# Nutritional Value of Acylated Oat Protein Concentrates

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Protein extract from oat groats was acylated with acetic (0.031 and 0.092 g/g of protein) and succinic (0.031 and 0.110 g/g of protein) anhydrides to produce acetyl protein concentrate (APC) and succinyl protein concentrate (SPC), respectively. With both the acylating agents, approximately 36 (APC-37, SPC-35) and 76% (APC-76, SPC-76) of the ϵ-amino groups of lysine were acylated. The nutritive value of acylated protein concentrates was compared with that of the untreated oat protein concentrate (UPC). The amino acid composition of the concentrate was not altered by acylation. The PER values of APC-37 and APC-76 were 84% and 47% that of UPC, whereas the PER values of SPC-35 and SPC-76 were only 62% and 0% that of UPC. The apparent digestibility coefficients (ADC) of nitrogen of APC-76, SPC-35, and SPC-76 were significantly ($p < 0.05$) increased when compared to UPC. Supplementation of SPC-76 with L-lysine hydrochloride to compensate for the amount succinylated gave a PER corresponding to 82% that of UPC. The blend (1:1 ratio on protein basis) of SPC-76 and whey protein concentrate gave a PER greater than that of UPC.

Oats are high in nutritional quality in comparison to other cereal grains (Youngs et al., 1982). However, only 2.3% of the total oat crop harvested is used for human consumption in Canada (Statistics Canada, 1985). In order to make oat protein more attractive as a food ingredient, it is desirable to improve their functional properties by chemical modification.

Chemical modification by acylation with acetic and succinic anhydrides has been widely used to improve functional properties of animal proteins (Creamer et al., 1971; Groninger and Miller, 1979; Thompson and Reyes, 1980) and plant proteins including those from soy (Melnychyn and Stapley, 1973; Franzen and Kinsella, 1976a), pea (Johnson and Brekke, 1983), wheat and wheat gluten (Grant, 1973; Ma et al., 1986), and oat (Ma, 1984). Ma (1984) reported that acylation of oat proteins increased the solubility, emulsifying properties, and fat-binding capacity. It has been observed that improvement in functional properties were more pronounced with succinylation than acetylation (Franzen and Kinsella, 1976a,b; Ma, 1984).

Acylation of animal proteins such as casein (Creamer et al., 1971), fish myofibrillar protein (Groninger and Miller, 1979), whey protein concentrate (Thompson and Reyes, 1980; Siu and Thompson, 1982b), and beef heart myofibrillar protein (Eisele et al., 1981) was found to reduce protein nutritive value as measured by net protein ratio (NPR) or protein efficiency ratio (PER). It has been reported that acetylated protein gave better growth response than succinylated protein (Groninger and Miller, 1979; King et al., 1981; Eisele et al., 1981).

There are number of reports on the effect of acylation on the functional properties of vegetable proteins. However, information on the nutritive value of modified proteins is limited.

The objective of the investigation was to examine a number of aspects of acylated oat protein relative to their suitability as foods with nutritional quality. These include the estimation of the extent of acylation on ϵ-$NH_2$ and sulfhydryl groups; the determination of PER and apparent digestibility coefficient (ADC) of nitrogen for oat proteins acylated to different extents; the effect of supplementation with lysine, lysine and cystine, or lysine and methionine to compensate for the amount succinylated on PER and ADC of nitrogen. Whey protein concentrate (WPC) contains a high content of lysine and sulfur amino acids. Hence, the effect of supplementation with WPC on the succinylated oat protein concentrate (SPC-76) was also determined by PER and ADC of nitrogen.

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## MATERIALS AND METHODS

Oat groats (*Avena sativa* L., variety Hinoat) were provided by Dr. V. D. Burrows, Ottawa Research Station, Agriculture Canada, Ottawa, Ontario. The groats were ground just before use in a hammer mill (Smalley Manufacturing Co.) to pass through a 3-mm screen. Whey protein concentrate (Savorpro 75) was provided by Express Foods Co. Inc., Louisville, KY. L-Lysine monohydrochloride and DL-methionine were purchased from Sigma Chemical Co., St. Louis, MO, while L-cystine was purchased from BDH Chemicals, Toronto, Canada. All other chemicals were of reagent grade.

**Preparation of Acylated Oat Protein Concentrates.** A slurry of ground groats (groat to water ratio 1:8) was passed through a Comitrol grinder equipped with a microcut head (Urshel Laboratories Inc., Valparaiso, IN) for further grinding. This slurry was adjusted to pH 9.5 with 3 N NaOH, stirred for 1 h at room temperature for extraction of proteins, and then centrifuged at 2000*g* for 10 min to remove the bran and the starch fractions (Ma, 1983). Acetic (0.031 or 0.092 g/g of protein in oat groats) or succinic anhydride (0.031 or 0.110 g/g of protein in oat groats) was added over a period of 1 h to the supernatant (protein extract), and the pH was maintained at 8.0 by adding 3 N NaOH. The suspension was maintained at pH 8.0 for another 1 h for the reaction to go to completion. Isoelectric precipitation (IEP) of acylated oat protein was performed at pH 4.5 with 5 N HCl, and the proteins were collected by centrifugation at 2000*g* for 15 min. The precipitate was adjusted to pH 7.0 and lyophilized. The untreated oat protein concentrate (UPC) was prepared in a similar manner except that no acylating agent was added and IEP was performed at pH 5.5. The freeze-dried products were ground in a cyclone mill (Cyclotec 1093 Sample Mill, Höganas, Sweden) to pass a 1.0-mm screen.

**Chemical Analyses.** The untreated and acylated oat protein concentrates and whey protein concentrate were analyzed for total nitrogen by the Kjeldahl method and lipid, moisture, and ash contents by the AACC procedures (1971). The extent of the ϵ-amino group of lysine-bound acyl groups was determined from the free lysine content

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Table I. Extent of Acylation of Oat Protein Extract Treated with Acetic or Succinic Anhydride[a]

| anhydride | anhydride concn, g/g protein | % of groups acylated | |
|---|---|---|---|
| | | $\epsilon$-$NH_2$ | SH |
| acetic | 0.031 | 37.1 ± 1.3 | 12.5 ± 0.3 |
| acetic | 0.092 | 76.4 ± 1.4 | 49.0 ± 2.8 |
| succinic | 0.031 | 35.1 ± 0.8 | 12.8 ± 0.1 |
| succinic | 0.110 | 75.7 ± 1.5 | 43.5 ± 0.7 |

[a] Mean ± SD of triplicate determinations.

of the acylated and untreated oat protein concentrates, using the dinitrobenzenesulfonate (DNBS) method of Concon (1975) without ether extraction. The diethyl ether extraction step was eliminated since the extent of modification of the $\epsilon$-amino group of lysine obtained was similar with and without ether extraction, and the reproducibility was much greater without ether extraction. L-Lysine hydrochloride was used as a standard in this method. The sulfhydryl group of cysteine bound to acyl groups was analyzed by Ellman's procedure as modified by Beveridge et al. (1974). Since the $\epsilon$-amino group of lysine was most acylated, the abbreviations used in the text will include percent $\epsilon$-amino group of lysine acylated to differentiate between different levels of acylated products. The amino acid compositions of the whey protein and oat protein concentrates were determined, after hydrolysis with 6 N HCl (powder to acid ratio 1:1000) under nitrogen atmosphere at 100 °C for 24 h, by an amino acid analyzer. Chromic oxide in the feces was measured according to the method of Christian and Coup (1954).

**Nutritional Evaluation.** Diets were formulated to contain 1.6% nitrogen (10% protein, N × 6.25), 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 with corn starch to adjust the digestible energy (DE) to 3.90 kcal/g, while Celufil, a nonnutritive fiber (U.S. Biochemical Corporation, Cleveland, OH), was used as a filler and given a DE value of zero.

The succinylated oat protein concentrate (SPC-76) was supplemented with L-lysine alone, L-lysine and L-cystine, or L-lysine and DL-methionine to compensate for the amount of $\epsilon$-$NH_2$ group of lysine and sulfhydryl group of cysteine succinylated. PER and ADC of nitrogen were determined on the mixture (1:1 ratio on protein basis) of succinylated oat protein (SPC-76) and whey protein concentrate (WPC) to see whether WPC would supplement adequately the limiting amino acids of succinylated oat protein and improve its nutritive value.

The feeding study was performed with Sprague–Dawley male rats allotted at random in groups of 10 with approximately equal mean weight (54.3 ± 2.9 g) and assigned at random to the diets for a 4-week period. Throughout the study 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 ± 0.3 °C), and a 12-h light–dark cycle. Feed and water were offered ad libitum.

During the 4-week experimental period, feed intake and weight gain were recorded. The protein efficiency ratio (PER) was calculated as the weight gained per unit weight of protein consumed.

Feces were collected from day 13 to day 20 to determine the apparent digestibility coefficient (ADC) of nitrogen. Chromic oxide (0.1%) was added to the diets and served as external indicator of digestibility.

**Statistical Analysis.** The results were subjected to analysis of variance, and treatment means were separated by Duncan's multiple-range test (Little and Hills, 1978).

## RESULTS AND DISCUSSION

**Extent of Modification.** The extent of acylation of oat protein concentrates estimated as the amount of $\epsilon$-amino groups of lysine and sulfhydryl groups of cysteine acylated are presented in Table I. With both acetic and succinic anhydrides $\epsilon$-amino groups of lysine were more acylated than sulfhydryl groups of cysteine. Similar observations have been reported on fish myofibrillar protein (Groninger, 1973) and on whey protein concentrate (Siu and Thompson, 1982a,b) with succinic anhydride. On the other hand, to achieve approximately 36 and 76% of $\epsilon$-amino group of lysine acylated with both the acylating agents, more succinic anhydride (0.110 g/g of protein in oat groats) was employed in comparison to acetic anhydride (0.093 g/g of protein in oat groats). This indicates that acetic anhydride is more reactive than succinic anhydride on both $\epsilon$-amino and sulfhydryl groups. A similar finding was reported by Ma (1984) on acylation of the $\epsilon$-amino group of lysine of oat proteins.

**Nitrogen Yield and Chemical Composition.** Nitrogen recovery for the untreated oat protein concentrate was 75.1% (Table II). This was increased slightly with acetylation and succinylation. There was no trend in the protein content with acylation, but the ash content increased with an increase in acylation. Succinylation resulted in higher ash content, partly due to an increased requirement for NaOH during acylation to maintain pH

Table II. Nitrogen Yield and Chemical Composition[a] of Untreated and Acylated Oat Protein Concentrates and Whey Protein Concentrate

| | UPC[b] | APC-37[b] | APC-76[b] | SPC-35[b] | SPC-76[b] | WPC[b] |
|---|---|---|---|---|---|---|
| nitrogen yield, % | 75.1 | 77.5 | 76.9 | 78.7 | 83.2 | [c] |
| protein, % | 75.5 | 76.6 | 74.1 | 74.8 | 75.4 | 78.1 |
| ash, % | 3.7 | 4.2 | 5.9 | 4.7 | 6.8 | 3.2 |
| ether extr (lipid), % | 5.5 | 5.6 | 1.3 | 5.1 | 0.9 | 8.4 |
| carbohydrates, % | 15.3 | 13.6 | 18.7 | 15.4 | 16.9 | 10.3 |
| essential AA, g/16 g N | | | | | | |
| lysine | 2.8 | 2.9 | 2.9 | 3.0 | 2.8 | 7.5 |
| cystine | 1.9 | 1.9 | 2.1 | 2.0 | 2.0 | 2.4 |
| methionine | 1.6 | 1.6 | 1.5 | 1.7 | 1.8 | 1.9 |
| threonine | 2.7 | 2.7 | 2.8 | 3.0 | 3.0 | 6.3 |
| isoleucine | 3.4 | 3.6 | 3.5 | 3.8 | 3.8 | 5.1 |
| leucine | 6.2 | 6.5 | 6.5 | 7.0 | 6.9 | 9.2 |
| phenylalanine | 4.8 | 5.0 | 5.0 | 5.3 | 5.4 | 3.0 |
| tyrosine | 3.4 | 3.5 | 3.5 | 3.7 | 3.8 | 2.8 |
| valine | 4.0 | 4.3 | 4.4 | 4.8 | 4.8 | 4.8 |

[a] Dry-weight basis. [b] UPC = untreated oat protein concentrate; APC-37 and APC-76 = acetylated oat protein concentrates; SPC-35 and SPC-76 = succinylated oat protein concentrates; WPC = whey protein concentrate. [c] Commercial product derived from sweet dairy whey with only 5% lactose.

8, than acetylation. SPC-76 had the highest ash content, and this is probably due to the amount of succinic anhydride used to achieve 76% acylation of the ε-amino groups of lysine. Lipid content decreased with an increase in acylation. One possible explanation for this observation may be that the increase in the extent of acylation altered the lipid–protein electrostatic interactions and thus reduced the lipid content (ether extract) in the precipitated proteins.

**Amino Acid Composition.** Essential amino acid profiles (except tryptophan) of the untreated and acylated oat protein concentrates and of whey protein concentrate (WPC) are presented in Table II. Under the same hydrolyzing conditions, some of the essential amino acids were released more with acylation when compared to UPC. For example, isoleucine, leucine, phenylalanine, tyrosine, and valine (hydrophobic amino acids) increased slightly with acylation, and this is probably due to dissociation of proteins, including a change in conformation that facilitates the acid hydrolysis of the proteins. Ma (1984) however observed a slight decrease in isoleucine, phenylalanine, and valine following acylation. On the whole acylation did not cause any destruction of amino acids in this study. Similar observations have been reported on succinylated leaf protein (Franzen and Kinsella, 1976b) and acetylated beef heart myofibrillar protein (Eisele et al., 1981), although others reported slightly lower values for lysine in acetylated or succinylated proteins (Groninger and Miller, 1979; Siu and Thompson, 1982a). The decrease in lysine content with acylation was attributed to incomplete deacylation of lysine during acid hydrolysis.

The amino acid profile of WPC indicates that it could serve as a good source of lysine, threonine, and sulfur amino acids, limiting amino acids in oat protein concentrates. Also WPC has a higher content of leucine and isoleucine and lower content of phenylalanine and tyrosine when compared to oat protein concentrates.

Since lysine, cystine, and threonine contents did not change significantly in the acylated oat proteins, it suggests that acylation is reversible upon acid hydrolysis.

**Nutritional Evaluation of Acylated Oat Protein Concentrates.** The data for feed intake, weight gain, protein efficiency ratios (PER), relative PER, and apparent digestibility coefficients (ADC) of nitrogen are summarized in Table III. Acylated oat protein concentrates gave significantly ($p < 0.05$) lower PER values compared to UPC, which indicates that chemical modification by acylation decreased its nutritive value. Also the PER decreased as the extent of modification of ε-amino group of lysine increased. The APC-76 gave a better growth response (relative PER 47%) than SPC-76 (relative PER 0%) for the same extent of modification of the ε-$NH_2$ group of lysine (76%), when compared to UPC (relative PER 100%). Similar findings were reported by several investigators for acylated animal protein concentrates (Groninger and Miller, 1979; King et al., 1981; Eisele et al., 1981).

Acylation of oat proteins, however, had a beneficial effect on the ADC of nitrogen (Table III). Acetylation at a lower level did not alter the ADC of nitrogen of the oat protein. But succinylation significantly ($p < 0.05$) increased the ADC of nitrogen in comparison to the untreated and acetylated oat proteins. The improvement in the ADC of nitrogen may be due to an increase in solubility and the dissociation or change in conformation of protein molecules making them more susceptible to proteolytic enzymes. Unpublished data from this laboratory indicated that both acetylation and succinylation increased the solubility of oat proteins, and the effect was more pronounced with succinylation. Similar findings were observed by Ma (1984). However, Siu and Thompson (1982a) reported that true protein digestibility was slightly lowered for highly succinylated cheese whey protein concentrates.

**Table III. Feed Intake, Weight Gain, PER, Relative PER, and ADC of Nitrogen Obtained after Feeding Rats with Experimental Diets[a]**

| diet description | feed intake, g | wt gain, g | PER | rel PER,[b] % | ADC of N, % |
|---|---|---|---|---|---|
| UPC, 1.68% N[c] | 298.0[b] | 64.0[d] | 2.05[d] | 100 | 86.0[e] |
| APC-37, 1.78% N | 277.9[bc] | 53.2[e] | 1.73[e] | 84 | 86.6[e] |
| APC-76, 1.72% N | 184.4[e] | 19.3[g] | 0.97[g] | 47 | 88.4[d] |
| SPC-35, 1.71% N | 223.3[d] | 30.1[f] | 1.26[f] | 62 | 89.8[ab] |
| SPC-76, 1.71% N | 129.3[f] | −3.3[h] | −0.25[h] | 0 | 89.1[bcd] |
| SPC-76 + L-Lys·HCl, 1.71% N | 279.3[bc] | 50.2[e] | 1.68[e] | 82 | 88.9[cd] |
| SPC-76 + L-Lys·HCl + L-Cys, 1.75% N | 260.0[c] | 48.2[e] | 1.69[e] | 82 | 89.5[abc] |
| SPC-76 + L-Lys.HCl + DL-Met, 1.75% N | 288.4[bc] | 52.8[e] | 1.68[e] | 82 | 89.9[ab] |
| SPC-76:WPC = 1:1,[d] 1.76% N | 385.6[a] | 112.2[c] | 2.63[c] | 128 | 90.3[a] |
| WPC, 1.69% N | 403.0[a] | 155.6[a] | 3.65[a] | 178 | 89.7[abc] |
| ANRC ref casein, 1.64% N | 394.4[a] | 139.6[b] | 3.43[b] | 167 | 89.1[bcd] |
| CV, % | 10.7 | 14.6 | 5.0 | | 1.0 |

[a] Mean values were calculated on 10 rats, except for SPC-76:WPC (1:1) where 9 rats were used. Means within a column followed by the same superscript letter are not significantly different ($p < 0.05$). [b] PER of UPC was given a value of 100%, and the others were calculated in comparison to UPC. [c] Feed nitrogen content. [d] 1:1 ratio on protein basis.

Ma (1984) reported that in vitro digestibility was improved significantly with highly acylated oat proteins in comparison to unmodified oat proteins using a multienzyme assay (trypsin, chymotrypsin, peptidase). However, a decrease in in vitro digestibility was demonstrated in a number of succinylated proteins, particularly in the release of lysine using pepsin and pancreatin or pepsin and trypsin (Matoba and Doi, 1979; Siu and Thompson, 1982a). Matoba and Doi (1979) also found that $N^{\epsilon}$-succinyllysyl bonds are resistant to hydrolysis by pancreatic proteases (α-chymotrypsin, trypsin, carboxypeptidases A and B) in vitro and the succinyl groups are not deacylated by pancreatin. The in vitro digestibility is dependent on the type of protein, extent of protein modification, and enzyme systems used. Even though the ADC of nitrogen measured by in vivo method was improved by acylation, further utilization of this nitrogen for the animal growth was greatly reduced as shown by PER values. The lower PER values of acylated oat protein concentrates in comparison to UPC may be due to partial utilization of acyllysine derivatives by the rat (Groninger and Miller, 1979) and other essential amino acids made partly unavailable due to acylation (Creamer et al., 1971). 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 (Leclerc and Benoiton, 1968) and since it can utilize only up to 50% (range 14–50%) of the ε-acetyl-L-lysine (Bjarnason and Carpenter, 1969; Jering et al., 1974; Groninger and Miller, 1979), the effect of acetylation was less severe than succinylation.

**Amino Acid Supplementation.** The nutritional effect of amino acid supplementation on SPC-76 is given in Table III. The addition of L-lysine hydrochloride to SPC-76, to compensate for the amount succinylated, significantly ($p < 0.05$) increased the PER from −0.25 to +1.68, a value corresponding to 82% that of the UPC. This might indicate that the lower nutritive value of SPC-76 is mainly

due to the unavailability of ε-succinyllysine to the rat. However, SPC-76 supplemented with lysine hydrochloride did not improve the PER to the value obtained for UPC. This indicates that not only lysine but other amino acids are also not available to the rat following succinylation. Creamer et al. (1971) observed sulfur amino acid deficiency in the experimental animal fed acetyl casein supplemented with L-lysine hydrochloride when compared to native casein.

The rat assay for PER conducted previously in this laboratory on oat groats indicated that lysine is the most limiting amino acid followed by sulfur amino acids and threonine. Siu and Thompson (1982a,b) reported that acylation of the hydroxyl group of threonine was lowest when compared to the ε-$NH_2$ group of lysine and the sulfhydryl group of cysteine. Since 36% of the sulfhydryl groups of cysteine were succinylated in SPC-76, L-cystine was supplemented to compensate for the amount acylated. Inoue et al. (1982) observed that supplementing soy protein isolate with L-cystine decreased the feed intake and growth, while DL-methionine increased feed intake and growth. This initiated us to evaluate the supplementation of SPC-76 with DL-methionine to compensate for the amount of sulfhydryl groups of cysteine succinylated, since rats can use D- and L-methionine equally well (Boggs et al., 1975).

Supplementation of SPC-76 with L-lysine hydrochloride and L-cystine or with L-lysine hydrochloride and DL-methionine did not change the PER values compared to the one with only lysine hydrochloride (Table III). This seems to indicate that even though 36% of the sulfhydryl groups was acylated, sulfur amino acids are not the second limiting amino acids in succinylated oat protein concentrate. Tang et al. (1958) reported that the coefficient of digestibility of threonine in oats was 72% while coefficients of digestibility of lysine and methionine were 84 and 85%, respectively. Since the digestibility of threonine is quite low in comparison to that of methionine, even a slight modification of the hydroxyl group of threonine will make threonine the second limiting amino acid following acylation.

The ADC of nitrogen was not altered following amino acid supplementation of SPC-76.

**Blend of SPC-76 and WPC.** The PER of SPC-76 was significantly ($p < 0.05$) increased from −0.25 to +2.63 with the addition of WPC, a value corresponding to 128% that of UPC (Table III). However, the PER of the blend (2.63) was significantly ($p < 0.05$) lower than that of WPC (3.65) and ANRC reference casein (3.43). Nevertheless, the PER value of the blend was 76.7% that of casein. Since lysine is the first limiting amino acid in oats, the improvement in nutritive value compared to UPC may be primarily attributed to the high lysine content of the WPC in comparison to SPC-76, which compensated more than the amount of ε-$NH_2$ group of lysine succinylated. WPC also supplied threonine and sulfur amino acids, which are limiting amino acids of oat protein concentrates. Finally, the higher content of leucine and isoleucine in WPC in comparison to UPC could have enhanced the effect of WPC on SPC-76 as shown by PER values (Table III).

The ADC of nitrogen of the blend was significantly ($p < 0.05$) higher than that of UPC, SPC-76, and casein. However, no significant difference was observed in ADC of nitrogen between the blend and WPC.

Results show that exhaustive acylation lowers the PER of oat proteins. This effect can be minimized by (1) reducing extent of acylation, (2) supplementing the diet with lysine, (3) fortification with animal protein such as whey protein concentrate that are rich in essential amino acids. The functional properties of the modified oat protein concentrates and the blend of SPC-76 and WPC are being investigated in our laboratory. However, modified proteins intended primarily as functional ingredients should be incorporated with animal protein.

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