

Effects of Electrolyte-Rich Foods on Acid-Catalyzed Protein Hydrolysis

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RESEARCH

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ABSTRACT

The hypothesis states that pairing nutrient-rich fruits and vegetables with animal protein can aid in protein digestion and increase metabolic bioavailability. Samples included 10 grams of chicken breast (control) paired with either white rice (low electrolyte), white potato (medium electrolyte), or dried daikon radish (high electrolyte). Samples were homogenized in 12M HCl while on ice and incubated at 100°C for various time points and the reaction stopped by neutralization with 12M NaOH. Samples were centrifuged, and the homogenates and supernatants were analyzed using a Bradford protein assay to measure protein digestion. Subsequent release of free (aromatic) amino acids was measured at absorbance 280nm. Sample combinations were also subjected to pepsin digestion and the resultant supernatants were analyzed for amino acid release. Results indicate that animal protein paired with higher levels of electrolyte-rich foods (e.g., dried daikon radish) released significantly more amino acids ($p < 0.05$) than animal protein digestion paired with foods having

lower levels of electrolytes. Practical applications of this research show that consuming electrolyte-rich, nutrient-dense foods with animal protein sources may increase the bioavailability of amino acids greater than eating the protein source alone or with empty calories.



Increasing levels of electrolyte-containing foods were analyzed with animal protein to measure overall amino acid release. Results indicate that pairing animal protein with electrolyte-rich food sources may increase the bioavailability of amino acids.

Key Words: Protein Metabolism; Digestion; Electrolytes; Nutrient-dense.

Introduction

Modern Western diets typically exceed recommended intakes of animal protein and processed foods, while lacking in electrolyte-rich foods such as fruits, vegetables, beans, and whole grains [1]. Processed foods currently make up the most concentrated source of sodium in our diets and are generally low in potassium. Potassium intake has decreased since the agricultural revolution, with more of a focus on processed foods and animal products rather than potassium-rich vegetables [2]. Consuming a wide variety of whole plant foods containing potassium could have a positive impact on overall health. Such potassium-rich foods include bananas, potatoes, beans, avocados, tomatoes, dried apricots, and spinach. The



Institute of Medicine (IOM) recommends daily potassium consumption of 4,700mg for Americans. The current average daily potassium intake is 2,600mg, with children meeting <10% of daily potassium needs [1]. Recommendations to increase potassium intake are for the generally healthy population. However, individuals with medical conditions such as chronic kidney disease, end-stage renal disease, severe heart failure, and adrenal insufficiency may not benefit from increased potassium, particularly those on angiotensin converting enzyme inhibitors and potassium-sparing diuretics [1].

The purpose of this study was to measure the effect of electrolyte-rich foods (e.g. fruits, vegetables, legumes) on the resultant amino acid release from digested animal protein. By completing an amino acid analysis of animal protein hydrolyzed with different sources of electrolyte-rich foods, one could measure the degree of amino acid release from dietary protein sources into the bioavailable supernatant to predict metabolic outcomes. Supporting electrolytes may change the effects of protein catabolism in the body [3]. The hypothesis states that pairing nutrient-rich fruits and vegetables with animal protein can aid in protein digestion and increase metabolic bioavailability. This research advances human nutrition by increasing the evidence that supporting electrolytes from whole food sources can alter protein digestion.

Materials and Methods

Protein and Electrolyte Sources

The animal protein was raw, skinless chicken breast. The experimental pairings were as follows: 10 grams chicken breast (control), 10 grams chicken breast + 10 grams cooked white rice (low electrolyte), 10 grams chicken breast + 10 grams raw white potato (medium electrolyte), 10 grams chicken breast + 10 grams dried (rehydrated) daikon radish (high electrolyte).

Triplicate samples of chicken and nutrient-rich/poor food items were homogenized in 10mL deionized water (dH₂O) and centrifuged at 5000rpm for 10 minutes at 4°C. Protein concentrations were determined using a

Bradford protein assay and pH values were determined of the whole homogenate and supernatants.

Acid-Catalysed Hydrolysis

Sample combinations were homogenized in 20mL of 12M HCl for five minutes while on ice. One mL samples were incubated at 100°C at zero-, one-, and four-hour intervals. Samples were neutralized at the respective time points with 1mL 12M NaOH and the final volume adjusted to 5mL with 3mL 8.2mM phosphate buffer at pH 7.2. Samples were centrifuged for 15 minutes at 4°C and the supernatants collected. Protein concentrations and subsequent amino acid release were measured using a JASCO V-530 UV/VIS spectrophotometer. Each sample combination was performed in triplicate. After the indicated time periods, the protein concentration in whole-cell homogenates and cell-soluble supernatants were analyzed in comparison to the slope of a standard bovine serum albumin (BSA) curve using a Bradford reagent protein assay. Amino acid release was followed by measuring the absorbance (Abs) of the supernatant at 280nm. Although not quantitative, the relative amount of amino acid release was based on Abs 280nm for aromatic amino acids (mainly tryptophan, and tyrosine to a lesser extent). Results are expressed in relative Abs units after correction for sample dilution.

Pepsin Enzymatic Assay

Under more physiologically-relevant conditions, tissue combinations were analyzed using the Worthington pepsin enzymatic assay method based on a stop-point assay of hemoglobin (Hgb) degradation developed by Anson (1938) [4]. Pepsin (Carolina Biological Supply Company, Item: 87-9379) was dissolved at a concentration of 1mg/mL in 0.01N HCl. Immediately prior to assay, the enzyme was diluted to the desired concentration in 0.01N HCl.

Each treatment was homogenized in 20mL in 0.01N HCl while on ice. Triplicate 2.5mL samples of the tissue combinations were added to separate test tubes and incubated at 37°C for five minutes to equilibrate. After five minutes, 0.5mL of the respective enzyme dilution (500µg)



was added. The reactions were stopped by adding 5mL of 5% trichloroacetic acid (TCA) at zero (control) and ten minutes. Samples were removed from incubation bath after five minutes and filtered through Whatman No. 1 filter paper. Filtrate Abs was recorded using a JASCO V-530 UV/VIS spectrophotometer at 280nm. Enzymatic hydrolysis was calculated according to the following Worthington Method Formula:

$$\text{Units / mg} = \frac{[A_{280}(\text{Filtrate}) - A_{280}(\text{Blank}) \times 1000]}{10 \text{ minutes} \times \text{mg enzyme reaction mixture}}$$

Statistics

Statistical analyses on the effects of the various test sample pairings on tissue protein hydrolysis versus chicken alone were carried out using ANOVA followed by a test of multiple comparisons.

Results

Table 1 shows the electrolyte content for each test sample. Results shown in Figure 1 show the release of soluble, cell-free proteins from test samples upon homogenization in 10mL dH₂O.

Table 1. Electrolyte content of samples per 10 grams.

Nutrition information based on †USDA National Nutrient Database and ‡Manufacturer's per 100 grams nutrition label (Eden Foods – Daikon Radish, Sundried and Sliced).

	Potassium	Calcium	Magnesium	Sodium	Phosphorous
Chicken[†] <i>Control</i>	33 mg	0.5 mg	2.8 mg	4.5 mg	21 mg
White Rice[†] <i>Low Electrolyte</i>	1 mg	0.2 mg	0.5 mg	0.5 mg	0.8 mg
White Potato[†] <i>Medium Electrolyte</i>	43 mg	1.2 mg	2.3 mg	0.6 mg	5.7 mg
Dried Daikon Radish[‡] <i>High Electrolyte</i>	287 mg	40 mg	12 mg	15 mg	29 mg

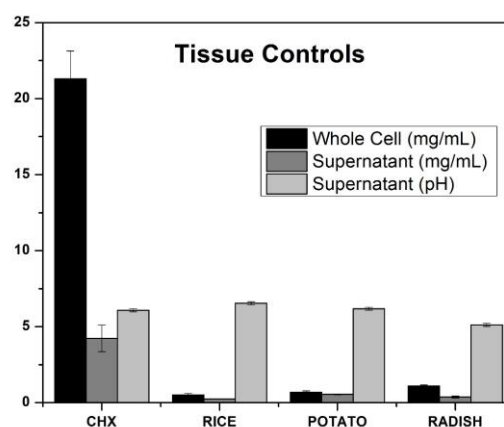


Figure 1: Figure 1 shows the comparison of protein concentrations (mg/mL) using a Bradford assay and pH values of individual supernatant samples. Values shown are the mean ± SEM for triplicate determinations. Samples of rice, potato, and radish did not contribute meaningful amounts of amino acids to skew potential results of soluble protein release of the chicken. The pH values of individual supernatants remain consistent among test samples, with the radish having a slightly lower pH.

Acid-Catalyzed Protein Hydrolysis

Figure 2 shows the time-dependent, acid-catalyzed release of soluble proteins and subsequent breakdown into individual amino acids from chicken alone. As a cautionary note, the prolonged exposure to high acid concentrations for extended periods of time (e.g. four-hour incubation) can begin to degrade individual amino acids [5, 6], resulting in inaccurate readings. Table 2 shows results of test samples on the initial soluble protein release from the tissue. Surprisingly, there were miniscule amounts of soluble proteins remaining in the supernatant at four hours for the chicken + rice pairing ($p < 0.005$).

The acid-catalyzed hydrolysis of cellular tissue protein results in the breakdown of amino acids into soluble peptide fragments. The supernatants of samples were filtered, Abs was measured at 280nm, and results were examined to determine if there was an additive effect of the treatments on chicken protein hydrolysis.

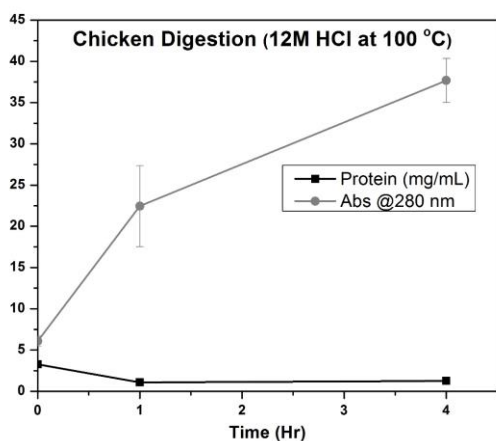


Figure 2. Results shown represent the mean \pm SEM for triplicate determinations.

Figure 2 shows the subsequent hydrolysis of cell-free proteins (mg/mL) into simple amino acids as measure by Abs at 280nm.

Table 2. Acid-catalyzed, cell soluble protein release.

Supernatant protein concentrations (mg/mL) determined by Bradford protein assay at 595nm.

	0 hour	1 hour	4 hour
Chicken	3.27 \pm 0.39	1.08 \pm 0.49	1.26 \pm 0.10
Chicken + Rice	2.66 \pm 0.44	1.27 \pm 0.52	0.00 \pm 0.05
Chicken + Potato	2.63 \pm 0.18	0.73 \pm 0.84	1.42 \pm 0.15
Chicken + Radish	2.53 \pm 0.43	1.77 \pm 0.42	1.08 \pm 0.20

Results shown in Table 3 demonstrate that electrolyte-poor food items (e.g. white rice) results in a significant loss of signal, thus indicating further amino acid degradation. Results also indicate that nutrient-dense food items (e.g. potato and radish) may either enhance soluble protein release or facilitate the stabilization of the released amino acids from further degradation (Table 3).

Table 3. Acid-catalyzed amino acid release (Abs 280nm).

*denotes statistically different from chicken alone ($p < 0.005$), §denotes that statistically different from other pairings using a two-tailed ANOVA ($p < 0.005$).

	0 hour	1 hour	4 hour
Chicken	6.07 \pm 0.62	22.45 \pm 4.91	37.69 \pm 2.67
Chicken + Rice	7.62 \pm 1.08	22.18 \pm 2.56	13.77 \pm 3.48*§
Chicken + Potato	8.04 \pm 0.60	25.31 \pm 2.68	29.03 \pm 4.81*§
Chicken + Radish	6.98 \pm 0.63	34.29 \pm 2.80*	22.06 \pm 1.78*§

Pepsin Enzymatic Assay

Table 4 demonstrates the standardization of the exogenously added pepsin enzyme activity using the Worthington assay method. Values represent the means of triplicate determinations. Based on these initial results, the mean activity of the pepsin solution was 92.68 Units/mg of protein. Sample treatments were then subject to pepsin hydrolysis as described by the above-mentioned protocol for the recommended time (ten minutes). Results shown in Table 5 from pepsin enzymatic assay demonstrate similar trends in amino acid release when compared to results from acid-catalyzed hydrolysis. At the ten-minute interval, chicken paired with dried daikon radish (high electrolyte) facilitated the highest release of amino acids from the whole cell into the supernatant, 847.7% higher than the theoretical yield (e.g. additive alone). Chicken paired with white potato (medium electrolyte) also facilitated amino acid release from the whole cell into the supernatant, 153.5% higher than the theoretical yield. Chicken paired with white rice (low electrolyte) facilitated the least amount, 1.2% higher than the theoretical yield.

Table 4. Standardization of pepsin activity with hemoglobin (Abs 280nm).

Absorbance values at zero and ten minutes were calculated using the Worthington method formula to establish units of pepsin proteolytic activity per mg enzyme activity. Therefore, based on these initial results, the mean activity of our pepsin solution was 92.68 Units of enzyme activity per mg protein.

Pepsin ($\mu\text{g}/\text{mL}$)	0 min	10 min	Δ	Units/mg
50	0.5761	0.6241	0.0480	96.00
100	0.5774	0.6709	0.0935	93.50
250	0.5808	0.7970	0.2162	86.48
500	0.5624	1.0361	0.4737	94.74

Table 5. Actual vs Theoretical Additive Effects of Pepsin Enzymatic Assay.

¶ The background readings of individual and paired rice samples are artificially high due to the light scattering effect of the colloidal suspension of high concentrations of soluble starch (Abs at 280nm ¶). This might also account for the somewhat higher background readings of tissue combinations with potato; however, to a lesser extent. **p < 0.005.

Percent Increase = ((Actual Value-Theoretical Value)/Theoretical Value*100)			
	Individual (Units/mg)	Paired (Units/mg)	% Increase from Individual Yield
Chicken	10.33±4.69		
Rice	111.64±49.58 [¶]	130.47±3.35 [¶]	1.2%
Potato	7.25±17.06	44.58±31.9	153.5%
Radish	-9.40±3.68	87.60±11.88 ^{**}	847.7%

Discussion

The purpose of this study was to measure the effect of electrolyte-rich foods (e.g. fruits, vegetables, legumes) on the resultant amino acid release from digested animal protein. Dietary protein metabolism occurs in the stomach under actions of pepsin and low stomach pH. Protein digestion results in the release of free amino acids, which can either, be utilized for protein synthesis or fulfillment of energy needs. Some amino acids (e.g. gluconeogenic) can be converted to glucose under conditions of starvation. Therefore, the estimated energy requirements and nutritional status of the individual must be considered to determine individual dietary protein needs. Macronutrients and micronutrients interact as a dynamic system, and nutrition research should continue to examine these interactions to determine potential overall effects on metabolism. This study was designed to measure if there is a biochemical difference between consuming proteins on its own versus protein paired with nutrient-dense food items.

Results shown in Figure 2 demonstrate an acid-catalyzed release of free amino acids from whole cellular protein (Abs 280nm). The inverse graphical relationship depicts that whole cell-soluble protein is broken down into subsequent amino acids. Interesting to note, the combination of chicken and rice (Table 2) results in an almost complete eradication of soluble protein at the four-hour mark. Expectedly, this would result in an increase in free amino acids. However, results shown in Table 3 demonstrate a significant decrease, or destabilization, of amino acid release from chicken and rice versus other treatments. This destabilization may be due to the expected behavior of the Maillard process, or increasing reaction rate with increases in pH, temperature, and reducing sugar content (e.g. white rice) [7]. For similar reasons, decreases are shown for the other treatments compared to chicken alone but to a lesser extent (Table 3).

The effect of the various food pairings was examined on pepsin-catalyzed hydrolysis of chicken protein. As shown in Table 5, there was negligible effect of rice on enzyme-catalyzed hydrolysis. This may be a real effect, or it

may be further complicated by the high colloidal activity of the rice-associated starches. The higher electrolyte treatments (potato and radish), seem to increase the amount of enzyme-catalyzed hydrolysis (radish $p < 0.005$) compared to chicken alone.

In summary, our results clearly suggest that high-electrolyte, nutrient-dense foods such as fruits and vegetables may help stabilize the acid-catalyzed amino acids from dietary protein sources, increasing the metabolic bioavailability of amino acids. This is in direct contrast to low-electrolyte, nutrient-poor foods (e.g. white rice) which may decrease amino acid stability and metabolic bioavailability.

Conclusion

Consuming nutrient-dense fruits and vegetables with protein sources may facilitate the release of amino acids to a greater degree than eating the protein source alone or with empty calories. Amino acids may become more bioavailable when paired with electrolyte-rich foods. Furthermore, pairing protein sources with nutrient-poor foods may have deleterious effects of amino acid bioavailability. This research is significant because individuals could potentially decrease protein consumption when consuming nutrient-dense plant foods (e.g. fruits, vegetables, legumes). This research advances the field of nutrition research by increasing the evidence that supporting electrolytes from whole food sources can alter protein digestion [7, 8].

Limitations

Results are based on a 1:1 ratio of protein to food pairing. Future studies should aim to use varying proportions of protein to electrolyte-containing foods to measure the degree of amino acid release. Furthermore, researchers acknowledge there could be other bioactive compounds in each sample that promote or hinder amino acid release. Future studies should compare protein groups with isolated electrolytes to confirm conclusions. Broad context applications for this small-scale pilot study could include amino acid interactions with electrolytes in various

forms of nutrition support (e.g. enteral and parenteral nutrition).

Conflicts of interest: Authors AEA and DMS report no conflicts of interest.

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