

Effect of succinylation of oil palm protein concentrates on the functional properties

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SUMMARY. Defatted kernel flour of oil palm, grounded to 60 mesh, was taken as raw material to produce protein concentrates (70,8% protein) which were succinylated at different levels (0.05; 0.2 and 0.6). The extent of acylation was measured as percentage of lysine modification reaching values from 18.4% to 48,6%. Protein concentrate functional properties were determined: Water solubility (pH 2-10); water absorption (320%); oil absorption (2,2 ml oil/g), emulsion activity and emulsion stability (28-46%). The functional properties were enhanced by succinylation if compared with the untreated protein concentrate, however, "in vitro" digestibility was not affected, by succinylation. In summary, the results of this study indicate that acylation using succinyl anhydride can improve the functional properties of oil palm protein concentrate over those without such treatment.

RESUMEN. Efecto de la succinilación en concentrados proteínicos de palma de aceite sobre las propiedades funcionales. La harina desgrasada de la almendra de la palma aceitera, fué molida a 60 mesh y por extracción alcalina de las proteínas se obtuvo un concentrado proteínico que contenía 68,2% de proteína el cual fué succinilado a diferentes niveles (0,05; 0,1; 0,2 y 0,6 gr anhídrido succínico/gr proteína), lográndose obtener concentrados 70% de proteína. La extensión de la succinilación se midió en términos de porcentaje de modificación de lisina disponible, la cual varió entre 18,4% a 48,6%. Los concentrados proteínicos elaborados se les determinaron: absorción de agua (320%), absorción de aceite (2,2 ml de aceite/gr de harina, actividad estabilidad de emulsión (28-46%) y porcentaje de solubilidad de proteína entre pH 2 a 10. Todas las propiedades funcionales fueron mejoradas con la succinilación (0,2 gr de anhídrido succínico/gr proteína) al compararlos con el concentrado proteínico sin modificación. La digestibilidad in vitro no fué afectada por la succinilación y los valores fueron entre 83,5% y 84,1%.

INTRODUCTION

During the last two decades studies concerning the development of new sources of non-conventional proteins have been conducted. Concentrate and protein isolates with valuable properties have been obtained, but such products have not had the expected impact at the commercial stage, since many of them have not offered the necessary functional properties which allow them to be incorporated as food ingredients (1); (2).

The oil palm (*Elaeis guineensis*) cultivated in the central region of Venezuela, after kernel oil extraction and through special process produces a flour which has only been used as cattle food; it contains 22% protein and by alkaline extrac-

tion it can be obtained a concentrate 66% protein. In this particular case solubility and emulsifying properties were low, which may be due to the use of high temperatures during oil extraction.(3).

Protein acylation using acetic or succinic anhydrides has remarkably improved the functional properties, specially in regard to solubility and emulsifying. Also, it has been observed that the acylation does not involve in toxic compounds and improves the organic properties (4-5).

In this research, the acylation effects were studied by using succinic anhydride on the functional properties of an oil palm protein concentrate, comparing it with the conventional also called untreated protein concentrate.

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MATERIAL AND METHODS

The oil palm deffated kernel flour used in this research work, was donated by Industria Bananera C.A (Bananera C.A Industry), which owns its cultivated lands in the central region of Venezuela. The flour mentioned before was ground to 60 mesh and a protein concentrate was obtained by alkaline extraction (3). The flour-solvent proportion was 1:10; the pH for extraction was 9.5 using mechanical stirring for 1 h. The isoelectric precipitation took place at pH 5.3; after centrifugation (10.000 g x 20 min.) the solid portion was dried out by freeze-dried. The protein content was 68,2% (N x 6.25).

Succinylated protein isolates.

The method used was reported by Choi et al (4). The flour was dispersed in distilled water at room temperature (25°C). Succinic anhydride was added to the suspension at different proportions (0,05; 0,1; 0,2; 0,6 g/g protein). During extraction the pH was kept at 8-8.5 by adding NaOH(2N) during 1 h. at constant stirring. Finally the pH was adjusted to 7.5 and the suspension was centrifuged at 5.117 g for 20 min at 25°C. The supernatant portion was filtered through fiberglass, and the filtrate was acidified to reach the protein isoelectric point by using HCl 2N. Then, it was centrifuged again and the precipitate was freeze-dried. The succinylated concentrates showed an average of 70,8% of protein. To establish a comparison, a soy protein concentrate was used, it contained 70% of protein.

Chemical modifications estimates.

The extent of chemical modification was estimated by determining the available lysine content, following the Hall et al (6) procedure which uses trinitrobenzone sulfonic acid (TNBS) reagent. Modification is expressed as percent reduction in available lysine.

Functional properties.

Solubility was determined using the method of Wang and Kinsella (7) and was calculated as the percentage crude protein (total N x 6.25) present in the supernatant portion. The solubility profile was studied under a pH range of 2-10, using HCl or NaOH solutions. The water absorption capacity was determined by the procedure of Wang and Kinsella (8). The fat absorption capacity was determined (9). Emulsifying activity and emulsion stability were determined by Dench et al (10).

"In vitro" digestibility index

The index "in vitro" digestibility was determined by Hsu et al (11). The multienzyme system of tripsin, chymotripsin and peptidase, as well as tripsine alone were used. Enzymes, porcine pancreas tripsin type IX, bovine pancreas chymotripsin type V, and hot intestinal mucosa peptidase grade III were purchased from Sigma Chemical Company, St. Louis, MO. The measures were expressed as percent of protein digested.

All measurements were made in triplicate and the reported values are the average of the three measurements.

RESULTS AND DISCUSSION

The solubility of protein concentrates from oil palm kernels flour is remarkably affected by succinylation reaction; evidences of such changes is noticed when compared to conventional protein concentrate. Figure 1 shows curves at pH 2 and 4 where the succinylated concentrates are less soluble than the non-succinylated samples. When the pH is within the range of 5 to 7, succinylated concentrates show a notable increase in solubility, specially at pH 6 where the highest increase takes place. Afterwards, when the pH is within the range of 8 to 10, the succinylated concentrates keep more soluble than the untreated proteins.

It is observed that the concentrates increase the solubility when acylation grade rises up to 0,2 g succinic anhydride/g protein; beyond this value there is no evidence of solubility increment. Succinylation reaction increases the concentrates negative charge which is evidenced by the change of the isoelectric point as a result of a pH decrease from 5 to 4. This change is probably due to an increase of the electrostatic repulsion forces and the rearrangement of the molecules (12-13).

Table 1 presents some functional properties of acylated concentrates compared to conventional ones. Lysi ϵ -amino groups coming from the proteins of oil palm deffated kernels flour reached a maximum acylation degree of 48.2%. After attaining this level acylation stays practically constant.

Water absorption by the succinylated concentrates increased in proportion with the percentage of lysine modification. Such results are expected as a consequence of the exposure of larger polar and non-polar protein groups on account of the rearrangement of their structures when reacting to anhydride (14). When comparing with the soya con-

EFFECT OF SUCCINYLATION OF OIL PALM PROTEIN CONCENTRATES ON THE FUNCTIONAL PROPERTIES

TABLE 1
SUCCINYLATION EFFECTS ON:
WATER AND ABSORPTION STABILITY AND ACTIVITY OF PROTEIN EMULSION CONCENTRATE
"IN VITRO" DIGESTIBILITY OF OIL PALM PROTEIN CONCENTRATE COMPARED
TO CONVENTIONAL CONCENTRATES

Succinic anhydride g/protein g	% of modified lysine	% water absorption	Oil absorption (ml oil/g)	% emulsion activity	% emulsion stability	In vitro%* digestibility
0.05	18.40 ± 4.50	270 ± 2.0	1.80 ± 0.02	28.80 ± 1.20	23.40 ± 0.55	83.91 ± 2.06a
0.10	28.00 ± 2.00	310 ± 3.8	2.38 ± 0.14	36.70 ± 1.10	30.1 ± 0.88	83.74 ± 1.70a
0.20	48.20 ± 1.70	318 ± 4.4	2.45 ± 0.20	46.40 ± 0.98	47.2 ± 1.4	83.65 ± 1.58a
0.60	48.60 ± 1.50	324 ± 3.2	2.21 ± 0.00	46.80 ± 1.30	36.1 ± 1.3	83.48 ± 1.50a
Oil palm conventional concentrate	0.00	225 ± 2.2	1.70 ± 0.15	26.8 ± 0.98	13.6 ± 0.99	84.12 ± 2.01a
Soy concentrate	0.00	310 ± 2.4	3.00 ± 0.15	90.80 ± 1.10	92.0 ± 1.00	--

* Values with equals superscript letters were not significantly different.
P < 0.005 (Duncan multiple ranager test)

centrate, it shows similar values to the succinylated concentrates.

In regard to oil absorption in general, the acylated concentrates showed a small increment in the oil absorption capacity when compared to conventional or untreated concentrates. Such behavior can be attributed to structural alterations of succinylated proteins and not to the increase of negative ions, it was also observed a trend to increase and then to decrease to higher succinylation levels.

Succinylation increased emulsion activity as acylation increased up to 48% of available lysine modification where such value was stabilized. Succinylated concentrates were more stable to heat than the non-treated concentrate emulsions. This increment might be related to the augmented swelling of the molecules protein capacity due to the high succinylation levels (15). However, Choi et al (13) suggest that protein molecules secondary changes during high succinylation is the factor which determines the emulsion capacity increment on selected cotton seed concentrates. It is observed that the emulsifying properties were low when compared with soy protein concentrate.

It can be said that succinylation did not affect the "in

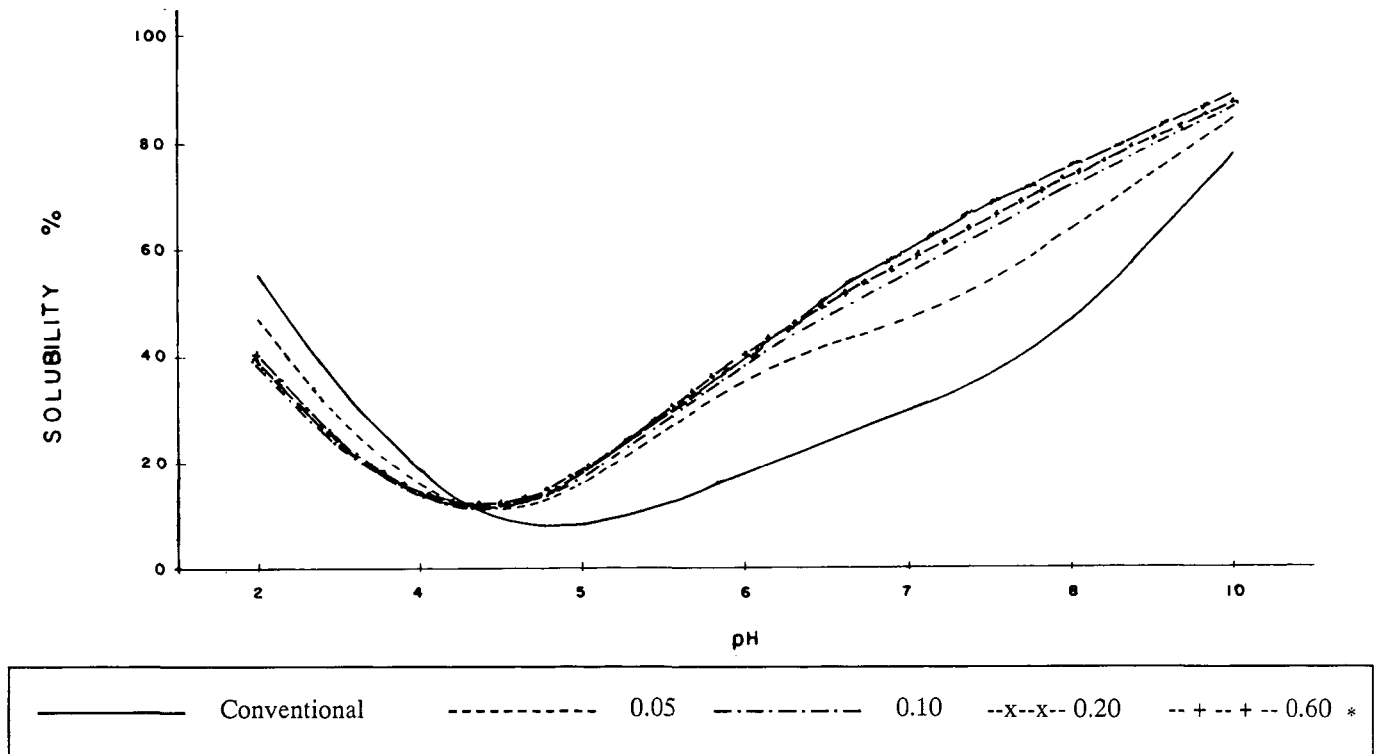
vitro" digestibility due to the fact that there were no significant differences between digestibility of conventional concentrates and succinylated concentrates. Ma and Wood (16) reported that succinylation did not induce significant changes in the essential aminoacid profile of oat protein with the exception of a small phenylalanine diminution; Likewise, Rahma (15) did not detect "in vitro" digestibility changes when defatted cotton flour protein was highly succinylated.

It can be concluded that succinylation significantly improves the functional properties, however high levels of anhydride are not necessary to reach the improvement of the functional properties studied. Wqual results are obtained using 0,2 g as well as 0,6 g of succinic anhydride/g protein. At industrial scale, it is desirable to employ the smallest amount possible of reagents in order to facilitate further purification and obtain a profitable process. From the nutritional standpoint, low acylation levels using succinic anhydride will not possibly cause nutritional damages to feeding systems where oil palm succinylated concentrates could be added.

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FIGURE 1
SOLUBILITY PROFILE OF OIL PALM SUCCINYLATED CONCENTRATES
AND CONVENTIONAL CONCENTRATE



* g succinic anhydride/g protein

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