to form amadori reaction products, which are not susceptible to attack by proteolytic enzymes during digestion. Loss of available lysine, which is the most negative nutritional consequence of the Maillard reaction, is particularly of significance in cereals which are limiting in this essential amino acids.

Loss of nutritive value in heated foods is due to block-

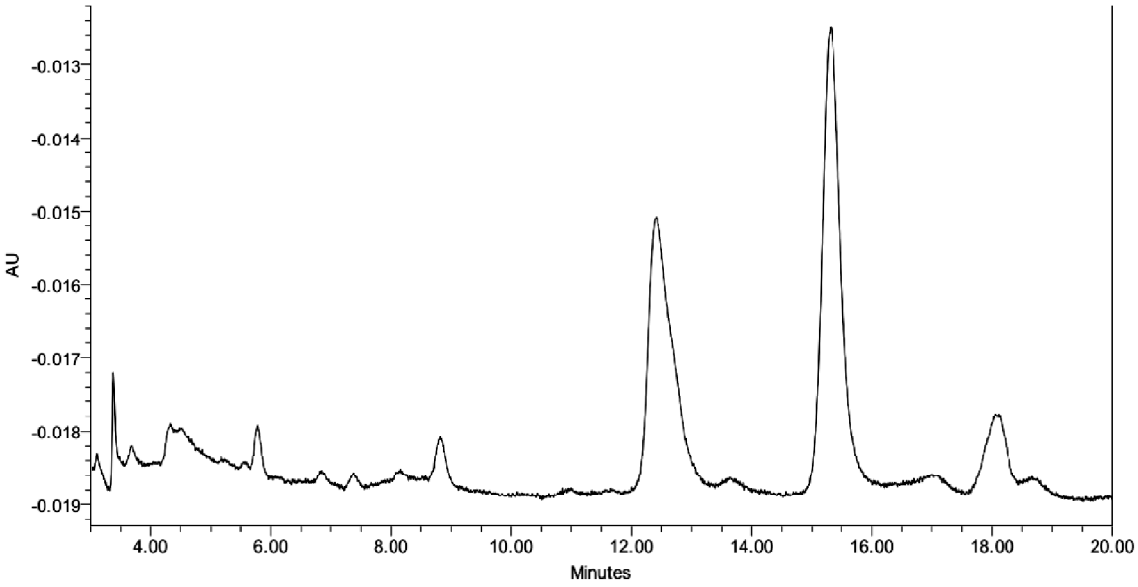

Fig. 7. Chromatogram of control with area 49445 at retention time 15 min.

age of lysine residues, which are not available for digestion due to complex formation at all stages of Maillard reaction[15]. Loss of available lysine may also attributed to reduced digestibility and inhibition of enzymes[16]. Available lysine was determined in order to ascertain the percent loss of lysine mainly through its involvement of environment in Maillard browning reactions. The changes in available lysine content of extrudates during storage at 25°C and 37°C are presented in Table 1.

It has been observed that the available lysine content of the product decreased significantly ($p < 0.05$) during the storage at 25°C and 37°C .The available lysine content of the product on the initial day was found to be 8.36 (g/kg of Sample) which reduced after one month of storage to 4.28 g/kg of Sample and 2.97 g/kg of Sample at 25°C and 37°C, respectively. The loss of available lysine was maximum 48% at 25°C and of 64% at 37°C in the extrudates after 1 month of storage. Lysine losses were greater at 37°C than at 25°C, which clearly indicates the influence of temperature on Maillard reaction. Maillard reaction once initiated remain continue during storage; however the rate of reaction depends on temperature. The reason behind it was due to the higher loss of available lysine at higher temperature.

Similar trend was reported in commercial infant cereals in which addition of milk solids doubled the loss of available lysine after storage at room temperature (25°C and 32°C), compared with the milk-free products. The loss of available lysine was 10% and 23% in commercial samples after 1 year of storage at 32°C and 55°C, respectively[7].

Table 1. Changes in available lysine content of extrudates during storage

Storage period	Available lysine content 25°C			Available lysine content 37°C		
(weeks)	g/kg of Sample	g/kg of protein	Lysine losses (%)	g/kg of Sample	g/kg of protein	Lysine losses (%)
0	8.36 ± 0.19^{d}	49.06	–	8.36 ± 0.19^{d}	49.06176	–
1	7.67 ± 0.15^{c}	45.02	8.23	6.23 ± 0.38^{c}	36.57181	25.45
2	6.19 ± 0.16^{b}	36.37	25.86	4.51 ± 0.14^{b}	26.45341	46.08
3	4.76 ± 0.24^{a}	27.94	43.04	3.60 ± 0.07^{c}	21.14166	56.90
4	4.28 ± 0.21^{a}	25.15	48.71	2.97 ± 0.35^{c}	17.42261	64.48

^{a,b,c,d}Means with the different letter within column are significantly different ($p < 0.05$).

Mean ±S.E. (n = 3).

Experimental

Raw materials and sample preparation:

Maize grains and milk protein sources were procured from Karnal Farmer and Modern Dairies Ltd., Karnal, Haryana, India respectively. Level of milk proteins in formulation was adjusted by using requisite quantity of rennet casein/WPC-70. Maize flour and milk proteins viz. WPC-70/ Rennet casein were dry-blended by passing through sieve (2 mm) and calculated water was sprayed over it to adjust 12, 14 and 16% moisture in pre-mix. The mixture was again passed through 2 mm size sieve and blended for 15 min to obtain a uniform mixture. The pre-mix was packaged in 1 kg Low Density Polyethylene (LDPE) bag and stored overnight for equilibration of moisture.

Extrusion cooking:

A twin screw extruder (Basic Technology Pvt. Ltd., Kolkata, India) was used for the preparation of extrudates. The conditioned mixture was fed to feed hopper equipped with screw augers to load materials into the barrel at uniform rate. The extruder screw speed was set to 340 rpm throughout the experiment. The temperature of inlet and outlet cooking section was set to 40°C and 100°C, respectively. The plasticized mass was passed through 4 mm die and extruded samples were dried for 30 min at 50°C. The dried samples were collected and stored in the appropriate laminated bags for further sensory and textural analysis.

Storage studies:

Optimized product prepared by extrusion was packed in metallized LDPE pouches and they were kept in incubator at 25±1°C and 37±1°C for 30 days. The samples were drawn at an interval of 1 week for analysis of available lysine content.

High performance liquid chromatography-Available lysine content:

The available lysine of the extrudates by HPLC method was determined by adopting the method of Peterson[17] with slight modifications. Five hundred milligram of sample was accurately weighed and placed in a 250 mL boiling flask. Glass beads along with 12.5 mL of 8% sodium bicarbonate was added and allowed it to wet for 10 min. After the sample was wetted by the sodium bicarbonate solution, 15 mL of an ethanol solution containing 0.4 mL of FDNB solution was added and the sample was shaken for 2 h in the orbital shaker at room temperature. The ethanol was then boiled off in a hot water bath. 30 mL of 8.1 *N* HCl were added to the sample and refluxed for 16 h. After hydrolysis, the sample was filtered while hot into a 250 mL volumetric flask and brought to volume with distilled water. Approximately 2 mL was then filtered through a 0.2 μm disc filter. Separation and quantification of DNP-lysine in the sample was then done by HPLC.

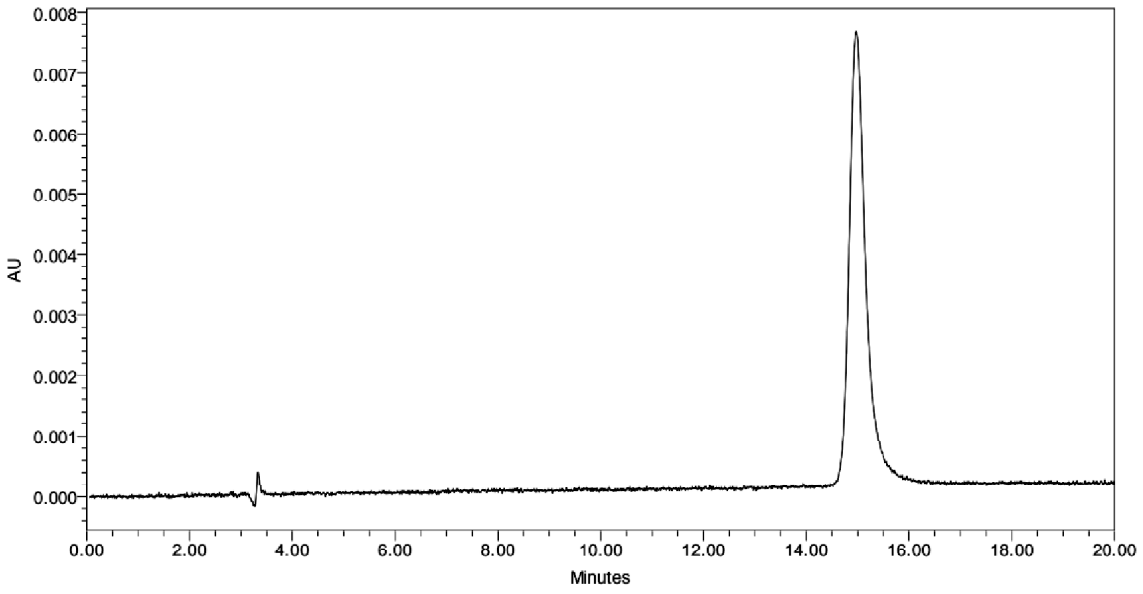

Fig. 8. Chromatogram of standard ε-lysine at retention time 15 min.

ε-DNP-lysine was determined by the external standard method. The concentration range was 5–25 μg/mL, with a correlation coefficient (r^2) of 0.926.

Chromatographic conditions:

Ten microliters of filtered solution were separated in a reverse-phase C_{18} HPLC column (250×2 mm i.d 5 μ particle size, Waters Alliance 2695 with PDA detector). The mobile phase was 20% acetonitrile and 80% 0.01 *M* sodium acetate buffer (pH 4.0). The elution was isocratic and the flow rate was 1 ml/min. The UV detector was set at 436 nm. The run time of ε-DNP-lysine was 20 min.

Statistical analysis:

Results of the analytical determinations were expressed as mean ± standard error (SE) of 3 measurements. The influence of independent formulation and processing variables on available lysine content of extrudates was analyzed using one-way analysis of variance. Significant differences were defined at $p < 0.05$. All the analyses were performed using IBM SPSS Statistics 20.

Conclusion

Incorporation of milk proteins into extrudates improved the nutritional value. Addition of Rennet Casein to the maize flour during extrusion showed significantly ($p < 0.05$) higher available lysine content of 8.84 g/kg compared with WPC-70. Storage stability of milk protein-maize based extrudates has resulted in significant difference in available lysine. Storing the samples at 25°C had the maximum retention of available lysine. The product developed will create new domestic and export market opportunities for the food and dairy industry. Research has also produced value added products from maize with good nutritional and acceptable product characteristics.

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References

1. L. Moœcicki and D. J. van Zuilichem, *Extrusion-cooking Techniques: Applications, Theory and Sustainability*, 2011, 1.
2. T. M. Masatcioglu, P. K. Ng and H. Koksel, *Journal of Cereal Science*, 2014, **60(2)**, 276.
3. Q. D. Tran, W. H. Hendriks and A. F. van der Poel, *Journal of the Science of Food and Agriculture*, 2008, **88(9)**, 1487.
4. P. Fernandez Artigas, B. Garcia Villanova and E. Guerra Hernandez, *Journal of the Science of Food and Agriculture*, 1999,**79(6)**, 851.
5. M. O. Iwe, D. J. Van Zuilichem, W. Stolp and P. O. Ngoddy, *Journal of Food Engineering*, 2004, **62(2)**, 143.
6. A. Makowska, D. O. R. O. T. A. Cais-Sokoliñska, A. G. N. I. E. S. Z. K. A. Wacœkiewicz, G. Tokarczyk and H. Paschke, *Czech Journal of Food Sciences*, 2011, **34(2)**, 154.
7. S. D. Hood-Niefer and R. T. Tyler, *Food Research International*, 2010, **43(2)**, 659.
8. A. Ramýìrez-Jiménez, E. Guerra-Hernández and B. Garcýìa-Villanova, *Food Chemistry*, 2003, **83(2)**, 219.
9. K. J. Carpenter, *Biochemical Journal*, 1960, **77(3)**, 604.
10. F. K. Saalia and R. D. Phillips, *LWT-Food Science and Technology*, 2011, **44(6)**, 1496.
11. A. K. Rathour, V. Rathore, B. M. Mehta, S. M. Patel and A. Chauhan and K. D. Aparnathi, *Journal of Food Processing and Preservation*, 2017, **41(3)**, e12882.
12. S. Wang and F. Zhu, *Food and Bioprocess Technology*, 2016, **9(1)**, 49.
13. J. A. Rufián-Henares and C. Delgado-Andrade, *Food Research International*, 2009, **42(3)**, 394.
14. S. Arena, G. Renzone, C. D'Ambrosio, A. M. Salzano and A. Scaloni, *Food Chemistry*, 2017, **219**, 477.
15. J. Gan, G. M. Bornhorst, B. M. Henrick and J. B. German, *Molecular Nutrition and Food Research*, 2018, **62(1)**, 1700231.
16. M. Friedman, *Journal of Agricultural and Food Chemistry*, 1996, **44(1)**, 6.
17. W. R. Peterson and J. J. Warthesen, *Journal of Food Science*, 1979, **44(4)**, 994.