

## Chemical and Nutritional Changes in Soybean During Germination

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### ABSTRACT

*Germination of soybeans for 6 days depressed the trypsin inhibitor activity by 32%. Oil content, acid value, iodine value and total unsaturated fatty acids of soybean oil decreased while peroxide value and total saturated fatty acids pronouncedly increased. Percentage of palmitic and linoleic fatty acids were highly affected during germination. Analysis of the hydrocarbon and sterol classes revealed n-tricosane and  $\beta$ -sitosterol to be, respectively, the major components. Marked changes in both hydrocarbon and sterol constituents occurred upon germination. Different periods of soybean germination increased non-protein nitrogen and decreased reducing sugars. However, total ash content decreased due to soaking; thereafter it was constant for the rest of the germination period. Different amino acid components increased due to germination when compared with their content in dry seeds. The protein solubility of germinated soybean was higher in  $H_2O$ , 5% NaCl and 0.02M NaOH than it was in ungerminated seeds. Water and oil absorption, emulsification and foaming capacities of soybean were increased after germination. A marked improvement in digestibility by pepsin was noticed while tryptic digestibility was still low (38%). PAGE patterns showed drastic changes in both low and high molecular weight protein fractions due to germination.*

## INTRODUCTION

Increased production and utilization of soybean (*Glycine max*) seeds in Egypt as a low-cost source of protein and fat have created an interest in its chemical composition and nutritive value. In Egypt many legume seeds are consumed by people after germination for different periods (e.g. fenugreek seeds and faba beans). Germination processes have been developed in some countries to overcome some of the disadvantages associated with ungerminated soybeans, such as undesirable flavour and odour and the presence of trypsin inhibitors (McKinney *et al.*, 1958; Suberbie *et al.*, 1981; Vanderstoep, 1981). Germination may also result in an increase in nutritive value relative to ungerminated seeds (Fordham *et al.*, 1975). Germinated seeds were rated high in protein, appearance, flavour and texture and could be consumed uncooked in salad, boiled in water with suitable seasoning or fried in fat (Smith & Circle, 1978). Germinated beans could also be used to replace, in part, wheat flour without affecting baking properties (Hsu *et al.*, 1980). Several studies confirmed the benefits of soybean germination (Kylan & McCready, 1975; Fordham *et al.*, 1975). These include an increase in ascorbic acid, hydrolysis of raffinose and stachyose, which are linked to flatulence problems (East *et al.*, 1972; Hsu *et al.*, 1973) and a decrease in the phytate content, which may increase availability of many essential elements (Reddy *et al.*, 1978). On the other hand, the germination process induced a general rise in metabolic activity leading to an initiation of seedling formation (Mayer & Poljakoff-Mayber, 1963). Such metabolic events could be affected by the quality of soybean seeds. However, limited data have been published relating to germination of seeds and their nutritive composition. Therefore, this study was undertaken to determine the effect of the germination on (a) the nutritive value of soybeans, (b) trypsin inhibitor activity, (c) *in vitro* digestibility, and (d) amino acid profile. We also wished to ascertain changes in oil properties, i.e. fatty acid, sterol and hydrocarbon composition during germination. The functional properties, as well as disc polyacrylamide gel electrophoresis of ungerminated and germinated soybean proteins, were also compared.

## MATERIAL AND METHODS

### Material

Soybean seeds (*Glycine max*, L.), variety Calland, grown in Minufiya Governorate during 1984 were supplied by the seed section, Agricultural Department, Shebin El-Kom, Egypt. The moisture content of the beans was 8.77%.

### *Germination of soybean seeds*

Mature, unbroken seeds were washed and soaked overnight in water (1:5 w/v). The soaked seeds were washed thoroughly and then spread in a layer on cellulose sponges and placed in plastic containers. Thereafter water was poured to one-half of the sample height to provide moisture during sprouting. The container was then covered with aluminium foil to exclude light and held at room temperature (23–25°C) for 1, 2, 3, 4, 5 and 6 days. The germinating seeds were washed once a day with water. Thereafter germinated beans were dried in an oven for 10 h at 40°C and ground in a Waring blender to pass through a 20 mesh sieve. The oil was extracted using *n*-hexane as a solvent, the extracted oil was separated by evaporating the solvent under high vacuum and oil was stored in brown glasses at 4°C for further analysis. The meal was re-extracted with *n*-hexane in a Soxhlet apparatus to remove oil residue and the defatted meal was desolventized at room temperature overnight then milled in a Waring blender for 3 min to pass through a 60 mesh sieve. The ground meal was stored in a Kilner jar at room temperature for further analysis.

## **Methods**

### *Analysis of oils*

*Oil properties.* Refractive index, acid value, iodine value, peroxide value and unsaponifiable matter percentage of oil, as well as moisture content of the seeds, were determined according to the methods described by the AOAC (1980).

*Fatty acid compositions.* Fatty acid methyl esters were prepared as noted by Anon (1966). The methyl esters were analyzed by gas-liquid chromatography using a PYE Unicam model 104 equipped with flame ionization detector; the stationary phase was 10% PEGA on Chromosorb W. The temperatures for injector, column and detector were 220, 190 and 220°C, respectively; gas flow rates for nitrogen, hydrogen and air were 30, 33 and 330 ml/min, respectively. Chart speed was 1 cm/min; attenuation was  $50 \times 10^{-2}$ . The identification of fatty acid methyl esters was based on their retention times and their relative percentages were calculated according to the method reported by Fryer *et al.* (1960) and Nelson *et al.* (1969).

*Identification of sterols and hydrocarbon components.* Hydrocarbons and sterols of the unsaponifiable matter were analyzed by gas-liquid chromatography under the following conditions: glass column packed with acid-alkali washed and silanized Diatomite C coated with 1% OV-17. The temperatures of injector, column and detector were 300°C, 270°C and

300°C, respectively. Gas flow rates were 30, 33 and 300 ml/min for nitrogen, hydrogen and air, respectively. A chart speed of 3 cm/min and an attenuation of  $32 \times 10^{-2}$  was used. Identification of sterols was carried out by comparing the relative retention times of each peak with that of the authentic samples. The results of Itoh *et al.* (1973) were also used for identification, since the same conditions were used. The relative percentages of each peak were calculated by using the triangular method of Nelson *et al.* (1969).

#### *Analysis of meal*

*Chemical analysis and amino acid profile.* Moisture, total protein and total ash were determined by AOAC methods (AOAC, 1980). The method of Dubois *et al.* (1956) was used to estimate total sugars in the ethanol extracts using glucose as standard. Total carbohydrates were calculated by difference. Non-protein nitrogen of the meals was determined according to Bahtty (1973). The acid hydrolyzate (6 N HCl) of the protein of soybean samples was prepared according to the method of Block *et al.* (1958). Quantitative determination of the amino acids was carried out using a Beckman amino acid analyzer model 121 as described by Morre *et al.* (1958). Microbiological assay was carried out to determine the methionine content using *Leuconostoc mesenteroides* as described by Helali *et al.* (1979).

*Trypsin inhibitor activity assay.* The method described by Roy & Bhat (1974) was used for determining the trypsin inhibitor activity in the crude preparations of both the phosphate buffer (0.1M, pH 7.6) and water extracts of the meal samples.

*In vitro digestibility.* The *in vitro*-digestibility index was determined using pepsin and trypsin enzymes after incubation at 37°C for 24 h as described by Abd El-Aal *et al.* (1986). The results are expressed as per cent protein digested. Pepsin and trypsin enzymes were from B.D.H., Great Britain.

*Polyacrylamide gel electrophoresis (PAGE).* Disc PAGE was carried out by the procedure described by Davis (1964) using 7.5% gel and 0.01M sodium phosphate buffer (pH 7.8). Germinated soybean flours were extracted in 1M NaCl solution (flour to solvent ratio, 1:10; extraction time, 1 h at room temperature ~28°C). The extracts were dialyzed against the buffer for 24 h with several changes of the buffer. About 100 mg of protein were loaded on the gel. Electrophoresis was performed for 2.5 h at a constant current of 3 mA/tube. The gels were stained with 0.5% amido black solution for 1 h, then destained with 7.5% acetic acid.

*Functional properties.* Nitrogen solubility indices of each flour in water, 5% NaCl and 0.02M NaOH solutions were determined according to the

method described by Rahma & Narasinga Rao (1979). For water and fat absorption capacities, the method of Rahma & Narasinga Rao (1984) was followed. Water absorption capacity is expressed as the weight of water bound per 100 g of dry flour. Fat absorption capacity is expressed as millilitres of oil bound per 100 g of dry flour.

Emulsifying capacity (EC) was determined by the method described by Webb *et al.* (1970). Two grams of flour were used and the collapsing point of the emulsion was determined visually. It is expressed as millilitres of oil emulsified per gram of dry flour. Refined corn oil was used for oil absorption and emulsifying capacity studies.

## RESULTS AND DISCUSSION

### Effect of germination on oil quality

#### *Physico-chemical properties*

Table 1 indicates the effect of germination treatment on some oil characteristics. A significant change in the oil content, due to the germination process, occurs during the first 4 days of germination, but slight decreases in the oil content of the beans amounting to 5.37% and 7.69%, respectively, occurred on the 5th and 6th days. This decrease could probably be ascribed to consumption of oil as energy and/or synthesis of certain structural constituents in the young seedling (Singh *et al.*, 1968). Similar observations were reported by McKinney *et al.* (1958). The acid value of soybean oil was gradually decreased during germination. The decreasing pattern in free fatty acid during germination is probably due to the greater rate of metabolism of fatty acids relative to their liberation (McKinney *et al.*, 1958). On the other hand, the peroxide value showed a noticeable increase during seed germination since it reached 8.28 meq/kg at the end of germination. The increase in the peroxide value indicates the formation of peroxides during germination. McKinney *et al.* (1958) found that the oil from the 5- and 6-day germinated soybean was dark and contained a petroleum ether-soluble chromogenic substance. The iodine value of the oils studied decreased from 131 to 120 due to germination (Table 1).

#### *Fatty acids composition*

Table 2 indicates the effect of germination on fatty acid composition of soybean seeds. In general, linoleic acid was the most abundant unsaturated fatty acid. The corresponding saturated fatty acid was palmitic acid. As germination progressed, more or less marked changes in fatty acid distribution occurred. Linoleic acid decreased while palmitic acid increased. Changes in other fatty acids were also observed. The ratio between total

**TABLE 1**  
Effect of Germination Process on Some Characteristics of Soybean Oil

| Properties                   | Germination period (days) |        |        |        |        |        |        |
|------------------------------|---------------------------|--------|--------|--------|--------|--------|--------|
|                              | Zero<br>(Control)         | 1      | 2      | 3      | 4      | 5      | 6      |
| Oil content (%) <sup>a</sup> | 20.67                     | 20.49  | 20.38  | 20.26  | 20.18  | 19.56  | 19.08  |
| Refractive index/25°C        | 1.4770                    | 1.4768 | 1.4768 | 1.4766 | 1.4737 | 1.4722 | 1.4711 |
| Acid value                   | 0.83                      | 0.67   | 0.65   | 0.41   | 0.40   | 0.39   | 0.35   |
| Peroxide value (meq/kg)      | 1.80                      | 2.99   | 3.96   | 4.44   | 4.65   | 6.62   | 8.28   |
| Iodine value                 | 131                       | 130    | 130    | 129    | 128    | 126    | 120    |
| Unsatifiable matter (%)      | 1.14                      | 0.86   | 0.90   | 0.93   | 0.91   | 0.94   | 1.10   |

<sup>a</sup> Calculated on dry weight basis.

**TABLE 2**  
Fatty Acids Composition of Soybean Oil during the Germination of Seeds

| Fatty acids                   | Germination period (days) |      |      |      |      |      |      |
|-------------------------------|---------------------------|------|------|------|------|------|------|
|                               | Zero<br>(Control)         | 1    | 2    | 3    | 4    | 5    | 6    |
| Palmitic                      | 10.8                      | 12.8 | 13.0 | 14.6 | 15.2 | 17.8 | 20.2 |
| Stearic                       | 2.75                      | 2.18 | 2.36 | 2.10 | 2.22 | 1.87 | 2.79 |
| Oleic                         | 18.0                      | 15.2 | 16.0 | 14.2 | 13.9 | 14.5 | 18.1 |
| Linoleic                      | 63.6                      | 64.4 | 63.1 | 63.4 | 64.4 | 60.2 | 55.0 |
| Linolenic                     | 4.98                      | 5.41 | 5.58 | 5.78 | 4.29 | 5.73 | 3.88 |
| Total saturated fatty acids   | 13.5                      | 15.0 | 15.4 | 16.7 | 17.4 | 19.6 | 23.0 |
| Total unsaturated fatty acids | 86.6                      | 85.0 | 84.6 | 83.4 | 82.6 | 80.4 | 77.0 |

unsaturated fatty acids ( $T_u$ ) and total saturated fatty acids ( $T_s$ ) was 6.42:1 prior to germination and decreased to 3.34:1 after 6 days of germination. The decrease in  $T_u$ , especially linoleic acid, coincided with the decreasing iodine value (Table 1) and was probably due to the desaturation of fatty acids which took place during  $\beta$ -oxidation (Dutton & Mounts, 1966). Generally, these results are in agreement with those obtained by Chen *et al.* (1975) and Yoshida *et al.* (1975).

#### *Unsaponifiable matter constituents*

Table 3 shows the changes in hydrocarbon and sterol fractions of soybean oil during the germination of seeds. The major hydrocarbon and sterol are *n*-tricosane and  $\beta$ -sitosterol, respectively. The germination process caused marked changes in both of these, but especially in *n*-tricosane. With regard to the hydrocarbon fraction, the results in Table 3 indicate that  $C_{20}$  and  $C_{23}$  compounds completely disappeared in the first and second days of germination then reappeared in small amounts on the third day for the former and the third and fourth days of germination for the latter then disappeared again for the remainder of the study. The  $C_{22}$  and  $C_{28}$  hydrocarbons showed a gradual increase with increase in germination time until the third day, then sharply decreased to the end of the germination period. On the other hand, the germination process induced a remarkable decrease in  $C_{32}$  and total hydrocarbons whereas other constituents, i.e.  $C_{25}$  and  $C_{27}$ , were identified only after the first and fourth days of germination.

Table 3 also demonstrates that the sterol fraction of soybean oil contained  $\gamma$ -tocopherol which decreased during germination. The rate of decrease was pronounced even after the first day of germination. Such a lowering of this natural antioxidant led to a shorter induction period of the oil and resulted in an increase in its peroxide value (see Table 1).  $\beta$ -sitosterol, campesterol + stigmasterol and  $\Delta$ -7 stigmasterol showed marked increases during the study. Cholesterol and fucosterol also increased whereas  $\Delta$ -7 avenasterol showed a variable decrease until the third day of germination then disappeared.

Generally, it can be concluded that the germination process of soybean seeds for 6 days resulted in quantitative and qualitative changes in the hydrocarbon and sterol fractions of the oil. These observations coincide with changes in the percentage of unsaponifiable matter (Table 1).

### **Effect of germination on meal quality**

#### *Chemical composition*

The proximate composition of germinated and ungerminated soybean meals (Table 4) indicates that there was a slight decrease in the total



TABLE 3  
Changes in Unsaponifiable Matter Components during the Germination of Soybean Seeds

| No. | Components                               | RRT  | Germination period (days) |       |       |       |      |      |      |   |
|-----|--|------|---------------------------|-------|-------|-------|------|------|------|---|
|     |  |      | Zero <sup>a</sup>         | 1     | 2     | 3     | 4    | 5    | 6    |   |
| 1   | <i>n</i> -Eicosane C <sub>20</sub>       | 0.02 | 1.41                      | —     | —     | 0.77  | —    | —    | —    | — |
| 2   | <i>n</i> -Docosane C <sub>22</sub>       | 0.04 | 3.05                      | 12.66 | 14.55 | 17.05 | 9.30 | 7.69 | 5.65 | — |
| 3   | <i>n</i> -Tricosane C <sub>23</sub>      | 0.05 | 13.77                     | —     | —     | 0.12  | 1.23 | —    | —    | — |
| 4   | <i>n</i> -Tetracosane C <sub>24</sub>    | 0.08 | 0.34                      | 0.51  | —     | —     | —    | 2.47 | 0.80 | — |
| 5   | <i>n</i> -Pentacosane C <sub>25</sub>    | 0.10 | —                         | 0.11  | 0.39  | 0.53  | 0.10 | 0.10 | 0.16 | — |
| 6   | <i>n</i> -Hexacosane C <sub>26</sub>     | 0.14 | 9.58                      | 1.60  | 2.52  | T     | T    | 2.72 | 2.49 | — |
| 7   | <i>n</i> -Heptacosane C <sub>27</sub>    | 0.16 | —                         | —     | —     | —     | 5.42 | —    | —    | — |
| 8   | <i>n</i> -Octacosane C <sub>28</sub>     | 0.18 | 0.43                      | 3.34  | 6.08  | 7.02  | 4.44 | 4.58 | 5.22 | — |
| 9   | Squalene C <sub>30</sub>                 | 0.22 | 4.30                      | 4.05  | 3.15  | 3.41  | 4.23 | 4.95 | 4.05 | — |
| 10  | <i>n</i> -Henetricontane C <sub>31</sub> | 0.30 | 0.48                      | 0.47  | 0.51  | 0.40  | 0.48 | 0.55 | 0.59 | — |
| 11  | <i>n</i> -Dotriacontane C <sub>32</sub>  | 0.42 | 4.20                      | 0.71  | 0.74  | 0.60  | 0.54 | 0.72 | 0.41 | — |
| 12  | $\gamma$ -Tocopherol                     | 0.51 | 6.29                      | 2.33  | 2.25  | 2.01  | 2.17 | 1.76 | 1.83 | — |
| 13  | Cholesterol                              | 0.61 | 1.17                      | 0.80  | 0.43  | 0.84  | 0.65 | 0.64 | 0.58 | — |
| 14  | Campesterol + Stigmasterol               | 0.82 | 12.3                      | 26.5  | 25.2  | 24.8  | 25.9 | 28.6 | 30.0 | — |
| 15  | $\beta$ -Sitosterol                      | 1.00 | 38.3                      | 44.4  | 41.9  | 40.4  | 42.2 | 41.9 | 45.1 | — |
| 16  | Fucosterol                               | 1.13 | 1.84                      | 0.72  | 0.66  | 0.54  | 0.58 | 0.26 | 0.41 | — |
| 17  | $\Delta$ -7 Stigmasterol                 | 1.25 | 1.52                      | 1.07  | 1.38  | 0.84  | 2.11 | 2.69 | 2.28 | — |
| 18  | $\Delta$ -7 Avenasterol                  | 1.37 | 0.57                      | 0.19  | 0.26  | 0.33  | —    | —    | —    | — |
| 19  | Unknown                                  | 1.55 | 0.53                      | 0.64  | —     | 0.36  | 0.60 | 0.39 | 0.47 | — |
|     | Total hydrocarbons                       |      | 37.6                      | 23.5  | 27.9  | 29.9  | 25.7 | 23.8 | 19.4 | — |
|     | Total sterols                            |      | 56.1                      | 74.2  | 69.8  | 68.1  | 72.1 | 74.5 | 78.8 | — |
|     | $\gamma$ -Tocopherol                     |      | 6.29                      | 2.33  | 2.25  | 2.01  | 2.17 | 1.76 | 1.83 | — |

<sup>a</sup> Control sample.

—, Not detected.

T, Traces (less than 0.1%).

RRT, Relative retention times of compounds as compared with that of  $\beta$ -sitosterol which is used as a reference.

**TABLE 4**  
Effect of Germination of Soybean for Different Periods on the Chemical Composition of the Produced Flour<sup>a</sup>

| Constituents (%)                       | Germination period (days) |      |      |      |      |      |      |
|--|---------------------------|------|------|------|------|------|------|
|  | Zero<br>(Control)         | 1    | 2    | 3    | 4    | 5    | 6    |
| Total protein (N × 6.25)               | 50.5                      | 49.7 | 50.2 | 50.3 | 50.5 | 50.8 | 51.8 |
| Non-protein nitrogen                   | 3.41                      | 1.97 | 2.30 | 3.25 | 4.37 | 4.92 | 5.25 |
| Reducing sugars (as glucose)           | 10.35                     | 9.88 | 9.48 | 8.92 | 8.66 | 8.27 | 7.53 |
| Total ash                              | 7.60                      | 6.29 | 6.33 | 6.45 | 6.36 | 6.30 | 6.31 |
| Total carbohydrates<br>(by difference) | 28.1                      | 31.7 | 31.7 | 31.1 | 30.1 | 29.7 | 29.6 |

<sup>a</sup> Mean of two determinations and expressed on dry matter basis.

protein, reducing sugars and total ash contents after one day of germination relative to the control sample. This was expected because the soluble fractions of these classes were leached out during the soaking period used usually before germination. As the germination period increased there was almost no variation in ash content. Reducing sugars showed slight decreases with increasing germination time. This may be due to utilization of simple sugars as a source of energy during the germination process. Total protein showed an apparent increase with the time of germination due to the oxidation and consumption of the other classes in the germination process. The same observation for the total protein content of fenugreek during germination was reported by El-Mahdy & El-Sebaiy (1982). Also Hsu *et al.* (1980) have reported a slight increase in total protein of dry peas, lentils and faba beans after 4 days of germination. The major change was found in the non-protein nitrogen fraction since about 42% was lost during soaking; thereafter, it increased again with increasing germination time. After 3 days of germination it became almost the same as in the ungerminated beans (Table 4). Meanwhile, after 4, 5 and 6 days of germination the non-protein nitrogen content became higher than that of the control sample. At 6 days of germination the increase in rate reached 54%. This could be ascribed to both the activity of proteolytic enzymes and the hydrolysis of protein molecules to simple fragments. These findings agree well with the data of El-Mahdy & El-Sebaiy (1982) for germinated fenugreek seeds.

#### *Amino acid profile*

Amino acid contents of the ungerminated and germinated soybean flours are presented in Table 5. It is clear from these results that the germination process resulted in a marked increase in the relative contents of both essential and non-essential amino acids. The rate of relative increase in essential amino acids was 8.9% after 3 days of germination, whereas this value reached 22.4% at the end of the germination period. The corresponding relative increases in non-essential amino acids were 17.6% and 17.5% after 3 and 6 days' germination, respectively. In this regard Chen & Thacker (1978) reported that during germination there probably is a turnover of proteins and amino acids with the balance between synthetic and degradative processes determining the resultant pattern. Results show also that the greatest increases appeared to be, in descending order, leucine > tyrosine > phenylalanine and glutamic acid whereas methionine content showed a slight decrease due to the germination process. These findings are partially in agreement with those of Chen & Thacker (1978) who pointed out that the germination process of pea seeds causes a large increase in glutamic acid.

**TABLE 5**  
Effect of Germination on the Amino Acid Contents of Soybean Meal (g per 16 g N)

| <i>Amino acid</i>    | <i>Germination period (days)</i> |          |          |
|----------------------|----------------------------------|----------|----------|
|                      | <i>Zero<br/>(Control)</i>        | <i>3</i> | <i>6</i> |
| <i>Essential</i>     |                                  |          |          |
| Arginine             | 5.68                             | 6.48     | 6.74     |
| Lysine               | 5.23                             | 5.57     | 6.22     |
| Tyrosine             | 2.49                             | 3.04     | 3.24     |
| Phenylalanine        | 3.88                             | 4.55     | 4.94     |
| Methionine           | 1.19                             | 1.13     | 1.14     |
| Leucine              | 5.88                             | 6.54     | 7.90     |
| Isoleucine           | 3.40                             | 3.22     | 4.09     |
| Threonine            | 3.34                             | 3.66     | 3.80     |
| Valine               | 3.40                             | 3.36     | 4.13     |
| Total EAA            | 34.5                             | 37.6     | 42.2     |
| Differences (%)      |                                  | (8.9%)   | (22.4%)  |
| <i>Non-essential</i> |                                  |          |          |
| Alanine              | 3.38                             | 3.94     | 4.27     |
| Aspartic acid        | 9.26                             | 10.8     | 10.0     |
| Glutamic acid        | 14.3                             | 17.2     | 17.8     |
| Glycine              | 3.29                             | 3.70     | 3.88     |
| Proline              | 4.35                             | 5.35     | 5.0      |
| Serine               | 4.61                             | 5.33     | 5.45     |
| Histidine            | 2.28                             | 2.41     | 2.32     |
| Total non EAA        | 41.4                             | 48.7     | 48.7     |
| Differences (%)      |                                  | (17.6%)  | (17.5%)  |
| Total AA             | 75.9                             | 86.3     | 90.9     |
| Differences (%)      |                                  | (13.6%)  | (19.7%)  |

Generally, it can be concluded that germination of soybean seeds leads to improved nutritive value of germinated beans when compared with raw seeds due to an increase in total amino acids. The relative changes in amino acid content may be due to a net synthesis of enzymic protein during germination, possibly accounting for a major portion of the reported protein increase (Young & Varner, 1959).

#### *Trypsin inhibitor activity (TIA)*

Table 6 shows the changes in TIA during the 6 days germination of soybean seeds. TIA was decreased with increasing germination time, being 26.9 and 26.5 after 6 days using water extract treatment and phosphate buffer extract, respectively. A rapid decrease in TIA was observed from the second to the fourth day of germination. Meanwhile, the decreasing rate tended to

**TABLE 6**  
Effect of Germination Process on Trypsin Inhibitor Activity of Soybean

| Day of germination | Water extract                       |               | Buffer extract |               |
|--------------------|-------------------------------------|---------------|----------------|---------------|
|                    | TIA <sup>a</sup> (TUI) <sup>b</sup> | Reduction (%) | TIA (TUI)      | Reduction (%) |
| Control (Zero)     | 39.8                                | —             | 39.6           | —             |
| 1                  | 38.4                                | 3.50          | 38.2           | 3.54          |
| 2                  | 35.4                                | 11.1          | 35.1           | 11.4          |
| 3                  | 30.8                                | 22.6          | 30.5           | 23.0          |
| 4                  | 28.1                                | 29.4          | 28.2           | 28.9          |
| 5                  | 27.9                                | 29.9          | 27.7           | 30.1          |
| 6                  | 26.9                                | 32.4          | 26.5           | 33.1          |

<sup>a</sup> TIA, Trypsin inhibitor activity.

<sup>b</sup> TUI, Trypsin units inhibited.

slow down starting from the fourth to the sixth day, since per cent reduction in TIA was only 3.01. The decrease in TIA due to the germination process could be attributed to the leaching out of some elements during the daily washing of the sprouts, such elements being involved in the mechanism of the trypsin inhibitor (Collins & Sand, 1976).

Generally, it may be concluded that germination of soybean seeds for 6 days had a slight effect on TIA when compared with other treatments of soybean such as using both dry and humid heating (Rady *et al.*, 1986).

#### *In vitro-protein digestibility*

The *in vitro* protein digestibility of germinated soybeans compared to the dry sample in the case of pepsin, as well as trypsin, is illustrated in Fig. 1. The general conclusions are:

- (1) The ungerminated beans were lower in digestibility than the germinated samples for both enzymes.
- (2) Digestibility by both enzymes increased as the germination time increased.
- (3) Pepsin digestibility was higher than that of trypsin.

The germinated samples undergo protein hydrolysis by proteolytic enzymes and this leads to improved digestibility compared with the dry sample. Also, as the time of germination proceeds, the rate of protein hydrolysis increases. However, pepsin digestibility was much higher for both ungerminated and germinated soybeans. It was 62% and 88% at 0 and 6 days of germination, respectively, compared with 23% and 38% for

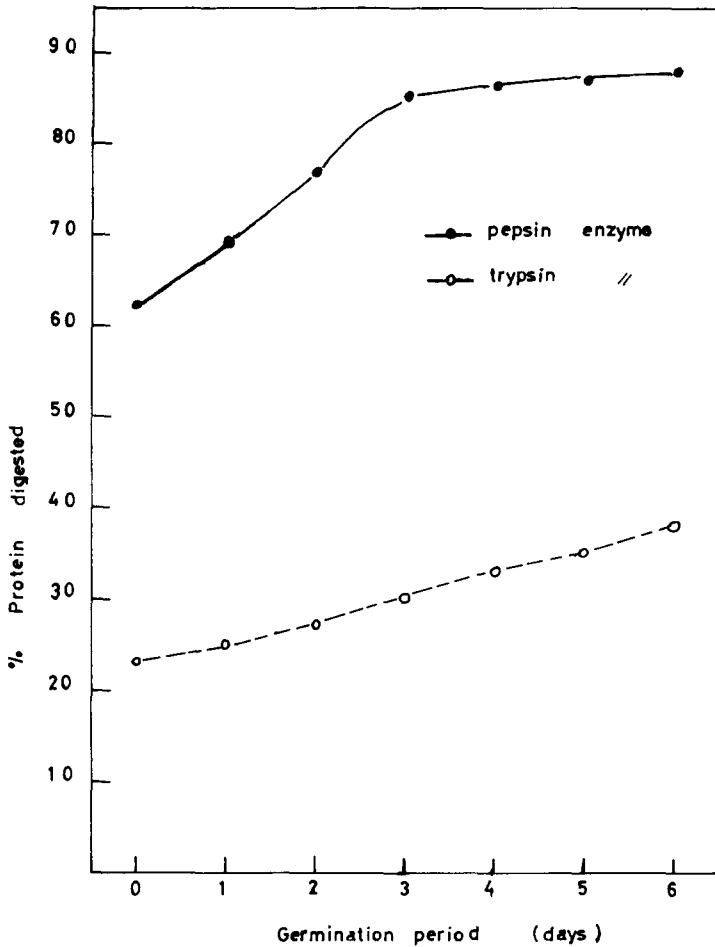
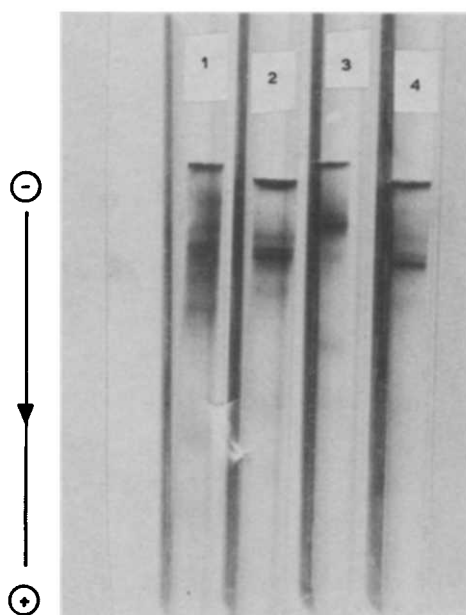


Fig. 1.

trypsin. The low trypsin digestibility could be due to the presence of trypsin inhibitor in soybean (Table 6). However, the germination process slightly reduced the inhibitor content and consequently the digestibility was not much improved. Steaming after germination may be an advantage by destroying the inhibitor and increasing the digestibility by trypsin.

#### *PAGE pattern*

The protein pattern of ungerminated and germinated soybean (Fig. 2) indicated limited variation in the protein pattern when compared with control up to 2 days of germination. There was only a slight decrease in the concentration of each fraction due to partial dissociation but the number of bands was the same. As the germination time increased to 4 and 6 days



**Fig. 2.** Polyacrylamide gel electrophoresis of soybean proteins: 1 = Control; 2 = 2 days after germination; 3 = 4 days after germination; 4 = 6 days after germination.

there were marked changes in the pattern. The two fast moving bands were very feint in colour, indicating their utilization during germination of the seeds. Also the major band of high molecular weight showed marked dissociation. Chen & Thacker (1978) and Suberbie *et al.* (1981) have reported an increase in the activity of proteolytic enzymes during soybean as well as pea seed germination.

The final conclusion drawn from the PAGE pattern is that there is utilization of albumins and the low molecular weight protein fraction at the beginning of the germination process. However, the globulin storage proteins and proteins of high molecular weight underwent hydrolysis in the latter stage of germination.

#### *Functional properties*

The solubilities of germinated soybean flours are shown in Fig. 3. Solubility was increased due to germination and it also increased with germination time. Solubility in 0.02M NaOH solution was higher than that in water and 5% NaCl solution. The solubilities after 6 days of germination were 74.0%, 78.8% and 91.6% in water, 5% NaCl and 0.02M NaOH solutions, respectively. Suberbie *et al.* (1981) found an increase in the solubility of soybean protein as the time of germination increased. As the germination

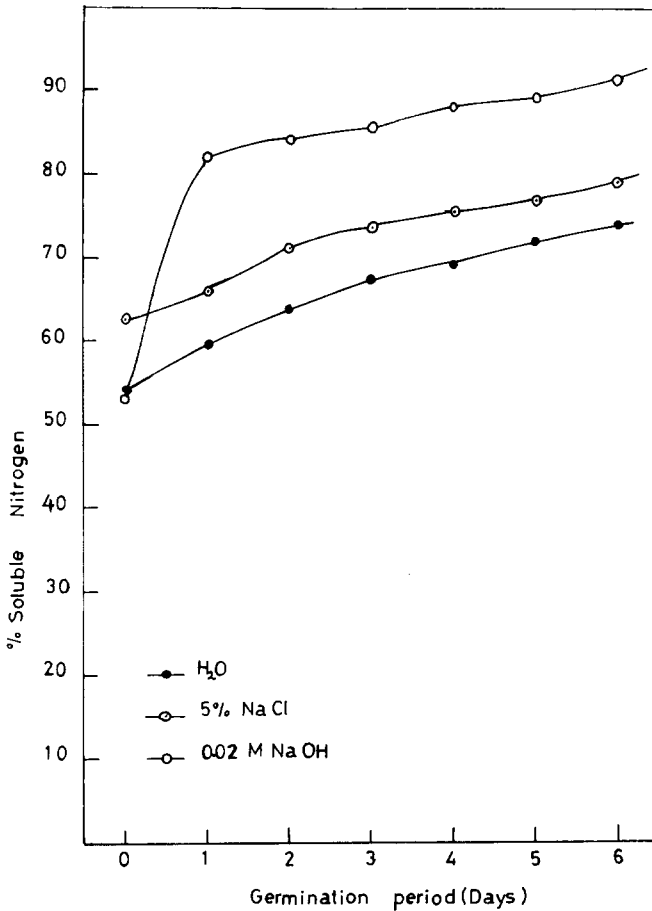


Fig. 3.

process proceeds, protein molecules undergo more hydrolysis by proteolytic enzymes, thereby improving protein solubility.

The water absorption capacity values (Fig. 4) showed a marked increase as the time of germination increased. Fat absorption, emulsifying capacity (EC) and foaming properties (Fig. 5) showed the same trend. The above-mentioned values were much better in the case of germinated flour samples when compared with the control. After 6 days of germination the rates of increase were 39.4%, 14.2%, 39.5%, 35.4% and 31.7% for fat and water absorption capacity, EC, foam capacity and foaming stability, respectively. Kinsella (1976) has reported that the EC of a protein depends upon the solubilized protein in the solution. Since the nitrogen solubility of the 6-day germinated flour was higher than the control (36.3% increase, Fig. 3), the EC of this sample could also be expected to be higher. However, there was



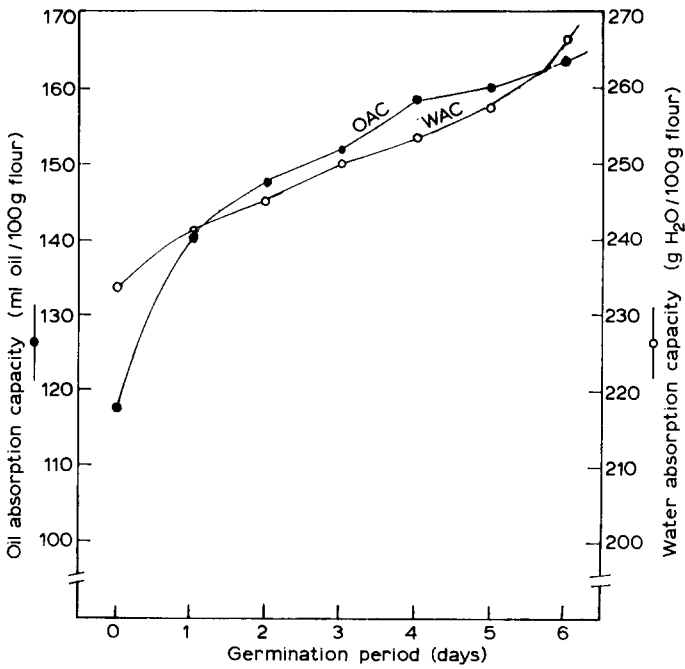


Fig. 4. Effect of germination of soybean for different periods on the water and oil absorption capacity of the defatted flour.

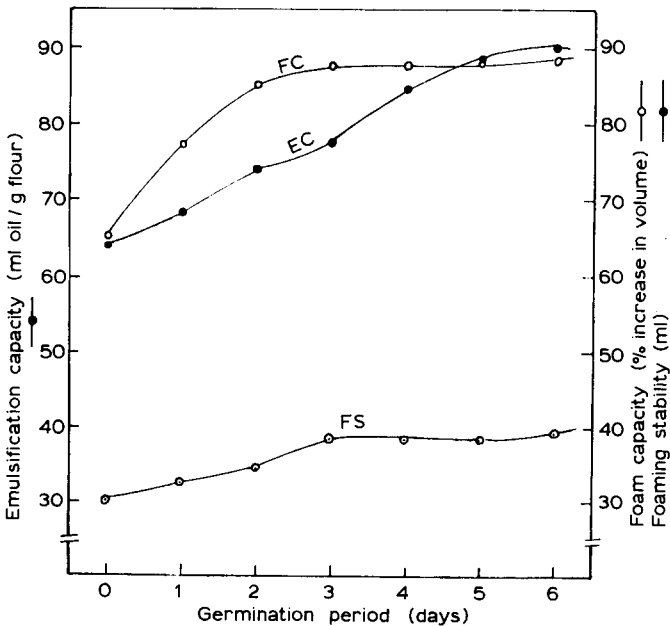


Fig. 5. Effect of germination of soybean for different periods on the emulsification and foaming capacity of the defatted flour.

not much change either in foam capacity or foam stability after 3 days of germination.

In general, germination of soybean improved the protein solubility pattern and the functional properties of the flour proteins.

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