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Some Functional Properties of Acetylated and Succinylated Oat Protein Concentrates and a Blend of Succinylated Oat Protein and Whey Protein Concentrates

Rathy Ponnampalam,[a] Gilles Goulet,[b] Jean Amiot,[a] Bruno Chamberland[a] & Germain J. Brisson[b]

[a] Département de Sciences et Technologie des Aliments.
[b] Département de Zootechnie, and Centre de Recherche en Nutrition, Pavillon Paul-Comtois, Université Laval, Ste-Foy, Québec, Canada G1K 7P4

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ABSTRACT

Protein extract from oat groats was acylated to various degrees with acetic and succinic 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, and changes in functional properties were monitored. Size exclusion-HPLC showed some dissociation of oat proteins with acylation. Nitrogen solubility, emulsifying properties, water hydration and fat binding capacities were improved by acylation, and the effect was more pronounced with succinylation. Although the nitrogen solubility of the blend (SPC-76 and whey protein concentrate, 1:1 ratio on protein basis) was slightly lower than that of the whey protein concentrate (WPC), the other functional properties such as emulsifying properties, water hydration, and fat binding capacities were improved in comparison with that of the WPC. The results suggest that acylated oat protein and the blend may serve as a valuable functional ingredient in emulsion food products.

INTRODUCTION

Oat protein concentrates are recognised to possess good nutritional quality (Goulet *et al.*, 1986) and unique physico-chemical and functional properties

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(Ma, 1983; Ma & Harwalkar, 1984). However, oat proteins are not used extensively for human consumption. In order to make oat proteins more attractive as potential food ingredients, it would be desirable to further improve their functional properties by chemical modification (Ma, 1984).

Acylation is one of the most effective methods used to improve protein functionality. It has been applied to various plant proteins including those from soy (Franzen & Kinsella, 1976), pea (Johnson & Brekke, 1983), wheat (Grant, 1973), 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 improvements in functional properties were more pronounced with succinylation than acetylation (Franzen & Kinsella, 1976; Ma, 1984).

Goulet *et al.* (1987) reported that acetylated oat proteins gave higher protein efficiency ratio (PER) values than succinylated oat proteins. However, both PER values were lower than that of acylation control. It was also reported that the product obtained by blending succinylated oat protein and whey protein concentrates gave a better PER value than the untreated oat protein concentrate.

The objective of the present study was to determine some chemical and functional properties of acylated oat protein concentrates, and the blend of succinylated oat protein and whey protein concentrates.

MATERIALS AND METHODS

Oat groats (*Avena sativa* L., variety Hinoat) were obtained from the Ottawa Research Station, Agriculture Canada, Ottawa, Canada. The groats were ground just before use in a hammer mill to pass through a 3-mm screen. Whey protein concentrate (Savorpro 75) was provided by Express Foods Company Inc., Louisville, Kentucky, USA. All other chemicals were reagent grade.

Preparation of acylated oat protein concentrates

Acylated oat protein concentrates were prepared according to the method described by Goulet *et al.* (1987). Protein extract from ground 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. The unmodified oat protein concentrate (UPC) was prepared in a similar manner except that no acylating agent was added. The freeze-dried products were ground in a cyclone mill (Cyclotec 1093 Sample Mill, Höganas, Sweden) to pass a 0·5-mm screen.

Chemical analyses

The unmodified and acylated oat protein concentrates and whey protein concentrate (WPC) were analysed for total nitrogen by the Kjeldahl method and lipid, moisture, and ash contents by the AACC (1971) procedures. Reactive groups in unmodified and acylated oat proteins were assayed as follows: available lysine with the dinitrobenzenesulphonate (DNBS) method of Concon (1975) without ether extraction; and sulphydryl groups with Ellman's reagent as modified by Beveridge *et al.* (1974). The abbreviations used in the text will include percentage ε-amino group of lysine acylated to differentiate between the different extents of acylated products.

The blend was prepared by mixing a 1:1 ratio (on protein basis) of succinylated oat protein concentrate (SPC-76) and whey protein concentrate (WPC). The proximate composition of the blend was determined by the above-mentioned methods.

Size exclusion-HPLC of water-soluble proteins

The protein concentrates were dissolved in HPLC water at pH 7·0, stirred for 1 h and filtered through a 0·45 μ filter to obtain water-soluble proteins. The molecular weight distribution profiles of the water-soluble proteins from unmodified and acylated oat protein concentrates were determined by HPLC (system consisted of a LKB 2150 pump and a Rheodyne injection valve equipped with a 20 μl loop) on a TSK-2000SW column according to the method described by Vijayalakshmi *et al.* (1986) with a methanol-modified mobile phase. TSK-2000SW is useful primarily for peptides and low molecular weight proteins.

Functional properties

Functional properties were determined on protein concentrates on an 'as is' basis except for the emulsifying properties (adjusted to an equal protein basis). Nitrogen solubility was measured according to the method described by Ponnampalam *et al.* (1987). Water hydration capacity (WHC) was determined according to the method of Quinn & Paton (1979). The method for determining fat-binding capacity (FBC) was that described by Lin *et al.* (1974). Bulk density was measured according to the method of Narayana & Narasinga Rao (1984). Emulsion stability was given as stability rating and was determined by the method of Acton & Saffle (1970). A turbidimetric method (Pearce & Kinsella, 1978) was used to assess the emulsion activity of the protein concentrates. All data on chemical analyses and functional properties were based on two or more replicates on each of the protein concentrates.

RESULTS AND DISCUSSION

Extent of modification

The extent of acylation of oat protein estimated as the amount of ε-amino group of lysine and sulphydryl group of cysteine acylated is presented in Table 1. With both acetic and succinic anhydrides, ε-amino groups were more acylated than sulphydryl groups. Similar observations have been

TABLE 1
Extent of Acylation of Oat Proteins Treated with Acetic or Succinic Anhydride[a]

Product[b]	*Anhydride*	*Anhydride concentration (g/g protein)*	*% of group acylated*	
			ε-NH_2	*—SH*
APC-37	Acetic	0·031	37·1 ± 1·3	12·5 ± 0·3
APC-76	Acetic	0·092	76·4 ± 1·4	49·0 ± 2·8
SPC-35	Succinic	0·031	35·1 ± 0·8	12·8 ± 0·1
SPC-76	Succinic	0·110	75·7 ± 1·5	43·5 ± 0·7

[a] Mean ± SD of three replicate experiments.
[b] APC-37 and APC-76: acetylated oat protein concentrates; SPC-35 and SPC-76: succinylated oat protein concentrates. The number represents the percentage ε-NH_2 groups acylated.

reported on fish myofibrillar protein (Groninger, 1973) and on cottonseed flour (Choi *et al.*, 1983) with succinic anhydride. The results also indicate that acetic anhydride is more reactive on both ε-amino and sulphydryl groups. A similar finding was reported by Ma (1984) on acylation of the ε-amino group of lysine of oat proteins.

Chemical composition

The protein content was not markedly changed by acylation (Table 2). However, ash content was increased due to a higher content of salt as a result of pH adjustment during acylation. The blend of SPC-76 and WPC was lower in protein and lipid content, but higher in ash content than that of WPC. Lipid content decreased with increase in acylation, and it was postulated 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 (Goulet *et al.*, 1987).

TABLE 2
Chemical Composition of Unmodified Oat Protein, Acylated Oat Protein, Whey Protein, and the Blend on Dry Weight Basis[a]

Product	*Protein* (%)	*Lipid* (%)	*Ash* (%)
UPC	75·5 ± 0·2	5·5 ± 0·3	3·7 ± 0·2
APC-37	76·6 ± 0·1	5·6 ± 0·2	4·2 ± 0·1
APC-76	74·1 ± 0·1	1·3 ± 0·2	5·9 ± 0·1
SPC-35	74·8 ± 0·2	5·1 ± 0·1	4·7 ± 0·1
SPC-76	75·4 ± 0·2	0·9 ± 0·1	6·8 ± 0·1
WPC	78·1 ± 0·2	8·4 ± 0·4	3·2 ± 0·1
Blend[b]	76·1 ± 0·1	4·2 ± 0·3	4·8 ± 0·1

[a] Mean ± SD of two replicate experiments.
[b] SPC-76:WPC = 1:1 on protein basis.

Size exclusion-HPLC of water-soluble proteins

The molecular weight distribution profiles of water-soluble proteins (pH 7·0) from the unmodified as well as acylated oat proteins are shown in Fig. 1. The large peak at elution time 16–17 min represents high molecular weight aggregates, greater than 67 000 daltons, which is beyond the exclusion limit of the column. However, acylated samples were resolved into one or two major peptide peaks with estimated MW of 5660–6400 and 3435–3520 daltons. This suggests that the APC-76 and SPC-76 consisted of two different sizes of protein agglomerates, while the APC-37 and APC-35 consisted of one protein agglomerate (Fig. 1). The lower molecular weight components are peptides and amino acids found in minor amounts in the unmodified as well as acylated concentrates. Grant (1973) reported that succinylation of protein frequently causes splitting of subunits from the quaternary structure of protein agglomerates due to repulsive forces between the negative succinyl groups. However, in this study both acetylation and succinylation caused some dissociation of protein subunits.

Functional properties

The solubilities of the unmodified and acylated oat proteins are presented in Table 3. All the oat proteins had minimum solubility between pH 4 and 5 (data for pH 4 not shown). Acetylation and succinylation improved solubility of oat protein at pH 5 and 7. The blend gave a similar solubility at pH 7 and intermediate solubility at pH 5 compared to SPC-76 and WPC.

The emulsion activity index (EAI) and emulsion stability (ES) of the

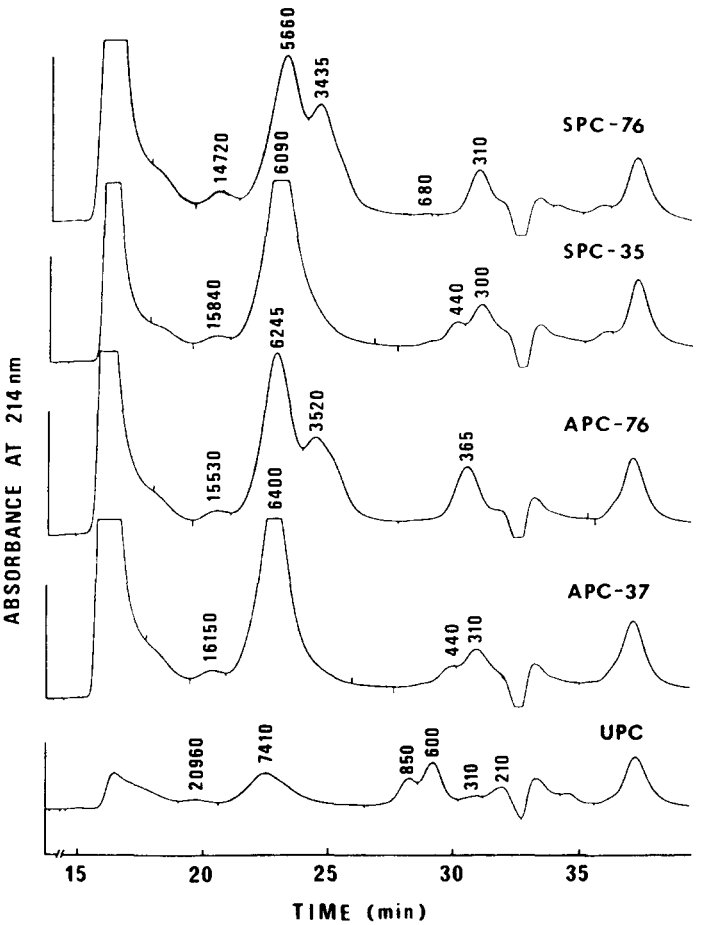

Fig. 1. Size exclusion-HPLC of water-soluble proteins in unmodified and acylated oat protein concentrates.

unmodified and acylated oat proteins, WPC, and the blend are presented in Table 4. EAI and ES of acylated oat proteins were greater than that of UPC. However, succinylated oat proteins had higher EAI and ES than the acetylated samples. The blend showed intermediate values for both EAI and ES in comparison to SPC-76 and WPC. Yamauchi *et al.* (1980) reported that stable emulsions were obtained with WPC at protein concentrations higher than 2%. This was further illustrated by testing the ES of the blend at the protein concentrations of 4% and 6%. The ES of 4% and 6% protein dispersions were 35% and 52%.

Table 4 lists the water hydration (WHC) and fat binding capacities (FBC) of the unmodified and acylated oat proteins. Both acetylation and succinylation resulted in increased WHC compared to that of the UPC, with

TABLE 3
Nitrogen Solubility of Oat Proteins, Whey Protein, and the Blend

Product	*% Nitrogen solubility*[a]		
	pH 3·0	*pH 5·0*	*pH 7·0*
UPC	58·3 ± 0·1	5·1 ± 0·1	23·0 ± 1·0
APC-37	67·3 ± 0·1	6·5 ± 0·1	75·1 ± 1·0
APC-76	50·9 ± 6·0	10·7 ± 0·1	82·3 ± 0·5
SPC-35	44·3 ± 5·0	11·6 ± 0·1	73·6 ± 1·2
SPC-76	39·5 ± 0·1	15·8 ± 0·1	88·0 ± 0·1
WPC	83·9 ± 0·1	79·8 ± 0·9	89·8 ± 1·1
Blend[b]	69·8 ± 2·3	40·9 ± 0·1	88·4 ± 0·1

[a] The data represent the mean of two or three replicate experiments.
[b] SPC-76:WPC = 1:1 on protein basis.

succinylation giving the greater increase. Johnson & Brekke (1983) reported that acetylation and succinylation of pea isolates resulted in increased water adsorption compared to that of the untreated isolate. Acylation can cause unfolding of the protein due to electrostatic repulsions between the added carboxyl groups and the neighbouring native carboxyl groups, producing more protein–water interactions and less protein–protein interactions

TABLE 4
Emulsifying Properties, Water Hydration and Fat Binding Capacities, Bulk Density of Oat Protein Concentrates, WPC and the Blend[a]

Product	*EAI*[b] (m^2/g)	*ES*[c] (%)	*WHC*[d]	*FBC*[e]	*Bulk density* (*g/ml*)
UPC	32·3 ± 1·6	24·6 ± 0·5	1·8–2·0	127·2 ± 0·7	0·45 ± 0·01
APC-37	40·2 ± 1·1	31·0 ± 1·2	2·0–2·2	166·4 ± 1·3	0·50 ± 0·02
APC-76	48·6 ± 1·0	33·3 ± 1·3	3·0–3·2	137·6 ± 1·2	0·52 ± 0·01
SPC-35	44·2 ± 1·4	33·9 ± 0·4	3·2–3·4	141·9 ± 1·4	0·50 ± 0·01
SPC-76	50·1 ± 1·8	36·6 ± 0·7	3·4–3·6	132·2 ± 0·5	0·53 ± 0·01
WPC	52·2 ± 1·2	17·8 ± 0·1	0·8–1·0	113·3 ± 2·0	—
Blend[f]	46·7 ± 1·0	24·0 ± 0·1	2·3–2·4	147·9 ± 13·8	—

[a] Mean ± SD of two or three replicate experiments.
[b] Emulsion activity index (EAI). 1% protein emulsion.
[c] Emulsion stability (ES) was determined using stability ratings.
[d] Water hydration capacity (WHC), g of H_2O/g of product.
[e] Fat binding capcity (FBC), g of oil/100 g product on a 14% moisture basis.
[f] SPC-76:WPC = 1:1 on protein basis.

(Franzen & Kinsella, 1976). In addition, the increased net negative charge of succinylated proteins would especially promote protein–water interaction (Kinsella, 1976). The WHC of WPC was low in comparison to all the oat protein concentrates. However, the blend showed an intermediate value for WHC in comparison to SPC-76 and WPC.

The FBC increased with acylation of oat proteins. The increase was more pronounced at lower extent of modification. The mechanism of oil absorption, according to Kinsella (1976) relies mostly on the physical entrapment of oil by capillary attraction. However, results obtained by Voutsinas & Nakai (1983) suggest that hydrophobicity of proteins is likely to play a major role in fat absorption. The improvement of fat binding and water hydration capacities of acylated oat protein concentrates may result from the unfolding of protein which allows the presentation of appropriate hydrophobic and hydrophilic groups at an oil–water interface as suggested by Morr (1979) for whey–protein molecules. The decrease in FBC with increase in extent of modification could be due to a slight increase in hydrophilic groups at an oil–water interface. In contrast, the FBC of the blend was considerably higher than would be expected on the basis of the data for the individual components. A similar observation was made by Patel *et al.* (1981) on pea protein concentrate–cheese whey blends.

The bulk density of oat protein increased with extent of acylation. Unfolding of polypeptides and replacement of short range attractive forces in the native molecule with short range repulsive ones by acylation could result in higher bulk density. Similar observations were made on soy protein and winged bean flour (Franzen & Kinsella, 1976; Narayana & Narasinga Rao, 1984).

The data on functional properties of the UPC and acylated proteins are in general agreement with those reported by Ma (1984). However, Ma (1984) observed a decrease in WHC and bulk density with acylation of oat proteins.

In general, the present data indicate that acylation improved some functional properties of oat protein, including solubility, emulsifying properties, water hydration and fat binding capacities. The improvement of oat protein functionality could enhance the value of oat protein and make it more competitive with other widely used food proteins. The acylated oat protein with the above functional properties should find application in many fabricated food systems such as meat emulsion products, salad dressings, and baked products (Ma, 1984).

The results of the whey–oat protein blend suggest favourable interaction between succinylated oat proteins and whey proteins. A 4% protein emulsion with 4% lipid in the final product pasteurised at 100°C for 30 min showed no evidence of protein precipitation or coagulation after one week of storage at room temperature. Addition of 10% sucrose showed no

evidence of protein precipitation or coagulation under similar conditions. Freeze-drying the blend with 10% sucrose increased the nitrogen solubility to 97%. Goulet *et al.* (1987) reported that acylation of oat protein gave a lower protein efficiency ratio (PER) value compared to that of the unmodified oat protein, and the effect was more pronounced with succinylation. It was also reported that the effect of supplementing SPC-76 with WPC improved the PER of the SPC-76. This was primarily attributed to the high lysine content of the WPC in comparison to SPC-76 which compensated more than the amount of ε-amino group of lysine succinylated. This demonstrates that the blend can serve as an excellent base for the preparation of emulsion food products such as protein beverages with good nutritional quality.

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