In Vitro and in Vivo Digestibilities of Succinylated Cheese Whey Protein Concentrates

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In vitro and in vivo amino acid digestibilities and true protein digestibility of cheese whey protein concentrates succinylated at various levels were determined. In vitro lysine, cysteine, methionine, and threonine digestibilities were greatly reduced at high levels of succinylation. The decrease in in vitro lysine, cysteine, and threonine digestibilities was related to the percent of these amino acids bound to succinic anhydride, suggesting that the succinyl amino acid bonds resist pancreatin hydrolysis. Contrary to the in vitro results, in vivo digestibility of almost all essential amino acids was slightly lowered at high levels of succinylation. This discrepancy may be explained by the absorption of succinyl amino acids in vivo. True protein digestibility reflected in vivo amino acid digestibility and was 3–6% lower for the highly succinylated proteins.

There is current interest on the chemical modification of proteins. Chemical modification such as succinylation affects the protein's physicochemical and functional properties and thereby improves its utilization in food systems. Numerous proteins including those from soy (Franzen and Kinsella, 1976a; Melnychyn and Stapley, 1973), fish (Chen et al., 1975; Miller and Groninger, 1976), leaf (Franzen and Kinsella, 1976b), single cell (McElwain et al., 1975), casein (Creamer et al., 1971), and whey (Thompson and Reyes, 1980) have been succinylated. In general, the succinylated proteins have improved solubility, emulsifying capacity, heat stability, and other functional properties.

The succinylation reaction follows the carbonyl addition pathway (Means and Feeney, 1971). Succinylation is possible on all nucleophilic groups of amino acid residues such as the ϵ-amino group of lysine, the hydroxyl group of serine and threonine, the sulfhydryl group of cysteine, the phenol group of tyrosine, and the imidazole group of histidine. However, the rates of reaction depend upon the rate of nucleophilic attack which in turn are dependent on the pK values of the nucleophiles and their steric availabilities. Lysine's ϵ-amino group reacts most readily due to its relatively low pK and steric availability for contact with the applied reagent. The tyrosine phenolic group is less reactive to succinylation due to higher pK value, steric hindrance to reaction (Means and Feeney, 1971), and spontaneous hydrolysis of tyrosyl ester linkage in aqueous media (Chang and Sun, 1978; Gounaris and Perlmann, 1967; Riordan and Vallee, 1964). Serine and threonine hydroxyl groups are weak nucleophiles and are not easily acylated. Succinylation of histidine and cysteine residues are seldom observed because the products readily hydrolyze in aqueous solution (Grant, 1973; Stadtman, 1955).

Franzen (1976) and Gounaris and Perlmann (1967) compared the rate of succinylation of lysine and other amino acid residues of bovine serum albumin and pepsinogen, respectively. Succinylation of sulfhydryl and hydroxyl groups were low when unreacted lysine residues were present, but the rate of reactions increased once lysine residues had completely reacted. When large molar excesses of succinic anhydride to protein were used, virtually all free amino groups and lesser numbers of sulfhydryl groups and other functional groups were succinylated. Indeed, 27–81% of the sulfhydryl groups and 66–97% of ϵ-amino groups in bovine serum albumin were succinylated when different levels of anhydride were used (Franzen, 1976). Twenty-one percent of the total hydroxy amino acids in bovine serum albumin were also succinylated under conditions whereby most of the lysyl residues were succinylated (Chang and Sun, 1978).

In vitro studies showed that succinylated proteins had low lysine digestibility due to the resistance of the succinyllysyl bonds to hydrolysis by pancreatic enzymes (Chen et al., 1975; McElwain et al., 1975; Matoba and Doi, 1979). In vivo, they also had low protein efficiency ratio (Groninger, 1973), which was attributed to the rat's inability to utilize succinyllysine. However, Creamer et al. (1971) attributed the low protein quality of a similar acylated protein (acetyl casein) to unavailabilities of lysine and sulfur-containing amino acids; when they supplemented acetyl casein with lysine, the added lysine did not make acetyl casein as nutritious as casein and signs of sulfur amino acid deficiency in the experimental animals were observed.

It is unclear from the previous studies on succinylated proteins whether availabilities of other amino acids are also affected, especially those that are capable of binding with succinic anhydride. In this paper, the amino acid availabilities of succinylated cheese whey concentrates were estimated by in vitro and in vivo tests.

EXPERIMENTAL SECTION

Materials. Cottage cheese whey was obtained from Gay Lea Foods Co., Weston, Ontario, Canada. Succinic anhydride was purchased from Matheson Coleman and Bell Co., a commerical heat-coagulated lactalbumin (LA) was from Champlain Industries, Stanbridge, Quebec, Canada, pepsin and pancreatin were from Fisher Scientific Co., Toronto, Ontario, Canada, and vitamin-free casein (CA) was from ICN, Cleveland, OH.

Sample Preparation. Cheese whey was heated at 95 °C for 15 min, at pH 4.6, and the heat-coagulated precipitate was freeze-dried to yield whey concentrates (WC). Succinylated whey concentrates (SWC) with four different levels of succinylation were prepared by using 0.05, 0.09, 0.46, or 2.04 g of succinic anhydride/g of WC and referred to respectively as SWC.05, SWC.09, SWC.46, or SWC2.04. Succinylation was according to the procedure of Thompson and Reyes (1980) with the following modifications. After succinylation at pH 8, the dispersion was adjusted to pH 4 and centrifuged. The precipitate was made into a 2.5% dispersion with distilled water, adjusted to pH 8, stirred for 15 min, adjusted to pH 4, and centrifuged. The precipitate was redispersed in distilled water to a 5% dis-

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Table I. Chemical Composition of Unmodified and Succinylated Cheese Whey Protein Concentrate (Percent Dry Basis)

	WC[a]	SWC.05[a]	SWC.09[a]	SWC.46[a]	SWC2.04[a]
protein	75.4	92.3	93.0	90.7	88.3
ash	2.5	0.4	0.5	0.5	0.5
carbohydrates	22.1	7.1	6.1	7.2	5.9
sodium	0.3	0.1	0.1	0.1	0.2
residual succinic anhydride[b]	0	0.2	0.4	1.6	5.3

[a] WC = unmodified control; SWC.05, SWC.09, SWC.46, and SWC2.04 = succinylated at 0.05, 0.09, 0.46, and 2.04 g of succinic anhydride/g of WC, respectively. [b] Analyzed as succinic acid.

persion, stirred for 15 min, centrifuged, and freeze-dried.

Chemical Analysis. Moisture, ash, nitrogen, and carbohydrates were determined by standard AOAC (1980) procedures, sodium content was determined by atomic absorption spectrophotometry, and residual succinic anhydride was determined as succinic acid according to Boehringer Mannheim (1979). Amino acids were measured by using a Beckman Model 120 amino acid analyzer after acid hydrolysis of the sample with 6 N HCl for 24 h at 110 °C. Lysine bound to succinic anhydride was determined by the fluoro-2,4-dinitrobenzene (FDNB) method as described by Franzen and Kinsella (1976b). Sulfhydryl groups bound to succinic anhydride were analyzed by Ellman's procedure as modified by Beveridge et al. (1974) and the succinylated hydroxy amino acids by the alkaline hydroxylamine reaction of Gounaris and Perlmann (1967).

In Vitro Digestibility Test. Pepsin and pancreatin digestibility tests were carried out in duplicate according to the method of Akeson and Stahmann (1964) as modified by Li-Chan et al. (1979). Amino acids released from the test proteins after 27 h of hydrolysis were measured by a Beckman amino acid analyzer.

In Vivo Digestibility Test. Ten percent protein diets were prepared with either WC, SWC with four levels of succinylation, CA, or LA as the protein source. A nitrogen-free diet was also prepared to determine metabolic N and amino acid excretion. The diet composition was according to AOAC (1980) specifications.

Six male weanling rats (Wistar strain, Woodlyn Farms, Guelph, Ontario Canada), 92.9 ± 0.21 g of mean body weight, were allocated to each experimental diets and fed for 14 days. All diets and water were provided ad libitum. The rats were housed in individual hanging, wire-meshed stainless steel cages in a room kept at 20–23 °C and on a 12 h (0800–2000 h) light–dark cycle. On day 7, rats were transferred to stainless steel metabolic cages which allow separate collection of urine and feces. Feces were collected on days 13 and 14 and stored at −20 °C until needed for analysis. Food intakes were recorded during the feeding trial.

Feces were pooled to provide two samples per treatment group. Each pooled sample, comprised of collections from three rats, was analyzed in duplicate for nitrogen and amino acid contents. Digestibility of individual amino acids was calculated by using the equation of Bragg et al. (1969):

$$\% \text{ digestibility} = \frac{\text{AAI} - (\text{FAA} - \text{MFAA})}{\text{AAI}} \times 100$$

where AAI = total amino acid intake, FAA = fecal amino acid, and MFAA = metabolic fecal amino acid.

The mean amino acid digestibility (MAA) was the average of the individual amino acid digestibility values of the protein.

True protein digestibility (TPD) was calculated as (Bressani, 1977)

$$\text{TPD} = \frac{\text{NI} - (\text{FN} - \text{MN})}{\text{NI}} \times 100$$

Table II. Essential Amino Acid Composition of Unmodified and Succinylated Cheese Whey Protein Concentrate (Grams per 100 Grams of Protein)[a]

amino acid	WC[b]	SWC.05[b]	SWC.09[b]	SWC.46[b]	SWC2.04[b]
Lys	9.3	9.6	9.0	8.2	7.7
Thr	4.5	4.3	4.5	4.2	4.3
Cys	2.3	2.1	2.3	2.5	2.2
Met	1.4	1.2	1.1	1.1	1.2
Val	5.9	5.8	5.7	5.5	5.4
Ile	4.8	4.9	5.0	4.6	5.5
Leu	11.4	11.4	11.7	11.6	11.8
Tyr	4.2	4.1	4.1	4.1	4.4
Phe	4.4	4.2	4.2	4.2	4.1

[a] Tryptophan not analyzed. [b] For the abbreviations, see Table I.

Table III. Extent of Succinylation of Cheese Whey Protein Concentrate (WC) When Reacted with Different Levels of Succinic Anhydride (SA)

g of SA/g of WC	% of groups succinylated		
	ϵ-NH_2	-SH	-OH
0.05	15	7	2
0.09	37	21	5
0.46	74	37	11
2.04	83	51	27

where NI = total nitrogen intake, FN = fecal nitrogen, and MN = metabolic fecal nitrogen.

The in vitro and in vivo digestibility data were subjected to analysis of variance and Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical Composition. All SWCs had higher protein and lower ash and carbohydrate contents than WC (Table I) due to the solubilization of carbohydrate and ash during pH manipulation and the washing process. The latter process also removed much of the residual sodium and succinic anhydride. More residual succinic anhydride in the highly succinylated than in the low succinylated samples was due to the larger quantities of succinic anhydride used in the preparation of the former. Obviously, additional washing steps are necessary for the highly succinylated samples. Increased residual succinic anhydride diluted the protein content of the SWC.

In general, succinylation did not affect the amino acid composition of the proteins except for lysine; it was lowered as the degree of succinylation increased (Table II). Groninger and Miller (1979) had similar observations and attributed this to incomplete deacylation of succinyllysine during acid hydrolysis. All SWCs yielded approximately the same amounts of hydroxy amino acids and cysteine after acid hydrolysis. Unlike lysine, therefore, succinylation did not affect the hydrolysis of the bound hydroxy amino acids and cysteine to free amino acids or destroy the amount of these amino acids in the WC.

The succinylated amino groups ranged from 15 to 83% as different levels of succinic anhydride reacted with WC (Table III). Sulfhydryl and hydroxy groups were also

Table IV. In Vitro Amino Acid Digestibilities[a]

amino acid	g of amino acid released/100 g of protein hydrolyzed				
	WC	SWC.05	SWC.09	SWC.46	SWC2.04
Lys	4.68 ± 0.10^{x}	3.33 ± 0.31^{y}	2.79 ± 0.10^{y}	0.94 ± 0.01^{z}	0.08 ± 0.01^{v}
Thr	0.47 ± 0.04^{x}	0.44 ± 0.04^{x}	0.38 ± 0.04^{x}	0.34 ± 0.04^{x}	0.26 ± 0.03^{x}
Cys	0.22 ± 0.01^{x}	0.16 ± 0.01^{y}	0.15 ± 0.0^{yz}	0.12 ± 0.01^{z}	*b*
Met	0.69 ± 0.05^{x}	0.72 ± 0.07^{x}	0.73 ± 0.05^{x}	0.49 ± 0.01^{y}	0.47 ± 0.02^{y}
Val	0.55 ± 0.04^{x}	0.56 ± 0.06^{x}	0.62 ± 0.04^{x}	0.73 ± 0.04^{x}	0.70 ± 0.01^{x}
Ile	0.44 ± 0.02^{x}	0.41 ± 0.04^{x}	0.47 ± 0.02^{x}	0.66 ± 0.02^{y}	0.76 ± 0.03^{y}
Leu	2.59 ± 0.12^{x}	2.61 ± 0.26^{x}	2.80 ± 0.08^{x}	2.85 ± 0.10^{x}	2.81 ± 0.07^{x}
Tyr	1.68 ± 0.07^{x}	1.49 ± 0.14^{x}	1.54 ± 0.07^{x}	1.80 ± 0.03^{x}	1.84 ± 0.06^{x}
Phe	1.62 ± 0.01^{x}	1.49 ± 0.13^{x}	1.55 ± 0.07^{x}	1.68 ± 0.01^{x}	1.72 ± 0.06^{x}

[a] For the abbreviations, see Table I. Mean ± SEM. Means with different superscripts in the same row are significantly different ($P < 0.05$). [b] Not detected.

succinylated but to a lesser extent than the amino groups at each level of succinylation. This reflected the lower reactivity of sulfhydryl and hydroxy groups to acylation. At the highest level of succinylation, approximately 50 and 27% of sulfhydryl and hydroxy groups, respectively, were bound compared to 83% of bound amino groups, in agreement with other researchers. Sulfhydryl and amino groups of myosin were succinylated at the 50 and 90% levels, respectively (Oppenheimer et al 1967). Groninger and Miller (1979) succinylated 32–35% of the sulfhydryl groups of myofibrillar proteins under conditions where 23–75% of the amino acid groups were acylated. Chang and Sun (1978) succinylated all of the lysine residues and 21% of the hydroxy amino acid residues of bovine serum albumin.

In Vitro Digestibility. Amino acid analysis after acid hydrolysis determines the amino acid content of a protein without any indication of the nutritional availability of the amino acids. The pepsin–pancreatin digestion test determines the relative availability of the amino acids for absorption, by measuring the relative ease of release of the amino acids after subjecting the test protein to enzymatic hydrolysis.

The four SWCs and WC were hydrolyzed according to the method described earlier. The grams of essential amino acid released per 100 g of protein hydrolyzed are presented in Table IV. From the comparison of the four SWCs and WC, succinylation affected the release of lysine, cysteine, methionine, isoleucine, and threonine. The lysine release was decreased drastically with increasing degrees of succinylation, while cysteine release was reduced more gradually. The methionine release was significantly lower and isoleucine release was higher for the two highly succinylated proteins (SWC.46 and SWC2.04). A trend of decreasing threonine release as degree of succinylation increased was observed, but the values were not significantly different from those of WC. This was due to the low level of succinylated threonine in the SWCs, resulting in their small decrease in in vitro digestibility. Succinylation had no significant effect on the release of the other essential amino acids.

The percent decrease in lysine released appeared to be related to the amount of FDNB-bound lysine (Table V). Although there was a progressive decrease in cysteine and threonine released as the degree of amino acid succinylated increased, the relationship was less obvious as that for lysine.

A low in vitro lysine digestibility of succinylated proteins was also observed by other investigators (Chen et al, 1975; McElwain et al., 1975; Groninger and Miller, 1979). Their values corresponded very well to the data obtained in this experiment. The low lysine digestibility was attributed to the resistance of succinyl–lysyl–peptide bonds to trypsin and carboxypeptidase hydrolysis (Matoba and Doi, 1979).

Table V. Comparison of Percent Bound and Decrease in in Vitro and in Vivo Amino Acid Digestibilities of Succinylated Cheese Whey Protein Concentrates

amino acid	SWC.05[a]	SWC.09[a]	SWC.46[a]	SWC2.04[a]
	Chemical Assay (Percent Bound)			
Lys	15	37	74	83
Cys	7	21	37	51
Thr[e]	2	5	11	27
	In Vitro Digestibility (Percent Decrease)[b]			
Lys	29	40	80	98
Cys	27	27	50	*d*
Thr	6	19	28	45
	In Vivo Digestibility (Percent Decrease)[c]			
Lys	0	0	12	14
Cys	0	0	0	6
Thr	0	0	2	6

[a] For the abbreviations, see Table I. [b] (Grams of amino acid released in WC – grams of amino acid released in SWC)/grams of amino acid released in WC × 100. [c] (Amino acid digestibility of WC – amino acid digestibility of SWC)/amino acid digestibility of WC × 100. [d] Data not available. [e] Chemical assay for bound hydroxy amino acids included threonine and serine; therefore the data represented the amount of bound threonine and serine in the SWCs.

Succinylated lysine was not released as free succinyllysine by pancreatin and presumably was present in the unhydrolyzed peptides (Matoba and Doi, 1979). However, succinylation has no effect on the protein susceptibility to chymotrypsin and pepsin hydrolysis (Matoba and Doi, 1979; Rao and Rao, 1979; Groninger and Miller, 1979).

Except for lysine, the release of the other amino acids in succinylated casein was unaffected (Matoba and Doi, 1979) in contrast to the data obtained in this experiment. The inhibition of cysteine and threonine released in this experiment may be related to the resistance of succinyl thioesters and succinyl hydroxyl esters to the hydrolysis by carboxypeptidase present in pancreatin. It may also be related to some of the cysteine and methionine of β-lactoglobulin and α-lactalbumin (main proteins of whey) positioned next to lysine (Brunner, 1977). If pancreatin enzymes cannot hydrolyze N^{ϵ}-succinyllysyl bonds to yield free succinyllsine, then the release of the amino acids adjacent to lysine will also be affected.

The enzyme digestion procedures have been criticized for not being able to duplicate the conditions in the gastrointestinal tract. Furthermore, the digestibility values are valid only for the conditions employed in the assay (Hackler, 1977). Szmelcman and Guggenheim (1967) reviewed a number of in vitro digestion techniques and concluded that such techniques can be used as a guide to the amounts of amino acids which may be released by enzymatic digestion in vivo.

Table VI. In Vivo Digestibilities of Unmodified (WC) and Succinylated Cheese Whey Protein Concentrates,[a] Lactalbumin (LA), and Casein (CA)[b]

amino acid	WC	SWC.05	SWC.09	SWC.46	SWC2.04	LA	CA
Lys	$96.5^{xy} \pm 0.14$	$95.7^{xy} \pm 0.55$	$95.3^{y} \pm 0.31$	$84.9^{z} \pm 0.88$	$83.0^{z} \pm 0.11$	$94.7^{y} \pm 0.45$	$97.7^{x} \pm 0.11$
Thr	$95.2^{x} \pm 0.21$	$94.8^{xy} \pm 0.54$	$95.4^{x} \pm 0.29$	$93.5^{y} \pm 0.21$	$89.8^{z} \pm 0.03$	$93.1^{y} \pm 0.54$	$95.8^{x} \pm 0.02$
Cys	$97.2^{x} \pm 0.03$	$96.8^{x} \pm 0.29$	$97.2^{x} \pm 0.03$	$96.2^{x} \pm 0.14$	$91.1^{y} \pm 0.12$	$96.9^{x} \pm 0.13$	$84.4^{z} \pm 1.28$
Met	$95.1^{x} \pm 0.10$	$96.2^{x} \pm 0.21$	$96.5^{x} \pm 1.47$	$96.6^{x} \pm 0.62$	$91.3^{y} \pm 2.10$	$91.5^{y} \pm 0.23$	$96.4^{x} \pm 0.44$
Val	$95.3^{x} \pm 0.13$	$95.5^{xy} \pm 0.33$	$95.0^{x} \pm 0.05$	$94.0^{z} \pm 0.06$	$90.3^{v} \pm 0.13$	$93.0^{w} \pm 0.18$	$96.1^{y} \pm 0.09$
Ile	$95.4^{x} \pm 0.11$	$94.7^{xy} \pm 0.36$	$95.1^{xy} \pm 0.01$	$92.6^{z} \pm 0.02$	$91.7^{v} \pm 0.32$	$94.2^{y} \pm 0.27$	$94.8^{xy} \pm 0.19$
Leu	$97.2^{x} \pm 0.13$	$96.7^{xy} \pm 0.33$	$96.9^{xy} \pm 0.04$	$95.9^{yz} \pm 0.13$	$94.6^{v} \pm 0.14$	$94.9^{zv} \pm 0.44$	$97.4^{x} \pm 0.16$
Tyr	$96.8^{x} \pm 0.39$	$97.3^{x} \pm 0.11$	$97.0^{x} \pm 0.02$	$95.0^{y} \pm 0.01$	$93.6^{z} \pm 0.12$	$94.2^{yz} \pm 0.35$	$98.7^{v} \pm 0.10$
Phe	$93.9^{x} \pm 0.61$	$97.4^{yz} \pm 0.35$	$97.0^{yz} \pm 0.04$	$96.6^{z} \pm 0.28$	$96.2^{zv} \pm 0.15$	$94.2^{xv} \pm 0.82$	$98.8^{y} \pm 0.04$
MAA[c]	$95.1^{x} \pm 0.17$	$94.9^{x} \pm 0.45$	$95.3^{x} \pm 0.18$	$92.8^{y} \pm 0.05$	$89.5^{z} \pm 0.0$	$93.2^{y} \pm 0.48$	$95.5^{x} \pm 0.23$
TPD[c]	$95.3^{x} \pm 0.17$	$95.2^{x} \pm 0.46$	$95.2^{x} \pm 0.10$	$92.4^{y} \pm 0.0$	$89.9^{z} \pm 0.0$	$93.3^{y} \pm 0.53$	$96.5^{x} \pm 0.17$

[a] For the abreviations, see Table I. [b] Mean ± SEM. Means with different superscripts in the same row are significantly different ($P < 0.05$). [c] MAA = mean amino acid digestibility; TPD = true protein digestibility.

On the basis of the above criticisms, in vivo digestion tests of the succinylated and unsuccinylated proteins were carried out to determine whether the proteins react in similar manner in vivo as in vitro. The values were compared with that of CA and LA, reference proteins commonly used in nutritional studies.

In Vivo Digestibility. In vivo digestibility of the essential amino acids, MAA, and TPD of WC, SWCs, LA, and CA are presented in Table VI. TPD closely paralleled the MAA values for all the proteins tested. WC, SWC.05, SWC.09, and CA had higher TPD and MAA values than LA and SWC.46, which in turn had higher values than SWC2.04. Therefore low and medium degrees of succinylation did not affect in vivo protein digestibility. When the percent decrease in TPD for the two highly succinylated protein concentrates (SWC.46 and SWC2.04) was calculated from the digestibility values in Table VI, there was a 3 and 6% decrease in TPD, respectively. A 10% decrease in TPD was also observed by Varnish and Carpenter (1975) when lactalbumin was exhaustively propionylated. In general, individual amino acid digestibility values reflected TPD for each protein tested except for a much lower lysine digestibility value than TPD for SWC.46 and SWC2.04. Lysine digestibilities for SWC.46 and SWC2.04 were 12 and 14% lower, respectively, as compared to a 3 and 6% decrease in their corresponding TPD. Lysine availability was not affected by a low and medium degree of succinylation. The highly succinylated proteins (SWC.46 and SWC2.04) had lower lysine, threonine, valine, isoleucine, leucine, and tyrosine digestibilities than WC. In addition, cysteine and methionine digestibilities of SWC2.04 were also significantly different from that of WC. Therefore the decrease in TPD of highly succinylated proteins was attributed to the decreased availability of many amino acids, with lysine having the lowest availability value. Varnish and Carpenter (1975) obtained a lower yet uniform amino acid digestibility pattern for propionylated lactalbumin. In general, the digestibility of amino acids in propionylated lactalbumin reflected overall protein digestibility. TPDs for CA and LA were comparable to the values reported by Sarwar et al. (1977) and Mitchell (1923).

GENERAL DISCUSSION

Both in vitro and in vivo data suggested that succinylation affected the amino acid availabilities. In the in vitro digestion test, only the release of lysine, cysteine, methionine, and threonine from the proteins was lowered by succinylation. However, in the in vivo test, availabilities of almost all of the essential amino acids of SWC.46 and SWC2.04 were affected. In the in vitro test, there was a drastic decrease in the release of lysine, cysteine, and threonine compared to the relatively high availability values obtained in the in vivo test (Table V). For example, when 83% of lysine was succinylated in SWC2.04, the percent inhibition of pepsin pancreatin released lysine was 98%. However, only a 14% decrease in lysine digestibility was measured by the in vivo method. In general, in vivo data showed succinylated proteins to have relatively high amino acid digestibilities whereas in vitro data would portray succinylated proteins to have low amino acid availabilities.

The discrepancies in results obtained between in vivo and in vitro digestion tests may be explained by the fact that in the latter, the protein was not completely hydrolyzed. Other digestive enzymes such as aminopeptidases and intestinal peptidases present in the rat's gut could modify the release of amino acids from succinylated proteins in vivo. Also the in vitro test does not take into account the absorption of succinyl amino acids via the intestine or the absorption of succinyl dipeptides if the peptide bonds are resistant to hydrolysis. Nor does the in vitro test take into account the effect of microorganisms present in the intestine that could modify the apparent amino acid digestibility. Therefore the actual amino acid availability value will vary according to which method is used for testing.

There are some indications that succinyllysine can be absorbed by the intestine. Groninger and Miller (1979) fed [^{14}C]succinyllysine and [^{14}C]succinyl myofibrillar protein to rats and observed little radioactivity in the rat feces 24 h after feeding. Large amounts of radioactivity were found in the urine, and some radioactivity were found in the expired CO_2. When rats were fed propionyllysine, 41% of lysine ingested was found in the urine as propionyllysine (Bjarnason and Carpenter, 1969). However, when rats were fed palmitoyllysine derivatives, little lysine absorption was observed (Stephens et al., 1977). As much as 90% of the palmitoyllysine ingested was found in the feces. It appears that succinyllysine and propionyllysine can be absorbed but were largely nonutilized and were excreted in the urine. The size of the acylating agent may have a role in determining the absorption properties of the acylated amino acid. Lysine derivatives with a long carbon chain such as palmitoyllysine may not be as readily absorbed as a short-chain derivative. It is possible for succinyl dipeptides to be transported through the intestinal mucosa, since mucosa had transport mechanisms for di- and tripeptides (Sleisenger and Kim, 1979). The data from this experiment lend support to the view that succinyllysine and possibly other succinyl amino acids and succinyl dipeptides can be absorbed by the intestine. This will explain the high digestibility value for lysine, cysteine, and threonine with the in vivo test and low availability values

for these amino acids with the in vitro test. The inhibition in the release of lysine, cysteine, and threonine observed by the in vitro test paralleled the degree of binding of these amino acids to succinic anhydride because the succinyl amino acid bonds were resistant to pancreatic enzymes hydrolysis. Such pattern was not observed in the in vivo test because other factors such as absorption of succinyl amino acids or succinyl dipeptides modified the apparent digestibility of these amino acids.

Bjarnason and Carpenter (1969) observed that propionyllactalbumin has higher lysine activity than propionyllysine. The authors postulated the different lysine activities to the hydrolysis of the acyl groups of propionyllactalbumin by gut enzymes. A protein could remain longer in the gut and be more exposed to the gut enzymes than propionyllysine, which could be absorbed as such. Groninger and Miller (1979) also observed acetylated myofibrillar protein to be 25% more utilizable than acetyllysine. However, no explanation was given for the variability of the different acetylated derivatives by these authors. The gut containing amino and intestinal peptidases, pepsin, and pancreatin enzymes might have acted on SWC in vivo in a manner similar to that of propionyllactabumin and acetylated myofibrillar protein, thereby making SWC more available in vivo than can be predicted by the in vitro test.

Other studies on the biological availability of Amadori compounds such as fructose–tryptophan and fructose–phenylalanine suggested the importance of intestinal microflora in the degradation and utilization of these compounds. In vitro tests showed that after 12-h incubation at 37 °C, fructose–tryptophan was degraded by cecal microflora and some free tryptophan was librated (Tanaka et al., 1975). Radioactivity studies with fructose–phenylalanine also suggested degradation of fructose–phenylalanine by the gut's microflora with one of the degraded products, possibly free phenylalanine (Johnson et al., 1979). Since microflora had high metabolic activity toward many nitrogeneous substances such as urea, amides, amino acids, and proteins, it may have a role in degrading unabsorbable amino acids or peptides that remained for a long time in the gut. It is not known whether the action of microorganisms in the cecum could alter the in vivo digestibility or the nutritional value of succinylated protein. It is also unknown how humans might handle the succinylated proteins in the absence of a large cecum.

If the succinyl amino acids are absorbed, their utilization depends on the ability of the organ's enzymes to deacylate the succinyl groups and give free amino acids. Mammalian tissues contain acylases which act on a number of *N*-acyl amino acids (Endo, 1978; Paik and Benoiton, 1963), but their specific activities toward succinylated amino acids have not been reported. Since highly succinylated proteins have a low protein efficiency ratio (Groninger, 1973; Creamer et al., 1971), the absorbed succinyl amino acids or dipeptides are probably not available for growth. The utilization, metabolic fate, and safety of the absorbed succinyl amino acids as well as the practical food application of the SWCs with improved functionality are discussed in another paper (Siu and Thompson, 1982).

The functional properties of SWCs with a low and medium degree of succinylation have not been determined. However, they are no doubt better than that of WC since others (Franzen and Kinsella, 1976a; Eiselle and Brekke, 1981; Choi et al., 1981) have observed improved functional properties with only a 30–55% degree of succinylation. SWCs with a low degree of succinylation can be used in food products where the nutritive value may be important, such as meat analogues. The highly succinylated proteins with good functional properties (Thompson and Reyes, 1980) but lower digestibilities could still be used as functional ingredients in products such as coffee whitener and salad dressings where nutritional value is of little concern.

LITERATURE CITED

Akeson, W. R.; Stahmann, M. A. *J. Nutr.* **1964**, *83*, 257.

AOAC "Official Methods of Analysis", 12th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.

Beveridge, T.; Toma, S. T.; Nakai, S. *J. Food Sci.* **1974**, *39*, 49.

Bjarnason, J.; Carpenter, K. J. *J. Nutr.* **1969**, *23*, 859.

Boehringer Mannheim "Methods of Enzymatic Food Analysis"; Boehringer Mannheim: Ville St. Laurent, Quebec, Canada, 1979.

Bragg, D. B.; Ivy, C. A.; Stephenson, E. L. *Poult. Sci.* **1969**, *48*, 2135.

Bressani, R. "Evaluation of Proteins for Humans"; Bodwell, C. E., Ed.; Avi Publishing Co.: Westport, CT, 1977; pp 81–117.

Brunner, J. R. In "Food Proteins"; Whitaker, J. R.; Tannenbaum, S. R., Ed.; Avi Publishing Co.: Westport, CT, 1977; pp 175–208.

Chang, T. S.; Sun, S. F. *Int. J. Pept. Protein Res.* **1978**, *11*, 65.

Chen, L.-F.; Richardson, T.; Amundson, C. H. *J. Milk Food Technol.* **1975**, *38*, 89.

Choi, Y. R.; Lusas, E. W.; Rhee, K. C. *J. Food Sci.* **1981**, *46*, 954.

Creamer, L. K.; Roeper, J.; Lahrey, E. H. *N.Z. J. Dairy Sci. Technol.* **1971**, *6*, 107.

Duncan, B. D. *Biometrics* **1955**, *11*, 1.

Eiselle, T. A.; Brekke, C. J. *J. Food Sci.* **1981**, *46*, 1096.

Endo, V. *Biochim. Biophys. Acta* **1978**, *523*, 207.

Franzen, K. L. Ph.D Thesis, Cornell University, Ithaca, NY, 1976.

Franzen, K. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1976a**, *24*, 788.

Franzen, K. L.; Kinsella, J. E. *J. Agric. Food Chem.* **1976b**, *24*, 914.

Gounaris, A. D.; Perlmann, G. E. *J. Biol. Chem.* **1967**, *242*, 2739.

Grant, D. R. *Cereal Chem.* **1973**, *50*, 417.

Groninger, H. S. *J. Agric. Food Chem.* **1973**, *21*, 978.

Groninger, H. S.; Miller, R. *J. Agric. Food Chem.* **1979**, *27*, 949.

Hackler, L. R. In "Evaluation of Proteins for Humans"; Bodwell, C. E., Ed.; Avi Publishing Co.: Westport, CT, 1977; pp 55–66.

Johnson, C. H.; Baker, D. H.; Perkins, E. G. *J. Nutr.* **1979**, *109*, 590.

Li-Chan, E.; Helbig, N.; Holbek, E.; Chau, S.; Nakai, S. *J. Agric. Food Chem.* **1979**, *27*, 877.

Matoba, T.; Doi, E. *J. Food Sci.* **1979**, *44*, 537.

McElwain, M. D.; Richardson, T.; Amundson, C. H. *J. Milk Food Technol.* **1975**, *38*, 521.

Means, G. E.; Feeney, R. E. "Chemical Modification of Protein"; Holden-Day: San Francisco, 1971.

Melnychyn, P.; Stapley, R. B. U.S. Patent 3 764 711, 1973.

Miller, R.; Groninger, H. S. *J. Food Sci.* **1976**, *41*, 268.

Mitchell, H. H. *J. Biol. Chem.* **1923**, *43*, 873.

Oppenheimer, H.; Barany, K.; Hamoir, G.; Fenton, J. *Arch. Biochem. Biophys.* **1967**, *120*, 108.

Paik, W. K.; Benoiton, L. *Can. J. Biochem. Physiol.* **1963**, *41*, 1643.

Rao, A. G. A.; Rao, M. S. N. *Int. J. Pept. Protein Res.* **1979**, *14*, 309.

Riordan, J. F.; Vallee, B. L. *Biochemistry* **1964**, *3*, 1768.

Sarwar, G.; Sosulski, F. W.; Bell, J. M. *Can. Inst. Food Sci. Technol. J.* **1977**, *10*, 31.

Siu, M.; Thompson, L. U., submitted for publication in *J. Agric. Food Chem.*, 1982.

Sleisenger, M. H.; Kim, Y. S. *N. Engl. J. Med.* **1979**, *300*, 659.

Stadtman, E. R. "Mechanism of Enzyme Action", McCollom Pratt Symposium; John Hopkins University Press: Baltimore, 1955.

Stephens, C. A.; Veen-Baigent, M. J.; Anderson, G. H. *Can. J. Physiol. Pharmacol.* **1977**, *55*, 434.

Szmelcman, S.; Guggenheim, K. *J. Sci. Food Agric.* **1967**, *18*, 347.

Tanaka, M.; Lee, T. C.; Chichester, C. O. *J. Nutr.* **1975**, *105*, 989.

Thompson, L. U.; Reyes, E. S. *J. Dairy Sci.* **1980**, *63*, 715.

Varnish, S. A.; Carpenter, K. J. *Br. J. Nutr.* **1975**, *34*, 339.

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