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In-vitro digestibility and microscopic appearance of germinated legume starches and their effect on dietary protein utilization

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The effect of germination on the in-vitro digestibility of some legume starches and their effect on dietary protein were studied. Starch was extracted from faba bean (*Vicia faba* L.), kidney bean (*Phaseolus vulgaris* L.) and chick pea (*Cicer artinum*) before and after germination for 3 days. Germination increased the starch digestibility of the above three legumes (33·2%–32·3%, 39·5–47·2% and 44·71–49·4%, respectively). The microscopic appearance of the isolated starch was conducted using a scanning electron microscopic technique. The size of the starch granules increased with slight damage in the starch isolated from germinated seeds in comparison to the ungerminated. The effect of the isolated legume starches on the utilization of casein as the sole protein in rat diets was evaluated using protein efficiency ratio (PER), digestibility coefficient (DC) and net protein utilization (NPU) as parameters. The utilization of casein significantly increased when the diets were supplemented with isolated starches from germinated legume seeds compared to ungerminated legume starches. Cooked starch from germinated chick pea had PER, DC and NPU values similar to those of a corn starch diet, being 2·4, 93·4 and 74·1%, respectively. This study found a good correlation between the nature of the starch granules and their in-vitro digestibilities and the utilization of the protein in the rat diets.

INTRODUCTION

Food legumes form an important source of protein, vitamins, carbohydrates and minerals in diets of large population groups in Egypt and some other developing countries (Aykroyd & Doughty, 1964). Legume starches have been found to be less digestible than cereal or cassava starches (Hoover & Sosulski, 1985). They showed that, during a 6 h period, corn starch was hydrolysed (75%) by porcine pancreatic α-amylase whereas the corresponding values for legume starches belonging to the species *Phaseolus vulgaris* ranged from 26 to 35%. On the other hand, the blood glucose responses of a wide spectrum of starchy foods were found to correlate with the in-vitro digestion rates of their starches. (Tappy *et al.*, 1986).

The microscopic structure of broad bean has been studied by El-Shimi *et al.* (1980) using scanning electron microscopy (SEM). They found that the size of the starch granules increased after soaking and germination. Moreover, the utilization of legume starches may have some effect on the utilization of legume protein. Gervani & Theophilus (1981) reported that the source of carbohydrates affects the utilization of the protein. They found that black gram and green gram starches promoted rat growth similarly to corn starch, but the growth of rats fed on Red gram and Bengal gram starches was significantly less ($P < 0{\cdot}05$). El-Shemi *et al.* (1992) reported that the nutritional value of legume seeds can be improved by germination. Germination induces biochemical changes which produce some changes in the physical properties and functionality of seed components (Wursch *et al.*, 1986).

This study was undertaken to explore the effect of germination on the in-vitro digestibility of some legume starches and the effects of their isolated legume starches on the utilization of the protein in the rat diets. Also, the structure of raw and germinated starches was investigated using an SEM technique to study the correlation between legume starch nature and its nutritional value.

MATERIALS AND METHODS

Materials

Faba bean (*Vicia faba* L.), chick pea (*Cicer artinum*) and kidney bean (*Phaseolus vulgaris* L.) were purchased

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from a local market in Alexandria, Egypt. The legume seeds were kept tightly in polyethylene bags at 4°C until used.

Methods

Germination

After soaking the beans in distilled water for 12 h at room temperature, the beans were washed and placed in stainless steel screen baskets, which were covered with a cheese cloth. During the germination period, the seeds were rinsed with tap water every 12 h. Samples were collected after 72 h of germination and air-dried. Ungerminated and germinated seeds were decorticated and ground to pass through a 60 mesh sieve.

Extraction of legume starch

Starch was extracted from the raw and germinated beans by method B described by Schoch & Maywald (1968). In the case of faba beans and kidney beans, 0·2% NaOH was used instead of distilled water for the isolation of starch. The extracted starch was air-dried and powdered before use. The isolated starches contained 98·8% starch based on dry matter, 0·28% nitrogen and 0·06% ash. The starch was determined by the anthrone method as described by (McCready *et al.*, 1950).

In vitro digestibility of legume starch

Ground samples (100 mg) were suspended in 9 ml of sodium phosphate buffer (0·2 M, pH 7·0) and incubated at 37°C for 15 mins. Further, 1 ml of α-amylase solution in 0·2 M sodium phosphate buffer (containing 200 units of enzyme activity and preincubated at 37°C for 15 mins) was added. At the end of 30 minutes the reaction was stopped by heating in a boiling water bath for 3 min (Deshpande & Salunkhe, 1982). The liberated sugars (expressed as maltose) were estimated by the method described in AACC (1975).

Scanning electron microscopy (SEM)

The starch specimen was mounted on circular stubs using double sided tape and coated with gold to a thickness of 4 nm. The specimen was then examined on a Joel-JSM 252 II, microscope, (Japan). Selected areas were photographed on panatomic × film.

Biological methods

The protein efficiency ratio (PER), digestibility coefficient (DC) and net protein utilization (NPU) were used as a parameters to assess the effects of legume starches on protein utilization in the diet. Eight groups of six weanling male albino rats, 3 to 4 weeks old (weight average 50 g), obtained from the High Institute of Public Health, University of Alexandria, were used. The composition of the diet is given in Table 1. Rats were fed *ad libitum* daily for 4 weeks. Uneaten and scattered feeds were estimated daily and rats were weighed daily. The PER was calculated according to the AOAC (1980). At the end of the feed period, faeces were collected daily and composited for 5 days. Food intake and body weight were also recorded to calculate the DC, as described by Mitchell (1924). Correction of faecal nitrogen excretion was made by calculating the faecal nitrogen on rats receiving a nitrogen-free diet. NPU was determined by the method of Miller & Bender (1955).

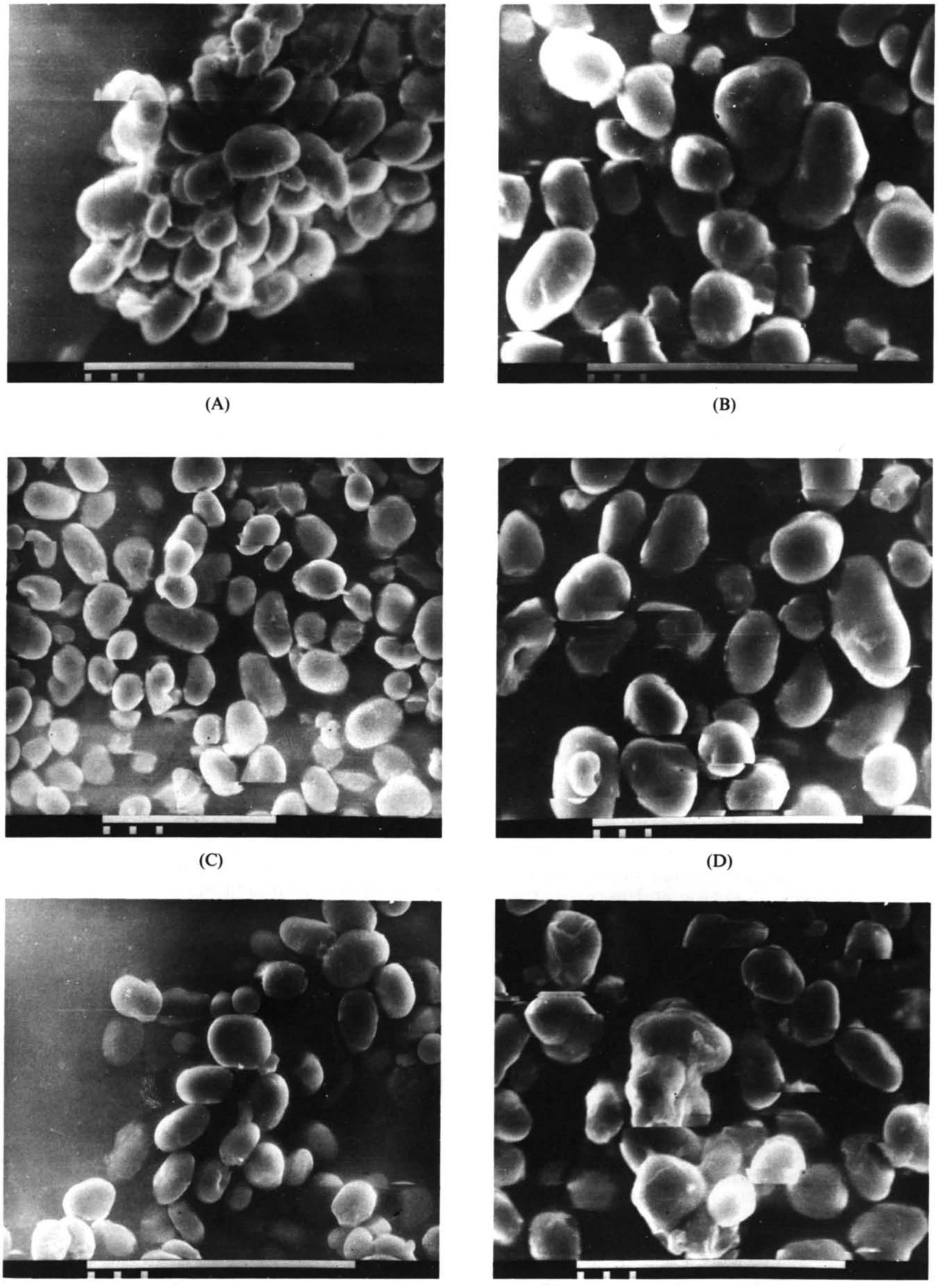
Table 1. Composition of casein diet supplemented with isolated starches from some legume seeds

Ingredient (g)	Legume starches (%)			
	Faba bean	Kidney bean	Chick-pea	Casein
Casein	10	10	10	10
Corn starch	75	—	—	—
Legume starch	—	75	75	75
Corn oil	10	10	10	10
Salt mixture[a]	4	4	4	4
Vitamin mixture[a]	1	1	1	1

[a] From INC Pharmaceuticals, OH, USA.
The composition of the salt mixture is (%): 54·3 calcium carbonate, 2·5 magnesium carbonate, 1·6 magnesium sulphate. $7H_2O$, 6·9 sodium chloride, 11·2 potassium chloride, 21·2 potassium phosphate (monobasic), 2·05 ferric phosphate, 0·008 potassium iodide, 0·035 manganese, 0·01 sodium fluoride, 0·017 aluminium sulphate and 0·9 copper sulphate.
The composition of the vitamin mixture is (mg/kg): 800 thiamin HCl, 1600 riboflavin HCl, 800 pyridoxin HCl, 5000 Ca pantothenate, 8000 niacinamide, 200 folic acid, 40 biotin, 100 menadione, 2000 a-tocopheryl succinate, 30 cyanocobalamin, 20 vitamin A, 32·5 IU vitamin D/g mixture and 961·43 g starch.

RESULTS AND DISCUSSION

In-vitro starch digestibility values are presented in Table 2. Starches isolated from germinated seeds were significantly ($P < 0·01$) more digestible than those from ungerminated samples. These values were 33·2, 32·3 and 39·5% for ungerminated faba bean, kidney bean and chickpea, respectively, and became 47·2, 44·7 and 49·4% after germination for 3 days. El-Faki *et al.* (1984) found a modest effect of germination on the in-vitro digestibility of legume starch. Moreover, Nnanna & Phillips (1990) reported that in-vivo digestibility of legume starch increased by germination. As indicated by Table 2, cooking of the isolated starches increased their digestibility significantly ($P < 0·01$) for both the ungerminated and germinated samples. It can be seen that the cooked germinated starch had the highest starch digestibility values (84·3–90·3%). Cooking was found to increase the digestibility of starches from germinated beans. The digestibilities of starches from cooked, germinated chickpea, cowpea and green gram were found to be two to four times higher than that seen with uncooked germinated seeds (Kumar & Venkataraman, 1976). The corresponding value for black gram and chick pea was 1·3 (Jood *et al.*, 1988). Swelling, gelatinization and destruction of the crystalline structure of starch granules are known to increase the action of amylase enzymes on them.



(A) (B) (C) (D) (E) (F)

Fig. 1. SEM micrographs of starches isolated from ungerminated and germinated legume seeds. (original magnification × 700). (A) ungerminated faba bean; (B) germinated faba bean (3 days); (C) ungerminated kidney bean; (D) germinated kidney bean (3 days); (E) ungerminated chick pea; (F) germinated chick pea (3 days).

Table 2. In vitro starch digestibility of ungerminated and germinated isolated legume starches in various forms

Treatment	Faba bean (%)	Kidney bean (%)	Chick-pea (%)	Corn starch (%)
Isolated starch				
A	33·2	32·3	39·5	
B	65·1	62·1	67·2	
C	47·2	44·7	49·4	
D	84·3	82·1	90·3	
Cooked corn starch				90·3

A: isolated starch from ungerminated seeds.
B: cooked isolated starch from ungerminated seeds.
C: isolated starch from germinated seeds.
D: cooked isolated starch from germinated seeds.
Cooking: the samples was steamed (115°C for 15 min).

Table 3. Effect of isolated legume starches on PER, DC and NPU of casein as a protein source in rat diets

Source of starch[a]	Actual PER	Adjusted[b] PER	DC (%)	NPU
Corn starch	2·21 ± 0·04	2·50	96·5	75·9
Faba bean				
Ungerminated	1·65 ± 0·09	1·87	69·4	60·3 ± 4·21
Germinated	1·98 ± 0·05	2·23	88·5	69·4 ± 3·09
Kidney bean				
Ungerminated	1·76 ± 0·06	1·99	66·3	61·1 ± 6·10
Germinated	1·90 ± 0·08	2·15	83·1	65·2 ± 5·07
Chick pea				
Ungerminated	1·94 ± 0·11	2·15	79·9	67·2 ± 4·04
Germinated	2·15 ± 0·08	2·43	93·4	74·1 ± 5·06

[a] Steamed (115°C for 15 mins).

[b] $\text{Adjusted PER} = \dfrac{\text{Actual PER} \times 2{\cdot}5}{\text{PER of corn starch diet}}$.

SEM micrographs of starch granules isolated from ungerminated and germinated faba bean, kidney bean and chickpea are shown in Fig. 1. It is clear that the size of the starch granules increased after 3 days of germination. The lengths of ungerminated legume starch granules ranged from 0·9 to 1·8 mm. After germination the granules averaged 1 to 2·9 mm for the three legume seeds. However, faba bean starch showed the highest swelling during the germination period (its starch length ranged from 1·1 to 1·8 mm and increased from 1·7 to 2·9 mm after germination). Some of the legume starch granules were broken while others maintained their integrity. Germinated kidney starches were more rigid than germinated chickpea starches, which were more fragile and some of the starch granules lost their structure. El-Shimi *et al.* (1980) stated that broad bean starch granules appeared to be very fragile after 8 days of germination. From the results of Table 2 and Fig. 1, germinated seeds which had large and somewhat broken starch granules had higher digestibility values than ungerminated ones. So it can be concluded that the digestibility of the legume starch is correlated to the nature of the starch. El-Faki *et al.* (1984) stated that the nature of starch may decide the type and degree of amylolysis in the *in-vitro* system. Further, Wursch *et al.* (1986) reported that disruption of the cells, especially before cooking, improves the susceptibility of starch to α-amylase digestion. Contradicting views have been expressed by other investigators on the relationship between granule size and rate of enzymolysis, some indicating that large granules are less readily attacked (Kulp & Mattern, 1973) and some not finding any association (Leach & Schoch, 1961). The effect of ungerminated starches on the utilization of casein as a sole source of protein in rate diets is given in Table 3. All the ungerminated legume starches under study were inferior to corn starch in promoting growth. The adjusted PER value for the rats fed on diets supplemented with corn starch was 2·5%, while it was 1·87, 1·99 and 2·15% for faba bean, kidney bean and chickpea starch diets, respectively. The protein DC values of the diets containing ungerminated legume starches were lower than that of the corn starch diet. Chickpea had the highest DC value among the three legumes under study, being 79·9%. NPU values for the legume starch diets confirmed the above results where NPU values for the diets containing ungerminated legume starches were lower than those containing corn starch. The results of Table 3 also show that the germination process significantly improves ($P < 0{\cdot}05$) the utilization of the protein in the diets. Adjusted PER, DC and NPU values increased by 19·3%, 20·3 and 15·1%, 12·9, 22·7 and 18·3%, and 13·0; 17·5 and 10·3% for faba bean, kidney bean and chick pea, respectively. Booher *et al.* (1951) reported that apparent digestibility of protein varies according to the component of the diet. Also, Yoshida & Morimoto (1955) pointed out that PER and DC of diets supplemented with potato starch were less than that of corn starch.

Gervani & Theophilus (1981) postulated that the influence of starches on protein utilization may be through their interference with digestibility. They also reported that the PER of red gram and Bengal gram protein can be improved if the digestibility of the respective carbohydrates is improved. The results of this study confirm their findings as re-enforced by the following: Table 2 shows that germination significantly improved the in-vitro digestibility of legume starches. Also, Table 3 shows that the diets supplemented with starches isolated from germinated seeds, which had high starch digestibility values, supported the utilization of the protein almost as well as corn starch (especially chick pea).

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