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INVESTIGATION OF SOME FUNCTIONAL AND NUTRITIVE PROPERTIES OF CEREAL GERM PROTEINS[1]

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Abstract

Proteins, protein concentrates and isolates were prepared from wheat, rye, barley, rice and maize germs, after defatting with hexane or supercritical carbon dioxide, by alkaline extraction (NaOH, pH=10) and acid precipitation (0.1 mol/l HCl). The chemical composition, amino acid composition and functional properties (emulsifying properties, foam activity and stability, water and oil absorption, solubility) were determined. The digestibility and biological value of protein preparations were also calculated.

Protein isolates had a high protein content (75.8 - 94.3%). The oil content was low, except in maize germ protein isolates, obtained after defatting with supercritical carbon dioxide. High digestibility (83 - 90%) and moderate biological value (63 - 75 FAO/WHO index) was characteristic of all isolates. Methionine and cysteine were the limiting amino acids.

Maize germ protein isolates showed the best functional properties, being comparable with those of soy protein isolates. Other germ protein isolates also had acceptable properties. No significant differences were observed between the functional properties of isolates prepared after defatting with hexane or supercritical carbon dioxide, except the foaming properties. The latter were adversely affected by supercritical fluid extraction.

Keywords: cereal germs, cereal germ protein, protein isolate, functional properties, nutritive value.

Introduction

The advantageous nutritive characteristics of cereal germs are well known. It is generally accepted that cereal germs are good sources of proteins, edible oils, vitamins and minerals [1], although it must also be noted that some germs contain antinutritive factors (e.g. natural enzyme inhibitors, hemagglutinins, etc.).

Cereal germs have long been used in feeds. In view of their large supply and high content of nutrients together with an adequate amount of fiber, the value of cereal germs can be greatly raised if their uses in foods

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can be expanded. It seems that the main field of such use of germs may be production of bread, biscuits, cakes, pasta products. In addition to bakery and pasta products germs may be ingredients of puddings, chocolates, infant foods and other special foods.

Of all cereal germs wheat, maize and rice germs have received the most attention. Experiments with use of wheat germ in bread started in the 1930s. As a result of these efforts, it was shown that acceptable bread with up to 12% wheat germ content can be prepared with the addition of surfactants such as sodium stearoyl lactylate, polysorbate and ethoxylated monoglycerides. The flavour and texture of 12% wheat germ bread was also found to be acceptable to consumers [1]. Rice germ has also been proposed to be used in different food products [2 – 5]. However, few rice germ foods have been commercialized. One major obstacle to the use of rice germ as a food ingredient is the rapid development of off-flavours as a result of oil rancidity [4]. More recent publications have reported about good possibilities of improving the storage technology of whole or broken rice germs. Breads and cakes were prepared from wheat flour fortified with 11% rice germ and granola containing 12% rice germ. Sensory panels reacted favourably to the order of rice germ as well as the taster of the germ fortified bread, cake and granola. Recently maize germ has received the most attention in research and development to explore its potential food uses.

The problems connected with the use of whole germs stimulated production and application of protein preparations (concentrates and isolates) as food ingredients. Concentrates and isolates have a number of advantages due to the following facts [6]:

- the protein-containing raw material may be unsuited for direct use in foods,
- the raw material may contain toxic substances,
- the raw material may contain antinutritive factors,
- the protein content of the majority of raw materials is not high enough for purposes of enrichment,
- in many cases protein-rich raw materials are by-products of other food industries,
- removal of proteins from waste-water could reduce environmental pollution,
- the isolates and concentrates make possible a more versatile use as food ingredients,
- the transport and storage of isolates and concentrates is much simpler and cheaper than that of the original raw materials.

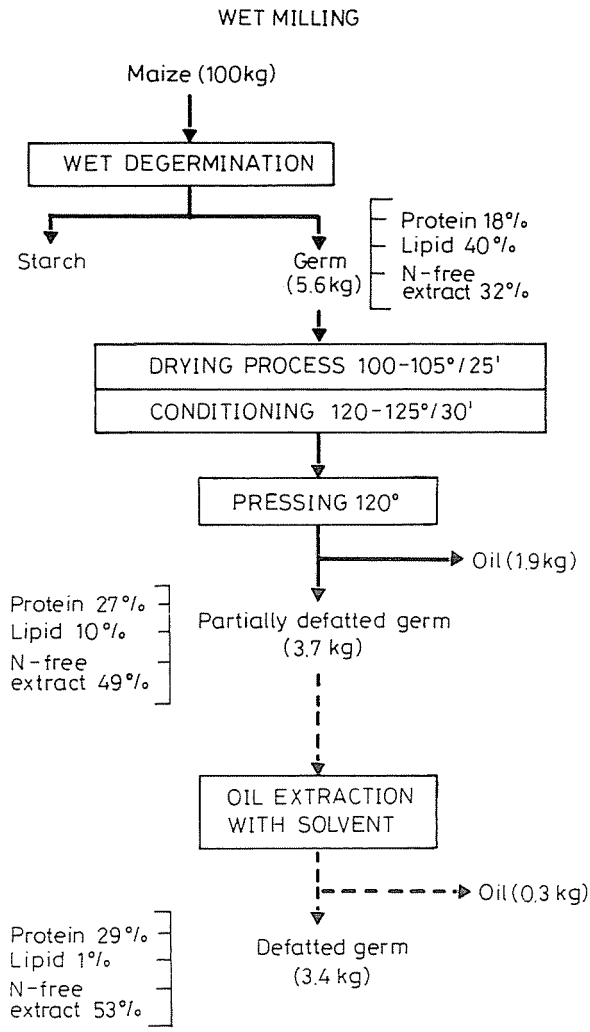

Fig. 1. Scheme of a wet milling process of maize

One of the most promising raw materials for the production of isolates and concentrates is maize germ [7, 8]. Maize germ is generally obtained by wet milling in starch industry and by dry milling in the flour industry. Both the above preparations are used for oil production and the defatted germ cakes are the raw materials of production of concentrates and isolates. The two typical processes of defatted maize germ production are shown in *Fig. 1* and *Fig. 2*.

The aims of research discussed in this paper are as follows:

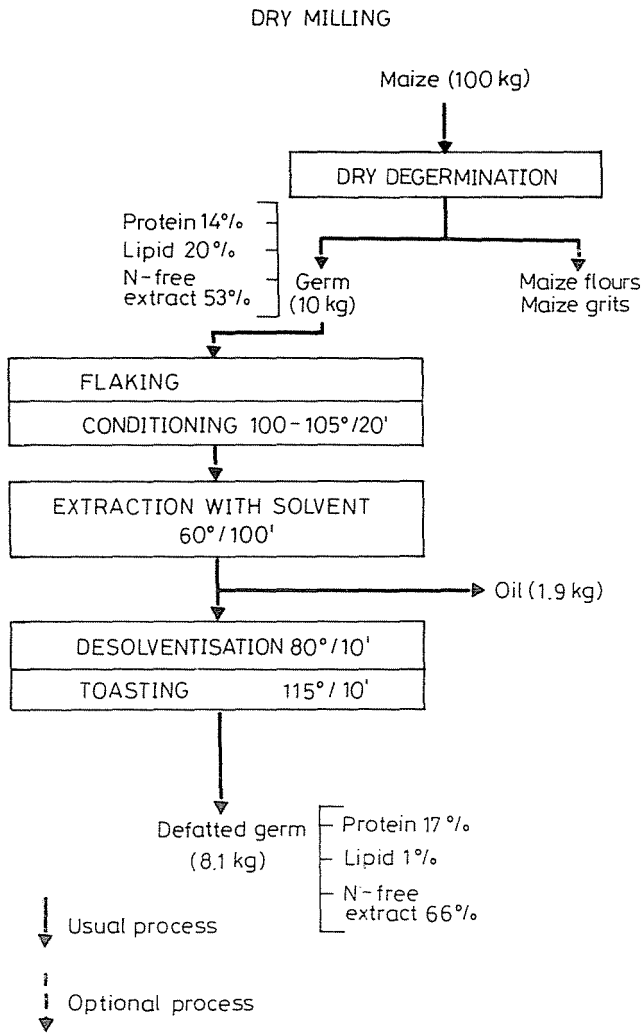

Fig. 2. Scheme of a dry milling process of maize

- to obtain additional data about the chemical composition of cereal germs grown in Hungary,
- to investigate the effect of the oil extraction process on the properties of protein concentrates and isolates,
- to study the functional properties of cereal germ protein preparations.

Materials and Methods

Wheat, rye, barley and rice germs were obtained from milling industry prepared by dry milling process. Maize germ was produced by wet milling process in starch, glucose, high-fructose and spirit producing factories.

Sample Preparation

Protein Meals

Extraction of commercially available germs and seeds for total fat removal was performed at the Technical University of Budapest. Depending on the oil content of the starting material, the fluid (hexane) extraction was performed in 3 – 5 steps in a 3 l solid-fluid extractor equipped with a stirrer, using a meal-to-hexane ratio of 1:4 wt/vol for 30 min at room temperature (20°C). For a supercritical fluid extractor, a 1 l laboratory vessel heatable to 200°C and loadable to 320 bar pressure was available. The parameters for the extraction of the plant materials were as follows: 24 h, 300 bar, 40°C; 60°C.

Protein isolates: The hexane- or supercritical CO_2-extracted protein meals were isolated by alkaline extraction. The protein meal was extracted with NaOH solution (pH 10) then with water for 30 min each in a stirred vessel at 30°C using a solid-to-liquid ratio of 1 : 3. The supernatants were separated by centrifugation (3000 rpm, 10 min). Then, the two solutions were combined. The pH of this solution was adjusted to 4.5 with 0.1 mol/l HCl. The proteins precipitated were centrifuged (3000 rpm, 20 min). The proteins separated were dialyzed and dried by lyophilization.

Analytical Methods

The chemical compositions of the proteins were determined with a TECATOR^R (Sweden) equipment, by using the practical modification of the Hungarian Standard (MSZ-6830/78). The determination of the protein content was performed by means of Kjeldahl's method with a Kjeltec-Lo30 type (N×6.25) analyzer. The at content was measured by extraction with hexane in a SOXTEX-1043 type apparatus. The fiber content was determined on the basis of acidic and alkaline hydrolysis in a FIBERTEC-1020 type apparatus. Moisture and ash were determined by gravimetric methods. The amino acid analysis was performed on an analyzer working on the principle of high-pressure ion exchange chromatography (AMINOCHROM II OE-914, LABORMIM, Hungary). The medium for the hydrolysis was

3 mol/l p-toluene-sulfonic acid. The determination of cysteine required a separate hydrolysis with 6 mol/l HCl.

In Vitro Biological Evaluation

The in vitro biological value was estimated by the Morup-Olesen Index on the basis of the essential amino acid composition [9].
Digestibility was determined by means of the method of SALGÓ et al. [10].

Measurement of Functional Properties

The emulsifying activity index was determined by means of the modified turbidimetric method [11], and the emulsion stability index by means of the conductivity method [12]. Foaming activity and foam stability were determined by measuring the conductivity of the foam immediately after stopping the aeration (foam activity = C_0), and determining the reciprocal rate of change in conductivity $\Delta t/\Delta C$ (time Δt needed for ΔC change of conductivity) (foam stability = $C_0 \times \Delta t/\Delta c$) [8]. Water and oil absorption were measured as the change of sample weight during a 30 min time interval [13]. The solubility profile of protein materials was determined as the protein solubility index using the suitable Sörensen buffer line from pH 1 to 13. The protein content of the solution was measured by the micro-Kjeldahl method (N×6.25).

Statistical Evaluation

The results published are mean values of three parallel measurements; the differences are significant at the $P = 99.5\%$ level.

Results and Discussion

General Observations

In accordance with the results reported in literature [14 – 16] the colour of oils obtained by supercritical fluid extraction proved to be lighter in every case. The protein products did not show substantial differences: all the proteins were light coloured, and after drying, they were odourless and neutral tasting.

Chemical Compositions

Whole Germs

The gross chemical composition of different germs is shown in *Table 1.* As it is seen from the table, rye and wheat germs have relatively higher protein contents. Oppositely, the lipid content is lower than that of maize germs. Ash and fiber contents are also relatively high in all types of germs.

Table 1
Gross chemical composition of cereal germs (dry weight basis) (%)

Type of germ	Protein	Fat	Ash	Fiber
Wheat	25.2	13.1	6.2	4.5
Maize	19.6	32.2	9.9	5.6
Rye	37.2	13.4	5.8	3.9
Barley	21.9	16.2	6.7	4.8
Rice	23.8	25.1	9.2	4.9

Proteins

The compositions of protein meals and isolates are shown in *Table 2.* It was found that the proportions of the constituents of individual protein meals or isolates are practically of the same order of magnitude.

It is impossible to draw general conclusions on the basis of the compositions of protein products obtained by supercritical fluid extraction and by hexane extraction, respectively. In the case of corn germ, the oil contents of the products obtained after supercritical fluid extraction are higher, while in the cases of the other materials hexane is less effective, which indicates that optimization of conditions is essential for all the materials.

Nutritional Values and Amino Acid Compositions of Proteins

The in vitro nutritional values and digestibilities of protein products calculated on the basis of the Morup-Olesen Index are shown in *Table 3.* The index values reflect the differences among the amino acid patterns of these materials. The protein meals and isolates generally have medium nutritional values.

It is particularly interesting that in the cases of all the materials examined, the protein meals obtained after supercritical fluid extraction show

Table 2
Chemical composition of defatted germ meals and protein isolates

Type of germ product	Oil extraction with	Protein %	Oil %	Ash %	Fiber %
Maize germ meal	hexane	29.2	2.3	2.2	8.6
	supercrit CO_2	30.5	4.4	1.9	13.4
Maize germ isolate	hexane	83.4	1.6	3.2	n.d.
	supercrit CO_2	75.8	7.2	4.0	n.d.
Wheat germ meal	hexane	34.1	1.6	5.5	5.4
	supercrit CO_2	35.0	0.1	5.3	3.9
Wheat germ isolate	hexane	90.8	4.3	4.2	n.d.
	supercrit CO_2	94.3	1.4	3.3	n.d.
Rice germ meal	hexane	37.2	3.1	6.9	5.6
	supercrit CO_2	38.5	3.8	5.1	5.1
Rice germ isolate	hexane	91.7	1.8	7.2	n.d.
	supercrit CO_2	90.6	1.2	5.9	n.d.
Rye germ meal	hexane	42.6	1.0	5.9	4.2
	supercrit CO_2	41.7	0.8	6.2	3.9
Rye germ isolate	hexane	93.2	1.2	5.5	n.d.
	supercrit CO_2	92.1	0.9	6.2	n.d.

slightly higher values than the corresponding ones made by extraction with hexane. The explanation of this phenomenon is not readily apparent. Examining the essential amino acid pattern, however, it appears that the cysteine and lysine contents of supercritical meals are somewhat higher. These characteristic differences, however, decrease during the isolation procedures.

Functional Properties of Proteins

For determining possible uses of new types of protein sources it is not enough to determine the chemical parameters and the nutritional values. The applicability of these proteins (e.g., for protein fortification or supplementation, for improvement of consistency, etc.) depends on the functional properties.

Table 3
Amino acid composition, digestibility and biological value of cereal germ protein isolates.
(Fat extracted with hexane)
(g amino acid/100 g protein)

Amino acid	Type of germ				
	Maize	Wheat	Rye	Rice	Barley
LYA	6.2	7.8	7.2	6.4	7.2
HIS	2.7	3.1	2.6	2.9	2.5
ARG	8.8	7.9	8.2	8.3	8.6
ASP	8.9	10.2	9.8	8.9	9.7
SER	4.6	4.1	4.3	4.6	4.2
GLU	15.4	15.3	14.9	13.7	15.6
PRE	4.3	3.9	4.4	4.7	4.8
CYS	1.6	1.2	1.1	1.2	1.1
MET	1.3	1.4	1.2	1.2	1.1
GLY	6.5	6.1	6.0	6.3	5.9
ALA	7.0	5.9	6.1	7.1	6.3
VAL	4.5	4.8	4.2	4.5	5.3
ILE	3.0	3.2	2.9	2.8	2.7
LEU	7.5	7.2	6.9	7.4	7.0
TYR	2.8	2.7	2.9	3.2	2.0
PHE	3.5	4.0	4.1	4.5	3.8
TRP	0.9	1.1	1.3	1.4	1.0
THR	4.2	5.0	3.9	4.1	3.9
Digestibility %	83.2	86.5	89.1	90.2	85.1
FAO/WHO 1973 index	75	74	67	70	63
Limiting A	ILE	MET+CYS	MET+CYS	MET+CYS	MET+CYS

Emulsifying Properties

The values of the emulsifying properties of the materials examined are summarized in *Fig. 3*. The emulsion activity values are nearly the same. Maize germ isolates and wheat germ meals show the most prominent values. As far as the stability of the emulsion formed is concerned, the picture is more variable. The stability of corn germ meals is low, and in turn, the supercritical wheat germ isolates as well as both corn germ isolates show high values.

It is impossible to draw a general conclusion on the basis of the values measured for the emulsifying properties of protein products of supercritical CO_2 and hexane extraction. It is also impossible to explain the extremely high emulsion stability of corn germ isolates obtained after supercritical

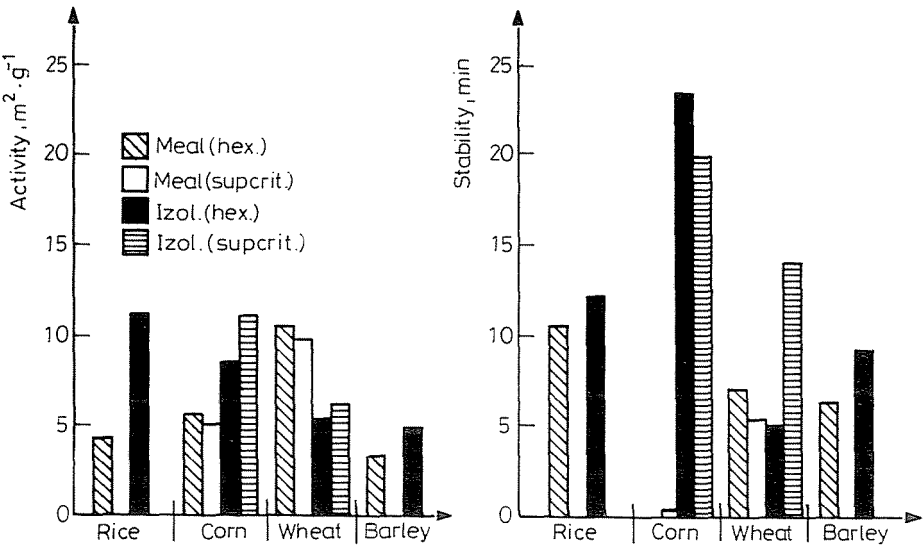

Fig. 3. Emulsion activity and stability of different cereal germ protein preparations

extraction. In earlier hypotheses [13], however, it seems likely that the oil content of the samples does not affect the emulsifying properties negatively.

Foam Activity and Stability

The foam formation properties can be followed in *Fig. 4*. Maize germ isolates show the best activity, but the foam formation of almost all of the materials is acceptable. Stability values are characteristic of the structure of the foam formed, and it is possible to draw similar conclusions as previously, maize germ ilotates made with hexane extraction and all the wheat germ preparations have the highest foam stability values. The latter is extremely interesting, because this strong foam structure develops during foam formation of smaller volume.

By comparing the foam properties of the supercritical fluid and the hexane-defatted preparations, it is possible to draw more general conclusions than before: supercritical fluid extraction adversely affects foam formation and foam stability. This phenomenon may be connected with the change of oil content of samples, but this statement must be verified by further experiments.

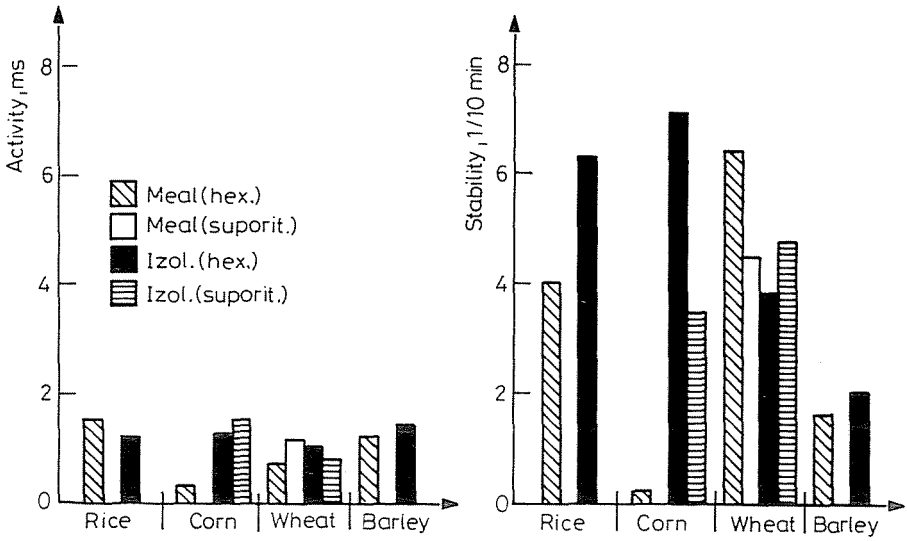

Fig. 4. Foam activity and stability of different cereal germ protein preparations

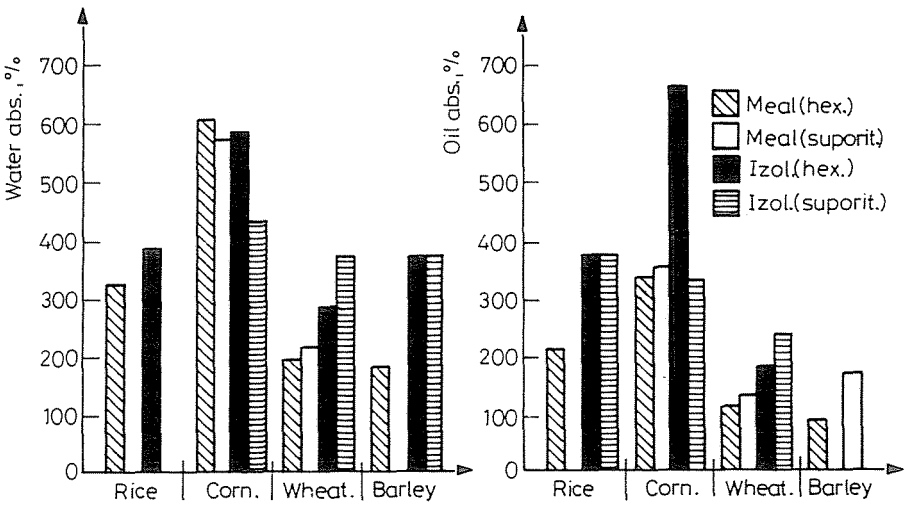

Fig. 5. Water and oil absorption of different cereal germ protein preparations

Water and Oil Absorption

The values of these parameters are shown in *Fig. 5.* Both absorption values range around 200 – 300%. Maize germ preparations show extreme values of

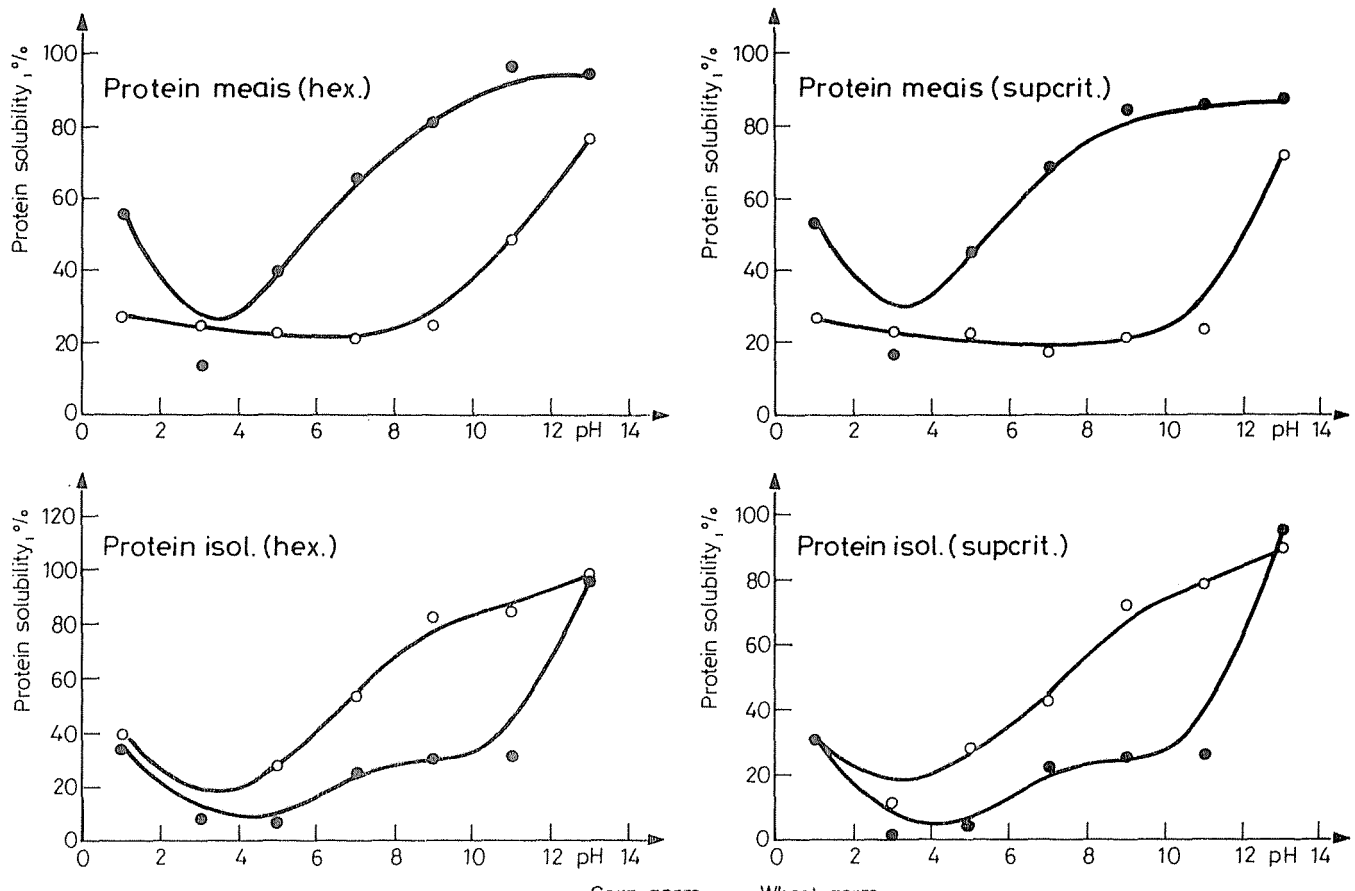

Fig. 6. Solubility of different cereal germ protein preparations

water binding, maize germ isolate obtained by the hexane procedure shows the highest oil binding. Comparing the results of the two types of products no uniform and significant differences could be observed. The absorption parameters are more closely related to the oil contents of the samples.

Protein Solubility

Figure 6 shows the solubility of proteins as a function of the pH. The minima of the curves are characteristic. It is worth noting that the protein solubility values are low at the same pH values in the case of isolates. The pH values of the isoelectric points shift depending on the way of the isolate production. This is particularly valid for maize germ preparations. These phenomena can be explained by the protein denaturation occurring during isolate formation. The effect of supercritical fluid extraction on the solubility of the individual proteins is not significant.

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