Functional Properties of Oat Proteins Modified by Acylation, Trypsin Hydrolysis or Linoleate Treatment

C.-Y. Ma* and **D.F. Wood**
Food Research Center, Agriculture Canada, Ottawa, Canada K1A OC6

Proteins extracted from defatted oats were chemically modified by acylation (succinylation and acetylation), potassium linoleate treatment or partial hydrolysis with trypsin. Total essential amino acid content was slightly lowered by acetylation, but unaffected by succinylation. Gel filtration chromatography showed some dissociation of oat polypeptides by succinylation, while trypsin hydrolysis caused considerable breakdown of the protein. Solubility and emulsifying properties were significantly improved by all the modifications. Fat binding capacity was improved by acylation and linoleate treatment, while water hydration capacity and foaming properties were improved by trypsin and linoleate modifications. The gelling property was improved by acylation. When meat protein was substituted with oat protein in model wieners, there was a decrease in cook yield, cohesiveness and firmness. However, when compared to the unmodified oat protein, succinylation led to an improvement in performance in an emulsified meat system.

Oats provide a potential source of low-cost proteins with good nutritional value, but are not used extensively for human consumption. Protein isolates and concentrates prepared from oats have been shown to possess good emulsifying and binding properties (1,2). In order to enhance the value of oat protein as a food ingredient, it would be desirable to improve its functionality, particularly solubility, which is poor near neutral pH. Chemical modifications such as acylation, enzymatic hydrolysis and surfactant treatment, have been found to be effective in improving the functionality of various food proteins (3-10). The present study reports on the effects of applying these modification procedures to oat proteins. The data on functionality changes have been reported elsewhere (11,12). The performance of the modified oat proteins on model weiner systems was assessed and compared to that of pea and soy protein isolates.

METHODS

Proteins were extracted from defatted oat groats in a weak alkaline suspension as described previously (1). Acylation, trypsin hydrolysis and linoleate treatment were performed directly on the alkaline protein extract as described previously (11,12). After modifications, the protein extracts were dialyzed against distilled water and freeze dried. The modified oat products had protein contents of around 60% and can be regarded as protein concentrates. Methods for all chemical analysis and functionality assessment have been described (11,12).

The effect of chemical modifications on the gelling property of oat protein was studied. Dispersions (10% w/v of oat protein at alkaline pH were heated at 100 C for 20 min in glass tubes. The hardness of the coagulum was measured by a back extrusion method (13) using an Instron Testing Machine.

To study the performance of oat and other plant proteins in meat emulsions, wiener batters were prepared with lean ground beef or pork. Meat protein was substituted with plant proteins at 5, 10 and 20% levels on a dry weight basis. Batters were prepared as described by Raymond et al. (14). Cook yield was determined by the method of Randall et al. (15), and cohesiveness and firmness were measured as described by Voisey and Randall (16). Two oat protein products, globulin and oat protein isolate, were prepared by salt and alkaline extraction, respectively, as described previously (2). Soy protein isolate (Supro 610) was from Ralston Purina, and pea protein isolate was a product of Woodstone Foods (Portage La Prairie, Manitoba).

RESULTS AND DISCUSSION

Amino acid composition/proximate composition. Succinylation did not cause significant changes in the essential amino acid profile of oat protein except a slight decrease in phenylalanine. Acetylated protein had slightly lower levels of valine, isoleucine and phenylalanine and a significantly lower cystine content. Consequently, the total essential amino acid content was lowered by acetylation (Table 1).

Linoleate treatment caused a considerable reduction in protein content, mainly due to the incorporation of 8% linoleate which also accounted for the higher fat content. Trypsin treatment did not cause a significant change in the proximate composition (Table 2).

TABLE 1.

Essential Amino Acid Content of Acylated Oat Proteins (g/100 g protein)[a]

Amino acid[b]	Native (0.0)[c]	Acetylated (50.2)[c]	Acetylated (86.1)[c]	Succinylated (32.8)[c]	Succinylated (61.9)[c]
Lysine	3.5	3.7	3.7	3.5	3.2
Threonine	3.0	3.2	3.2	3.1	3.6
Cystine	2.9	1.2	1.3	2.7	3.0
Methionine	1.4	1.5	1.5	1.6	1.5
Valine	5.1	4.2	4.0	5.1	5.2
Isoleucine	3.7	3.1	2.9	3.4	3.5
Leucine	7.5	7.4	7.2	7.5	7.6
Tyrosine	4.5	4.4	4.2	4.0	4.4
Phenylalanine	6.2	5.3	5.4	5.4	5.5
Total	37.8	34.0	33.4	36.3	37.5

[a]Average of duplicate determinations.
[b]Tryptophane was not determined.
[c]% modified.

*To whom correspondence should be addressed.

TABLE 2.

Proximate Composition of Native and Modified Oat Protein Concentrates (% dry wt basis)[a]

Protein concentrate	Protein (N X 6.25)	Crude fat	Carbo-hydrate	Ash
Native	64.0	0.5	32.0	4.4
Linoleate-treated	49.8	9.2	33.4	7.6
Trypsin-hydrolyzed	59.9	0.5	34.2	5.2

[a]Average of duplicate determinations.

Gel chromatography. To study the molecular weight distribution of the modified oat proteins, gel filtration chromatography was carried out on a Sephacryl S-200 column using 2 M sodium thiocyanate as eluant.

The native proteins were fractionated into six peaks (Fig. 1A). The void volume peak represents high molecular weight aggregates, and the 58, 36 and 22 kDa peaks correspond, respectively, to the dimer and two monomers of oat globulins, the major protein fraction in oats. Both succinylation (Fig. 1B) and acetylation (Fig. 1C) caused some dissociation of aggregated proteins in the void volume peak, and in the case of succinylation, the globulin dimers.

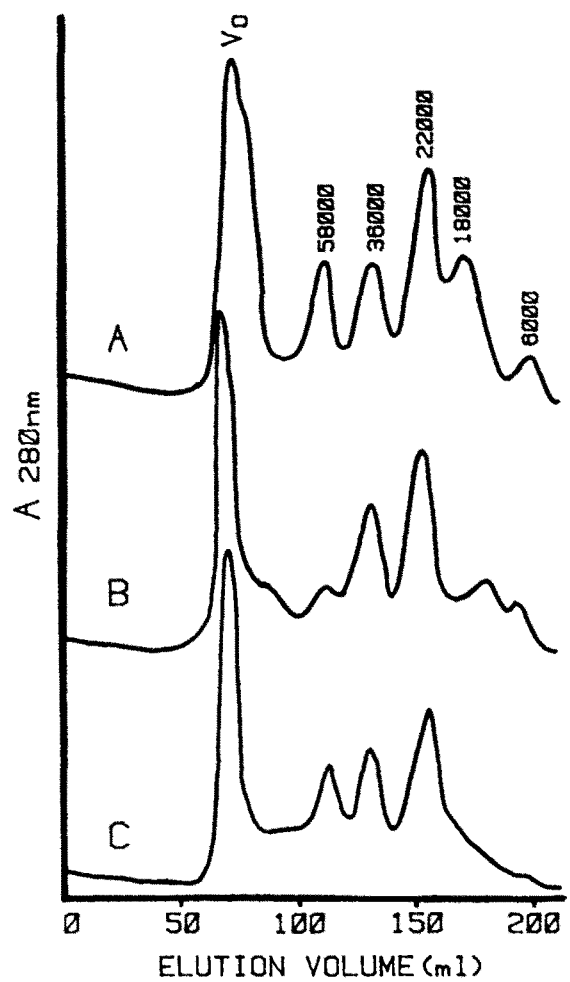

FIG. 1. Gel filtration chromatography of oat protein on Sephacryl S-200 column (2.5 X 45 cm), using 2M sodium thiocyanate as eluant. Flow rate was 25 ml/hr. The numbers above the peaks represent the estimated molecular weights of the fractions. V_o, void volume; A, native; B, succinylated (61.9%); C, acetylated (86.1%).

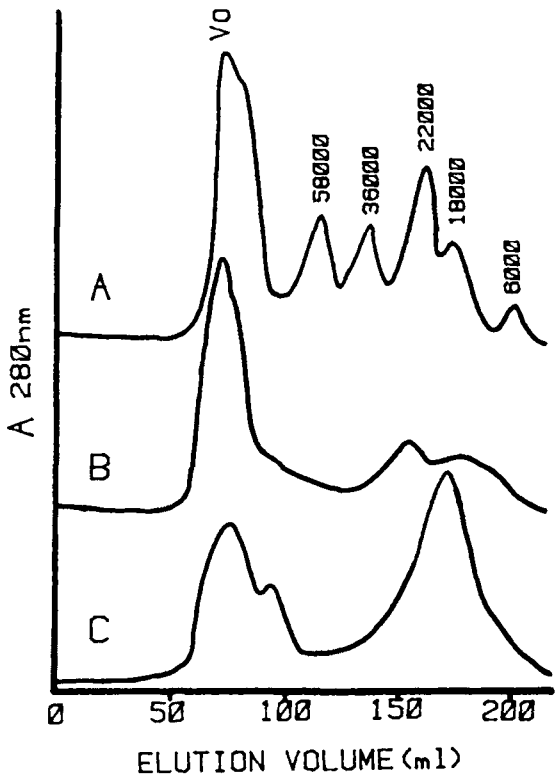

FIG. 2. Gel filtration chromatography of oat protein on Sephacryl S-200 column (2.5 X 45 cm), using 2M sodium thiocyanate as eluant. Flow rate was 25 ml/hr. The numbers above the peaks represent the estimated molecular weights of the fractions. V_o void volume; A, native; B, linoleate-treated; C, trypsin-hydrolyzed.

Linoleate treatment (Fig. 2B) led to very poor resolution, probably due to an increase in net negative charge from the fatty acid carboxyl groups, and a disruption of hydrophobic forces by the fatty acid salts; both conditions can promote interactions between the protein and gel matrix. Trypsin hydrolysis (Fig. 2C) caused a general reduction in the molecular size of the polypeptides, suggesting that trypsin did not act preferentially on any specific class of oat protein.

Solubility. Figure 3 shows the pH-solubility curves of unmodified and acylated oat proteins. Typical bell-shaped curves were observed with minimum solubility near pH 5. When compared to the control, acetylation caused some increase in solubility while succinylation led to significant improvement in solubility, particularly near neutral pH.

When compared to the control, linoleate-treated oat protein had slightly better solubility above pH 4, while trypsin treatment led to a significant improvement in solubility at acidic pH, and a slight reduction at alkaline pH (Fig. 4). The improvement in solubility could be due to a reduction in molecular size by

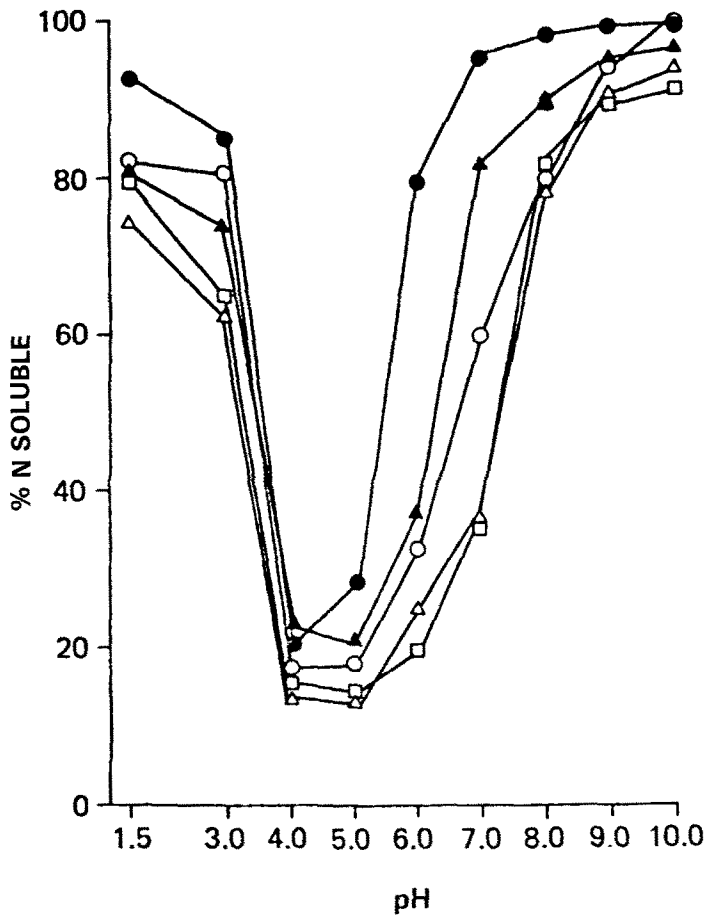

FIG. 3. Nitrogen solubility curves of acylated oat protein concentrate. □, native; ○, succinylated (32.8%); ●, succinylated (61.9%); △, acetylated (50.2%); ▲, acetylated (86.1%).

enzyme hydrolysis and a disruption of hydrophobic interaction by linoleate treatment which affects solubility by increasing interfacial area (10).

Emulsifying properties. Data on emulsifying capacity (EC), emulsifying activity index (EAI) and emulsion stability index (ESI) of the acylated oat proteins are presented in Table 3. Acylation improved both EC and EAI, and ESI was greatly improved even at a lower level of modification.

Linoleate and trypsin treatments also led to a significant improvement in emulsifying properties of oat proteins, particularly the emulsion stability (Table 3).

TABLE 3.

Emulsifying Properties of Native and Modified Oat Proteins[a]

Protein concentrate	EC[b] (ml oil/g protein)	EAI[c] (m^2/g)	ESI[d] (min)
Native	1993	40.4	6.2
Acetylated, 50.2%[e]	2116	40.8	13.0
Acetylated, 86.1%[e]	2280	43.6	16.5
Succinylated, 32.8%[e]	2132	44.0	19.2
Succinylated, 61.9%[e]	2352	56.2	21.0
Linoleate-treated	2372	56.8	120.0
Trypsin-hydrolyzed	2128	49.4	35.0

[a]Average of three determinations.
[b]Emulsifying capacity.
[c]Emulsifying activity index.
[d]Emulsion stability index.
[e]% modified.

Water and fat absorption. Acylation caused a marked decrease in bulk density which could contribute to the high fat binding capacity. The mechanism of fat absorption has been attributed mostly to physical entrapment of oil (17). In contrast, water hydration capacity was decreased by acylation, which could be attributed to the increase in protein solubility and the elimination of the charged ε-amino groups of lysine (Table 4).

The water hydration capacity of oat protein was improved by both linoleate and trypsin treatments. The fat binding capacity was increased by linoleate treatment and decreased by enzyme hydrolysis, which may also be attributed to the changes in bulk density of the samples (Table 4).

Foaming properties. Foamability of oat proteins was increased by both acetylation and succinylation, but foam stability was decreased. Both foamability

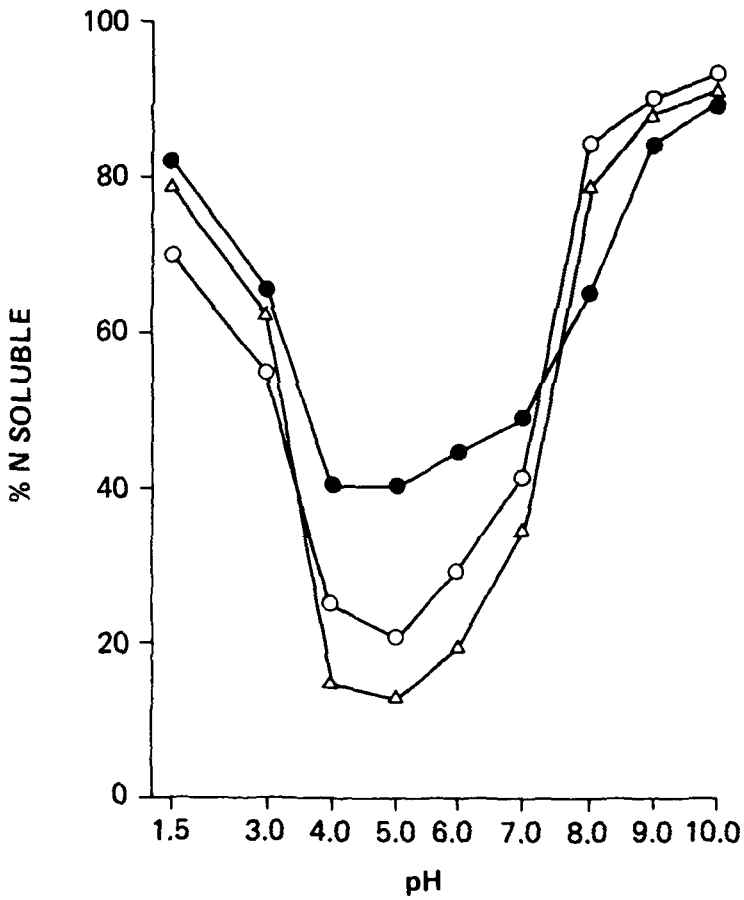

FIG. 4. Nitrogen solubility curves of linoleate-and trypsin-treated oat concentrates. △, native; ○, linoleate-treated; ●, trypsin-hydrolyzed.

TABLE 4.

Water and Fat Binding Properties of Native and Modified Oat Proteins[a]

Protein concentrate	Bulk density (g/ml)	WHC[b] (ml/g)	FBC[c] (ml/g)
Native	0.31	2.00	2.10
Acetylated, 50.2%[d]	0.14	1.95	4.95
Acetylated, 86.1%[d]	0.11	1.35	6.35
Succinylated, 32.8%[d]	0.13	1.65	5.25
Succinylated, 61.9%[d]	0.10	1.45	6.30
Linoleate-treated	0.20	2.15	3.50
Trypsin-hydrolyzed	0.46	2.20	1.40

[a]Average of duplicate determinations.
[b]Water hydration capacity.
[c]Fat binding capacity.
[d]% modified.

TABLE 5.

Foaming Properties of Acylated Oat Proteins[a]

Protein concentrate	Foamability (%)	Foam stability (%)	
		30 min	60 min
Native	85	70	53
Acetylated, 50.2%[b]	100	56	50
Acetylated, 86.1%[b]	90	42	35
Succinylated, 32.8%[b]	125	35	25
Succinylated, 61.9%[b]	95	30	22
Linoleate-treated	95	78	67
Trypsin-hydrolyzed	110	72	60

[a]Average of duplicate determinations.
[b]% modified.

and foam stability decreased with an increase in the extent of modification (Table 5). This could be due to an excessive increase in charge to hinder protein-protein interaction at the interface. For linoleate and trypsin treatments, both foamability and foam stability were improved (Table 5).

Gelling property. Oat protein forms a self-supporting gel under certain pH, ionic strength and heating conditions. Table 6 shows that unmodified oat protein concentrate formed a fairly weak gel at both pH 8.5 and 9.5. The gel hardness was greatly increased by both acetylation and succinylation, particularly at lower pH. Trypsin treatment, on the other hand, led to a weak gel structure, probably due to reduction in

TABLE 6.

Effect of Chemical Modification on Gel Hardness[a]

Protein concentrate	Gel hardness, Newtons	
	pH 8.5	pH 9.5
Native	1.59	1.75
Acetylated protein concentrate	3.88	1.93
Succinylated protein concentrate	4.28	2.02
Trypsin-treated protein concentrate	1.02	0.09

[a]Average of 6 determinations.

the size of the protein molecules which may no longer be able to associate to form a strong gel matrix.

Performance in comminuted meat system. Since acylated proteins have good emulsifying and binding properties, a study was conducted to assess the performance of succinylated oat proteins in beef and pork wiener systems.

The cook yield in beef wiener formulations decrased with increasing level of substitution (Fig. 5). When compared to the unmodified oat protein, subsitution with succinylated protein led to an improvement in cook yield, particularly at the 5 and 10% levels of substitution. In the pork wiener system (data not shown), succinylation did not improve the cook yield over the unmodified protein.

Succinylation appeared to improve the texture of the substituted pork wiener batters as indicated by cohesiveness and firmness measurements. Substitution with acylated protein led to an initial increase in cohesiveness, followed by a decrease at higher levels. When compared to the unmodified sample, succinylation improved the cohesiveness at all levels of substitution (Fig. 6). In pork wieners, substitution with either succinylated or unmodified oat protein led to a progressive decrease in firmness (Fig. 7). However, pork wieners substituted with succinylated protein had firmness values higher than those substituted with unmodified protein, indicating an improvement by succinylation. In the beef system, however, succinylation caused a slight decrease in both cohesiveness and firmness (data not shown). The results indicate a difference between beef and pork in response to oat protein substitution.

Two succinylated oat protein products, globulin and oat isolates, were compared to pea and soy protein isolates with respect to their performance in the model wiener systems (Table 7). A good cook yield was obtained for all proteins, except succinylated oat isolate in the pork wiener system. Similar to oat proteins, pea and soy proteins gave better texture in pork than in beef wiener system. In general, the modified oat protein had performance characteristics quite comparable to the other two plant proteins studied.

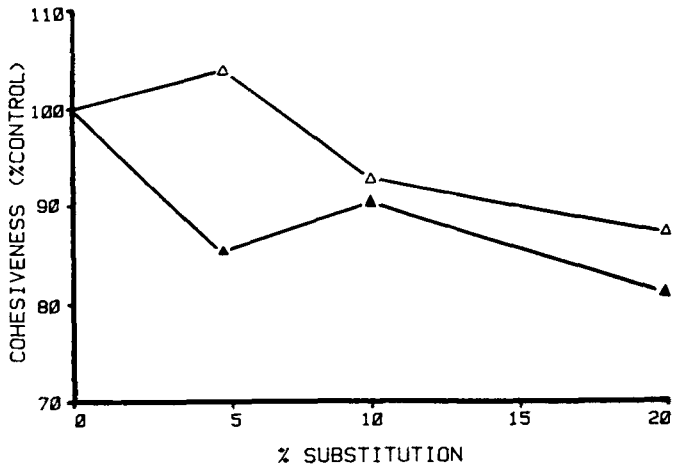

FIG. 6. Effect of substituting meat with native and succinylated oat protein on cohesiveness of pork wieners. ▲, native; △, succinylated.

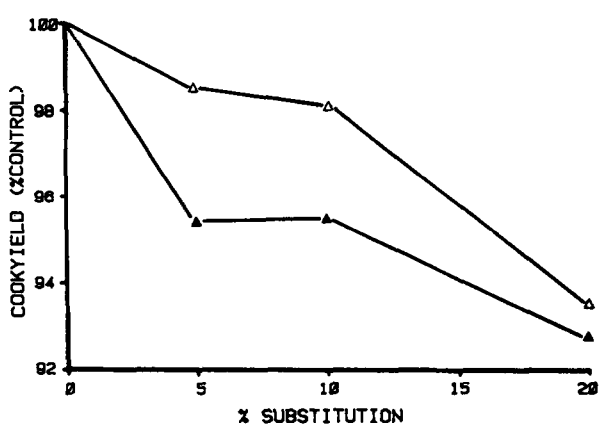

FIG. 5. Effect of substituting meat with native and succinylated oat protein on cook yield of beef wieners. ▲, native; △, succinylated.

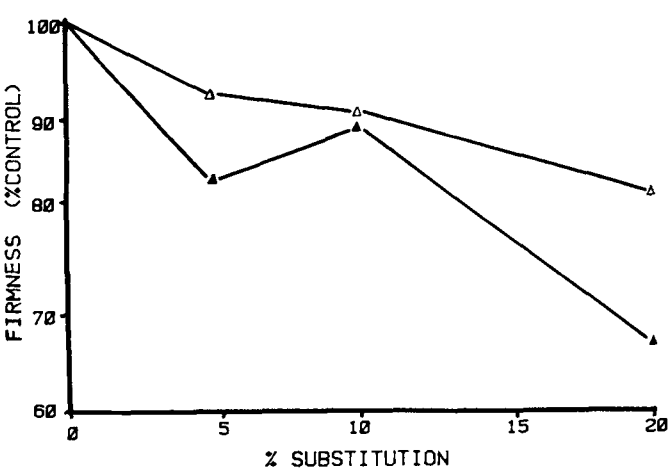

FIG. 7. Effect of substituting meat with native and succinylated oat protein on firmness of pork wieners. ▲, native; △, succinylated.

TABLE 7.

Performance of Some Vegetable Proteins in Model Wiener Systems[a]

Protein	Wiener	Cook yield (% control)	Cohesiveness (% control)	Firmness (% control)
Pea protein isolate	Beef	95.6	82.6	77.5
	Pork	95.6	81.7	82.8
Soy protein isolate	Beef	96.5	81.0	83.0
	Pork	95.2	105.3	96.2
Succinylated oat globulin	Beef	94.2	75.2	76.9
	Pork	96.1	78.7	88.1
Succinylated oat isolate	Beef	95.5	73.3	70.5
	Pork	91.2	103.8	94.0

[a]Average of three determinations.

The present data show that some functional properties of oat proteins, particularly solubility and emulsifying properties, can be significantly improved by acylation, linoleate or trypsin treatment. Most of these improvements can be attributed to changes in the physicochemical properties of oat proteins resulting from altered conformation and an increase in net charge. The functionality improvement will enhance the use of this nutritionally superior protein in many fabricated food systems.

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