AMINO ACID PROFILE AND IN VITRO PROTEIN DIGESTIBILITY-CORRECTED AMINO ACID SCORE (PDCAAS) OF READY-TO-EAT BREAKFAST CEREALS: AN ASSESSMENT OF PROTEIN QUALITY

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***Abstract*— The aim of this study was to determine the amino acid content and in vitro protein digestibility of breakfast cereals and evaluate their protein quality using in vitro protein digestibility-corrected amino acid score (PDCAAS) methods. Statistically big differences were found in the proportions of essential amino acids between breakfast cereals. Higher protein digestibility was found in samples containing rice and corn than those containing oats. The first limiting amino acid score (AAS) in 6 out of 12 samples was methionine + cysteine, in 4 out of 12 samples was lysine, and in 2 out of 12 samples was tryptophan. According to the first limiting AAS, the in vitro PDCAAS ranged from 0.19±0.01 to 0.86±0.02 in breakfast cereals. When we evaluated the first limiting AAS, lysine, methionine+cysteine and tryptophan predominantly determined PDCAAS. As a result, the PDCAAS, which is an indicator of protein quality, was generally low in breakfast cereal products.**

***Keywords*— Nutrition, Breakfast Cereal, Protein quality, *In vitro*, Digestibility.**

I. INTRODUCTION

Breakfast, which is part of an adequate and balanced diet, is an important component of a healthy diet. Consumption of breakfast cereal products has gradually increased due to changing eating habits (Gibney *et al*., 2018; Williams, 2014). Breakfast cereal is considered a good source of protein in daily nutrition (Devi *et al*., 2014; Louie *et al*., 2012). Protein is composed of amino acids and is essential for life. Proteins act as enzymes in many metabolic reactions, serve as hormones, skeletal muscle proteins, antibodies, and receptors, and facilitate tissue repair (Collins, 2009). According to the Acceptable Macronutrient Distribution Range (AMDR), 10-35% of daily calorie intake from protein was developed to express dietary recommendations in the context of a complete diet. The recommended dietary allowance for both men and women is set at 0.8 g per kg of body weight (Wolfe *et al*., 2017). The protein quality is evaluated based on the essential amino acids (EAAs) content and EAAs are not synthesized by human metabolism and must be taken with diet (Collins, 2009; Leser, 2013).

Breakfast cereals are produced through extrusion, which changes the biochemical properties of food (Patil *et al*., 2016). Extrusion greatly affects the nutritional value of breakfast cereals (Yaman *et al*., 2019). This process takes place at high temperatures and pressures and divides proteins and starches into smaller structures to increase digestibility (Oliveira *et al*., 2015; Moreno *et al*., 2017). In addition, extrusion can cause the loss of essential amino acids, particularly lysine (Paes and Maga, 2010).

In 1991, the FAO/WHO suggested using the PDCAAS for assessing protein quality (FAO/WHO, 1991). The PDCAAS grades the quality of a protein based on the content of EAAs contained therein (Collins, 2009). The PDCAAS uses a digestibility value based on total digestibility of crude protein determined in rats and multiplies this value by the concentration of the first limiting AA, which is identified by comparing the AA profile of the test protein with the profile of the presumed AA requirement for 2- to 5-year-old children (Mathai *et al*., 2017). A PDCAAS of 1 represents that after digestion, the protein provides 100 percent of the essential amino acids required by the organism (Mathai *et al*., 2017; Rutherfurd *et al*., 2014). The highest PDCAAS is 1, and scores above 1 are truncated to 1. The rate of digested protein is important in determining the PDCAAS of a food protein. Low digestibility will give low PDCAAS (Wolfe *et al*., 2016). According to the WHO/ FAO/UNU (2007) and some studies, the most limiting amino acids are lysine, sulphur-containing amino acids, threonine, and tryptophan (Pérez-Conesa *et al*., 2002), valine, threonine, isoleucine and leucine (Almeida *et al*., 2015) when determining PDCAAS.

There is limited data about PDCAAS of proteins because clinical studies are time consuming, expensive, and have ethical problems. However, studies report con sistent results between in vivo and in vitro PDCAAS (Rozan *et al*., 1997; Tavano *et al*., 2016). The aim of this study was to determine the amino acid profile, in vitro protein digestibility, and protein quality using the PDCAAS for breakfast cereals.

Table 1. Product list, main contents, and declared and measured amounts of protein in breakfast cereals

Sample	Main product contents	Declared (g/100 g)	Measured (g/100 g)	% of declared
Breakfast Cereal 1	Whole grain and rice flakes	1	7.6 ± 0.3^{ef}	81.3 ± 2.8^{g}
Breakfast Cereal 2	Whole grain and rice flakes	9	7.9 ± 0.3^{de}	87.3 ± 2.9^{efg}
Breakfast Cereal 3	Whole grain and rice flakes	9.4	8.7 ± 0.3^{bcd}	92.3 ± 2.7^{def}
Breakfast Cereal 4	Wheat and rice flakes	9	8.8 ± 0.3^{bc}	97.4 ± 2.8^{bcd}
Breakfast Cereal 5	Whole corn flakes	7.1	6.8 ± 0.2^{f}	95.8 ± 2.8^{cde}
Breakfast Cereal 6	Corn and wheat flakes	6	4.8 ± 0.3^{g}	79.4 ± 2.5^{g}
Breakfast Cereal 7	Corn and wheat flakes	7.9	7.6 ± 0.3^{ef}	95.8 ± 3.2^{cde}
Breakfast Cereal 8	Oats, barley, rye, corn, and whole wheat	7.9	8.1 ± 0.3^{cde}	103 ± 3.9^{bc}
Breakfast Cereal 9	Oat fiber,barley malt extract and whole grains	10.6	9 ± 0.3^{b}	85.2 ± 2.9^{fg}
Breakfast Cereal 10	Oatmeal	10.9	9.2 ± 0.3^{b}	84.7 ± 2.8^{fg}
Breakfast Cereal 11	Oatmeal, flax seed and dried fruits	7.6	8.1 ± 0.3^{cde}	107 ± 4.0^{b}
Breakfast Cereal 12	Oatmeal with hazelnut	7.1	11.4 ± 0.4^{a}	161 ± 5.7^{a}

Values are means ± standard deviation, n = 3. The different letters in the same column indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).

Table 2. HPLC Gradient program for amino acid analysis

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.01	100	0
13.00	84	16
22.00	64	36
26.00	60	40
26.01	40	60
33.00	100	0
40.01	100	0

II. METHODS

A. Sampling

In this research, 12 different extruded breakfast cereals were obtained from local markets in Istanbul, Turkey. Product list and main contents are shown in Table 1.

B. Amino Acid Analysis

Determination method for amino acids described by Bidlingmeyer *et al*. (1984) was used with some modifications. 0.5 g sample was added to a 50 ml schott glass bottle. Then, 20 ml 6 N HCl was added and hydrolyzed in an oven at 120 °C for 24 h. Next, the samples were cooled to room temperature and filtered. Then, 0.2 ml filtrate was evaporated at 50 °C under nitrogen in a 10 ml test tube. The sample was washed two or three times with 0.1 mL distilled water and 0.1 mL acetonitrile (ACN) to remove the acid under nitrogen. The Edman's Reagent reagent phenylisothiocyanate (PITC) was used for precolumn derivatization of amino acids. The derivatization phase utilized 0.5 ml coupling solution (ACN:MeOH:TEA, 100:50:20, v/v) and 0.1 mL derivatization reagent (1.2% PITC in ACN) added to the test tube and kept in an oven at 40 °C for 30 min. The derivatized sample was evaporated at 40 °C under nitrogen and then washed two times with 0.2 ml ACN. Finally, 5 ml 0.02 M ammonium acetate buffer solution was added and the sample was filtered with a 0.22 µm cellulose acetate (CA) filter.

C. HPLC Determination of Amino Acids

Separation method for amino acids described by Siebert *et al*. (1991) was used with some modifications. A Shimadzu Nexera-i HPLC with a Shimadzu UV-20A UV-Vis detector (Shimadzu Corporation, Kyoto, Japan) was used to separate amino acids. The mobile phase consisted of buffer solution (A) and acetonitrile (B). Mobile Phase A: 0.78 g NaH2PO4.2H2O and 0.88 g Na2HPO4.2H2O were weighed in a 1 L flask and dissolved with deionized water. Then, the pH of the mobile phase A solution was adjusted to 6.8±0.1 and filtered through a 0.22 µm CA filter under vacuum. The mobile phase gradient program used in the separation of amino acids is given in Table 2. The wavelength was 254 nm. The separation was achieved with a Gemini-NX 5u C18 110 Å, 4.6 x 250 mm column (Phenomenex, CA, USA) with a flow rate of 1 ml/min. The column oven temperature was 40 °C.

D. Cysteine Analysis

Cysteine analysis was performed according to the method specified by DS EN ISO 13903 (ISO, 2005). First, the oxidation mixture was prepared with 4.5 mL performic acid-phenol solution (4.73 g phenol in 89% formic acid and 11% water) mixed with 0.5 mL hydrogen peroxide. This solution was incubated at room temperature for 1 h in order to form performic acid. Then, the mixture was cooled in an ice-water bath before addition to the sample. Next, 0.5 g homogenized sample was weighed in a 10 ml glass test tube and 5 ml oxidation mixture was added and placed in a refrigerator at 0 °C for 16 h. After this step, the sample was hydrolyzed, derivatized and analyzed by HPLC (Section II.B).

E. Tryptophan Analysis

Tryptophan has an indole structure, which completely decomposes under acidic conditions. Therefore, basic hydrolysis is applied during analysis. Determination and separation method for tryptophan described by Çevikkalp *et al*. (2016) was used with some modifications. First, 0.5 g sample was added to a 50 ml schott glass bottle. Next, 20 ml 5 N NaOH solution was added and hydrolyzed in an oven at 120 °C for 12 h. The samples were cooled to room temperature. Then, samples were filtered through filter paper and 1 ml of filtered sample was put into a 250 mL beaker and adjusted to pH 6.3±0.1 using 1 M HCl solution. Then, the volume was completed to 100 mL with deionized water and filtered with a 0.22 µm CA filter and injected into the HPLC.

F. HPLC Determination of Tryptophan

A Shimadzu Nexera-i HPLC with a RF-20A detector (Shimadzu Corporation, Kyoto, Japan) was used to analyze tryptophan (Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of 90% buffer

Table 3. Amount of amino acids (mg/100 g) in breakfast cereals products

Amino acid mg/100 g	BC.1	BC.2	BC.3	BC.4	BC.5	BC.6	BC.7	BC.8	BC.9	BC.10	BC.11	BC.12
Asp	193±11^{d}	44±3^{f}	323±16^{c}	580±29de	151±7^{ef}	172±9^{b}	171±8de	108±5de	367±18^{c}	781±39^{a}	846±42^{a}	818±40^{a}
Glu	1789±63^{g}	695±24^{f}	2259±79^{b}	317±11^{cd}	1097±38^{e}	810±28^{g}	1959±69^{d}	1143±40^{b}	2034±71^{c}	2214±78^{f}	255±8.9^{e}	2862±100^{a}
Ser	379±15de	376±15^{g}	344±14^{ab}	441±18^{e}	275±11^{f}	152±6^{bc}	334±13^{d}	353±14de	430±17^{c}	477±19de	372±15de	504±20^{a}
Gly	431±16^{ef}	803±29^{i}	491±18^{cd}	560±20^{g}	291±10^{h}	181±6^{c}	362±13^{f}	470±17de	563±20^{c}	519±19^{a}	451±16^{def}	622±22^{b}
His	215±8^{d}	62±2^{f}	267±10^{b}	330±12^{d}	231±9^{e}	184±7^{c}	265±10^{e}	93±3^{d}	347±13^{c}	381±14^{h}	285±11^{g}	444±17^{a}
Arg	349±14^{b}	296±12^{g}	463±19^{c}	554±23^{ef}	205±8^{fg}	193±8^{b}	254±11^{d}	102±4^{c}	488±20^{c}	455±19^{e}	543±23^{h}	704±29^{a}
Thr	238±10^{d}	343±15^{g}	203±9^{c}	333±15^{fg}	149±7^{g}	148±7^{b}	173±8de	435±19^{ef}	315±14^{bc}	288±13^{b}	246±11^{a}	344±15^{b}
Ala	405±18^{c}	349±16^{f}	421±19^{c}	580±26de	634±29^{a}	286±13^{ab}	359±16^{cde}	572±26^{cd}	432±19^{c}	457±21^{e}	456±21^{ab}	563±25^{b}
Pro	813±39^{cd}	1702±81^{i}	690±33^{gh}	995±47de	791±38^{def}	395±19^{c}	820±39^{def}	1238±59^{fg}	542±26^{h}	569±27^{a}	909±43^{b}	762±36^{ef}
Tyr	348±16^{e}	338±15^{f}	340±15^{cde}	478±21^{e}	350±16de	186±8^{a}	317±14de	396±18^{e}	399±18^{bc}	360±16^{e}	343±15^{bcd}	424±19^{b}
Val	513±24^{c}	467±22^{e}	544±26^{c}	770±37^{c}	409±19^{d}	291±14^{a}	517±25^{c}	686±33^{c}	657±31^{b}	539±26^{cd}	534±25^{b}	733±35^{ab}
Met	130±6^{d}	194±9^{e}	191±9^{d}	80±4^{e}	155±7^{c}	72±3^{e}	88±4^{d}	229±11^{b}	115±5^{d}	122±6^{b}	112±5^{a}	44±2^{f}
Ile	365±9^{d}	247±6^{h}	408±10^{e}	565±14de	291±7^{f}	159±4^{a}	381±9^{e}	462±11^{d}	442±11^{c}	361±9^{g}	397±10^{bc}	489±12^{b}
Leu	723±25^{cde}	826±29^{f}	726±26^{e}	1060±37de	1108±39^{a}	452±16^{a}	753±27^{e}	1082±38^{e}	850±30^{c}	731±26^{cd}	802±28^{a}	961±34^{b}
Phe	515±16^{cde}	600±19^{h}	639±20^{ef}	803±25^{fg}	547±17^{fg}	250±8^{a}	561±17^{g}	678±21^{bcd}	680±21^{b}	574±18^{def}	623±19^{bc}	792±25^{a}
Lys	240±9^{a}	201±7^{b}	484±18^{c}	422±15^{e}	153±6^{g}	927±34^{d}	340±12^{f}	201±7^{c}	508±18^{c}	539±20^{fg}	1113±40^{fg}	513±19^{c}
Trp	66±4^{cd}	69±4^{g}	53±3^{a}	79±5^{ef}	28±2^{g}	22±1^{bc}	54±3de	85±5^{f}	79±5^{bc}	93±6^{cd}	68±4^{ab}	96±6^{a}
Cys	68±3^{cd}	102±5^{b}	101±5^{b}	40±2^{d}	66±3^{a}	35±2^{e}	44±2^{ef}	130±6^{e}	69±3^{cd}	78±4^{c}	72±3^{cd}	27±1^{f}

Values are means ± standard deviation, n = 3. The different letters in the same rows indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).

Table 4. Proportion of amino acids (per 100 g protein) in breakfast cereals products

A.A.	BC.1	BC.2	BC.3	BC.4	BC.5	BC.6	BC.7	BC.8	BC.9	BC.10	BC.11	BC.12
Asp	2.56±0.23^{ef}	0.37±0.27^{g}	3.73±0.30^{de}	6.62±0.52^{f}	2.23±0.18^{fg}	3.61±0.30^{c}	2.27±0.19^{de}	1.33±0.11^{f}	4.07±0.34^{d}	8.47±0.71^{b}	10.42±0.92^{a}	7.16±0.61^{bc}
Glu	23.66±0.17^{c}	8.85±0.07^{h}	26.04±0.26^{a}	3.61±0.03^{f}	16.13±0.10^{g}	16.98±0.16^{i}	25.89±0.18^{e}	14.06±0.14^{a}	22.52±0.21^{d}	23.98±0.22^{c}	3.14±0.03^{i}	25.04±0.20^{b}
Ser	5.01±0.04^{b}	4.78±0.04^{c}	3.97±0.05^{f}	5.03±0.06^{f}	4.04±0.04^{e}	3.18±0.03^{b}	4.41±0.03^{g}	4.34±0.03^{e}	4.76±0.04^{c}	5.16±0.05^{a}	4.57±0.03^{d}	4.41±0.03^{e}
Gly	5.70±0.04^{de}	10.21±0.07^{a}	5.66±0.06^{def}	6.38±0.06^{i}	4.28±0.03^{d}	3.79±0.04^{b}	4.79±0.03^{j}	5.78±0.05^{h}	6.23±0.06^{c}	5.62±0.05^{ef}	5.55±0.05^{fg}	5.44±0.04^{g}
His	2.85±0.02^{g}	0.79±0.01^{i}	3.07±0.03^{f}	3.77±0.04^{e}	3.40±0.03^{h}	3.87±0.04^{c}	3.50±0.02^{b}	1.15±0.01^{d}	3.85±0.03^{bc}	4.13±0.04^{a}	3.50±0.03^{d}	3.88±0.03^{b}
Arg	4.61±0.04^{f}	3.77±0.04^{h}	5.33±0.08^{d}	6.32±0.09^{j}	3.01±0.04^{k}	4.05±0.05^{b}	3.35±0.03^{g}	1.25±0.01^{i}	5.40±0.06^{d}	4.93±0.06^{e}	6.68±0.06^{a}	6.16±0.06^{c}
Thr	5.36±0.07^{e}	4.44±0.06^{b}	4.85±0.09^{h}	6.61±0.11^{j}	9.33±0.15^{a}	6.01±0.10^{c}	4.74±0.06^{f}	7.04±0.09^{i}	4.78±0.07^{d}	4.95±0.07^{f}	5.61±0.07^{g}	4.93±0.06^{g}
Ala	10.74±0.16^{e}	21.65±0.33^{g}	7.96±0.16^{f}	11.34±0.22^{a}	11.63±0.21^{b}	8.28±0.14^{c}	10.83±0.16^{d}	15.22±0.20^{f}	5.99±0.10^{f}	6.16±0.10^{f}	11.17±0.15^{e}	6.66±0.09^{f}
Pro	4.60±0.06^{e}	4.31±0.06^{a}	3.92±0.07^{f}	5.45±0.09^{c}	5.14±0.08^{b}	3.91±0.06^{cd}	4.19±0.06^{f}	4.87±0.06^{de}	4.42±0.06^{h}	3.90±0.06^{gh}	4.22±0.05^{cde}	3.71±0.05^{g}
Tyr	3.19±0.00^{d}	4.43±0.00^{ef}	2.38±0.00^{g}	3.87±0.01^{b}	2.22±0.01^{c}	3.17±0.00^{a}	2.32±0.00^{g}	5.42±0.03^{f}	3.54±0.01^{de}	3.17±0.01^{gh}	3.06±0.02^{f}	3.05±0.01^{h}
Val	6.89±0.04^{d}	6.03±0.03^{ij}	6.40±0.05^{g}	8.95±0.08^{hi}	6.12±0.07^{b}	6.22±0.03^{a}	6.93±0.04^{h}	8.55±0.01^{d}	7.39±0.03^{c}	5.94±0.02^{j}	6.65±0.00^{e}	6.50±0.03^{f}
Met	1.74±0.01^{e}	2.51±0.01^{b}	2.25±0.02^{d}	0.93±0.01^{c}	2.32±0.03^{a}	1.53±0.01^{k}	1.19±0.01^{f}	2.86±0.00^{j}	1.29±0.00^{i}	1.34±0.01^{h}	1.40±0.00^{g}	0.39±0.00^{l}
Ile	4.80±0.17^{c}	3.13±0.11^{f}	4.70±0.16^{cd}	6.45±0.20^{de}	4.27±0.12^{b}	3.32±0.12^{a}	5.01±0.18^{f}	5.65±0.23^{c}	4.88±0.18^{c}	3.90±0.14^{e}	4.85±0.20^{c}	4.25±0.16^{de}
Leu	8.92±0.41^{cd}	9.82±0.44^{c}	7.86±0.34^{de}	11.35±0.46^{a}	15.25±0.58^{b}	8.89±0.41^{b}	9.30±0.42^{cd}	12.41±0.63^{c}	8.80±0.42^{cd}	7.41±0.35^{e}	9.20±0.47^{c}	7.85±0.37^{de}
Phe	6.76±0.36^{de}	7.58±0.40^{bcd}	7.34±0.37^{bcde}	9.13±0.44^{abc}	8.00±0.37^{ab}	5.21±0.28^{a}	7.35±0.38^{f}	8.26±0.48^{bcd}	7.48±0.41^{bcd}	6.18±0.33^{ef}	7.59±0.44^{bcd}	6.86±0.37^{cde}
Lys	3.15±0.18^{de}	2.54±0.15^{e}	5.57±0.31^{c}	4.81±0.26^{e}	2.24±0.11^{e}	19.39±1.13^{c}	4.47±0.26^{a}	2.46±0.16^{cd}	5.60±0.34^{c}	5.82±0.35^{c}	13.59±0.86^{b}	4.46±0.27^{cd}
Trp	0.90±0.00^{d}	0.91±0.00^{d}	0.64±0.00^{g}	0.93±0.01^{i}	0.42±0.00^{a}	0.47±0.00^{e}	0.74±0.00^{h}	1.07±0.00^{f}	0.90±0.00^{d}	1.04±0.00^{b}	0.86±0.00^{e}	0.87±0.00^{e}
Cys	0.29±0.00^{d}	0.30±0.00^{c}	0.50±0.00^{a}	0.12±0.00^{b}	0.44±0.00^{c}	0.24±0.00^{j}	0.25±0.00^{g}	0.30±0.00^{f}	0.22±0.00^{h}	0.27±0.00^{e}	0.29±0.00^{d}	0.08±0.00^{j}

Values are means ± standard deviation, n = 3. The different letters in the same rows indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).

solution (0.033 M) and 10% ACN. Then, the pH was adjusted to 6.3±0.1 with 1 M HCI solution and filtered through a 0.22 µm CA filter under vacuum. Excitation and emission wavelengths were 280 and 340 nm, respectively. The separation was achieved with a Gemini-NX C18 110 Å, 5µ, 4.6 x 250 mm column (Phenomenex, CA, USA). The flow rate was 1 mL/min. The column oven temperature was set to 30 °C.

G. *In Vitro* protein digestibility (IVPD) and PDCAAS

The in vitro protein digestibility was performed based on method described by Pasini *et al*. (2001) with some modifications. Samples containing 250 mg protein were weighed in a 50 ml plastic falcon tube. Because breakfast cereals are extruded products, they can form viscous suspensions in digestion. Therefore, samples were first treated with α-amylase to hydrolyze gelatinized starch (Stuknytė *et al*., 2014; Hejazi *et al*., 2016). First, 20 ml distilled water was added to the samples and incubated at 90 °C for 5 min with α-amylase (0.1 ml, termamyl). Then, the pH was adjusted to 1.9-2.0 with 0.05 NaOH and pepsin (2800 units/mg, enzyme/protein ratio of 1:30 (w/w)) was added. The digestion occurred at 37 °C for 30 min in a shaking water bath. Afterwards, 5 ml phosphate buffer solution (1 M) was added and adjusted to pH 7 with 0.05 NaOH solution. Then, pancreatin (4x U.S.P., enzyme/protein ratio of 1:25, (w/w)) was added and incubated at 37 °C for 2 h in a shaking water bath. Finally, the digestion was stopped with 1 ml TCA (20%, w/v), centrifuged at 80000 g for 10 min. The nitrogen content of pellet was analyzed using the Kjeldahl method (AOAC, 2012).

In vitro protein digestibility (%) = ((total protein-pellet protein) / total protein))*100

H. *In Vitro* Protein Digestibility-Corrected Amino Acid Scoring (PDCAAS)

PDCAAS = ((mg of first limiting amino acid in 1 g test protein) / (mg of the same amino acid in 1 g reference protein))*protein digestibility (Joint WHO/FAO/UNU Expert Consultation, 2007).

I. Statistical Analysis

All analyses were performed in triplicate, and the average value was used with standard deviation. Significant differences were assessed using ANOVA Tukey's test ($p < 0.05$).

Table 5. Amount and proportions of essential amino acids (EAA) and branched chain amino acids (BCAA) in breakfast cereals

Sample no	EAA (mg/100 g)	BCAA (mg/100 g)	EAA (%)	BCAA (%)
BC.1	2790±41^{f}	1601±62^{i}	36.9±0.5^{cd}	21.2±0.1^{e}
BC.2	2947±41^{e}	1539±37^{j}	37.5±0.5^{c}	19.6±0.0^{f}
BC.3	3249±54^{d}	1678±82^{g}	37.4±0.6^{c}	19.3±0.1^{fg}
BC.4	4112±60^{a}	2395±104^{a}	46.9±0.7^{a}	27.3±0.1^{a}
BC.5	2841±25^{ef}	1809±153^{e}	41.8±0.4^{b}	26.6±0.2^{b}
BC.6	2320±46^{g}	902±14^{k}	48.7±1.0^{a}	18.9±0.0^{h}
BC.7	2867±42^{ef}	1651±59^{h}	37.9±0.5^{c}	21.8±0.1^{c}
BC.8	3860±53^{b}	2230±52^{b}	47.5±0.7^{a}	27.4±0.1^{a}
BC.9	3645±59^{c}	1949±95^{d}	40.3±0.7^{b}	21.6±0.1^{cd}
BC.10	3247±55^{d}	1631±68^{h}	35.2±0.6^{de}	17.7±0.1^{i}
BC.11	3895±72^{b}	1734±57^{f}	47.9±0.9^{a}	21.3±0.1^{de}
BC.12	3973±62^{ab}	2184±101^{c}	34.7±0.5^{e}	19.1±0.1^{gh}

Values are means ± standard deviation, n = 3. The different letters in the same column indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).

Table 6. Amino acid score, protein digestibility, and protein digestibility-corrected amino acid score (PDCAAS) of breakfast cereals

A.A.	Ref.*	B C . 1	B C . 2	B C . 3	B C . 4	B C . 5	B C . 6	B C . 7	B C . 8	B C . 9	B C . 1 0	B C . 1 1	B C . 1 2
His	15	1.85±0.04^{b}	0.51±0.01^{f}	2.00±0.04^{b}	2.45±0.05^{b}	2.21±0.05^{c}	2.52±0.05^{b}	2.28±0.05^{b}	0.75±0.02^{f}	2.50±0.05^{b}	2.69±0.06^{a}	2.28±0.05^{b}	2.52±0.05^{b}
Ile	30	1.60±0.00^{c}	1.04±0.00^{e}	1.56±0.00^{c}	2.14±0.00^{c}	1.42±0.00^{d}	1.11±0.00^{g}	1.68±0.00^{c}	1.89±0.00^{d}	1.63±0.00^{d}	1.30±0.00^{d}	1.63±0.00^{c}	1.42±0.00^{de}
Leu	59	1.66±0.03^{c}	1.82±0.03^{b}	1.45±0.03^{d}	2.10±0.04^{c}	2.82±0.05^{b}	1.64±0.03^{d}	1.72±0.03^{c}	2.30±0.04^{b}	1.63±0.03^{d}	1.37±0.03^{cd}	1.71±0.03^{c}	1.46±0.03^{d}
Lys	45	0.71±0.01^{f}	0.57±0.01^{f}	1.25±0.01^{e}	1.08±0.01^{f}	0.51±0.00^{g}	4.37±0.04^{a}	1.01±0.01^{e}	0.56±0.01^{g}	1.26±0.01^{f}	1.31±0.01^{d}	3.07±0.03^{a}	1.01±0.01^{g}
Met + Cys	22	1.15±0.03^{e}	1.66±0.05^{c}	1.48±0.04^{cd}	0.61±0.02^{g}	1.43±0.04^{d}	0.99±0.03^{h}	0.77±0.02^{f}	1.94±0.06^{d}	0.89±0.03^{g}	0.95±0.03^{e}	0.99±0.03^{e}	0.27±0.01^{h}
Phe + Tyr	38	2.99±0.02^{a}	3.13±0.01^{a}	2.96±0.01^{a}	3.83±0.02^{a}	3.45±0.02^{a}	2.39±0.02^{c}	3.04±0.01^{a}	3.46±0.02^{a}	3.13±0.01^{a}	2.65±0.01^{a}	3.11±0.01^{a}	2.79±0.01^{a}
Thr	23	1.33±0.03^{d}	1.85±0.05^{b}	0.99±0.02^{f}	1.60±0.04^{d}	0.93±0.02^{e}	1.32±0.03^{f}	0.97±0.02^{e}	2.26±0.06^{b}	1.47±0.04^{e}	1.32±0.03^{d}	1.28±0.03^{d}	1.27±0.03^{f}
Try	6	1.40±0.05^{d}	1.41±0.05^{d}	0.99±0.03^{f}	1.44±0.05^{e}	0.65±0.02^{f}	0.73±0.02^{i}	1.15±0.04^{d}	1.67±0.06^{e}	1.40±0.05^{e}	1.61±0.05^{b}	1.33±0.05^{d}	1.35±0.05^{ef}
Val	39	1.68±0.05^{c}	1.47±0.04^{d}	1.56±0.04^{c}	2.18±0.06^{c}	1.49±0.04^{d}	1.52±0.04^{e}	1.69±0.05^{c}	2.09±0.06^{c}	1.80±0.05^{c}	1.45±0.04^{c}	1.63±0.05^{c}	1.59±0.05^{c}
Digestibility (%)		87.9±5.9^{ab}	91.4±6.2^{a}	85.2±5.8^{abc}	87.6±5.9^{ab}	64.8±4.4^{d}	85.8±5.8^{ab}	95.0±6.4^{a}	95.4±6.4^{a}	69.1±4.7^{cd}	90.1±6.1^{a}	62.6±4.2^{d}	71.9±4.9^{bcd}
PDCAAS		0.63±0.01^{c}	0.47±0.01^{e}	0.84±0.02^{a}	0.53±0.02^{d}	0.33±0.00^{f}	0.63±0.02^{c}	0.73±0.02^{b}	0.53±0.00^{d}	0.62±0.02^{c}	0.86±0.02^{a}	0.62±0.02^{c}	0.19±0.01^{g}

The different letters in the same column for A.A. indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).
The different letters in the same rows for digestibility and PDCAAS indicate statistical differences between samples (ANOVA $p < 0.05$, Tukey's test).
*Reference values (mg/kg) for adult according to WHO/FAO/UNU (2007) report.

III. RESULTS AND DISCUSSION

The measured and declared amounts of total protein in breakfast cereals are shown in Table 1. The amount of measured total protein ranged between 4.8 ± 0.3 to 11.4 ± 0.4 g/100 g in breakfast cereals. The declared amount of protein on the labels ranged from 6 and 10.9 g/100 g. The measured total protein amount ranged from 79.4 ± 2.5 to 161 ± 5.7 % of the amount listed on the breakfast cereal packaging. The protein content in 5 of 12 samples was less than 10% of the declared value, whereas it was more than 10% of the declared amount in only one sample.

The measured amounts of each amino acid in 100 g of breakfast cereal and the ratio of each amino acid in 100 g protein are shown in Table 3 and Table 4, respectively. In this study, breakfast cereals were evaluated into 3 groups according to their main contents (rice, corn, and oats). The 1st group contains rice (samples 1, 2, 3, and 4), the 2nd group contains corn (samples 5, 6, and 7), and the 3rd group contains oats (samples 8, 9, 10, 11, and 12). As seen in Table 1, 4 of the 12 samples contained rice flakes with whole grains. Similar results were found for the amounts of amino acids other than aspartic acid (44-580 mg/100 g), glutamic acid (317-2259 mg/100 g), and histidine (62-330 mg/100 g) in these samples. The amount of protein in these samples was similar and ranged between 7.6 and 8.7 g/100 g. However, there were a big statistical differences in the ratios of aspartic acid (0.37-6.62%), glutamic acid (3.61-26.04%), histidine (0.79-3.77%), and proline (7.96-21.65%) to total protein ($p < 0.05$). As seen in Table 1, three of the 12 samples contained corn flakes with or without whole grain. Although the amount of protein in these samples ranged between 4.8 and 7.6 g/100 g, there was no big difference between the ratios of each amino acid in the total protein except for lysine (2.24-19.39%). As seen from Table 1, 5 out of 12 samples contained mainly oats. The total amount of protein in 4 out of 5 samples ranged from 8.1 to 9.2 g/100 g, whereas this amount was 11.2 g/100 g in the other. Similar results were found in the amounts 12 out of 18 amino acids other than aspartic acid (108-818 g/100 g), glutamic acid (255-2862 g/100 g), histidine (93-444 g/100 g), arginine (102-704 g/100 g), methionine (44-209 g/100 g), and lysine (201-1113 g/100 g) in these samples. Although the amount of protein in these samples ranged between 8.1 and 11.2 g/100 g, there were a big statistical differences between the ratios of aspartic acid (1.33-10.42%), glutamic acid (3.14-25.04%), histidine (1.15-4.13%), arginine (1.25-6.68), proline (5.99-15.22%), methionine (0.39-2.86%), and lysine (2.46-13.59%) ($p < 0.05$). As seen from the results, the proportion of lysine in 10 out of 12 samples ranged from 2.24 to 5.82%, whereas the other two samples ranged from 13.59 to 19.39%. In addition, in the other breakfast cereal

groups, there was a difference in the amounts and proportions of glutamic acid, aspartic acid, proline, and histidine.

Essential amino acids (EAAs) include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. The branched chain amino acids (BCAAs) are leucine, isoleucine, and valine. As seen in Table 5, the amount of essential amino acids (EAAs) ranged from 2790 to 4112 mg/100 g in group 1, 2320 to 2867 mg/100 g in group 2, and 3247 to 3973 mg/100 g in group 3. The proportion in 100 g protein ranged from 36.9 to 46.9%, 37.9 to 48.7%, and 35.2 to 47.9%, respectively. Although samples 10 and 12 in group 3 contained high amounts of protein, a lower proportion of EAAs were found than in the other samples. In group 1 breakfast cereals, there was no big difference observed in the proportion of EAAs in 3 of the 4 samples, whereas the other sample had a high rate of EAAs. Although sample 6 in group 2 contained a low amount of protein, a high rate of EAAs was found compared to the other group 2 breakfast cereals.

The amount and proportion of branched chain amino acids (BCAAs) ranged from 1601 to 2395 mg/100 g in group 1, 902 to 1809 mg/100 g in group 2, and 1631 to 2184 mg/100 g in group 3, whereas the proportion in 100 g protein ranged from 19.3 to 27.3%, 18.9 to 26.6%, and 17.7 to 27.7%, respectively (Table 5). Although group 1 breakfast cereal contained similar amounts of protein (7.6-8.8 g/100 g), only one sample contained a high amount (2395 mg/100 g) and proportion (27.3%) of BCAAs compared to the other breakfast cereals. In group 3 breakfast cereals, sample 8 contained a low amount of protein but a high proportion (27.4%) of BCAAs. In group 2, two samples contained both a low protein and BCAAs amount, whereas the other sample contained a low amount of protein but a high proportion of BCAAs compared to other groups.

Breakfast cereals products are manufactured through extrusion. When the extrusion technique and its relationship with nutrient losses are evaluated, extrusion causes amino acid loss due to the heat and shear, therefore reducing protein quality (Tiwari and Jha, 2017). Paes and Maga (2010) reported that extrusion causes a decrease in the amount of isoleucine, leucine, lysine, threonine, and valine in maize flour. As seen in different studies, lysine is the most adversely affected EAAs by the extrusion technique. According to the TURKOMP (2019), the ratio of lysine in 100 g protein is between 3.5 and 5.8 (5.6 in wheat, 3.5 in rice, 5.8 in corn, and 5.4 in oats). As seen from Table 4, the ratio of lysine was below 3.5 in 4 out of 12 samples. This decrease might have occurred during the extrusion (low moisture and high temperature) degradation of starch resulting in the formation of a reducing sugar. Because lysine has a reactive amino group (ε-amino group) in the end of its side chain, it can react with reducing sugar and may cause the formation of maillard reaction products (Mesías *et al*., 2019). According to Csapó *et al*. (2008), extrusion causes a significant loss of lysine, arginine, histidine, aspartic acid, and serine in mixtures of extruded soy and sweet potato flours. The ratio of aspartic acid is between 4.0 and 8.3 (wheat, corn, rice, and oats) in TURKOMP (2019). When evaluating our results, the ratio of aspartic acid was below 4 in 6 out of 12 samples. In addition, the ratio of histidine and glutamic acid were below reference values (1.9-2.1) in 2 out of 12 samples and (14.4-22.8) in 4 out of 12 samples, respectively. Therefore, there was a decrease in lysine, glutamic acid, histidine, and aspartic acid ratios in breakfast cereals produced using the extrusion technique. Abdel-Aal and Hucl (2002) reported that the ratio of EAAs was 31-33% in flour and breakfast cereals. We found that the ratio of EAAs ranged between 34.7±0.5 and 48.7±1.0. The extrusion processes did not change the sum of the EAAs.

The protein digestibility results of breakfast cereals are summarized in Table 6. As seen in Table 6, the digestibility ranged from 85.2 to 91.4% in group 1 breakfast cereals, 64.8 to 95.0 % in group 2 breakfast cereals, and 62.6 to 95.4% in group 3 breakfast cereals. The highest protein digestibility was observed in group 1 breakfast cereals whereas the lowest digestibility was observed in group 3 breakfast cereals. In group 3, the digestibility ranged between 62.6 to 71.9 in 4 out of 5 samples, but the other one was high (95.4%). When we compared the results, the digestibility of breakfast cereals in group 1 (>87.6%) was higher than other the groups.

Since extrusion occurs at high pressure and temperature, proteins are separated into smaller structures. (Tiwari and Jha, 2017). Thus, digestibility increases in both structures. Oats contain a certain amount of beta glucan and it has the highest water holding and water binding capacity (Rosell *et al*., 2009). The high water holding and water binding capacity of oats may reduce the digestibility of this breakfast cereal group (Decker *et al*., 2014; Ahmad *et al*., 2010). As seen from our results, the average protein digestibility was low in group 3 breakfast cereals. This group of breakfast cereals mainly contained oats. The protein digestibility of extruded legumes and wheat-based snacks is higher than that of non-extruded flour (Patil *et al*., 2016). Previous studies show that the presence of anti-nutritional compounds such as tannins may reduce protein digestibility (Gilani *et al*., 2012). Abdel-Aalc and Hucl (2002) reported that the protein digestibility of breakfast wheat flakes was 86%. In another study, the fecal nitrogen digestibility of corn-based breakfast cereal was 81.8% (Rutherfurd *et al*., 2014). As seen from these studies the protein digestibility of corn and wheat based breakfast cereals over %80. However, protein digestion was low in breakfast cereals containing oats. The low protein digestibility in breakfast cereal products may be explained through anti-nutritional compounds, containing oats and the degree of extrusion.

The PDCAAS values were calculated according to the reference values suggested by joint FAO/WHO/UNU Expert Consultation (2007). The calculated corrected amino acid score and the first limiting amino acid values are presented in Table 6. Considering the scores, isoleucine, phenylalanine+tyrosine, and valine values were

higher than 1.0 in all breakfast cereals. As seen from the table, the first limiting AAs were lysine, threonine, tryptophan, and methionine+cysteine (sulfur AA) in group 1 breakfast cereals, lysine, tryptophan, and methionine+cysteine in group 2, and lysine and methionine+cysteine in group 3. The first limiting AA in six samples was methionine+cysteine, in four samples was lysine, and in two samples was tryptophan (or threonine for sample 3). Although there are many methods for determining and evaluating dietary protein quality, the WHO/FAO/UNU recommends PDCAAS as a suitable method. According to the WHO/FAO/UNU report, the most limited amino acids are lysine (cereal), sulphur amino acids (legumes), threonine (some cereals), and tryptophan (maize). Pérez-Conesa *et al*. (2002) reported that sulphur amino acids, lysine, and tryptophan were the limiting amino acids in all infant cereals. Abdel-Aal and Hucl (2002) reported that the first limiting amino acid was lysine in processed cereals. In another study, lysine was the limiting amino acid in cereal-based products and amino acid scores were ranged between 0.15 to 0.54 (Caire-Juvera *et al*., 2013). In the same study, methionine±cysteine was limiting amino acids in legume products and scores were ranged between 0.41 to 0.47. In our study, according to the first limiting AAs, the lowest calculated corrected AAs ranged from 0.57 to 0.99 in group 1 breakfast cereals, 0.51 to 0.77 in group 2, and 0.27 to 0.99 in group 3 (Table 6). As seen from our results, amino acid scores were similarly low. When these obtained values are multiplied by IVPD, the PDCAAS ranged from 0.47 to 0.84 in group 1, 0.33 to 0.73 in group 2, and 0.19 to 0.86 in group 3 (Table 6). As seen from these results, the low protein digestibility of some type of breakfast cereals affects the PDCAAS values. When we evaluated the limiting AAs, lysine, methionine+cysteine (sulfur AA), and tryptophan predominantly determined PDCAAS.

IV. CONCLUSIONS

The consumption of breakfast cereals, which are considered a good source of protein, is gradually increasing as eating habits change. Extrusion has an adverse effect on the amount of certain amino acids. In this study, there were differences between the amounts and proportions of lysine, aspartic acid, and histidine in breakfast cereals produced using the extrusion technique. Higher protein digestibility was found in samples containing rice and corn than those oats containing. As the limiting AAs, lysine, methionine+cysteine (sulfur AA), and tryptophan predominantly determined PDCAAS. When we evaluated the amino acid content, digestibility, and limiting AAs, the PDCAAS was generally low in breakfast cereal products. PDCAAS of a food which provides a way to predict how efficiency protein will meet a human's amino acid needs. In general, it is considered that breakfast cereals do not adequately meet human protein requirement due to low PDCAAS. Therefore, new formulations are needed to improve protein quality in breakfast cereals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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