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Effect of processing on the in vitro digestibility of proteins and carbohydrates in some Indian legumes

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Abstract. The effect of different types of processing such as boiling, pressure cooking, puffing, frying, germination, and germination followed by cooking on the protein and carbohydrate digestibility of chickpea, horsegram and cowpea were studied in in vitro systems. In the case of chickpea, the protein digestibility was not significantly improved by any of the treatments. However, for horsegram and cowpea, improvement in protein digestibility was observed after some of the different processing treatments. Frying decreased the protein digestibility in all of the pulses. All of the treatments, except germination, caused a marked increase in in vitro carbohydrate digestibility.

Introduction

Legumes are commonly used in Indian diets. They are consumed in different parts of India after processing them in various ways based on tradition and taste preferences. However, information from comprehensive evaluations of different processing methods is limited.

Legumes contain a large amount of carbohydrates varying from 55 to 60% and protein 15–20%. The composition of the carbohydrate differs in various legumes, though starch is the major constituent [12, 13]. One of the important factors determining the availability of nutrients in protein foods is their digestibility. This can be determined either in vitro using suitable hydrolytic enzyme systems or in vivo using experimental animals (usually rats). The in vivo techniques are time consuming and expensive compared to in vitro methods and so the latter have been used by several workers [3] since generally satisfactory agreement with in vivo results from rat bioassays were obtained. The effect of processing of these legumes on the in vitro digestibility of protein and carbohydrate has been studied and the results are reported here.

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Materials and methods

Legumes and processing

Chickpea (Cicer arietinum) horsegram (*Dolichos biflorus*) and cowpea (*Vigna sinesis*) used in the present study were obtained locally. After cleaning, the legumes (either raw or processed) were milled to pass through a 60-mesh sieve and kept in closed air tight bottles for further analysis. The processing included six treatments for each legume viz., boiling in water, pressure cooking, puffing, deep frying, germination and germination followed by cooking [6]. The conditions of processing are summarised in Table 1. Untreated raw legume served as control in each case.

In vitro digestibility of protein

This was determined by the method of Akeson and Stahman [2]. The legume flour samples were incubated with pepsin (Sigma Chemical Co., Sr. Louis, Ma., USA) at pH 2.0 for 3 h. followed by pancreatin (Centron Research Laboratory, Bombay, India) at pH 8 for 24 h at 37°. The protein was precipitated with 10% TCA at the end of the experiment (27 h) and the nitrogen in the supernatant was determined using the microkjeldahl method [1]. Digestibility was calculated as protein hydrolysed as a percent of the total protein in the sample. Means and standard errors for each mean were calculated from values obtained from analyses of three samples of each product.

In vitro digestibility of carbohydrate

Legume flour was incubated with bacterial α-amylase (0.35 units/mg) in 0.1 M citrate buffer (pH 6.9, at 20°C), aliquots were withdrawn after 1, 30, 60 and 240 min and added to ethanol and centrifuged. The supernatant was analyzed for reducing sugars by the modified Somogyi method [11]. Results for three samples were averaged and the standard error for each mean calculated.

α-amylase activity

A solution of soluble starch (2%) in citrate buffer (0.1 M, pH 6.9) was incubated with bacterial α-amylase, at 20°C. Reducing sugar in the hydrolysate was estimated by the modified Somogyi's method [11]. One unit of enzyme activity is defined as the amount of enzyme required to release 1 μ mole of reducing sugar expressed as maltose per min. under the above conditions.

Results

Table 2 shows the results of in vitro digestibility of proteins in chickpea, horsegram and cowpea. None of the processing treatments improved the digestibility of chickpea protein. There was a decrease in the digestibility

Table 1. Conditions of processing used for chickpea, horsegram and cowpea

	Processing	Conditions
1	Boiled	Boiled in water (1:10 w/v) until soft. Dried in a through-flow air-dried at 60°C. Cooking times: chickpea (100 min), horsegram (150 min), and cowpea (35 min).
2	Pressure cooked	At 15 lbs until soft and dried as above. Cooking times: chickpea (40 min) horsegram (45 min), and cowpea (15 min).
3	Puffed	Soaked in water in wet jute bag for 60 min followed by roasting in hot iron pan with sand at 250°C for 30–40 sec.
4	Fried	Flours made into a dough, extruded through a hand press, and deep fried in oil at 230°C for 2 min
5	Germinated	Soaked in water (6–10 h), germinated on wet jute bag for 24 h at room temp (28–30°C), and freeze dried.
6	Germinated and cooked	Freeze-dried germinated legume seeds were cooked in boiling water until soft and dried. Cooking times: chick-pea (100 min), horsegram (120 min), and cowpea (30 min)

following frying. Other treatments, such as germination, followed by cooking, puffing, and boiling in water did not affect the digestibility.

In horsegram, most of the treatments improved the digestibility as compared with the raw sample, only frying decreased the digestibility. Germinated and cooked samples showed the highest improvement followed by pressure cooking, puffing and cooking.

The protein digestibility of cowpea showed a pattern similar to that of horsegram. All the heat treatments caused an improvement in digestibility, except frying. Maximum digestibility was observed in legumes which had been germinated and then cooked. Frying lowered the digestibility considerably.

The carbohydrate digestibility (α-amylolysis) of the legumes is summarised in Table 3. In all legumes, there was a progressive increase in maltose liberation up to 4 h. The digestibility of cowpea was lower compared to chickpea and horsegram. In all the legumes, pressure cooked and germinated

Table 2. In vitro digestibility of proteins of raw and processed legume flours[a]

Process	Chickpea (%)	Horsegram (%)	Cowpea (%)
Raw	80.63 ± 0.718	78.60 ± 1.112	79.00 ± 0.677
Boiled	79.75 ± 0.332	84.00 ± 0.457	83.20 ± 1.122
Pressure cooked	80.80 ± 0.665	86.00 ± 0.393	86.90 ± 0.987
Puffed	80.96 ± 0.818	84.57 ± 0.487	86.80 ± 0.864
Fried	78.44 ± 0.447	76.56 ± 0.229	76.70 ± 0.567
Germinated	80.51 ± 0.662	79.34 ± 0.556	79.48 ± 0.459
Germinated and cooked	80.04 ± 0.554	87.85 ± 0.198	86.74 ± 0.466

[a]Digestibility = Protein hydrolysed as percent of total proteins. Values are means ± SE of three independent observations.

Table 3. Effect of processing on α-amylosis of chickpea, horsegram and cowpea[a] (mg maltose released/100 mg legume flour)

Process	Chickpea				Horsegram			
	1	30	60	240	1	30	60	240
Raw	2.9 ± 0.171	5.2 ± 0.181	5.4 ± 0.181	7.8 ± 0.145	2.9 ± 0.166	5.7 ± 0.184	6.3 ± 0.198	7.4 ± 0.210
Boiled	8.7 ± 0.512	22.3 ± 0.556	26.5 ± 0.447	37.8 ± 0.347	7.8 ± 0.182	25.5 ± 0.314	27.8 ± 0.287	37.3 ± 0.222
Pressure cooked	7.6 ± 1.011	22.9 ± 0.989	25.8 ± 0.339	35.7 ± 0.414	6.9 ± 0.177	24.2 ± 0.516	26.3 ± 0.714	35.6 ± 0.786
Puffed	8.6 ± 0.717	20.1 ± 0.616	23.2 ± 0.717	29.1 ± 1.002	8.8 ± 0.616	23.1 ± 0.939	25.8 ± 0.919	29.0 ± 1.014
Fried	8.1 ± 0.818	20.3 ± 0.514	21.7 ± 0.818	28.2 ± 1.112	7.7 ± 0.444	22.1 ± 1.002	23.1 ± 0.764	28.4 ± 0.910
Germinated	3.5 ± 0.133	5.2 ± 0.252	5.8 ± 0.998	9.3 ± 1.131	3.6 ± 0.231	6.4 ± 0.166	6.7 ± 0.156	9.7 ± 0.223
Germinated and cooked	8.4 ± 0.414	30.0 ± 0.888	30.7 ± 1.002	34.6 ± 0.998	8.7 ± 0.177	24.9 ± 0.858	25.9 ± 0.699	33.8 ± 0.719

	Cowpea			
	1	30	60	240
Raw	2.5 ± 0.222	5.4 ± 0.188	5.8 ± 0.191	7.0 ± 0.256
Boiled	7.8 ± 0.414	19.7 ± 0.515	27.5 ± 0.714	30.9 ± 0.667
Pressure cooked	5.9 ± 0.914	21.6 ± 1.411	26.8 ± 1.112	35.6 ± 1.010
Puffed	9.3 ± 0.256	29.2 ± 0.911	29.6 ± 0.887	32.0 ± 0.934
Fried	8.1 ± 0.444	21.6 ± 0.897	23.1 ± 0.653	29.1 ± 0.677
Germinated	3.9 ± 0.319	7.9 ± 0.578	9.2 ± 0.433	15.1 ± 0.718
Germinated and cooked	8.6 ± 0.876	27.4 ± 1.011	29.3 ± 0.891	36.5 ± 0.674

[a]Values are means ± SE of three independent observations.

cooked samples had improved digestibility. However, germinated legumes had lower digestibilities. None of the legumes had a carbohydrate digestibility greater than 38%.

Discussion

Legumes are known to have a low protein digestibility. Processing can improve the digestibility of legume protein by destruction of trypsin inhibitors and by opening of protein structure through denaturation. Recently, however, Murthy and Urs [10], while studying the effect of roasting and puffing on the digestibility of bengal gram, observed that the in vitro digestibility of raw sample which was 79% decreased to 57 and 67% after roasting and puffing, respectively. Slight increases in the digestibility of horsegram was observed by Ray [14] following autoclaving at 15 lbs for 30 min. Germination was reported to enhance protein digestibility, by Subbalakshmi et al., [16] in horsegram and mothbean. While studying the effect of cooking on the digestibility of redbeans, blackbeans and whitebeans, Jaffe [8] reported an improvement in digestibility.

Enhancement of protein digestibility of horsegram and cowpea through heat treatment may be attributed to destruction of trypsin inhibitors or other factors which are present in these legumes. In chickpea, where the trypsin inhibitors are in low concentration [9], no beneficial effect of heat treatment is observed. The reason for the large decrease in digestibility of chickpea, horsegram and cowpea proteins after frying may be attributed to the fat imbibed by the legumes during frying. This has been observed for bengalgram proteins by Hsu et al. [7] and by Murthy and Urs [10]. A decrease in the protein digestibility can also occur during processing due to the non-enzymatic browning (Mallard reaction) and thermal cross linking. Improvement in protein digestibility on germination may be attributed to the modification and degradation of storage proteins.

Significant differences in amylosis rates were noticeable in the processed legumes as compared to the raw. This is explainable on the basis that the processed samples contain gelatinised starch which is readily attacked by α-amylase, whereas the untreated samples (i.e., the controls) have starch in the ungelatinised or granular form which is less readily hydrolysed. Germinated samples (without cooking) were comparable to the control in their rates of amylolysis. Among the heat processed samples, puffed ones exhibited lower starch digestibility; this tendency was also more pronounced in the fried sample, probably due to differences in the extent of starch gelatinisation. Geervani and Theophilus [5] have reported that the digestibility of fermented and germinated legumes was comparable with that of legumes subjected to boiling, pressure cooking and parching. Subbulakshmi et al., [16] in their studies on the effect of cooking and germination on the in vitro carbohydrate digestibility of horsegram and mothbean, observed a

significant increase after cooking in both the ungerminated and germinated samples.

The rate of amylolysis of the cooked samples was nearly three times higher than that of the uncooked samples. A similar trend was observed in the carbohydrate digestibility of both ungerminated and germinated greengram chickpea and cowpea [4]. Subbulakshmi et al. [16] have reported that a cooking period of 40–60 min caused a marked improvement in the digestibility of horsegram carbohydrate.

The exact reasons for the poor digestibility of legume carbohydrates as compared with those of cereals are not known at present. However, it has been claimed that the content and chain length of the amylose components of the starch may play a part in causing such low digestibilities [15]. The higher the content of amylose, the lower was the digestibility of the starch. Bengalgram and blackgram, which are least digestible, are found to have an amylose with higher chain lengths than the amylose from greengram and redgram [15]. The presence of other various non-starchy carbohydrates may also influence the starch digestibility in vitro.

Summary

Different types of processing are beneficial in increasing the digestibility of legumes. Processes which involve cooking gelatinise starch and thus improve its digestibility by α-amylase. As the cooking destroys the trypsin inhibitors and denatures the protein, proteolytic digestion is also improved. In foodstuffs in which trypsin inhibitors are highly active, the beneficial effect of cooking is high (e.g., horsegram and cowpea). Deep fat frying has been found to cause slightly lowered carbohydrate and protein digestibility as compared with other forms of cooking.

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