Functional and Nutritional Properties of Acylated Rapeseed Proteins

R. Ponnampalam[a,*], **J. Delisle**[b,c], **Y. Gagné**[a] and **J. Amiot**[a,b]
[a]Departement de Sciences et Technologie des Aliments, [b]Groupe de Recherche en Sciences et Technologie du Lait (STELA), and [c]Departement de Zootechnie, Université Laval, Quebec, Canada, G1K 7P4

Rapeseed flour was acylated to various degrees with acetic and succinic anhydrides to produce acetylated rapeseed protein concentrates (ARPC) and succinylated rapeseed protein concentrates (SRPC), respectively. With both acylating agents, approximately 5–82% (ARPC-5 to ARPC-82) and 5–56% (SRPC-5 to SRPC-56) of ε-amino groups of lysine were acylated. Changes in functional properties were monitored; nitrogen solubility, emulsifying properties and specific viscosity were improved by acylation. The effects on functional properties were more pronounced with succinylation than acetylation. The nutritive value of succinylated rapeseed protein concentrates was determined and compared with those of unmodified rapeseed protein concentrate and rapeseed flour. Succinylation reduced the availability of lysine and decreased ($p<0.05$) the net protein ratio (NPR) and apparent digestibility coefficient (ADC) of nitrogen in rapeseed protein concentrates. Supplementation of SRPC with lysine improved the NPR compared to that of SRPC-56.

KEY WORDS: Rapeseed protein, acetylation, succinylation, acylation, functional properties, nutritional evaluation, canola flour, rapeseed flour.

Rapeseed is the dominant oilseed crop grown in Canada. It is a valuable source of protein with a well-balanced amino acid composition and a high content of sulfur-containing amino acids (1). Nevertheless, the presence of antinutrients such as glucosinolates, phytic acid, and phenolic compounds in the meal has limited its food uses (2). In order to make rapeseed proteins more attractive as potential food ingredients, it would be desirable to improve their functional properties, while at the same time retaining the nutritional value of the protein.

There are a number of reports on functional properties of rapeseed protein concentrates and isolates (3,4); good emulsification, whipping and fat-holding properties were observed, with poor water-holding and gelation properties. In addition, the nitrogen solubility of rapeseed protein concentrate was shown to be low at the pH values usually encountered in most food systems. Chemical modification is one approach to improve nitrogen solubility and expand the range of functional properties offered by rapeseed proteins.

Acylation has been widely used to improve protein functionality. It has been applied to various plant proteins, including those from oat (5,6), wheat (7), soy (8), peanut (9), pea (10), cottonseed (11) and rapeseed (12). Acetylation and succinylation of plant proteins have been reported to increase protein solubility (5,6), improve emulsification and foaming properties (11), increase water-holding and oil-holding capacities (5) and improve flavor (13). In addition, acylation of rapeseed flour improved nitrogen yield, decreased phytic acid extractability, and provided protein concentrates and isolates with improved solubility, emulsifying and oil-holding properties (14,15). Furthermore, acetylated rapeseed flour was less susceptible to tryptic and peptic hydrolysis, but the water-soluble protein fragments were devoid of phenolic constituents (16).

There are numerous reports on the effect of acylation on the nutritive value of animal proteins. However, this information is limited on plant proteins. Acetylation decreased the nutritive value of rapeseed proteins as measured by net protein ratio, and the effect was more pronounced as the extent of modification increased (17). Acylation of oat proteins was found to reduce protein nutritive value as measured by protein efficiency ratio, but supplementation with lysine improved the nutritive value (18).

The objectives of the present investigation were to examine some functional (acetylated and succinylated proteins) and nutritional (succinylated proteins) properties of acylated rapeseed proteins relative to their suitability as foods.

EXPERIMENTAL PROCEDURES

Materials. Dehulled, hexane-extracted, nonheat-treated rapeseed flour (*Brassica napus*, var. Tower) was provided by J.D. Jones, Food Research Institute, Ottawa, Canada. Casein was obtained from ANRC, Humko Sheffield Chemical (Memphis, TN). Acetic anhydride was purchased from Anachemia, Montreal, Canada, and succinic anhydride was purchased from Eastman Kodak Co. (Rochester, NY). All other chemicals were reagent grade.

Preparation of acylated rapeseed protein concentrates. Acylation of rapeseed flour (RF) was carried out by a procedure similar to that of Franzen and Kinsella (8) to evaluate functional properties. A control was treated in the same manner except that no acylating agent was added. Five percent (W/V) of RF/water (distilled) suspension was adjusted to pH 8.5 with 3N NaOH, and acetic or succinic anhydride (0.02, 0.06, 0.10, 0.14 and 0.20 g of anhydride/g of protein) was added slowly with constant stirring for 1 hr. The mixture was stirred for another 2 hr with the pH maintained at 8.5 by addition of 1N NaOH. The suspension was dialyzed against distilled water at 4°C (Spectrapor membrane no. 1, Spectrum Medical Industries, Inc., Los Angeles, CA) for 40 hr to remove excess reagent, and the acetylated concentrate was recovered by lyophilization.

Succinylated rapeseed protein concentrates (SRPC) (0.05, 0.10, 0.15 and 0.25 g succinic anhydride/g protein in RF) were prepared as above for nutritional quality evaluation. The proteins were precipitated at the

*To whom correspondence should be addressed.

isoelectric point (pH 3.95) with 5N HCl (17). The suspension was left at 4°C for 12 hr for complete precipitation and centrifuged at 2500 × *g* for 15 min at room temperature, followed by decanting the supernatant. The protein residue was redispered in distilled water, adjusted to pH 7.0, lyophilized, and stored until further analysis.

Chemical analysis. The RF and unmodified (URPC), acetylated (ARPC), and succinylated (SRPC) rapeseed protein concentrates (RPC) were analyzed for protein content (N × 6.25) by the Kjeldahl method with a Kjel-Foss Automatic 16210 apparatus (A/S N. Foss Electric, Denmark) and the energy content was determined by the AOAC method using an adiabatic Parr instrument. The extent of ε-amino group of lysine bound acetyl or succinyl groups was determined from the available lysine content of the acetylated/succinylated and control (unmodified) rapeseed protein concentrates, using the dinitrobenzenesulfonate (DNBS) method (19). The extent of sulfhydryl group of cysteine bound to acyl groups was analyzed by Ellman's procedure as modified by Beveridge *et al.* (20). Succinylation of tyrosine residues of rapeseed protein and their hydrolysis at pH 8.0 and 25°C were followed by measuring the absorbance at 280 nm (21). Abbreviations used in the text indicate percentage ε-amino group of lysine acylated to differentiate between the various acylated products.

The amino acid compositions of the samples were determined with an amino acid analyzer (LKB Biochrom Ltd., Cambridge, England) equipped with an HP 3392A integrator, following hydrolysis with 6N HCl (powder:acid ratio, 1:1000) under nitrogen at 110°C for 24 hr. Chromic oxide in the feces was measured according to the method of Christian and Coup (22).

Functional properties. Functional properties were determined on an as is basis, except for emulsifying properties (adjusted to an equal protein basis). Nitrogen solubility between pH 3.0 and 9.0 was determined in a 1% (W/W) dispersion according to the method described by Ponnampalam *et al.* (16). Emulsion activity index (EAI) was measured by the turbidimetric method of Pearce and Kinsella (23) with slight modification. Protein dispersion of 2.5% was used and pH was adjusted to 6.5. Corn oil was used instead of peanut oil. Emulsion stability (ES) was determined by the procedure of Yasumatsu *et al.* (24). However, the emulsion was prepared by the method of Pearce and Kinsella (23) with slight modification, as mentioned above. Specific viscosities of protein dispersions were measured by using a Epprecht Rheomat 15 viscosimeter and the method described by Childs and Park (11).

Nutritional evaluation. Diets were formulated to contain 1.6% nitrogen (10% protein, N × 6.25) supplied as RF, URPC, or succinylated rapeseed protein concentrates (SRPC). Casein was used as the reference protein. Diets also contained 10% corn oil, 4% mineral mix (USP XVII, Teklad Test Diets, Madison, WI) and 1% vitamin fortification mix (Teklad Test Diets). All diets were made isocaloric on a digestible energy (DE) basis; corn starch was used to adjust DE to 3.93 kcal/g, and Celufil, a non-nutritive fiber (U.S. Biochemical Corp., Cleveland, OH), was used as a filler and given a DE value of zero. A protein-free diet was prepared for the net protein ratio (NPR) measurement. In addition, one of the succinylated rapeseed protein concentrates (SRPC-56) was supplemented with L-lysine hydrochloride alone and L-lysine hydrochloride and L-cystine to compensate for the amount of succinylated ε-NH_2 group of lysine and sulfhydryl group of cysteine (17%).

The feeding study was performed with Sprague-Dawley male rats, allotted at random in groups of 10 with approximately equal mean weights (54.9 ± 2.7 g), and randomly assigned to the diets for a 10-day period. The rats were housed in individual, stainless-steel, wire screen cages equipped to collect feces and kept in a room with constant relative humidity (48 ± 1%), temperature (21.8 ± 0.4°C), and a 12-hr light-dark cycle. Feed and water were offered *ad libitum.* During the 10-day period, feed intake and weight gain were recorded and NPR was calculated. Feces were collected from day 3 to day 10 to determine the apparent digestibility coefficient (ADC) of nitrogen. Chromic oxide was added to diets at 0.1% and served as external indicator of digestibility.

Statistical analysis. The results were subjected to analysis of variance (general linear models procedure), and treatment means were separated by Duncan's multiple-range test (25).

RESULTS AND DISCUSSION

Extent of chemical modification. Treatment of rapeseed flour with increasing quantities of acetic or succinic anhydride progressively acylated the ε-amino group of lysine as shown in Figure 1. The addition of 0.2 g of acetic or succinic anhydride/g of rapeseed protein resulted in approximately 82% acetylation and 45% succinylation. Similar observations have been reported for oat protein (5,6) and for fish myofibrillar protein (26). To achieve approximately the same level of succinylation as that of acetylation, one must employ more succinic anhydride and increase the reaction time to facilitate dissolution of succinic anhydride.

Effect of chemical modification on protein functionality-solubility. Acylation markedly enhanced protein solubility above and below pH 5.0 when compared

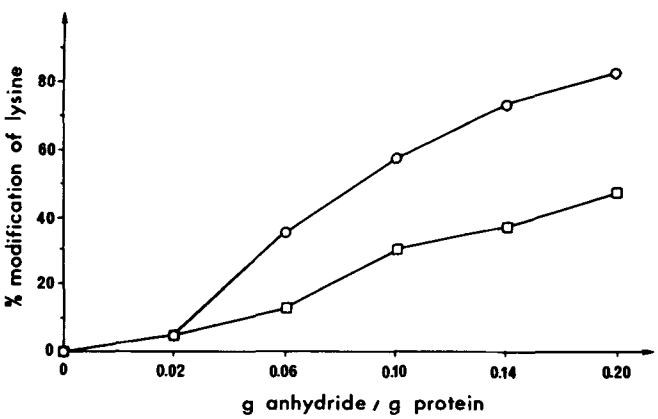

FIG. 1. Effect of anhydride concentration on modification of ε-amino group of lysine of rapeseed proteins. ○, acetylation; □, succinylation.

TABLE 1

Nitrogen Solubility[a] of Acylated Rapeseed Protein Concentrates (%)

RPC	pH		
	4	6	8
URPC	22	32	64
ARPC-35	33	55	75
ARPC-57	32	75	83
ARPC-82	34	83	88
SRPC-13	33	55	75
SRPC-30	32	75	83
SRPC-47	35	79	79

[a]Each analysis was done in triplicate. Abbreviations used in the text will include % of ε-amino group of lysine acetylated or succinylated to differentiate between products. URPC, unmodified rapeseed protein concentrate; ARPC-35, ARPC-57 and ARPC-82, acetylated rapeseed protein concentrates; SRPC-13, SRPC-30 and SRPC-47, succinylated rapeseed protein concentrates.

TABLE 2

Emulsifying Activity Index (EAI) and Emulsion Stability (ES) of Acetylated and Succinylated Rapeseed Protein Concentrates.

RPC	EAI (m^2/g)	ES (%)
URPC	12.5 ± 0.5^{a}	$60 \pm 3^{b,c}$
ARPC-5	14.4 ± 0.6^{d}	$65 \pm 4^{b,c}$
ARPC-35	19.7 ± 0.7^{c}	$67 \pm 3^{a,b}$
ARPC-56	$22.9 \pm 0.6^{b,c}$	$72 \pm 4^{a,b}$
ARPC-73	24.4 ± 0.7^{b}	74 ± 4^{a}
ARPC-82	$24.9 \pm 0.8^{a,b}$	74 ± 3^{a}
SRPC-5	16.1 ± 0.8^{d}	66 ± 3^{b}
SRPC-13	21.0 ± 0.7^{c}	$69 \pm 4^{a,b}$
SRPC-30	24.0 ± 0.6^{b}	$72 \pm 4^{a,b}$
SRPC-37	$26.5 \pm 0.8^{a,b}$	$72 \pm 3^{a,b}$
SRPC-47	27.3 ± 0.8^{a}	74 ± 4^{a}

[a,b,c,d]Means with different superscript are significantly different ($p < 0.05$).

Abbreviations used in the text will include % of ε-amino group of lysine acetylated or succinylated to differentiate between products. URPC, unmodified rapeseed protein concentrate; ARPC-5, ARPC-35, ARPC-56, ARPC-73 and ARPC-82, acetylated rapeseed protein concentrates; SRPC-5, SRPC-13, SRPC-30 and SRPC-37, SRPC-47, succinylated rapeseed protein concentrates.

to unmodified concentrate (Table 1). Acylated proteins were more soluble at the isoelectric point than the URPC. The solubilities of acetylated and succinylated proteins increased as the extent of modification increased. Protein solubilities at pH values above 5.0 of ARPC-35 and ARPC-57 were similar to SRPC-13 and SRPC-30, respectively.

Acylation has been found to increase protein solubility, alter protein conformation by promoting unfolding and increasing dissociation of subunits from quaternary structure, as well as shifting the isoelectric point to lower values. The change in conformation of succinylated proteins results from their high net charge and replacement of short range attractive forces (ammonium, carboxyl) in the native molecule with short range repulsive forces (succinate carboxyl, native carboxyl) (27). In addition, intra- and intermolecular electrostatic repulsion promote unfolding of the protein reducing protein-protein interactions and increase protein-water interactions, thus enhancing aqueous solubility. Since net negative charge is proportional to the extent of succinylation, the solubility of rapeseed concentrate increased as the extent of chemical modification increased. However, the solubilities of SRPC-30 and SRPC-56 (results not shown) were similar. This demonstrates that extensive succinylation was not required to substantially increase solubility. A similar effect was observed by Franzen and Kinsella (8) with soy protein.

Acetylation of protein replaces cationic amino groups with neutral acetyl groups, thus producing fewer electrostatic repulsions. Hence, the aqueous solubility of acetylated rapeseed concentrate was intermediate in magnitude between those of unmodified and succinylated rapeseed concentrate. Similar observations have been made by other researchers on acylated plant proteins (5,6,8,28).

Emulsifying properties. The emulsion activity index (EAI) and emulsion stability (ES) of the unmodified and acylated rapeseed proteins are presented in Table 2. EAI of acylated proteins were significantly ($p < 0.05$) higher as compared to URPC. In addition, EAI of SRPC-37 was much higher than ARPC-35. Furthermore, EAI values of SRPC-30, ARPC-73, and ARPC-82 were similar. This demonstrates that high EAI could be achieved with moderate succinylation rather than extensive acetylation. A high positive correlation ($r=0.83$ for acetylation and $r=0.98$ for succinylation) was obtained at pH 6.5 between solubility and EAI of acylated rapeseed proteins. Exhaustive acylation increased the ES of rapeseed protein significantly ($p < 0.05$) from 60–74% as compared to URPC. Acylation increased EAI and ES of rapeseed protein concentrate, probably due to enhanced solubility. The positive correlation between solubility and the emulsification capacity has been documented by others (24,29). As the protein becomes more soluble, it forms layers around the fat droplet to facilitate association with the aqueous phase. In addition, soluble proteins encapsulate the fat globule and render the emulsion more stable to heat treatment.

Viscosity. Acylated rapeseed proteins exhibited higher specific viscosities compared to unmodified proteins (Fig. 2). The relationship was similar in 5% sample dispersions. In addition, specific viscosity increased as the extent of acetylation increased. However, all the succinylated RPC gave similar specific viscosity values, therefore, the values represent average specific viscosities of 2.5 and 5.0% dispersed concentrates.

It is generally recognized that the viscosity of a dispersion depends on the concentration of the solute and molecular weight, shape, and size of the polymer molecule (3,11). Since acylation alters protein conformation by promoting unfolding, it can cause an increase in volume of protein molecule and, thus, increase viscosity. Furthermore, increased aqueous solubility of acylated proteins due to unfolding, as well as dissociation of subunits, may be responsible for the increase in viscosity (5,6).

Nutritional properties—amino acid composition. In general, succinylation did not affect the amino acid composition of the rapeseed proteins except for lysine,

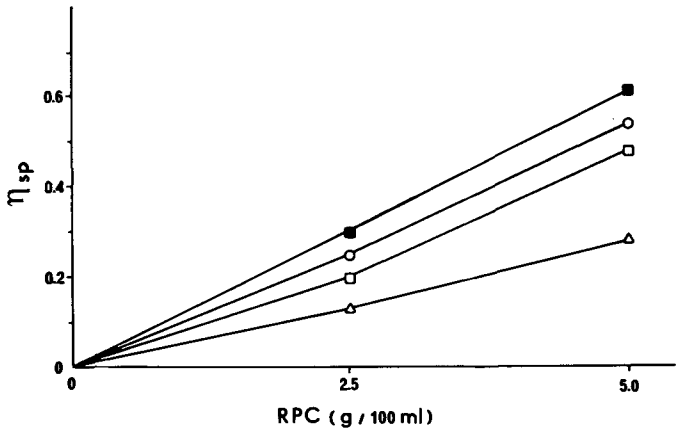

FIG. 2. Specific viscosity of acylated and unmodified rapeseed protein concentrates at pH 6.5. Δ, URPC; □, ARPC-5; ○, SRPC (Ave.); and ■, ARPC-82. Abbreviations used in the text will include % of ε-amino group of lysine acetylated or succinylated to differentiate between products. URPC, untreated rapeseed protein concentrate; ARPC-5 and ARPC-82, acetylated rapeseed protein concentrates; and SRPC, succinylated rapeseed protein concentrates.

which was lowered as the degree of succinylation increased (Table 3). Lysine content of URPC (5.8 g/100 g of protein) was much higher than that of SRPC-56 (4.6 g/100 g of protein). Similar observations have been reported on acylated fish (30) and whey proteins (31), although others have reported no destruction of amino acids (13,18). The decrease in lysine content with acylation may be attributed to incomplete deacylation of lysine during acid hydrolysis.

Nutritional evaluation. The data for weight gain, feed intake, net protein ratio (NPR) and apparent digestibility coefficient (ADC) of nitrogen are summarized in Table 4. Succinylated rapeseed protein concentrates gave significantly ($p < 0.05$) lower NPR values, ADC of nitrogen and weight gain as compared to URPC, which indicates that succinylation decreased nutritive value. Similar findings were reported for acylated oat proteins (18) and acetylated rapeseed proteins (17). Weight gain, feed intake and NPR decreased significantly ($p < 0.05$) as the extent of succinylation increased, but no significant difference was observed in ADC of nitrogen. Furthermore, URPC gave significantly ($p < 0.05$) higher weight gain, feed intake, NPR and ADC of nitrogen as compared to RF. This may be due to removal of antinutrients such as phenolic compounds, phytic acid and glucosinolates (16). The lower NPR values of succinylated proteins in comparison to URPC may be due to unavailability of ε-succinyl-lysine to rats (32), since the rat does not have a deacylase that will hydrolyze acyl groups larger than the acetyl group attached to the ε-NH_2 group of lysine.

Amino acid supplementation. The nutritional effect of amino acid supplementation on SRPC-56 is given in Table 4. The addition of L-lysine hydrochloride to SRPC-56 to compensate for the amount succinylated, significantly ($p < 0.05$) improved weight gain, feed intake, NPR and ADC of nitrogen. The NPR of SRPC-56 with lysine hydrochloride was 166% and 89% as compared to SRPC-56 and URPC, respectively. This might indicate that the lower nutritive value of SRPC is mainly due to unavailability of ε-succinyl-lysine to rats. However, SRPC-56 supplemented with lysine hydrochloride did not improve the NPR compared to the value obtained for URPC. This indicates that not only lysine, but other amino acids are also not available to the rat following succinylation. Similar findings were reported by Goulet *et al.* (18). Supplementation of SRPC-56 with L-lysine hydrochloride and L-cystine did not change the NPR value or other parameters compared to one supplemented with only L-lysine hydrochloride (Table 4). This seems to indicate that even though 17% of the sulfhydryl groups was acylated, sulfur amino acids are not the second limiting amino acids in succinylated rapeseed protein concentrates. Similar ob-

TABLE 3

Essential and Limiting Amino Acid Content of Unmodified and Succinylated Rapeseed Protein Concentrates (g/100 g protein)

Amino acid[a]	URPC	SRPC-17	SRPC-30	SRPC-37	SRPC-56
Lysine	5.8	5.7	5.1	5.0	4.6
Cystine	1.8	1.8	1.8	1.7	1.8
Methionine	2.3	1.9	2.0	2.2	2.3
Isoleucine	3.8	3.9	3.7	3.8	3.9
Leucine	7.5	7.6	7.6	7.5	7.6
Phenylalanine	4.4	4.5	4.3	4.4	4.5
Tyrosine	3.5	3.5	3.3	3.4	3.4
Threonine	4.7	4.8	4.3	4.4	4.7
Valine	5.1	4.9	5.2	5.0	5.0
Arginine	6.8	6.7	6.9	6.6	6.8
Histidine	3.1	2.9	2.8	3.0	2.9

[a]Tryptophan was not determined.

Abbreviations used in the text will include % of ε-amino group of lysine acetylated or succinylated to differentiate between products. URPC, unmodified rapeseed protein concentrate; SRPC-17, SRPC-30, SRPC-37 and SRPC-56, succinylated rapeseed protein concentrates.

TABLE 4

Nutritional Evaluation of Rapeseed Flour, Untreated Rapeseed Protein Concentrate and Succinylated Rapeseed Protein Concentrates

Protein source	Weight gain (g)	Feed intake (g)	NPR	ADC of N (%)
RF	10.9^{e}	54.6^{d}	$3.49^{c,d}$	$82.6^{c,d}$
URPC	33.4^{b}	95.7^{b}	4.36^{a}	84.3^{b}
SRPC-17	26.3^{c}	93.1^{b}	$3.63^{b,c}$	81.0^{e}
SRPC-30	21.5^{d}	91.0^{b}	3.20^{d}	81.2^{e}
SRPC-37	18.2^{d}	82.0^{c}	3.23^{d}	$81.6^{d,e}$
SRPC-56	6.3^{f}	62.2^{d}	2.35^{e}	$81.6^{d,e}$
SRPC-56 + L-lys.HCl	30.5^{b}	96.1^{b}	3.89^{b}	$83.6^{b,c}$
SRPC-56 + L-lys.HCl + L-Cys	31.1^{b}	97.2^{b}	3.95^{b}	83.0^{c}
Casein	41.1^{a}	105.3^{a}	4.43^{a}	91.9^{a}

^{a,b,c,d,e,f}Means with different superscript are significantly different ($p < 0.05$).

Abbreviations used in the text will include % of ε-amino group of lysine acetylated or succinylated to differentiate between products. RF, rapeseed flour; URPC, untreated rapeseed protein concentrate; SRPC-17, SRPC-30, SRPC-37 and SRPC-56, succinylated rapeseed protein concentrates.

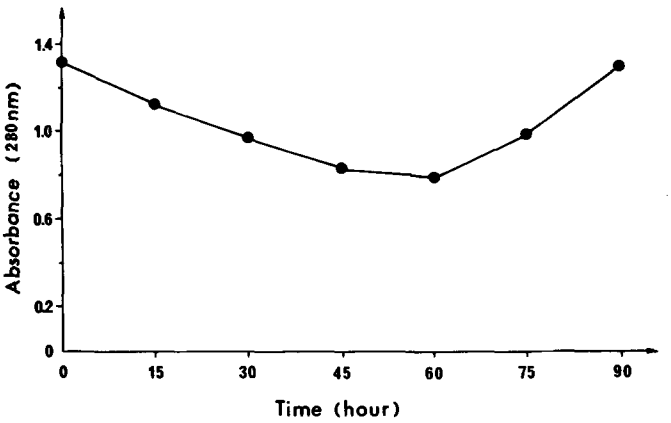

FIG. 3. Succinylation of tyrosine residues of rapeseed protein followed by hydrolysis at pH 8.0 and 25°C.

servations were made by Goulet *et al.* (18) with succinylated oat protein concentrates.

In rapeseed proteins, methionine and tyrosine are considered to be limiting amino acids. Delisle *et al.* (33) found that supplementation of rapeseed 2S fraction with tyrosine improved nutritive value (NPR and ADC of nitrogen), but supplementation of rapeseed protein concentrate with methionine did not alter the nutritive value. However, succinylated bovine serum albumin (BSA) resulted in 62% ε-amino groups of lysine residues, 13% hydroxyamino acid (e.g., threonine) and 12% tyrosine residues succinylated (21). Chang and Sun (21) also observed that deacylation of succinyl-tyrosyl residues took place after 4 hr of standing. However, rapeseed protein requires approximately 45 hr for complete succinylation of tyrosine residues and for complete hydrolysis (Fig. 3). This is probably due to differences in protein conformation of BSA and rapeseed proteins. Furthermore, the time of exposure of rapeseed proteins to succinic anhydride was 18 hr and the transit time in the rat gut was about 12 hr (34). Therefore, it is probable that the succinyl-tyrosyl residues were not completely deacylated since the proteins were exposed to alkaline conditions for only 30 hr. This illustrates that slight chemical modification of the phenolic group of tyrosine could make tyrosine the second limiting amino acid.

The results indicate that succinylation lowers NPR of rapeseed proteins (28). This effect can be minimized by reducing the extent of succinylation and supplementing the diet with limiting amino acids such as lysine and probably tyrosine.

ACKNOWLEDGMENTS

The research was supported by the National Sciences and Engineering Research Council of Canada (NSERC), Grant No. G1306. We thank A. Roy for performing amino acid analysis and G. Goulet for providing valuable advice.

REFERENCES

1. Ohlson, R., and K.J. Anjou, *J. Am. Oil Chem. Soc. 56*:431 (1979).
2. Jones, J.D., *Ibid. 56*:716 (1979).
3. Sosulski, F.W., E.S. Humbert, K. Bui and J.D. Jones, *J. Food Sci. 41*:1349 (1976).
4. Thompson, L.U., R.F.K. Liu and J.D. Jones, *Ibid. 47*:1175 (1982).
5. Ponnampalam, R., G. Goulet, J. Amiot, B. Chamberland and G.J. Brisson, *Food Chem. 29*:109 (1988).
6. Ma, C.-Y., *J. Food Sci. 49*:1128 (1984).
7. Grant, D.R., *Cereal Chem. 50*:417 (1973).
8. Franzen, K.L., and J.E. Kinsella, *J. Agric. Food Chem. 24*:788 (1976).
9. Sundar, R.S., and D.R. Rao, *Lebensm. Wiss. Technol. 11*:188 (1978).
10. Johnson, E.A., and C.J. Brekke, *J. Food Sci. 48*:722 (1983).
11. Childs, E.A., and K. Park, *Ibid. 41*:713 (1976).
12. Paulson, A.T., and M.A. Tung, *Ibid. 52*:1557 (1987).
13. Franzen, K., and J.E. Kinsella, *J. Agric. Food Chem. 24*:914 (1976).
14. Thompson, L.U., and Y.S. Cho, *J. Food Sci. 49*:771 (1984a).
15. Thompson, L.U., and Y.S. Cho, *Ibid. 49*:1584 (1984b).
16. Ponnampalam, R., M.A. Vijayalakshmi, L. Lemieux and J. Amiot, *Ibid. 52*:1552 (1987).
17. Delisle, J., B. Chamberland and A. Roy, *Plant Foods Hum. Nutr. 37*:265 (1987).
18. Goulet, G., R. Ponnampalam, J. Amiot, A. Roy and G.J. Brisson, *J. Agric. Food Chem. 35*:589 (1987).
19. Concon, J.M., *Anal. Biochem. 66*:460 (1975).
20. Beveridge, T., S.J. Toma and S. Nakai, *J. Food Sci. 39*:49

(1974).
21. Chang, T.-S., and S.F. Sun, *Inst. J. Peptide Protein Research 11*:65 (1978).
22. Christian, K.R., and M.R. Coup, *N.Z.J. Sci. Technol. 36*:328 (1954).
23. Pearce, K.N., and J.E. Kinsella, *J. Agric. Food Chem. 26*:716 (1978).
24. Yasumatsu, K., K. Sawada, T. Wada and K. Ishu, *Agric. Biol. Chem. 36*:737 (1972).
25. Little, T.M., and F.J. Hills, *Agricultural Experimentation Design and Analysis*, Wiley, New York, 1978.
26. Groninger, H.S., *J. Agric. Food Chem. 21*:978 (1973).
27. Habeeb, A.F.S.A., H.G. Cassidy and S.J. Singer, *Biochim. Biophys. Acta 29*:587 (1958).
28. Gagné, Y., *Propriétés fonctionnelles et nutritionnelles des protéines de colza acylées, Mémoire de maitrise*, Université Laval, Québec, Canada, 1987, p. 85.
29. Wang, J.C., and J.E. Kinsella, *J. Food Sci. 41*:286 (1976).
30. Groninger, H.S., and R. Miller, *J. Agric. Food Chem. 27*:949 (1979).
31. Siu, M., and L.U. Thompson, *Ibid. 30*:743 (1982).
32. Leclerc, J., and L. Benoiton, *Can. J. Biochem. 46*:471 (1968).
33. Delisle, J., J. Amiot, F. Dauphin, A. Roy, G.J. Brisson and J.D. Jones, *Plant Foods Hum. Nutr. 37*:109 (1987b).
34. Fleming, S.E., and B. Lee, *J. Nutr. 113*:592 (1983).

[Received September 22, 1989; accepted March 23, 1990]