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## IMPROVING OF FUNCTIONAL PROPERTIES OF OILSEED AND LEGUME PROTEINS BY STRUCTURAL CHANGES

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The structure modification of the main proteins from oilseeds and legumes by means of alkali- or acid-denaturation and succinylation, respectively, and its influence on the protein functionality is discussed. This kind of modification enables to improve the functional properties with minimal energy input.

Oilseeds and legume seeds contain two types of storage proteins as a main protein fractions. This are the so-called 11 S and 7 S globulins — or legumin- and vicilin-like proteins — with molecular weights of 300 000–400 000 and 150 000–190 000 g/mol, respectively [1]. Both types represent oligomeric proteins with a very similar arrangements of their subunits. Thus, the 6 subunits of the 11 S globulins are arranged in a trigonal antiprism with the point group symmetry  $32$  (Fig. 1) [3, 14]. This is valid for a great number of proteins from different botanical families [4]. There is not only a high structural similarity in the quaternary structure but in the secondary structure, too [11, 19]. Both types of proteins belong to the group of proteins rich in  $\beta$ -sheet structure and poor in  $\alpha$ -helix conformation.

Owing to these structural similarity we can expect certain similarities in those functional properties which are related to the spatial structure. On the other side, there must be specific functional differences in the properties which are determined by the distribution of surface charge and hydrophobic surface regions.

Let us discuss the influence of structural changes of the proteins on functional properties and look for three main possibilities to change the spatial structure:

- the heat-induced denaturation,
- the denaturation by changes of the pH,



Fig. 1. Quarternary structure model of the 11 S globulin from sunflower seed, according to [2]

— the chemical modification by succinylation.

According to the main topic of this symposium we will omit the energy-consuming heat-denaturation processes. Both the denaturation by pH-changes and the succinylation can be performed under mild conditions without a high energy input.

Since the protein structure is stabilized mainly by noncovalent forces and hydrogen bonds, ionic and hydrophobic interactions, we can destabilize it by disturbing the latter ones. An increase of the positive or negative charge results in a disturbance of the charge equilibrium of the native protein and in a partial or extensive unfolding of the structure. It has been found that a distinct pH-range exists in the acidic and basic region, at which a non-reversible unfolding takes place. It is necessary to carry out this denaturation under such conditions which avoid a strong aggregation of the denatured proteins. In this way we can improve the water and fat absorption, the emulsifying properties and the behaviour of plant proteins in meat model systems (Fig. 1-4) [8, 13, 16, 17]. Examples are shown for protein isolates from sunflower seed, faba bean and soybean.

Contrary to the acid- or alkali-induced denaturation the acylation allows to change the properties of the proteins in a more differentiate manner by introducing acyl groups. Succinylation of the 11 S globulins from sunflower and

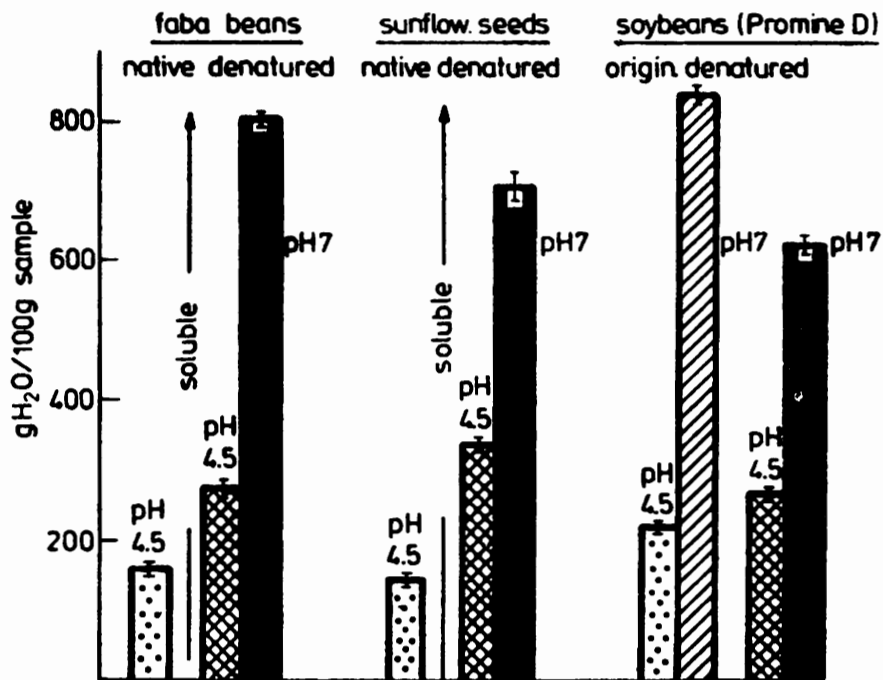


Fig. 2. Water absorption capacity of protein isolates from faba beans, sunflower seeds and soybeans before and after denaturation by acid treatment, according to [8, 9]

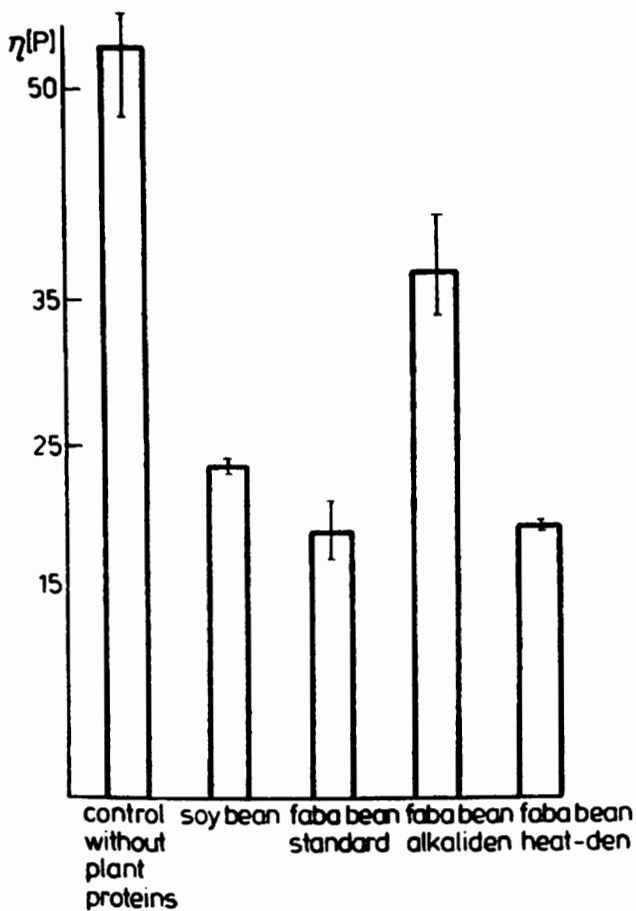


Fig. 3. Apparent viscosity of a meat model system containing 20% of a plant protein; comparison of the influence of a commercial soybean protein (Promine R), a standard faba bean protein isolate, and alkali- and heat-denatured faba bean protein isolates, according to [10]

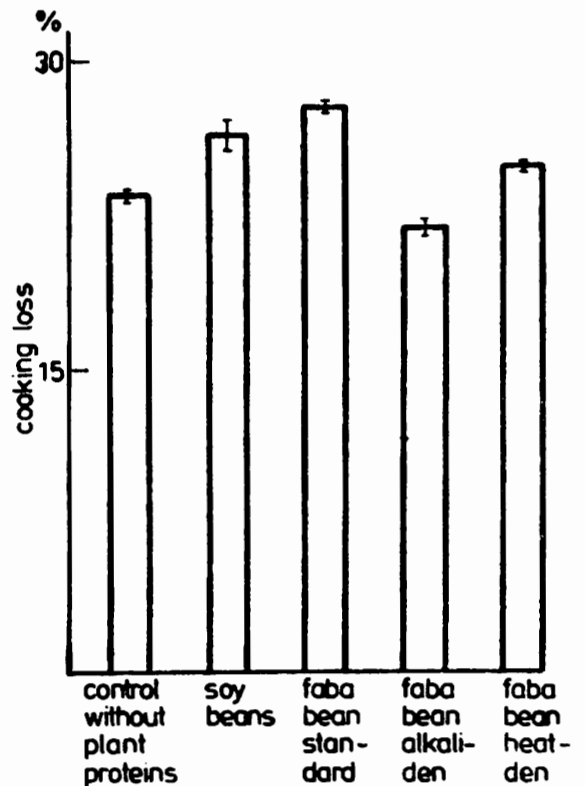


Fig. 4. Cooking loss of a meat model system containing 20% of a plant protein; comparison of the influence of a commercial soybean protein (Promine R), a standard faba protein isolate, and alkali- and heat-denatured faba bean protein isolates, according to [10]

rapeseeds [10, 15] as well as from peanuts [18] with increasing amounts of reagent results in step by step dissociation of the proteins (Fig. 5). After blocking 60-70% of the reactive amino groups the final step of dissociation of the

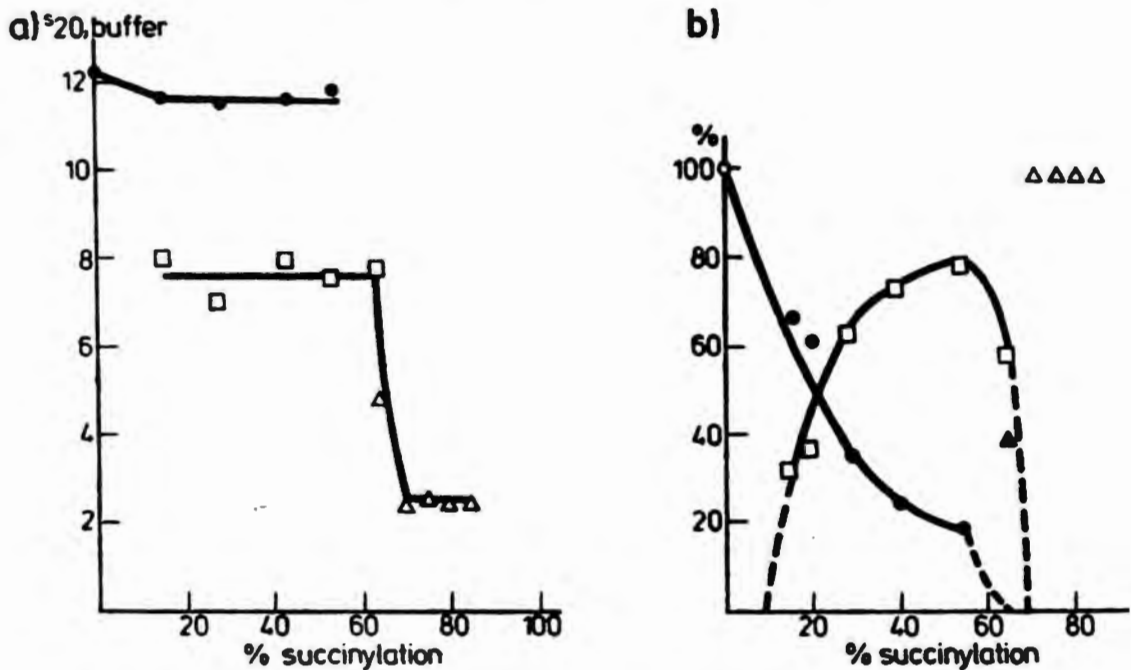


Fig. 5. Dependence of sedimentation coefficients (a) and percentage of components of dissociation (b) of the 11 S globulin from sunflower seed (helianthinin) on the degree of succinylation ●, 12 S, □ 7 S, ▲ 5 S, △ 2.5 S, according to [11]

sunflower globulin has been attained and a 2.5 S component appears [7, 15]. This degree of succinylation is a critical one for all studied 11 S proteins [10, 15, 18] as well as for the 7 S protein from vicia faba [2]. It represents a level of modification at which a sudden unfolding of the proteins takes place, a process which can be seen from the drastic change of the intrinsic viscosity and the spectroscopic properties (Fig. 6, 7).

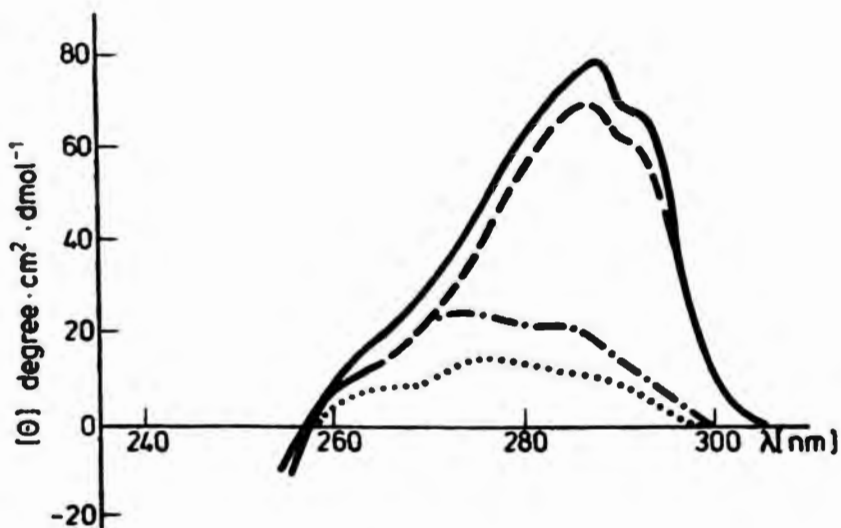


Fig. 6. Near ultraviolet CD spectra of the 11 S globulin from sunflower seed at various degrees of succinylation (— 0%, --- 32%, - · - 68%, ... 83%, according to [11])

Let us have a look at the change of some functional properties which depend on the degree of succinylation.

1. The emulsifying properties of faba bean protein isolates increase continuously with the degree of succinylation (Fig. 8) [6].

Fig. 7. Dependence of the intrinsic viscosity ( $\eta$ ) and the mean residue ellipticity at 280  $\mu\text{m}$  ( $\Theta$ ) taken from the CD spectra of the 11 S globulin from rapeseed on the degree of succinylation, according to [12]

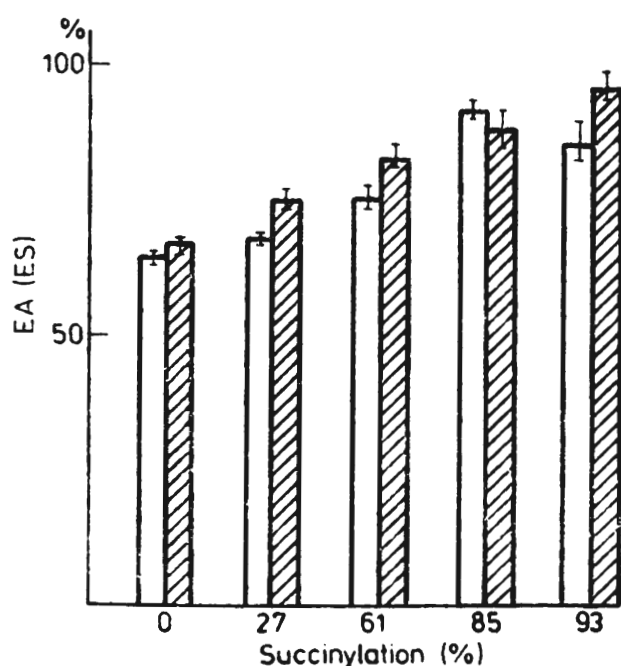
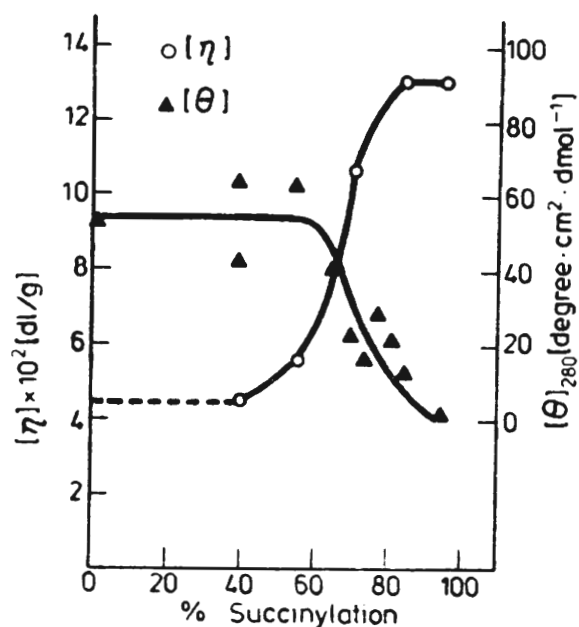


Fig. 8. Emulsifying activity (EA — □) and emulsion stability (ES — ▨) of faba bean protein isolates at various degrees of succinylation, according to [16]

2. The water absorption of sunflower protein isolate increases drastically at a succinylation degree above the critical one (Fig. 9) [9].

3. The foam capacity of faba bean isolates reaches their highest value at a moderate degree of succinylation only after heating. At a high level of modification, i.e. above the critical one, the maximum foam capacity develops without heating (Fig. 10) [12]. That means, that the unfolding of the protein structure by strong succinylation allows to avoid additional heat input for an optimum foaming.

4. The rheological properties of concentrated solutions or dispersions of succinylated faba bean protein isolates depend highly on the degree of modification [5]. The flow curves show a transition at the critical level of succinylation (Fig. 11).

The dispersions of highly succinylated proteins reveal structural viscosity and high yield values at room temperature and tend to jellify without heating. We can see a transition of the flow curves of moderately modified proteins at a temperature of about 65°C. This transition lacks in the exhaustively modified

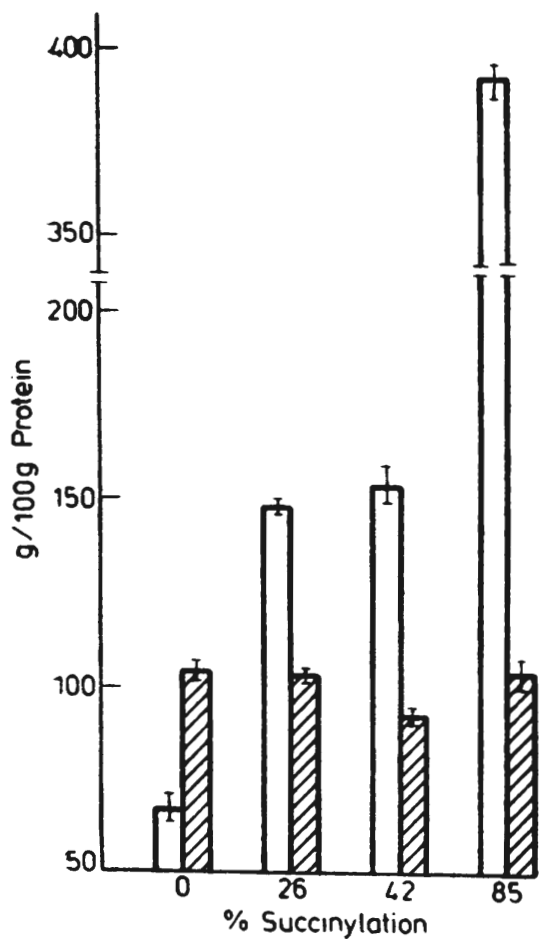


Fig. 9. Water ( $\square$ ) and oil absorption ( $\blacksquare$ ) capacity of sunflower protein isolates at various degrees of succinylation, according to [17]

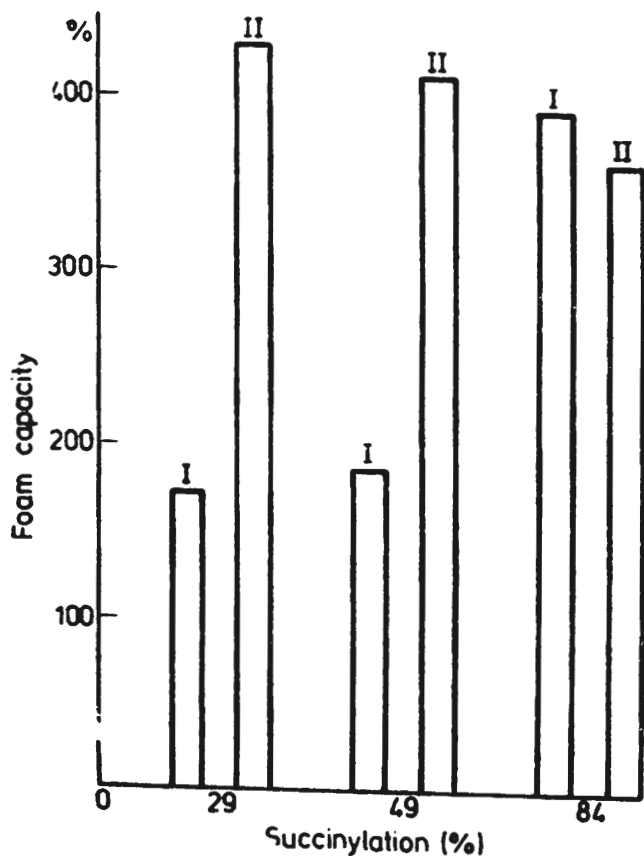


Fig. 10. Foam capacity of 10% dispersion of faba bean protein isolates at various degrees of succinylation, before (I) and after (II) heating, according to [18]

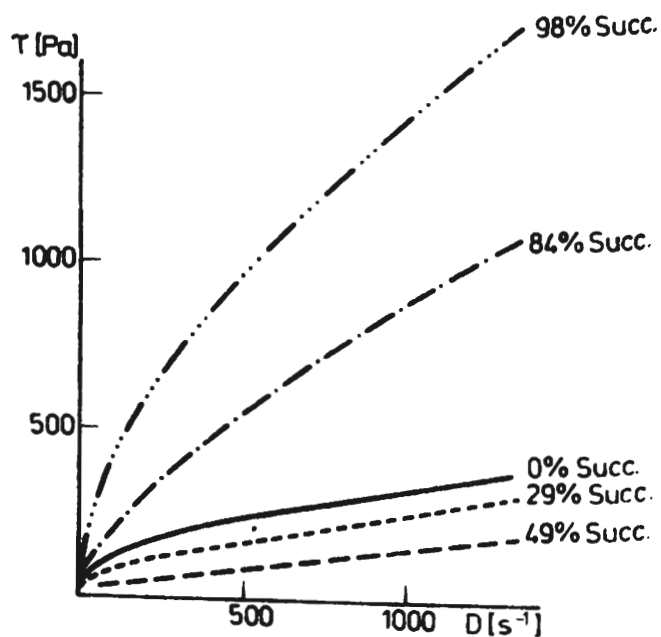


Fig. 11. Flow curves of 10% dispersions of faba bean protein isolates at various degrees of succinylation at 20°C, according to [19]

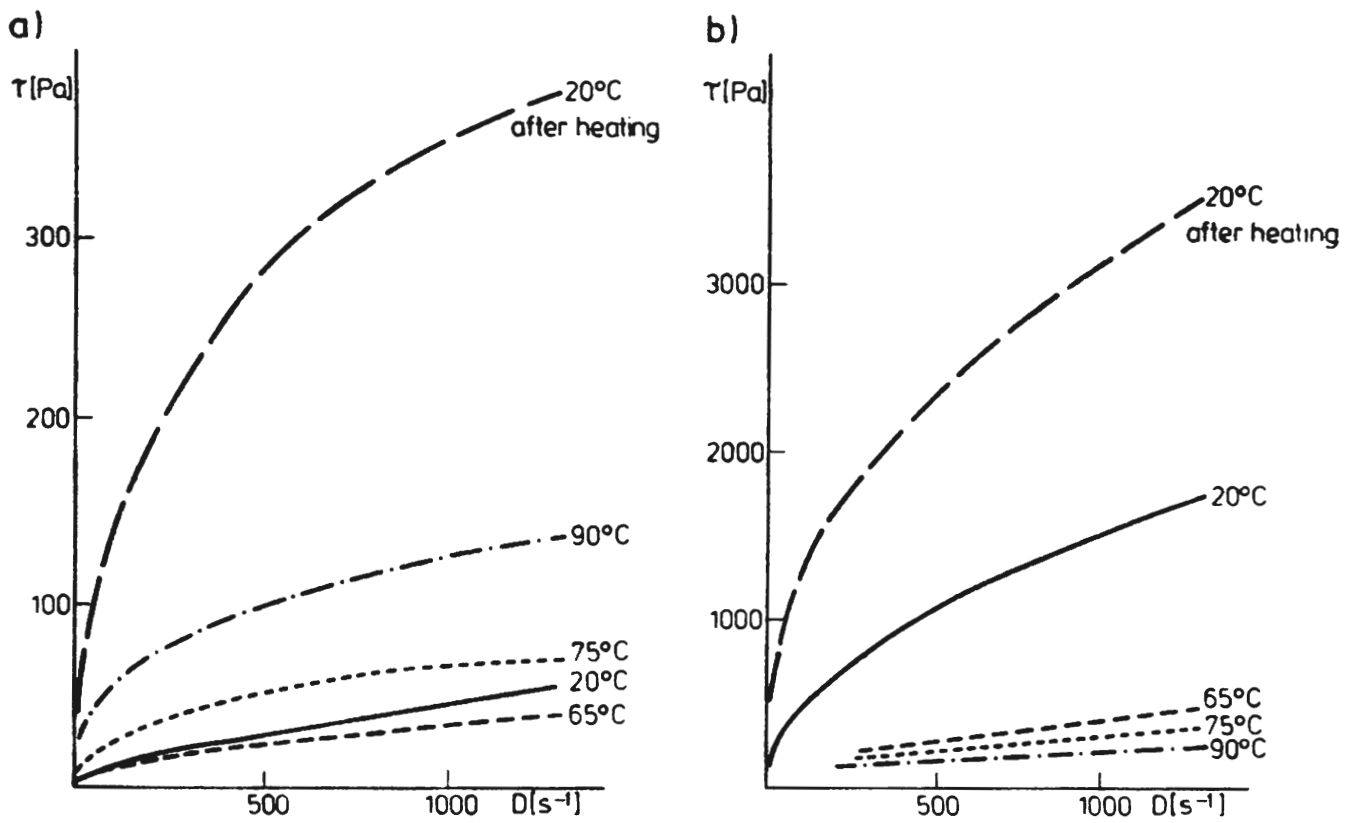


Fig. 12. Flow curves of 29% (a) and 96% (b) succinylated faba bean protein isolates at various temperatures ( $c = 10\%$ ), according to [19]

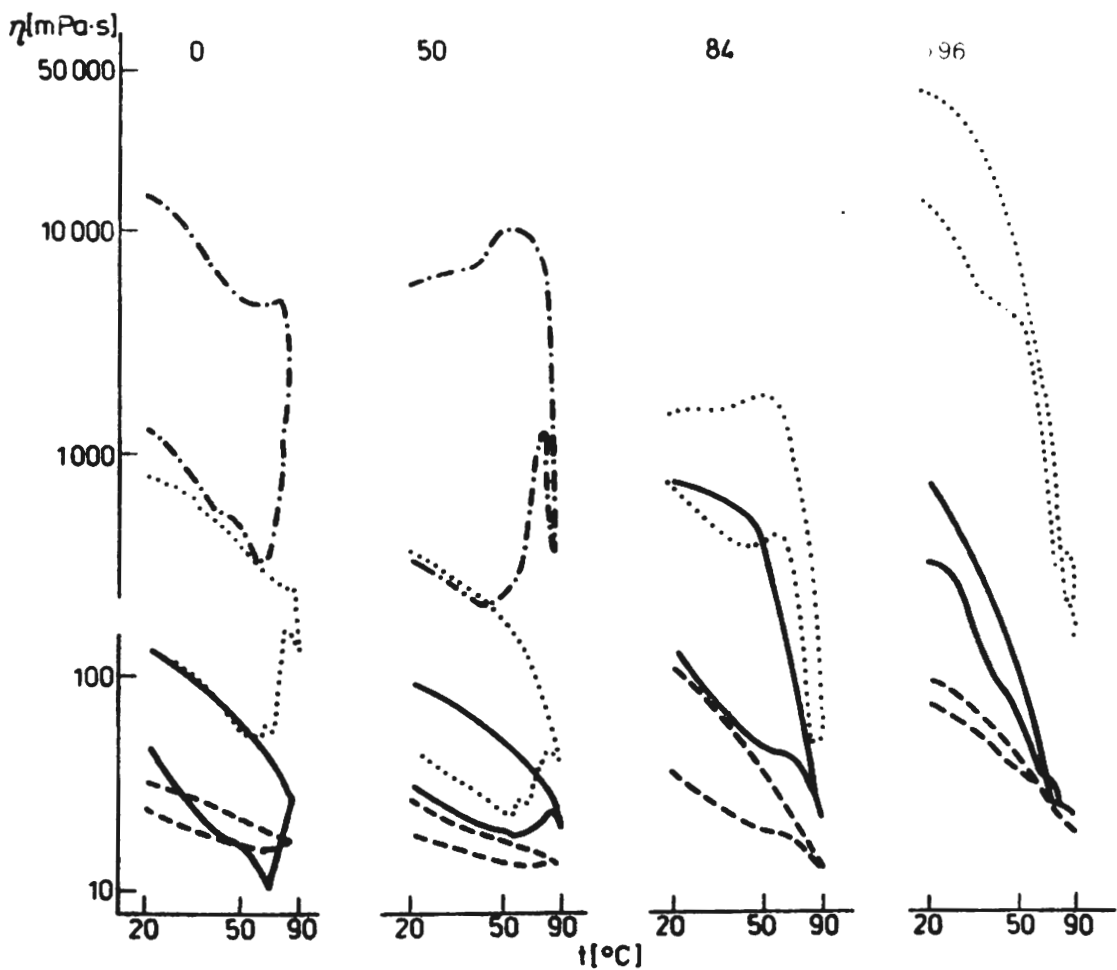


Fig. 13. Viscosity dependence of unmodified and differently (50%, 84%, 96%) succinylated faba bean protein isolates in 8% (---), 10% (—), 12.5% (...), 15% (-·-) dispersions, according to [19]



protein (Fig. 12). At the transition temperature the unfolding of the protein structure takes place. This can be seen in a viscosity increase in the cyclic flow curves which describe the viscosity changes during heating and cooling (Fig. 13). The latter one lacks totally in the exhaustively succinylated and therefore unfolded protein. The protein-protein interaction in this unfolded protein are effective enough to overcome the electrostatic repulsion. Therefore this modified protein can form gels at room temperature without any heat input.

## CONCLUSIONS

The results presented here show the possibilities to influence the functional properties by a directed change of the native structure without a high energy input. Both the pH-induced denaturation and the chemical modification by succinylation are suitable basis for chemical procedures.

Though the succinylation enables the structure and functionality modification in a more defined and versatile manner, its application for the production of protein foods could be limited from a physiological point of view, if the modified protein is chosen as replacer for a main part of the protein in a food.

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## POPRAWA WŁAŚCIWOŚCI FUNKCJONALNYCH BIAŁEK NASION ROŚLIN OLEISTYCH I STRĄCZKOWYCH PRZEZ ZMIANY STRUKTURALNE

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### Streszczenie

Główne białka z nasion roślin oleistych i strączkowych reprezentowane są przez frakcje globulinowe 11S i 7S o bardzo podobnej strukturze oligomerycznej w każdej z tych frakcji. Przez zmianę struktury przestrzennej można osiągnąć poprawę właściwości funkcjonalnych tych białek. Przedyskutowane są podstawowe kierunki modyfikacji struktury: łagodna denaturacja w środowisku kwaśnym lub alkalicznym i sukcylinowanie. Ostatnia z wymienionych metod pozwala na stopniową zmianę struktury białka i jego właściwości funkcjonalnych. Obydwie metody umożliwiają poprawę właściwości funkcjonalnych bez wysokiego nakładu energii.