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Some Chemical and Biological Properties of a Protein Concentrate from Sunflower Seeds*

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A protein concentrate from sunflower seeds containing about 55 % of protein has been prepared by conventional methods and analyzed for the amino acid content. In contrast to some earlier statements this preparation seems to contain a weak trypsin inhibitor activity demonstrable in rat experiments. The protein quality will be improved by removing substances soluble in water at 100°C. This antinutritional material which is responsible for significant lower values for growth and PER ** in the raw sunflower protein concentrate has so far not been investigated.

An improvement of the supplementary mixture at present produced in Ethiopia by an Ethio-Swedish nutrition project has been suggested by incorporation of a sunflower protein concentrate. This will increase the protein content of that blend to more than 20 % which is the level now being recommended by the international organizations in this field. Further improvements in quality are possible.

Formulation of low-cost blends of leguminous and cereal foods in which various compounds compensate each other's amino acid deficiencies is one of several useful approaches to combating the malnutrition which is characteristic of the emerging nations.[1,2] Similar mixtures have also been formulated in connection with an Ethio-Swedish nutrition project (CNU).[3] The content of crude protein (total nitrogen $\times 6.25$) in those mixtures hardly exceeds 14 %.

Recently it has been stated that diets for preschool children in developing countries should contain not less than 18 % high quality protein on a dry weight basis.[4] Simultaneously WHO has developed a protein-rich diet (CSM) for the same age group.[5] It has also been urgent for CNU to increase the protein content of the present low-cost supplementary food (SM8 B) from 14 up to about 20 %. Enrichment with a protein concentrate from oil seeds seemed to be a promising alternative since there is a considerable cultivation of such crops in Ethiopia.[6]

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** Protein Efficient Ratio.

Table 1. Proximate analysis of protein concentrates from sunflower seed.

Protein [a]	Ether extractable fraction	Crude fiber	Moisture	N-free extract
%	%	%	%	%
48–55	1–5	2–4	7–9	31–38

[a] Determined by Kjeldahl analysis. Nitrogen to protein conversion factor 6.25.

Many oil seeds contain toxic factors which complicate the use of such protein concentrates for human consumption. The sunflower is one of the few species whose seeds seem to be comparatively free from antinutritional factors.[7,8] The seed production is sufficient to cover immediate needs.

EXPERIMENTAL

Materials. Flours from tef (*Eragrostis abyssinica* Trotter), dehulled (split) peas (*Pisum sativum* L.) and chick peas (*Cicer arietinum* L.) were shipped from Ethiopia. Sunflower seeds were bought from a local shop for Weibull Inc. which imports these seeds mainly from Eastern Europe.

Amino acid analysis. Hydrolysis was carried out for 20 and 70 h, respectively, on samples containing about 200 mg of protein. Tubes containing 1 liter of 6 N HCl were brought to boiling by evacuation and sealed during boiling. The acid was rapidly removed by use of a rotary evaporator. Cystine and methionine were determined on oxidized samples using the method described by Moore.[9] The amino acids were determined according to Spackman *et al.*[10]

Tryptophan was determined on alkaline hydrolysates performed as described by Lunven.[11] The amino acid was analyzed by a microbiological procedure using *L. plantarum* (8014).

Biological evaluation. Evaluation of protein quality in foods was carried out according to Müller.[12] For each diet 10 rats of the Sprague-Dawley race were used. The animals were obtained from a local breeding farm at Norrviken near Stockholm.

Table 2. Amino acid content of sunflower protein concentrate. All figures based on dry weight. essential amino acid

Foods		Iso-leucine	Leucine	Lysine	Methi-onine	Cystine	Phenyl-alanine	Tyrosine	Thre-onine	Trypto-phan
Sunflower										
Concentrate I	A	302	434	246	161	120	311	179	256	90.2
Concentrate II	A	263	390	199	114	101	293	156	225	—
Concentrate I	B	2630	3790	2140	1400	1050	2710	1560	2230	787
Concentrate II	B	3740	5540	2830	1630	1440	4160	2220	3190	—
Concentrate I	C	121	175	98.8	64.7	48.2	125	71.9	103	36.3
Concentrate II	C	129	191	97.5	56.0	49.6	143	76.4	110	—
Concentrate I	D	2490								
Concentrate II	D	2040								

Preparation of protein concentrate. The husks were removed by means of a seed refiner from Termenius Inc., Halmstad, equipped with a scourer. This stage was facilitated by a low moisture content in the seeds. At the procedure some flour was formed from the dehusked seeds.

The whole kernels were extracted over night with ethyl ether. After drying it was possible to remove a thin yellow membrane from the shrunken kernels by means of blowing. Flakes from the kernels were then extracted three times with ethyl ether at room temperature in a counter current system. The dried material was ground into a fine flour by passing it through a large coffee bean grinder. This preparation is called (I).

The coagulated material was obtained by suspending the fine flour in 10 volumes of water and heating by circulating steam through a steel vessel. The coagulated material was removed by centrifugation and dried with ethanol and ethyl ether. This preparation is called (II). Up to now about 100 kg of I has been prepared. Some of it has been sent down to Ethiopia for acceptability tests.

RESULTS AND DISCUSSION

Table 1 shows the composition of some protein concentrates prepared from different batches of sunflower seed. The range of values reflects the well-known inverse relationship found between the content of oil and seed coat, oil and protein, and oil and cellulose.[13,14]

Table 2 summarizes the amino acid analysis carried out on different sunflower materials. The values are calculated as the proportion of each essential amino acid to the total of essential amino acids (A/E ratio).[15] This facilitates a comparison of the essential amino acid composition with that of egg or human milk. These two materials were recently adopted for reference purposes in preference to the FAO provisional standard.[15] In comparison with egg standard lysine is the first limiting amino acid. It is easy to calculate how much of synthetic lysine must be added to bring the sunflower lysine content up to that of egg. After heat coagulation the amino acid composition did not change much except for a slightly lowered content of lysine.

The character of lysine as the first limiting amino acid has been shown by others.[15,16] With the exception of lysine the amino acid composition of the sunflower protein concentrate is well balanced, rich in both methionine and

A: mg/g total nitrogen. B: mg/100 g of food. C: mg/g of total essential amino acid. D: Total mg /g total nitrogen.

Valine	Arginine	Histi-dine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine	% of protein	Conver-sion factor
376	612	171	301	621	1380	377	301	347	54.5	6.25
301	570	160	249	548	1272	324	249	306	88.8	6.25
3280	5340	1490	2620	5410	12000	3290	2490	3020		
4270	8100	2270	3530	7780	18070	4610	3530	4350		
151										
147										

tryptophan in accordance with previous observations.[16,17] When the sunflower protein concentrate is considered as a component in a protein-rich supplementary mixture for human consumption it must be remembered that the ratio of its essential amino acid content in terms of g per g total nitrogen (E/T ratio) is a little low as in most protein concentrates from oil seeds.[15] Since the total nitrogen fraction in the sunflower protein concentrate is practically identical with the total amino acid nitrogen, the ratio of total essential amino acids to total non-essential amino acids is also low.

Table 3 summarizes the growth and PER values of a series of diets where the single protein source consists of protein concentrates from sunflower seeds. These concentrates were tested either as flour, porridge of flour, heat coagulated flour or porridge of heat coagulated flour. The reasons for choosing these alternatives were the following.

It has been shown that the kernels from sunflower seeds contain a trypsin inhibitor which is active in *in vitro* tests. However, it should not inhibit the intestinal proteolysis of flour from kernels free from fat.[18] From a comparison of the growth and PER values of diets 283 *v.* 286 the following conclusion

Table 3. Weight gain and protein efficiency ratio (PER) of rats fed protein concentrate from sunflower seeds.

Diet No.	Diet	Protein [a] in diet %	Average gain in weight g	PER Mean	PER SD [b]
241.	Porridge of protein concentrate [c]	10.3	40	1.97	0.44
251.	Porridge of protein concentrate [c]	11.5	72	2.38	0.16
261.	Flour of protein concentrate,[c] heat coagulated	11.4	94	2.48	0.23
277.	Porridge of protein concentrate [c]	10.3	30	1.61	0.28
279.	Porridge of protein concentrate [c]	10.1	38	1.87	0.11
286.	Porridge of protein concentrate [c]	10.0	43	2.04	0.13
281.	Porridge of protein concentrate,[c] heat coagulated	10.2	55	2.29	0.21
285.	Porridge of protein concentrate,[c] heat coagulated	10.4	51	2.20	0.24
280.	Porridge of protein concentrate [d]	10.3	58	2.18	0.21
278.	Porridge of protein concentrate,[d] heat coagulated	10.3	70	2.49	0.16
287.	Porridge of protein concentrate,[d] heat coagulated	10.1	59	2.51	0.49
283.	Flour of protein concentrate,[c] experimental time 8 weeks [f]	10.2	43	1.88	0.16
	Casein+methionine (reference diet) [e]	10.1	118 (100—128)	4.23 (4.00—4.50)	

[a] Determined by Kjeldahl analysis of diets as fed. Nitrogen to protein conversion factor 6.25.
[b] SD=Standard deviation.
[c] Protein concentrate prepared from sunflower kernels.
[d] Protein concentrate prepared from kernel-flour formed during dehusking.
[e] Average of 8 experiments. The values within parenthesis are the range values.
[f] Values calculated per 3 weeks.

can be drawn. The growth for flour and porridge are the same but the PER value for porridge is probably significantly higher than for flour, $0.05>P>0.02$.[19] The increase in PER corresponds to what could be expected as a result of a heat-inactivation of a weak trypsin inhibitor when the flour is boiled for 10 to 15 min. The soya trypsin inhibitor is inactivated by a similar procedure.[4]

The main reason for the preparation and testing of the heat coagulated protein concentrates from sunflower seeds was as follows. If a protein concentrate is to be used for human consumption it is important that we can remove the presence of other types of antinutritional factors such as toxic glucosides and other types of toxic substances found in some protein-rich material from oil seeds.[7] These are substances of low molecular weight and should be removed by heat coagulation of the proteins in the concentrate.

A further reason is that the sunflower belongs to the Compositae family and it has been found that protein concentrates from two other oil seeds belonging to that family, namely nug (*Guizotia abyssinica* Cass.) and safflower (*Carthamus tinctoria* L.) contain a factor of high molecular weight which provokes diarrhoea in rats eating such concentrates.[20] This factor can be partially inactivated by heat coagulation of the concentrates from these seeds. If a similar factor is present in the protein concentrates from sunflower it must be in very small amounts since loose stools are only very rarely found in rats eating the flour before heat coagulation.

However, the values in Table 4 indicate that there is a definite improvement in quality reflected in significant increases in growth as well as in PER after heat coagulation.

The protein concentration in the heat coagulated material is more than 70 % and the recovery of protein after this process is about 80 %. The dark colored solution left after heat coagulation has not yet been analyzed for antinutritional factors. It might contain some of the glucosides with hemolytic activity which have been isolated from sunflower plants.[21]

The present supplementary food produced by CNU in Ethiopia consists of a balanced mixture of tef and split chick peas with addition of dried skim milk (*cf.* Diet 255, Table 5). Some alternative diets were composed where a sunflower protein concentrate was added to the components of SM8 B to increase the protein concentration of the final mixture to more than 20 %.

Table 4. Statistical evidence for improved protein quality by heat coagulation of protein concentrates from sunflower seeds. Dietary numbers are the same as in Table 3.

Dietary number for compared pairs	Degree of significance for differences in growth values	Degree of significance for difference in PER values
281 v. 286 [a]	$P<0.01$	$P=0.01$
281 v. 279 [a]	$P<0.01$	$P<0.01$
285 v. 279 [a]	$P<0.01$	$P<0.01$
278 v. 280 [b]	$0.05>P>0.02$	$P<0.01$

[a] Indicates a protein concentrate prepared from whole kernels.
[b] Indicates a protein concentrate prepared from kernel-flour formed during dehusking.

Table 5. Weight gain and protein efficiency ratio (PER) of cereal-legume blends supplemented with a protein concentrate from sunflower seed. For comparison values obtained with CSM,[a] Diet No. 269, are included, as well as values for SM8-B, Diet 255.

Diet No.	Dietary Composition						Protein[d] in diet %	Average gain in weight g	PER		Protein in original mixture %	Fed in form of
	Cereal %		Pulses %		DSM[b] %	SPC[c] %			Mean	SD[e]		
276	Tef	(47.3)	Split chick peas	(30.5)	11.1	11.1	9.9	67	2.71	0.14	25.0	Porridge
274	Tef	(46.8)	Split chick peas	(40.7)	0	12.5	10.1	63	2.88	0.32	21.6	Porridge
273	Tef	(22.0)	Split chick peas	(55.0)	0	23.0	10.0	59	2.62	0.40	36.2	Porridge
290	Tef	(50.0)	Split peas	(27.8)	5.5	16.7	10.0	77	2.99	0.13	22.9	Flour
255	Tef	(61.1)	Split chick peas	(27.8)	11.1	0	10.1	72.6	3.07	0.36	14.4	Porridge
269	Corn	(70.0)	Defatted soya	(25.0)	5.0	0	10.1	73.7	3.23	0.19	18.7	Porridge

[a] CSM was generously supplied by Dr. Lester Teply, UNICEF.
[b] DMS=Dried skim milk.
[c] SPC=Sunflower protein concentrate. In diets 273 and 274 a heat coagulated concentrate was used.
[d] Determined by Kjeldahl analysis of diets as fed. Nitrogen to protein conversion factor 6.25.
[e] SD=Standard deviation.

Since the decrease in the world supply of skim milk powder is expected to continue this material was excluded in some of the alternatives.

Table 5 summarizes the growth and PER values of the proposed alternatives to the present SM8 B with its protein content of about 14 % raised to more than 20 %. It seems clear that of the four proposed alternatives with diet numbers 273, 274, 276, and 290 the last one appears to be the best. It did give about the same weight increase and PER value as the present SM8 B (Diet 255) with the difference that the Diet 290 had a protein content of 22.9 %.

When comparing the two last discussed diets with the WHO diet CSM (269 in Table 5) it is quite evident that all three give about the same gain in weight. However, the PER value for CSM is significantly higher than that for diet 290, with $P<0.01$, while there was no significant difference between the PER values for CMS and SM8 B, $0.3>P>0.2$.

The composition of SM8 B and of the diet 290 are similar in some respects. The percentage of leguminous products are the same but of different types. The reason for choosing split peas in diet 290 is that previous experience has shown that the chick pea variety cultivated in Ethiopia seems to be of inferior nutritional values.[22] The question is what else can be done to improve the quality of Diet No. 290 in order to obtain a product of at least the same quality as CSM. One definite improvement would be to increase the content of DMS to the same amount as in SM8 B, 11.1 %. Another improvement could be to decrease the content of split peas and instead add lysine as free amino acid.

The effect of such a measure is illustrated by the values given in Table 6. Lysine has been added in amounts to bring the content of the dietary proteins up to that of the egg standard. Comparing Diet 283 (Table 3) with Diet 288 the effect of adding lysine results in significant increases in both weight gain and PER value, P in both cases <0.01. In fact the PER value of the lysine-enriched sunflower protein concentrate is the same as found for SM8 B. A comparison of the lysine enriched mixture of tef and sunflower protein concentrate (Diet 289, Table 6) and the CSM blend (Diet 269, Table 5) shows no significant difference in weight gain, $P=0.9$, but probably a significant higher PER value for CSM, $0.05>P>0.02$.

Table 6. Weight gain and protein efficiency ratio (PER) of a sunflower protein concentrate and a blend of protein concentrate and tef supplemented with lysine.

Diet No.	Dietary Composition			Protein [b] in diet %	Average gain in weight g	PER Mean	PER SD [c]	Protein in original mixture %	Fed in form of
	Tef %	SPC [a] %	Lysine %						
288	0	100	0.78	9.8	71	3.01	0.26	54.3	Flour
289	75	25	0.58	10.1	73	2.83	0.49	21.8	Flour

[a] SPC=Sunflower protein concentrate.
[b] Determined by Kjeldahl analysis of diets as fed. Nitrogen to protein conversion factor 6.25.
[c] SD=Standard deviation.

REFERENCES

1. Bressani, R. L. G., Elias, A. A. and Scrimshaw, N. S. *J. Nutr.* **74** (1961) 201.
2. Sirimil, K., Soliman, A. M., Van Lov, A. T. and King, K. W. *J. Nutr.* **8** (1965) 415.
3. *Children's Nutrition Unit, An Ethio-Swedish Project in the Field of Health, Addis Ababa, Ethiopia*, Almquist & Wiksell, Uppsala 1967.
4. Bressani, R. *Proc. Intern. Conf. Soybean Protein Foods*, ARS-71–35, (May 1967) 28. Northern Utiliz. Res. Devel. Div., U.S.D.A., Peoria, Ill. 61604.
5. *WHO/UNICEF Note on CSM.* WHO/Nutr./67. 127.
6. *Statistical Abstract*, (1964), Central Statistical Office, Addis Ababa.
7. Altschul, A. M. *World Protein Resources, Advan. Chem. Ser.* **57** (1966) 52; Chem. Soc. Washington, D. C.
8. Mauron, J. *VIIth Intern. Cong. Nutr. Abstr. Pap.* (1966) 79.
9. Moore, S. *J. Biol. Chem.* **238** (1963) 235.
10. Spackman, D. H., Stein, W. H. and Moore, S. *Anal. Chem.* **30** (1958) 1190.
11. Lunven, P. *Qualit. Plant. Mater. Vegetabiles* **10** (1963) 276.
12. Müller, R. *Z. Tierphysiol. Tierernähr. Futtermittelk.* **19** (1964) 257.
13. Kopeikovskii, V. M. and Garbuzova, G. V. *Chem. Abstr.* **62** (1964) 6801 D.
14. Khokhlenko, A. F. *Chem. Abstr.* **61** (1964) 4700 H.
15. FAO/WHO Expert group, WHO Techn. Rep. Ser. No. 301 (1965) 34.
16. Yecsai, G. *Chem. Abstr.* **62** (1964) 4532 D.
17. Nehring, K., Hoffman, B. and Nerge, I. *Arch. Tierernähr.* **15** (1965) 195.
18. Bielorai, R. and Bondi, A. *J. Sci. Food Agr.* **14** (1963) 124.
19. Mainland, D. *Element. Med. Stat.* W. B. Saunters Comp., London 1964.
20. Ågren, G. *Unpublished results.*
21. Kaprzyk, Z., Fonberg, M., Polus, E. and Raczynski, G. J. *Chem. Abstr.* **63** (1965) 11249 h.
22. Ågren, G. *Unpublished results.*

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