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Effect of domestic processing and cooking methods on in-vitro starch digestibility of different pea cultivars (*Pisum sativum*)

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There were significant ($P < 0.05$) varietal differences in the starch digestibility (*in vitro*) and contents of reducing sugars, non-reducing sugars, and starch in four cultivars of field as well as vegetable peas. Various domestic processing and cooking methods, including soaking for 6, 12 and 18 h, soaking (12 h) followed by dehulling, ordinary and pressure cooking of unsoaked, soaked and soaked-dehulled seeds, and sprouting for varying periods, i.e. 12, 24 and 48 h, brought about significant increase in starch digestibility of peas. Pressure cooking was found to be the most effective method followed by ordinary cooking, sprouting, dehulling and soaking. Cooking may gelatinise starch and sprouting may mobilise starch, thereby resulting in improved starch digestibility by pancreatic amylase.

INTRODUCTION

Legume grains are recognised as a major source of complex carbohydrates, including dietary fibre, in the diets of developing countries like India. Legumes contain large amounts of carbohydrates (55–60%) (El-Faki *et al.*, 1984). Although compositions of carbohydrates differ in various legumes, starch is the major constituent of available carbohydrates (Nigam & Giri, 1961) in most of the food legumes. Starch of food legumes is known to possess low digestibility (Geervani & Theophilus, 1981; El-Faki *et al.*, 1984) which may be ascribed to chain length and amount of amylose (Rao, 1976) and the presence of amylase inhibitors, i.e. phytate and polyphenols (Thompson & Yoon, 1984), in legumes including peas (Savage & Deo, 1989).

In India, legume grains are processed and consumed in a variety of forms, depending on cultural and taste preferences. The most common domestic methods for processing of legumes include soaking, ordinary cooking, pressure cooking and sprouting, which may affect antinutrients like phytates (Khokhar & Chauhan, 1986), tannins (Rao & Deosthale, 1982) and starch, as well as other available carbohydrates of some legumes (Jood *et al.*, 1988). In the present paper, an attempt has been made to report the effects of various domestic processing and cooking methods on the contents and digestibilities of available carbohydrates of some new high-yielding varieties of field and vegetable peas, as these cultivars may behave differently after processing and cooking.

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MATERIALS AND METHODS

Materials

The seeds of two varieties each of the vegetable (Bonneville and Arkel) and field peas (HFP4 and Rachna) were procured from the Departments of Vegetables and Plant Breeding, College of Agriculture, Haryana Agriculture University, Hisar, India.

Processing and cooking methods

Soaking. Seeds were soaked in double-distilled water for 6, 12 and 18 h at 30°C in an incubator, the seed-to-water ratio used being 1:5 (w/v).

Dehulling. After soaking the seeds overnight (12 h), hulls were removed manually.

Ordinary cooking. The soaked seeds (12 h) were rinsed in double-distilled water and put into tall beakers fitted with condensers connected to running water. Water was added three times to the soaked seeds which were cooked on a hotplate until they became soft (as felt between fingers). Similarly unsoaked seeds were also cooked until soft using a seed-to-water ratio of 1:4 (w/v).

Pressure cooking. Both soaked (12 h) and unsoaked seeds were pressure-cooked (15 lb/in^2 for 10 min). The ratio of dry seeds to cooking water was 1:3 (w/v) whereas it was 1:2 (w/v) for soaked seeds (12 h, 30°C).

Sprouting. The soaked seeds (12 h) were placed in sterile Petri plates lined with wet filter papers and kept in an incubator at 30°C for 12, 24 and 48 h for sprouting.

Preparation of processed samples

All the processed (i.e. soaked, soaked dehulled, ordinarily cooked, pressure-cooked and sprouted) samples were dried in a hot air oven at 60°C to a constant weight. The dried samples were ground in an electric grinder (Cyclotec, M/s. Tecator, Höganäs, Sweden) using a 0·5 mm sieve size and kept in air-tight plastic containers stored at room temperature for further chemical analysis.

Chemical analysis

Total soluble sugars were extracted in 80% ethanol according to the procedure of Cerning and Guilhot (1973). Starch from the sugar-free pellet obtained after centrifugation was extracted in 52% perchloric acid at room temperature (Clegg, 1956). Quantitative determinations of total soluble sugars and starch were carried out according to the colorimetric method of Yemm and Willis (1954). Reducing sugars were estimated by Somogyi's modified method (Somogyi, 1945). The amount of non-reducing sugars was calculated from the difference between the total soluble sugars and the reducing sugars. The total available carbohydrates were calculated as the sum of total soluble sugars and starch.

Starch digestibility (*in vitro*) was assessed by employing pancreatic amylase and then measuring maltose liberated by using dinitrosalicylic acid reagent (Singh *et al.*, 1982). Each of the processing treatments and analyses for each variety of sample was carried out in three replicates.

Statistical analysis

The data were subjected to statistical treatment for analysis of variance to determine the significant differences among various treatments, and correlation coefficients were derived according to standard methods (Panse & Sukhatme, 1961).

RESULTS AND DISCUSSION

Carbohydrates

Significant ($P < 0·05$) varietal differences were noticed for the reducing and non-reducing sugars, starch and total available carbohydrate contents (Table 1).

The content of total soluble sugars ranged from 5·81 to 6·98 g/100 g in difference pea cultivars. Both the vegetable pea varieties, i.e. Arkel and Bonneville, had significantly ($P < 0·05$) higher levels of total soluble sugars than field peas. Bonneville had the maximum total soluble sugars followed by Arkel, HFP4 and Rachna.

The contents of reducing and non-reducing sugars among all the four pea varieties differed significantly ($P < 0·05$) among themselves. Arkel and HFP4 had the maximum and minimum amounts of reducing sugars, respectively. The field pea varieties had significantly ($P < 0·05$) lower concentrations of reducing sugars when compared with Bonneville and Arkel, the vegetable pea cultivars.

Significant varietal differences existed in starch contents of peas. Field peas had more starch than vegetable peas. Rachna had the maximum starch content followed by HFP4, Arkel and Bonneville (Table 1). Similarly, field peas also appeared to contain higher levels of total available carbohydrates. Rachna and Bonneville had the highest and the lowest levels of total available carbohydrates, respectively (Table 1). The range of carbohydrate contents in peas reported in the present study is consistent with those reported earlier (Fleming & Reichert, 1983; Wills *et al.*, 1984; Savage & Deo, 1989).

Table 1. Total soluble sugars, reducing sugars, non-reducing sugars, starch and total available carbohydrate contents of peas (on dry matter basis).[a]

Variety	Total soluble sugars (g/100 g)	Reducing sugars (mg/100 g)	Non-reducing sugars (g/100 g)	Starch (g/100 g)	Total available carbohydrates (g/100 g)	Starch digestibility (mg maltose released/g meal)
Vegetable peas						
Bonneville	6·98 ± 0·03	531 ± 0·04	6·44 ± 0·03	53·4 ± 0·03	60·4 ± 0·03	36·5 ± 0·03
Arkel	6·81 ± 0·03	577 ± 0·04	6·23 ± 0·03	61·4 ± 0·02	68·2 ± 0·02	35·3 ± 0·01
Field peas						
HFP4 (Aparna)	5·97 ± 0·05	410 ± 0·06	5·56 ± 0·04	62·8 ± 0·01	68·7 ± 0·02	30·5 ± 0·04
Rachna	5·81 ± 0·05	433 ± 0·08	5·38 ± 0·06	63·8 ± 0·01	69·6 ± 0·01	31·2 ± 0·02
SE (m)	± 0·07	± 0·86	± 0·02	± 0·20	± 0·11	± 0·12
CD ($P < 0·05$)	0·21	2·82	0·06	0·36	0·35	0·36

[a] Values are means ± SD of three independent determinations.

Table 2. Effect of soaking and dehulling on in-vitro starch digestibility of peas (mg maltose released/g meal on dry matter basis)[a]

Variety	Period of soaking			Soaked and dehulled
	6 h	12 h	18 h	
Vegetable peas				
Bonneville	39·3 ± 0·05 (+8)	42·6 ± 0·01 (+17)	49·7 ± 0·01 (+36)	47·3 ± 0·02 (+29)
Arkel	38·2 ± 0·02 (+8)	41·6 ± 0·05 (+18)	48·3 ± 0·03 (+37)	46·6 ± 0·14 (+32)
Field peas				
HFP4 (Aparna)	33·6 ± 0·03 (+10)	36·2 ± 0·01 (+19)	43·1 ± 0·01 (+41)	41·4 ± 0·03 (+35)
Rachna	35·2 ± 0·02 (+13)	38·4 ± 0·07 (+23)	46·3 ± 0·06 (+48)	44·5 ± 0·05 (+42)
SE (m)	± 0·45	± 0·04	± 0·09	± 0·04
CD ($P < 0·05$)	1·35	0·13	0·30	0·13

[a] Values are means ± SD of three independent determinations. Figures in parentheses indicate per cent increase (+) over control values.

In-vitro starch digestibility

Starch digestibility (in vitro), expressed as mg maltose released/g flour, was 30·5–36·5 in raw unprocessed seeds of different pea cultivars. The in-vitro starch digestibility of field pea cultivars, viz. HFP4 and Rachna, was significantly ($P < 0·05$) lower than that of vegetable peas (Table 1).

Effect of domestic processing and cooking on in-vitro starch digestibility

Soaking and dehulling

A significant ($P < 0·05$) increase in starch digestibility occurred when pea seeds were soaked in water for different time periods; this also increased with an increase in the period of soaking (Table 2). Soaking for 12 h enhanced the starch digestibility which was 17–23% higher than the corresponding values in raw unprocessed pea seeds. Among all the time periods, maximum increase in starch digestibility was noticed when the seeds were soaked for 18 h. The in-vitro starch digestibility of soaked seeds of field peas was increased to a greater extent than that of soaked vegetable peas. The enhancement in starch digestibility of field as well as vegetable pea seeds which were soaked (12 h) and dehulled seemed to be higher than the seeds which were only soaked for 12 h.

Enhancement of starch digestibility as a result of soaking and dehulling may be attributed to loss of antinutritional factors such as phytic acid and polyphenols, which inhibit the activity of α-amylase and thus lower the starch digestibility (Deshpande & Cheryan, 1984). On the other hand, prolonged soaking of intact peas may have allowed the mobilisation of phenolics, which are known to interfere with starch digestion (Deshpande & Salunkhe, 1982) from the seed coat to the cotyledons.

Table 3. Effect of ordinary cooking on in-vitro starch digestibility of peas (mg maltose released/g meal, on dry matter basis)[a]

Variety	Unsoaked and cooked	Soaked and cooked	Soaked, dehulled and cooked
Vegetable peas			
Bonneville	51·8 ± 0·01 (+42)	54·5 ± 0·05 (+49)	78·4 ± 0·02 (+115)
Arkel	50·6 ± 0·03 (+43)	53·3 ± 0·02 (+51)	77·2 ± 0·01 (+119)
Field peas			
HFP4 (Aparna)	44·2 ± 0·01 (+45)	48·1 ± 0·02 (+58)	72·3 ± 0·03 (+137)
Rachna	48·5 ± 0·02 (+55)	52·3 ± 0·01 (+67)	77·4 ± 0·03 (+148)
SE (m)	± 0·11	± 0·05	± 0·18
CD ($P < 0·05$)	0·35	0·15	0·54

[a] Values are means ± SD of three independent determinations. Figures in parentheses indicate per cent increase (+) over control values.

Ordinary cooking

After cooking the unsoaked pea seeds, in-vitro starch digestibility increased to a greater extent in all the pea cultivars. The maximum increase was observed in Rachna (55%) followed by HFP4 (45%), Arkel (43%) and Bonneville (42%) (Table 3). However, cooking of soaked seeds brought about further significant ($P < 0·05$) increase in starch digestibility (more than 1·5–2-fold increase). Rachna had a significantly ($P < 0·05$) higher starch digestibility than the other three varieties.

There were significant ($P < 0·05$) differences among the values for starch digestibility of different field and vegetable pea cultivars when the seeds were cooked after soaking and dehulling. An increase in starch digestibility of soaked-dehulled cooked seeds appeared to be significantly higher when compared to that of unsoaked as well as soaked-cooked seeds. Ordinary cooking of soaked-dehulled seeds brought about maximum increase in the starch digestibility of Rachna, the field pea cultivar.

Pressure cooking

Pressure cooking had a more pronounced effect on in-vitro starch digestibility than ordinary cooking. The starch digestibility increased markedly when the unsoaked, soaked and soaked-dehulled pea seeds were autoclaved (Table 4). Pressure cooking of unsoaked seeds enhanced the starch digestibility but to a relatively lesser extent when compared to soaked (12 h) and pressure-cooked seeds. Among all the processing treatments, soaked and dehulled seeds gave maximum increase in starch digestibility. The starch digestibility was more than doubled in the soaked-dehulled pressure-cooked seeds of field and vegetable peas.

Processing of legumes, involving heat treatment, may gelatinise starch which is then readily attacked by

Table 4. Effect of pressure cooking on in-vitro starch digestibility of peas (mg maltose released/g meal, on dry matter basis)[a]

Variety	Unsoaked and pressure-cooked	Soaked and pressure-cooked	Soaked, dehulled and pressure-cooked
Vegetable peas			
Bonneville	53·2 ± 0·02 (+46)	71·5 ± 0·03 (+96)	82·3 ± 0·03 (+125)
Arkel	52·4 ± 0·02 (+49)	70·3 ± 0·03 (+99)	81·2 ± 0·01 (+130)
Field peas			
HFP4 (Aparna)	47·4 ± 0·02 (+55)	65·1 ± 0·01 (+113)	76·6 ± 0·02 (+151)
Rachna	51·4 ± 0·03 +65	69·3 ± 0·04 +112	81·2 ± 0·01 +169
SE (m)	± 0·13	± 0·21	± 0·16
CD ($P < 0·05$)	0·39	0·65	0·48

[a] Values are means ± SD of three independent determinations. Figures in parentheses indicate per cent increase (+) over control values.

α-amylase. Starch in untreated samples is ungelatinised and less readily hydrolysed. This may explain partly the better starch digestibility of ordinarily cooked and pressure-cooked seeds. Differences in starch digestibility during different heat treatments may be due to differences in extent of starch gelatinisation. During cooking, swelling and rupturing of starch granules of the legumes takes place and this may contribute towards the improvement in starch digestibility in cooked legumes. This facilitates a more randomised configuration for α-amylase to effect hydrolysis. Cooking has also been reported to inactivate amylase inhibitors, phytates and tannins which may be responsible for the increase in starch digestibility. The initial soaking treatment ensures uniform expansion and seed coat and cotyledon matrix and cellulose hydration to aid in heat transfer and subsequent tenderisation. This may be the reason for the greater starch digestibility in soaked-cooked seeds than in the unsoaked-cooked ones.

Previous workers have also reported improved starch digestibility in various legumes including chick pea, cow pea, black gram, red gram and faba beans (Rao, 1969; El-Faki *et al.*, 1984; Jood *et al.*, 1988; Sharma, 1989).

Table 5. Effect of sprouting on in-vitro starch digestibility of peas (mg maltose release/g meal, on dry matter basis)[a]

Variety	Period of sprouting		
	12 h	24 h	48 h
Vegetable peas			
Bonneville	45·5 ± 0·02 (+25)	56·5 ± 0·04 (+55)	64·3 ± 0·01 (+76)
Arkel	45·5 ± 0·05 (+23)	55·5 ± 0·29 (+57)	63·5 ± 0·03 (+80)
Field peas			
HFP4 (Aparna)	38·4 ± 0·04 (+26)	50·3 ± 0·01 (+65)	58·7 ± 0·01 (+92)
Rachna	41·6 ± 0·02 (+33)	54·5 ± 0·07 (+75)	63·3 ± 0·01 (+103)
SE (m)	± 1·73	± 0·17	± 0·14
CD ($P < 0·05$)	5·64	0·51	0·47

[a] Values are means ± SD of three independent determinations. Figures in parentheses indicate per cent increase (+) over control values.

Sprouting

There was an appreciable increase in starch digestibility of sprouted pea seeds; it increased with an increase in the period of germination (Table 5). After 12 h germination, Rachna and Arkel had the highest and the lowest increase in starch digestibility, respectively. There were significant ($p < 0·05$) differences in the starch digestibility of different pea cultivars sprouted for 12, 24 and 48 h. When the sprouting was done for 24 h, a 55–75% increase was observed in starch digestibility compared to the raw values. Further increase in the germination period from 24 to 48 h had a pronounced effect on the digestibility of starch. About a 2-fold increase was observed in different pea cultivars when sprouted for 48 h.

An increase in digestibility upon germination is expected because of the pre-digestion of starch by amylolytic enzymes. Amylases and phosphorylases may become active during the germination process. The resulting enhanced concentration of oligosaccharides in the sprouts may contribute to better starch digestibility (Jaya & Venkataraman, 1980; Nnanna & Phillips, 1990).

Germination improves the nutritive value of legumes by introducing the formation of enzymes which eliminate or reduce the antinutritional and undigestible factors in legumes (Nnanna & Phillips, 1990). In addition, germination affected changes in starch digestibility (Geervani & Theophilus, 1981) which probably also resulted from enzyme action.

The beneficial effect of sprouting for varying periods on starch digestibility of some food legumes has been reported earlier by some workers (Kataria *et al.*, 1990; Nnanna & Phillips, 1990).

CONCLUSIONS

In conclusions, peas are good sources of dietary carbohydrates like many food legumes, but have relatively low starch digestibility. All domestic processing and cooking treatments improved the in-vitro digestibility of starch of field as well as vegetable peas to varying extents. Among all the treatments, pressure cooking was the most effective method for increasing starch digestibility, followed by ordinary cooking, sprouting, dehulling and soaking. Various domestic processing and cooking methods might have caused a reduction in the level of antinutrients, thereby increasing the starch digestibility.

Table 6. Correlation coefficients of antinutritional factors with in-vitro starch digestibility of peas

Treatment	Antinutritional factors			
	Phytic acid	Polyphenols	Saponins	Trypsin inhibitor activity
Raw	−0·723 2**	0·968 1**	−0·822 0**	0·832 8**
6 h soaking	−0·287 8	−0·500 4	0·719 0**	−0·103 1
12 h soaking	−0·755 4**	−0·506 9	−0·853 2**	0·856 7**
18 h soaking	0·898 1	−0·486 8	−0·052 6	−0·737 2**
Soaked (12 h) ad dehulled	−0·843 3**	−0·713 4	0·750 7	0·863 4**
Unsoaked and cooked	−0·768 2**	0·508 0	0·799 8**	−0·109 0
Soaked (12 h) and cooked	0·857 6**	−0·708 1**	−0·330 5	0·855 0**
Soaked (12 h), dehulled and cooked	0·976 8**	0·966 3	0·729 8**	−0·390 3
Unsoaked and pressure cooked	−0·786 9**	−0·502 1	0·735 2**	0·853 7
Soaked (12 h) and pressure cooked	0·325 2	0·962 4**	0·288 7	−0·405 6
Soaked (12 h) dehulled and pressure cooked	−0·780 7**	0·956 1**	0·722 8**	0·397 4
12 h sprouting	−0·850 7**	−0·502 2	−0·855 4**	0·858 2**
24 h sprouting	−0·293 7	−0·605 6*	0·282 7	−0·652 0*
48 h sprouting	0·870 4**	0·956 1**	0·574 7*	−0·816 7**

* Significant at 5% level.
** Significant at 1% level.

This was further established by the fact that significant ($P < 0·05$) negative correlations were obtained between the antinutrients (e.g. phytic acid, polyphenols, saponins and trypsin inhibitors) and starch digestibility (in vitro) of soaked, dehulled, ordinarily cooked, pressure-cooked and sprouted peas (Table 6). Effects of processing and cooking on the levels of antinutrients of peas will be reported in due course.

REFERENCES

Cerning, J. & Guilhot, J. (1973). Changes in carbohydrate composition during maturation of wheat and barley kernel. *Cereal Chem.*, **50**, 220–5.

Clegg, K. M. (1956). The application of the anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agric.*, **7**, 40–3.

Deshpande, S. S. & Cheryan, M. (1984). Effect of phytic acid, divalent cations and their interactions on alpha amylase activity. *J. Food Sci.*, **49**, 516–19.

Deshpande, S. S. & Salunkhe, D. K. (1982). Interactions of tannic acid and catechin with legume starches. *J. Food Sci.*, **47**, 2080–1.

El-Faki, H. A., Venkataraman, L. V. & Desikachar, H. S. R. (1984). Effect of processing on the *in vitro* digestibility of protein and carbohydrates in some Indian legumes. *Plant Foods Hum. Nutr.*, **34**, 127–33.

Fleming, S. E. & Reichert, R. D. (1983). A comparison of flatulence potential of field pea and soybean seed fraction. *Can. Inst. Food Sci. Technol. J.*, **16**, 30–4.

Geervani, P. & Theophilus, F. (1981). Studies on digestibility of selected legumes carbohydrates and its impact on the pH of the gastro intestinal tract in rats. *J. Sci. Food Agric.*, **32**, 71–5.

Jaya, T. V. & Venkataraman, L. V. (1980). Effect of germination on the nitrogenous constituents, essential amino acid, carbohydrates, enzymes and antinutritional factors in chick pea and green gram. *Indian Food Packer*, **34**, 3–11.

Jood, S., Chauhan, B. M. & Kapoor, A. C. (1986). Saponin content of chick pea and black gram. Varietal differences and effects of processing and cooking methods. *J. Sci. Food Agric.*, **37**, 1121–4.

Jood, S., Chauhan, B. M. & Kapoor, A. C. (1988). Contents and digestibility of carbohydrates of chick pea and black gram as affected by domestic processing and cooking. *Food Chem.*, **30**, 113–27.

Kataria, A., Chauhan, B. M. & Punia, D. (1990). Effect of domestic processing and cooking methods on the contents of carbohydrate of amphidiploids (black gram × mung bean). *Food Chem.*, **36**(1), 63–7.

Khokhar, S. & Chauhan, B. M. (1986). Antinutritional factors in moth bean (*Vigna aconitifolia*): Varietal differences and effects of methods of domestic processing and cooking. *J. Food Sci.*, **51**(3), 591–4.

Nigam, V.N. & Giri, K.V. (1961). Sugar in pulses. *Can. J. Biochem. Physiol.*, **39**, 1847–53.

Nnanna, I. A. & Phillips, R. D. (1990). Protein and starch digestibility and flatulence potential of germinated cowpeas (*Vigria unguiculata*). *J. Food Sci.*, **55**(i), 151–3.

Panse, Y. G. & Sukhatme, P. V. (1961). *Statistical Methods of Agricultural Workers*, 2nd edn. Indian Council of Agricultural Research, New Delhi, pp. 12, 18.

Rao, P. S. (1969). Studies on the digestibility of carbohydrates in pulses. *Indian J. Med. Res.*, **57**(ii), 215–17.

Rao, P. S. (1976). Nature of carbohydrates in pulses. *J. Agric. Food Chem.*, **24**, 958–62.

Rao, P. U. & Deosthale, Y. G. (1982). Tannin content of pulses. Varietal differences and effects of germination and cooking. *J. Sci. Food Agric.*, **33**, 1013–16.

Savage, G.P. & Deo, S. (1989). The nutritional value of peas (*Pisum sativum*): A literature review. *Nutr. Abst. Rev.* (Series A), **59**, 66–83.

Sharma, A. (1989). Effect of processing and cooking methods on nutrient composition and antinutritional factors of Bakla (*Vicia faba*). MSc Thesis, Haryana Agricultural University, Hisar, India.

Singh, U., Kherdekar, M. S. & Jambunathan, R. (1982). Studies on desi and kabuli chick pea (*Cicer arietinum* L.) cultivars. V. The level of amylase inhibitors, level of

oligosaccharide and *in vitro* starch digestibility. *J. Food Sci.*, **47**, 510–12.

Somogyi, M. (1945). A new reagent for the determination of sugars. *J. Biol. Chem.*, 160–1.

Thompson, L. U. & Yoon, J. H. (1984). Starch digestibility as affected by polyphenols and phytic acid. *J. Food Sci.*, **49**(4) 1228–9.

Wills, R. B. H., Evans, T. J., Lim, J. S. K., Serivan, F. M. & Greenfield, H. (1984). Composition of Australian foods. 25. Peas and beans. *Food Technology in Australia*, **36**, 512–14.

Yemm, E. W. & Willis, A. J. (1954). The estimation of carbohydrates in plant extract by anthrone. *Biochem. J.*, **57**, 508–10.