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Effect of Heating on Apparent Digestibility of Some Infant Formulations and Cereal-Legume Blends Available in Bangladesh

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Abstract: The apparent digestibility index (DI) of infant formulations obtained from the local market of Bangladesh were studied by monitoring the change in absorbance at 280 nm during enzyme action. Acetone powder of the samples were used as substrate and the enzyme was pepsin (EC.3.4.23.1). In every case, the enzyme protein ratio was 1:12.5. The highest DI was for a milk powder based product called "Product 103" and the least DI was observed for a wheat and fruit based "Product 102" having a DI of 7.57×10^{-4} and 2.29×10^{-4}, respectively. There were no significant differences in the apparent digestibility of most of the infant formulations. Effect of heat treatment on digestibility was assessed after heating for 5 minutes at 100°C. There were significant differences in DI before and after heating. The DI of wheat and fruit based product, "Product 103" was 5.63 fold greater(12.91×10^{-4}) greater than the value before heating.

Key words: Digestibility, *in vitro*, protein, pepsin

Introduction

Protein is one of the five basic components in an adequate diet (Lehninger, 1982). Protein undernutrition is common for millions of people of underdeveloped countries, Bangladesh is one such example. Children of growing age are the worst sufferers. They suffer from many nutritional diseases due to lack of adequate quantity of good quality protein (Lehninger, 1982). On the other hand, it is found that a number of weaning foods are marketed in the local market of Bangladesh.

The nutritive values of dietary protein depends primarily upon the concentration and distribution pattern of their constituent amino acids (Bodwell *et al.*, 1980). Amino acid composition data thus generally indicate the protein nutritive value of various protein sources. However, nutritive value as estimated by animal assays is often lower than that predicted from amino acid data. This is attributed, in part, to a lack of complete availability of all of the amino acids due to incomplete digestion of the protein.

In vitro methods of protein evaluation are useful in screening new protein foods and processing methods because of their rapidity (Dimes and Haard, 1994; Walter and Mark, 1964). Reviews of laboratory methods of protein quality evaluation have been reported by Swaisgood and Catignani (1982). An *in vitro* digestion is a convenient and rapid way to assess the potential bioavailability of proteins by enzymatic digestion under model conditions. Food proteins are digested by proteolytic enzymes. These enzymes catalyze the hydrolysis of specific peptide bonds (Cheftal and Cug, 1985; Fennema, 1985). Numerous methods have been used to mimic the *in vitro* digestion of proteins (Madisetty, 1991).

The objective of the present study was to assess the *in vitro* digestibility of commercial infant formulations commonly available in Bangladesh before and after heat treatment. Enzymatic proteolysis was used as the tool to quantitate digestibility.

Materials and Methods

Infant formulations (Products 101-108)(Table 1) and fresh wheat and chickpea were obtained from the local market of Bangladesh. Blended cereal-legume mixes comprising of roasted wheat and chickpea, with (Complete Blended Food) and without vitamin/mineral mixture (Blended Food)(Table 1), were donated by the infant food production facilitiy of Ganashastha Kendra at Teknaf, Bangladesh. Sodium phosphate dibasic, sodium phosphate monobasic and diethyl ether were obtained from Aldrich Chemical Company, Inc., Milwaukee, WI; glycine and acetone were obtained from Fisher Chemicals, Fisher Scientific; The reference food protein bovine serum albumin(BSA), ovalbumin (OVA, and pepsin (EC.3.4.23.1) were obtained from Sigma Chemicals Company, St. Louis, MO. All other reagents were analytic grade.

Buffers: Extraction buffer (33 mM phosphate buffer pH 6.8). The extraction buffer that was used for solubilizing the infant formulations was 33 mM sodium phosphate at pH 6.8.
Digestion buffer used for the digestion experiment was 0.2 M glycine-HCl at pH 2.6.

Preparation of acetone powder: Acetone powder of the samples were obtained by an improvement of the method as described by Haque and Kito (1982). Three grams of food sample was dissolved in 20 ml of extraction buffer (33 mM phosphate buffer pH 6.8) and mixed thoroughly with shaking. The tube was then centrifuged at 4000 RPM (3077 x g) for 10 minutes in a TECHNOSPIN R (DuPont) centrifuge . After centrifugation, the pellet was discarded, supernatant was collected and kept in a salt-ice bath of -2°C. Acetone (1 fold v/v) chilled to -20°C overnight was added to the cooled supernatant. The supernatant was allowed to stand for 45 minutes in the salt-ice bath in order to get good precipitation. The sample was centrifuged at 4000 RPM (3077 X g) for 15 minutes. The supernatant was discarded by decanting, and the protein precipitate was collected. An equal volume of diethyl ether (ice cold) was added to the precipitate to wash away residual lipids. Protein precipitate thus collected was thoroughly dried of solvent under a stream of air, redispersed with distilled water, and lyophilized in a LABCONCO (Labconco Corp., Kansas City, MO) freeze dryer system. Each acetone powder was stored in sealed containers and kept at -10°C until needed.

Preparation of heat treated samples: Heat treatment of the infant formulations and food samples were carried out at 100°C in a thermostated water bath (Isotemp Refrigerated Circulator, Model 90, Fisher Scientific) for 5 minutes. The samples were then transferred to an ice bath (at 4°C) for another 5 minutes. After that, the samples were equilibrated to room temperature for further experiment.

Determination of apparent *in vitro* digestibility: *In vitro* digestibility was assessed by the method described by Haque and

Kinsella (1988) and Haque and Khalifa (1992). Enzyme to protein ratio was kept constant at 1:12.5 for all experiments. A freshly prepared pepsin solution (25 μL) was injected into a temperature equilibrated (37°C) micro-cuvette containing 500 μL of acetone powder solution in digestion buffer. A high precision HPLC grade syringe (Hamilton Co, Reno, NV) was used for injecting the enzyme solution. Temperature was controlled by a water-jacketed micro-cuvette holder connected to a temperature controlled water bath. A micro-stirrer (Instech Labs Precision Controller, Plymouth Meeting, PA) was used to stir the content of the cuvette (the acetone powder solution and enzyme) constantly at a minimum attenuation using a Teflon probe. Care was taken to ensure that the stirrer was not in the path of the light beam. Change in absorbance at 280 nm was recorded every 15 sec. after the enzyme was injected into the acetone powder solution. A computer-assisted (SpecScan Software) Spectronic 1201 Milton Roy Dual Beam Spectrophotometer was used for all experiments. The spectrophotometer electronically compensated for possible instrument drift with time (Haque and Khalifa, 1990). Results are the mean values of three separate determinations.

Calculation of apparent digestibility: The curve was plotted taking absorbance (X-axis) and time (Y-axis) (Fig.1).
Absorbance values were obtained from the computerized printout of data obtained from Spectronic 1201 Spectrophotometer. The absorbance value for each sample was plotted every 0, 3, 6, 9, 12, and 15 min. Then the slope of the curve was calculated. The slope was used as the index of digestibility. Each value was mean of three separate determinations.

Results

Roasted milled chickpea had the higher digestibility (87.80X 10^{-4}) (Table 1). Raw chickpea followed roasted milled chickpea with a digestibility index of 62.93 X 10^{-4}. The least digestible reference food was BSA.
Among the reference foods, it was observed that roasted wheat was more digestible compared to raw wheat (Table 1). This may be due to conformational change of proteins during roasting which made it more susceptible to digest. This is also true for roasted milled chickpea, it was even more digestible than reference protein, OVA and BSA.
The most digestible infant formula was "Product 103" having a digestibility index of 7.57 X 10^{-4} (Table 1). The weaning food composed of wheat and fruit named "Product 102" was the least digestible in our study, with a DI of only 2.29 X 10^{-4}.
The infant formulations studied here are milk-based weaning foods of different combinations. It was found that *in vitro* digestibility did not vary significantly. Product 106, Product 108, and Product 107 are manufactured by the same manufacturers and they ranked 11th, 12th, and 14th, respectively. The decreaed digestibility of " Product 107" may be due to the wheat proteins. There was significant difference between digestibility of food samples before and after heating (Table 1, Fig. 2). Product 102 showed highest percentage of change in DI after heat treatment. It increased to 12.91 from 2.29 reflecting a 463% change. "Product 103" showed the least percentage change in DI before and after heating (Table 1).
Product 103, product 101, and product 105 are infant formulations that are not combined with other plant proteins. These samples had DI of 7.57 X 10^{-4}, 5.10 x 10^{-4}, and 3.97 X 10^{-4}, respectively (Table 1). They ranked 5th, 8th, and 10th. Product 104 had a much lower digestibility index compared to these samples. It ranked at 13th with a slope value of 2.80 X 10^{-4} (Table 1).

Discussion

In vitro digestibility is a useful method for protein quality evaluation (Walter and Mark, 1964). There were two procedures widely used for screening potential protein-food stuffs based on the total amino acid composition - chemical score and essential amino acid index (Swaisgood and Catignani, 1982). But nutritive value of proteins depends on the complete availability of all the amino acids, so a complete digestion of a protein is necessary. In our study, we determined the *in vitro* digestibility of infant formulations as a measure of protein quality.
In vitro digestibility of protein can be estimated by various means (Madisetty, 1991). Our method (Haque and Kinsella, 1988) was rapid compared to the method described by Walter and Mark (1964) and later by Alaknani *et al.*, 1994. This is a spectrophotometric method where the change in absorbance were monitored during enzyme action.
In an experiment, (Alaknani *et al.*, 1994) showed the comparison between different digestion procedures for the multi-elemental analysis of human milk and a representative variety of infant formulas. The effects of digestion procedures and the mass of reference samples on the recovery, precision, and accuracy of multi-elemental analysis were examined. The digestibility was determined by using 0.05 g samples digested in 1.0 ml of concentrated HNO_3 on a hot plate set at 70°C for 5 days and measured directly.
Protein and amino acid digestibility and protein quality of liquid concentrate and/or powder forms of infant formulas were studied by rate balance and growth methods (Sarwar *et al.*, 1989). In this experiment, Sarwar *et al.* (1989) did not compare the digestibility

Table 1: Effect of Heat Treatment on Digestibility Index (DI)

Name of Sample	DI Before Heating X 10^{-4}	DI After Heating X 10^{-4}	% Change
Product 101 (Bebelac)	5.10^{ef}	9.00	+76
Product 102 (Dano Infant-Wheat & Fruit)	2.29^{g}	12.91	+463
Product 103 (Dano Milk Powder)	7.57^{d}	7.73	+2
Product 104 (Biomil Milk Formula)	2.80^{fg}	2.33	-16
Product 105 (My Boy Eldorin)	3.97^{efg}	1.49	-62
Product 106 (Nestle Rice)	3.93^{efg}	2.87	-26
Product 107 (Nestle Wheat)	2.52^{g}	4.00	+59
Product 108 (Nestle Lactogen)	3.73^{efg}	3.20	-14
Blended Food	6.00^{de}	11.20	+86
Roasted Milled Chickpea	87.80^{a}	57.30	+34
Raw Chickpea	62.90^{b}	18.50	-70
Complete Blended Food	4.17^{efg}	8.44	+102
Raw Wheat	5.83^{de}	2.23	-61
Roasted Wheat	7.70^{d}	3.61	-53
Ovalbumin (OVA)	14.73^{c}	1.29	-91
Bovine Serum Albumin (BSA)	1.83^{g}	1.33	-27

$^{a, b, c, etc}$Means followed by the same letter are not significantly different at $P<0.05$

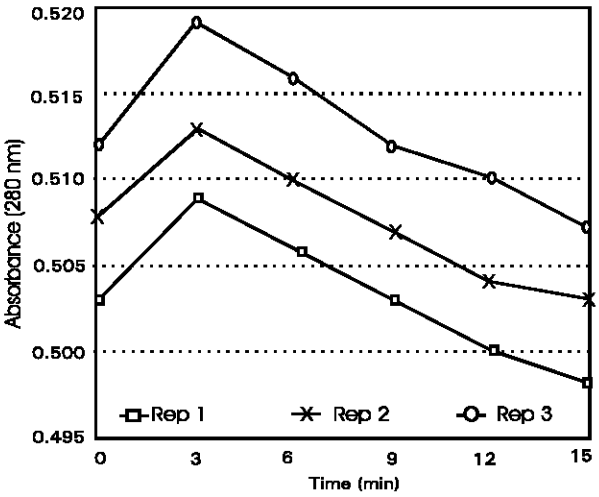

Fig. 1: Absorbance vs Time curve for BEBELAC

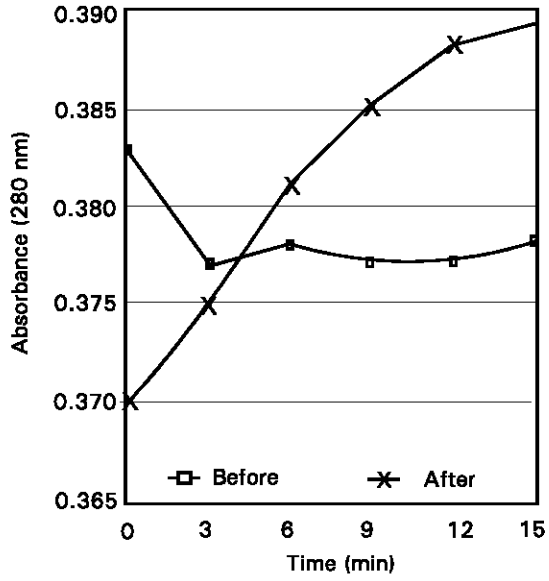

Fig. 2: Effect of heating on digestibility

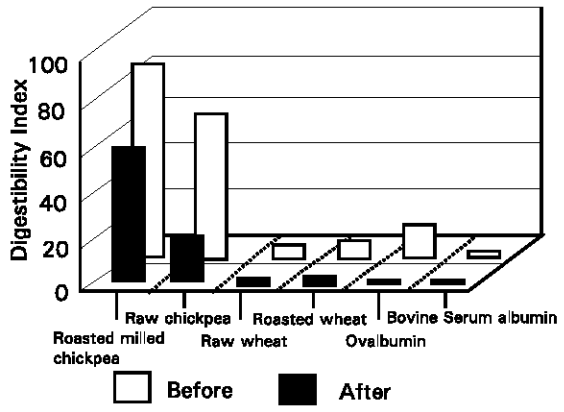

Fig. 3.1: Effect of heat treatment on digestibility index (DI)

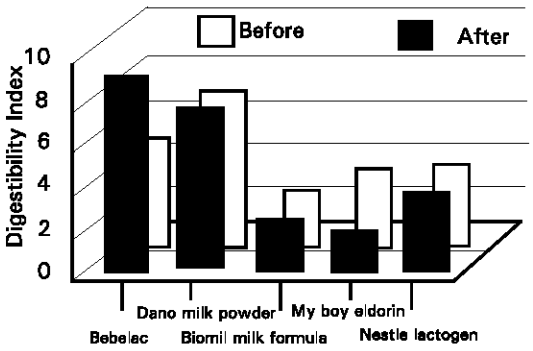

Fig. 3.2: Effect of heat treatment on digestibility index (DI)

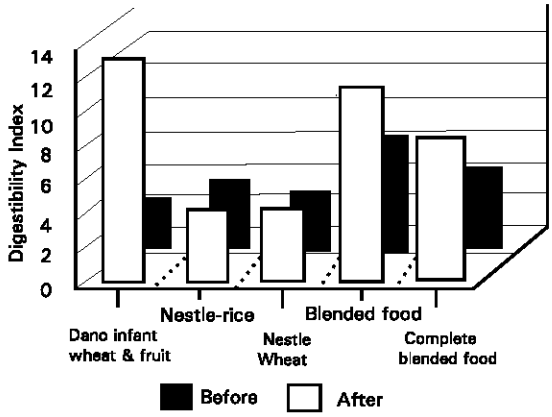

Fig. 3.3: Effect of heat treatment on digestibility index (DI)

of different infant formulations. They compared the difference in protein digestibility of liquid concentrates and powder forms of some different infant formulas. He found that digestibility of protein in liquid concentrates were up to 13% lower than those in powders.

An *in vitro* enzymatic digestion is a convenient and rapid method to assess potential bioavailability of protein under model condition. It was found that protein digestibility were not varied significantly among the infant formulations studied with some exception but there was a significant difference between the DI value of infant formulation before and after heating (Table 1, Fig. 3.1,3.2, and 3.3). Some become more digestible and some less digestible. It was found that legume based infant formulations showed a marked rise in DI after heat treatment (Fig. 3.3). Since all of the infant formulations are prepared from milk based proteins, we can conclude that the difference between digestibility may be due to the presence of non milk based proteins or due to the different types of processing techniques. Detailed *in-vivo* studies are required to establish these observations.

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