Food Chemistry **39** (1991) 13–26

Phosphorylation of Food Proteins with Phosphorus Oxychloride—Improvement of Functional and Nutritional Properties: A Review

G. Matheis

DRAGOCO, Dragocostrasse, D-3450 Holzminden, FRG

(Received 3 July 1989; accepted 28 February 1990)

ABSTRACT

Phosphorylation with $POCl_3$ (in the absence and presence of essential amino acids) could be a promising tool for improving functional and nutritional properties of food proteins (e.g. yeast protein, zein, soybean protein). The amount of phosphorus covalently bound to proteins can reach up to 3·9%, but is usually in the order of 1–2%. The in-vitro digestibility of food proteins phosphorylated with $POCl_3$ is not adversely affected. The in-vivo digestibility (using the Tetrahymena thermophili *bioassay) has been studied only in two cases (casein and zein). While digestibility was not affected in the case of casein, the growth rate of the microorganism showed an 11-fold improvement on modified zein (phosphorylated in the presence of limiting amino acids) over that of the original zein. Phosphoproteins are abundant in nature and some, e.g. milk casein, egg white albumin, egg yolk phosvitin, are part of the regular human diet. Experiments with animals are necessary to determine the digestion of proteins phosphorylated with $POCl_3$ by mammals, and to study the introduction of potentially toxic residues into the proteins that may prevent their direct utilization in foods.*

INTRODUCTION

In the last 15 years, the deliberate modification of food proteins in order to change their properties has become increasingly popular as a research area

Food Chemistry 0308-8146/90/$03·50

(Feeney & Whitaker, 1977, 1982, 1985*a*,*b*, 1988; Matheis & Whitaker, 1984*a*, 1987; Chobert *et al.*, 1987; Kinsella, 1987). The purposes of the modifications are several. Modification may be a means of eliminating the undesirable toxic and/or antinutritional properties of some proteins or other constituents; increasing or decreasing the solubility of proteins; changing the functional properties of proteins; improving the nutritional properties by the covalent attachment of limiting essential amino acids; and protecting the protein against process-induced modification, such as the Maillard reaction.

The solubility of proteins can be improved by limited hydrolysis or by increasing the number of hydrophilic groups of proteins. Limited hydrolysis with proteinases has been reviewed extensively (Fox *et al.*, 1982; Whitaker & Puigserver, 1982; Chobert *et al.*, 1987). Increase in the number of hydrophilic groups of a protein could be accomplished by converting glutaminyl (Gln) and asparaginyl (Asn) residues to glutamyl (Glu) and aspartyl (Asp) residues by deamidation, or by covalent attachment of hydrophilic amino acid residues, glyco groups or phosphate groups.

Replacement of neutral amino acid residues with acidic amino acid residues would be expected to have a major effect on the properties of proteins from wheat and other cereals, where up to one-third of the total amino acids is Gln. Deamidation can be accomplished at pH 8 to 9 at elevated temperatures. Unfortunately, some racemization and β-elimination are also likely to occur. An enzymatic approach would be preferred to overcome the limitation of the chemical approach. Kikuchi *et al.* (1971) identified an enzyme, peptidoglutaminase (PGase), in the soil micro-organism *Bacillus circulans* that can catalyze the deamidation of the carboxyamide group of Gln when the latter is part of a peptide. Attempts to apply this enzyme to casein, whey protein (Gill *et al.*, 1985) and soybean protein (Hamada *et al.*, 1988) were of limited success.

Hydrophilic amino acids have been covalently attached to food proteins by use of the active *N*-hydroxysuccinimide ester method (Puigserver *et al.*, 1979). The most extensively modified casein, for example, contained 10 additional aspartyl groups per mol of casein.

Glyco groups have been covalently attached to proteins by several methods (Gray, 1974; Marshall & Rabinowitz, 1975; Krantz *et al.*, 1976; Lee *et al.*, 1979). The carbohydrate–protein conjugates were reported to be soluble, but none of the workers reported any quantitative data on solubility.

Chemical phosphorylation of proteins using various phosphorylating reagents has been reviewed by Frank (1984, 1987) and Matheis and Whitaker (1984*b*), covering the literature up to 1984. Of the phosphorylating reagents used so far (Table 1), only phosphorus oxychloride ($POCl_3$) seems

TABLE 1
Phosphorylation of Proteins by Various Phosphorylating Reagents[a]

Phosphorylating reagent	*Examples of phosphorylated proteins*		
	Protein	*Maximum phosphorus covalently bound*	
		%	*Mol per mol of protein*
$POCl_3$	Casein	1·0	7·4
	β-Lactoglobulin	1·2	13·8
H_3PO_4[b]	Soybean protein	1·1	73·3[c]
P_2O_5/H_3PO_4	α_{s1}-Casein	0·8	6·3
H_3PO_4/Cl_3CCN	α_{s1}-, β- and κ-Casein	1·5	11·2
Monophenyl phosphodichloride	Gelatin	0·5	15·6[d]
Phosphoramidate	Insulin	1·0	1·9
Diphosphoimidazole	Ribonuclease	1·5	6·0

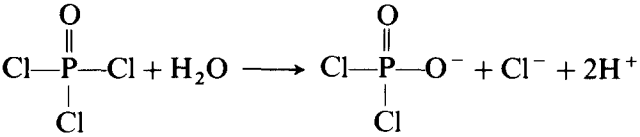
[a] Compiled from Frank (1984, 1987) and Matheis and Whitaker (1984*a*).
[b] In the presence or absence of urea.
[c] Molecular weight taken as 360 000.
[d] Molecular weight taken as 100 000.

to prove an economical and practical reagent for large scale application to food proteins. Although Sung *et al.* (1983) reported the successful phosphorylation of soybean protein with trisodium trimetaphosphate, Matheis and Whitaker (1984*b*) were unable to reproduce the work. It is difficult to remove all the adsorbed phosphate from the proteins, thereby showing the phosphate to be covalently bound. The purpose of the present review is to give a brief account of the reactions of $POCl_3$ with proteins, and on recent progress on phosphorylation of food proteins with $POCl_3$.

REACTION CONDITIONS AND REACTION PRODUCTS

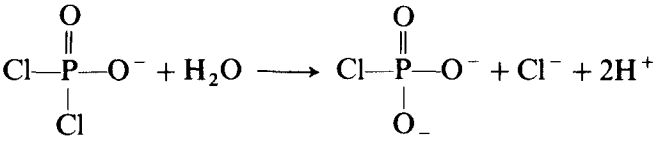
In an aqueous system, $POCl_3$ reacts rapidly with H_2O in an exothermic fashion (eqns (1)–(3)). When $POCl_3$ is added directly to an aqueous protein solution, the low pH (eqns (1)–(3)) and the heat produced lead to denaturation of the protein. In order to minimize these problems, $POCl_3$ is usually dissolved in an organic solvent (e.g. carbon tetrachloride or *n*-hexane) and added in small portions to aqueous protein solutions. The pH is kept between 5 and 9 by adding sodium hydroxide as required. The temperature is controlled in an ice bath.

Because Cl^- is a good leaving group, $POCl_3$ and the two intermediates (eqns (1) and (2)) are very reactive and phosphorylation of functional protein groups can be achieved as shown in eqns (4)–(7) (PRO = protein). Evidence for the phosphorylation of serine and threonine (eqn (4)), lysine (eqn (5)), histidine (eqn (6)) and tyrosine (eqn (7)) residues by $POCl_3$ has been reported for various proteins, as analyzed by pH stability studies, ^{31}P nuclear magnetic resonance (NMR) spectroscopy, reactive protein groups, and



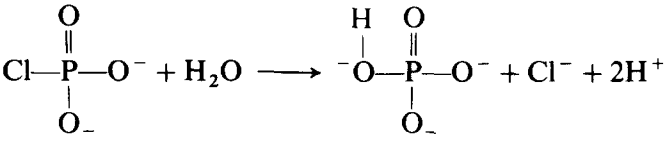
(1)

Intermediate 1



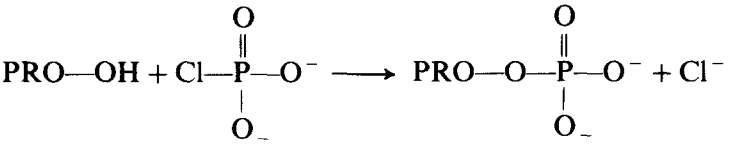
(2)

Intermediate 2

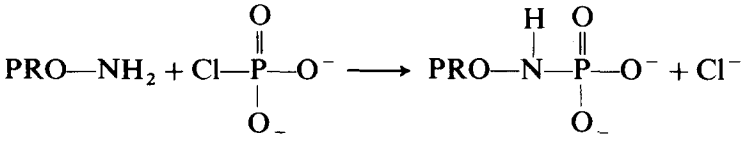


(3)

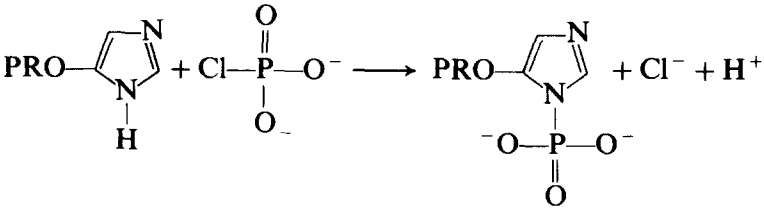
Phosphate ion



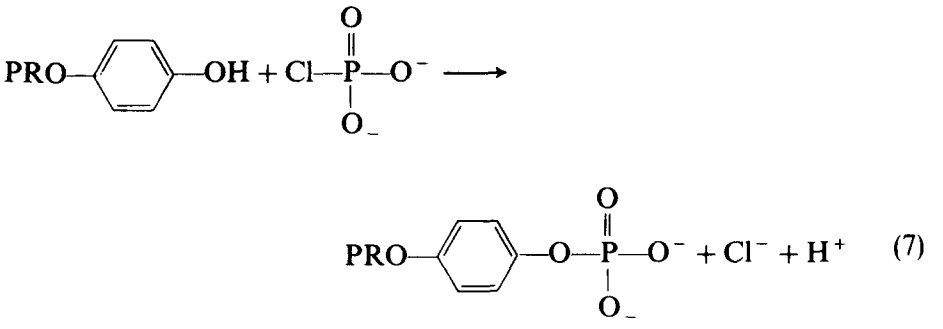
(4)



(5)

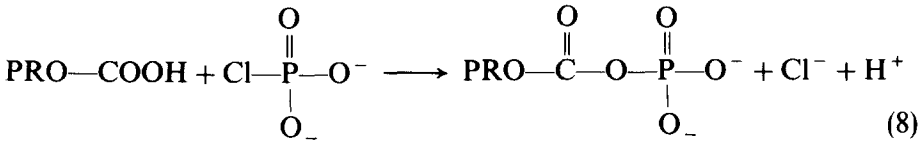


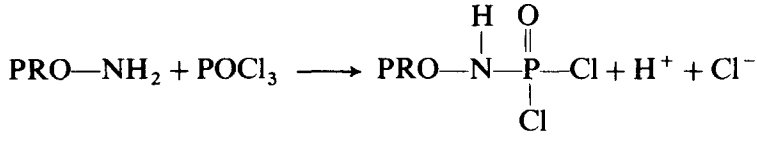
(6)

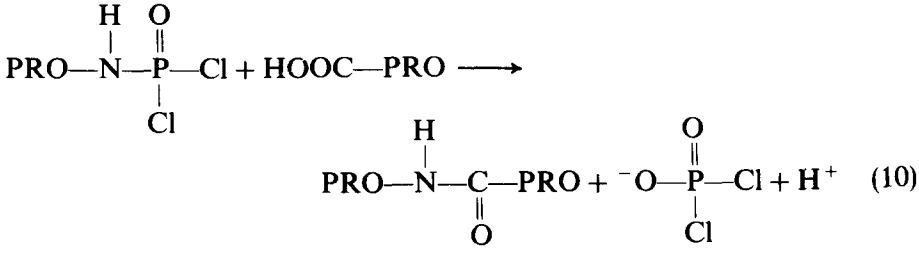


digestion of proteins followed by analysis of phospho amino acids (Frank 1984, 1987; Hirotsuka *et al.*, 1984; Matheis & Whitaker, 1984*b*,*c*; Huang & Kinsella, 1986*a*).

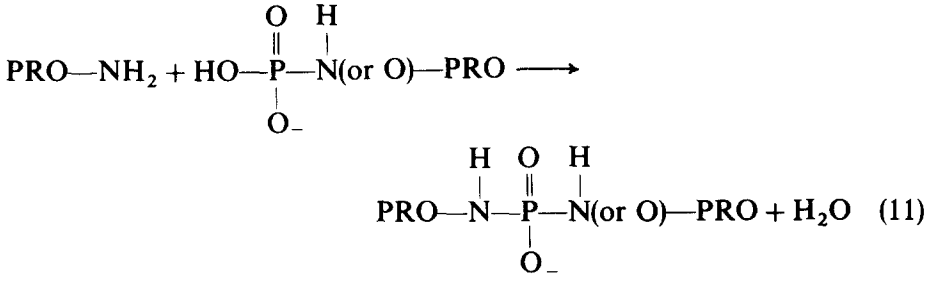
Aspartic and glutamic acid residues are both capable of forming phospho anhydrides (eqn (8); PRO = protein). This could be considered for phosphorylation of lysozyme with $POCl_3$, since one of the ^{31}P NMR signals (−14·5 ppm at pH 8·0) was close to the signal of phosphoaspartic acid in the phosphorylated intermediate of adenosine triphosphatase (−17·4 ppm at pH 7·4) (Matheis & Whitaker, 1984*c*). The presence of diphosphate and tri(or poly)phosphate residues in proteins phosphorylated with $POCl_3$ has also been demonstrated (Frank, 1984, 1987; Matheis & Whitaker, 1984*b*,*c*: Huang & Kinsella, 1986*a*).



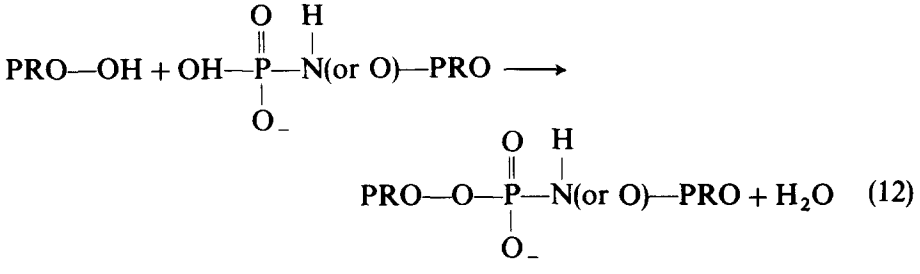




$POCl_3$ can cause intermolecular cross-linking of proteins as was shown by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) and mercaptoethanol (Matheis & Whitaker, 1984*b*; Huang & Kinsella, 1986*a*). The nature of these cross-links is not known. Possibilities include *O,O'*-phosphodiester and *N,N'*-phosphodiamidate bridges as well as isopeptide bonds. The latter are thought to be formed according to eqns (9) and (10) (PRO = protein). Free amino or hydroxyl groups of the protein can also react with the phosphoryl group of the phosphorylated protein as shown in eqns (11) and (12) (PRO = protein) (Huang & Kinsella, 1986*b*).



(11)



(12)

$POCl_3$ has also been used in a totally nonaqueous system (Willmitzer & Wagner, 1975). In this case, clupeine (a herring protein) was first converted to clupeine capronate and then suspended in trimethyl phosphate. When $POCl_3$ was added, the suspension became a clear solution. The reaction was terminated by pouring the solution into water.

The proteins discussed in the following sections have all been phosphorylated in aqueous solutions, essentially according to the early method of Rimington (1927), i.e. the pH was kept between 5 and 9 with sodium hydroxide and the temperature was below 25°C.

Yeast protein

The high content of nucleic acid, which may range from 8 to 25 g per 100 g of protein, is a potential problem associated with the consumption of large

amounts of yeast nucleoprotein as human food. Therefore, before yeast may be used as a major source of food protein, the nucleic acid content should be reduced so that daily intake of nucleic acid from yeast may not exceed 2 g (Sinskey & Tannenbaum, 1975). Various approaches for reducing nucleic acid content of yeast nucleoproteins have been reviewed by Kinsella (1987), including phosphorylation with $POCl_3$.

As shown in Table 2, phosphorylation removed approximately 85% of the contaminant nucleic acid from the nucleoprotein. This indicates that phosphorylation of yeast protein induced dissociation of nucleoprotein complexes by increasing the net negative charges on the protein followed by electrostatic repulsion within the nucleoprotein complexes (Damodaran & Kinsella, 1984). Since the dissociated nucleic acids have an isoelectric pH around 1·5–2·0, the nucleic acids remain soluble during precipitation of protein at pH 4·2.

TABLE 2
Proximate Chemical Composition of Yeast, Yeast Nucleoprotein and Yeast Proteins Prepared by Different Methods[a]

Sample	*Protein* (%)	*Nucleic acid* (%)	*Carbohydrate* (%)	*Lipid* (%)
Whole yeast cell	42·4	11·4	28·8	4·0
Nucleoprotein	64·0	20·0	6·0	5·1
Succinylated protein	90·0	1·8	3·0	3·5
Citraconylated protein	84·0	2·2	3·7	5·1
Phosphorylated protein	82·0	2·7	5·1	5·7

[a] Kinsella (1987).

The amount of phosphorus esterified to proteins prepared at various $POCl_3$:protein ratios increased with $POCl_3$ concentration (Huang & Kinsella, 1986*a*). At a ratio of 1·0, approximately 3·6 mol of phosphorus were esterified per 10^4 g of yeast protein (Table 3). Higher $POCl_3$:protein ratios did not increase the extent of phosphorylation.

The nature of phosphate linkages was characterized by ^{31}P NMR spectroscopy, pH stability studies, and determination of disappearance of reactive lysine groups (Huang & Kinsella, 1986*a*). All three methods indicated that lysine was the major amino acid phosphorylated, while minor amounts of phosphorus were bound to serine or threonine hydroxyl groups (Table 3). ^{31}P NMR spectroscopy also indicated trace amounts of di- or polyphosphates (Table 3).

Improved functional properties of phosphorylated yeast protein include water-solubility, water-holding capacity, viscosity, emulsifying activity and

TABLE 3
Amount of Phosphorus Covalently Bound and Nature of Phosphate Linkages in Yeast Protein, Zein and Soybean Protein Phosphorylated with $POCl_3$

Phosphorylated protein	*Phosphorus covalently bound*		*Nature of phosphate linkage*[a]
	%	*Mol/mol of protein*	
Yeast protein[b]	1·2[c]	Not determined	—NH—PO_3^{2-}, —O—PO_3^{2-} and —NH(or O)—$P_2O_6^{3-}$ (A, B, C)
Zein[d,e]			Not determined
Z1		0·3	
Z2	0·2	2·6	
Z3	0·8	9·5	
Z4	1·7	20·0	
Z5	3·5	40·0	
T1	0·1	1·1	
L	0·1	1·2	
M1	0·1	1·0	
M2	1·1	12·3	
Soybean protein[f]	1·4	140·0[g]	—NH—PO_3^{2-} (A)

[a] Method of analysis: A = pH stability studies, B = ^{31}P NMR spectroscopy, C = disappearance of functional groups.
[b] Huang and Kinsella (1986*a*).
[c] Corresponding to 30% of the lysine residues.
[d] Chobert *et al.* (1987).
[e] Z1–Z5:in the absence of amino acids; T1 and L: in the presence of Trp and Lys, respectively; M1–M2: in the presence of mixtures of Trp and Lys.
[f] Hirotsuka *et al.* (1984).
[g] Molecular weight taken as 360 000.

foaming properties (Table 4). The in-vitro digestibility of yeast protein, using trypsin and pancreatin, was not affected by phosphorylation (Huang & Kinsella, 1987).

Zein

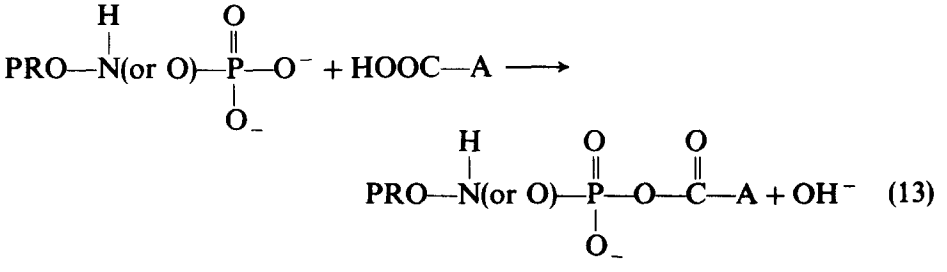
Chobert *et al.* (1987) phosphorylated zein in the absence and presence of some limiting essential amino acids with $POCl_3$. Reaction conditions are given in Table 5. In the absence of amino acids, up to 40 mol of phosphorus per mol of zein could be covalently attached (Table 3). In the presence of tryptophan, lysine or a mixture of both, up to 12 mol of phosphorus per mol of zein were covalently bound (Table 3). In addition, both amino acids were covalently attached (Table 5). Threonine, the third limiting amino acid in zein, was not covalently bound when added to the zein/$POCl_3$ reaction mixture (Chobert *et al.*, 1987).

TABLE 4
Changes in Functional Properties of Food Proteins Phosphorylated with $POCl_3$ as Compared to the Native Proteins

Change in functional properties	*Phosphorylated protein*	*Reference*
Increased water-solubility	Yeast protein[a]	Huang and Kinsella (1986*b*)
	Zein[b,c]	Chobert *et al.* (1987)
	Soybean protein	Hirotsuka *et al.* (1984)
Decreased water-solubility	Yeast protein[d]	Huang and Kinsella (1986*b*)
Increased water-holding capacity	Yeast protein[e]	Huang and Kinsella (1986*b*)
Increased viscosity	Yeast protein	Huang and Kinsella (1986*b*)
Increased gel-forming properties	Soybean protein	Hirotsuka *et al.* (1984)
Increased emulsifying activity	Yeast protein[a]	Huang and Kinsella (1987)
	Zein[f]	Chobert *et al.* (1987)
	Soybean protein	Hirotsuka *et al.* (1984)
Increased foaming properties	Yeast protein[e]	Huang and Kinsella (1987)

[a] Between pH 5·5 and 7·0.
[b] Zein phosphorylated in the absence of amino acids (samples Z1–Z5) between pH 1 and 10, except for sample Z3 (9·5 mol P/mol zein) which was less soluble than native zein between pH 1 and 4.
[c] Zein phosphorylated in the presence of amino acids (samples T1, L, M1 and M2).
[d] Between pH 7·5 and 8·0.
[e] Between pH 5·0 and 8·0.
[f] All zein samples (Z1–Z5, T1, L, M1, and M2) between pH 1 and 10, except around pH 4.

The mechanism by which $POCl_3$ activates the covalent incorporation of tryptophan and lysine into protein is not known and has not been reported previously. Chobert *et al.* (1987) suggest that the amino acids are incorporated either through forming a mixed phosphate/carboxyl anhydride (eqn (13); PRO = protein; A = amino acid) or by the amino group of



(13)

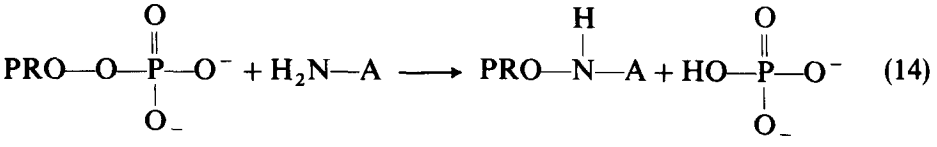
the amino acids acting as a nucleophile to displace phosphate from the phosphorylated protein (eqn (14); PRO = protein; A = amino acid). One could also speculate that the amino acids are first phosphorylated by $POCl_3$

TABLE 5
Phosphorylation of Zein by $POCl_3$ in the Absence and Presence of Free Amino Acids[a]

Sample[b]	*Zein concentration* (%)	*Molar ratio*[c] *$POCl_3$/zein*	*Molar ratio free amino acid/zein*	*Amino acid bound*[d] (%)
Z1	1·20	100	—	—
Z2	4·75	100	—	—
Z3	5·00	400	—	—
Z4	5·00	800	—	—
Z5	5·00	1 600	—	—
T1	5·00	200	10	0·61
T2	5·00	200	30	0·86
L	5·00	200	40[e]	0·46
M1	5·00	200	5 (Trp) and 10 (Lys)	1·05 (Trp) and 0·24 (Lys)
M2	5·00	400	5 (Trp) and 10 (Lys)	0·63 (Trp) and 0·22 (Lys)

[a] Chobert *et al.* (1987).
[b] Z = in the absence of free amino acids, T and L = in the presence of Trp and Lys, respectively, M = in the presence of mixtures of Trp and Lys.
[c] Molecular weight of zein: 38 000.
[d] Including small amounts (0·16% Trp and 0·09 % Lys) in the original zein.
[e] Increasing the ratio of Lys to zein to 80 did not improve the extent of Lys incorporation.

and then attached to the protein (eqn (5); substitute PRO = protein for A = amino acid). Using essentially the same method that was used for phosphorylation of zein, both amino and hydroxyl groups of amino acids (cysteine, serine, tyrosine) have been phosphorylated (Frank, 1984).



$$\text{PRO—O—}\overset{\overset{\text{O}}{\|}}{\underset{\underset{\text{O}^-}{|}}{\text{P}}}\text{—O}^- + \text{H}_2\text{N—A} \longrightarrow \text{PRO—}\overset{\overset{\text{H}}{|}}{\text{N}}\text{—A} + \text{HO—}\overset{\overset{\text{O}}{\|}}{\underset{\underset{\text{O}^-}{|}}{\text{P}}}\text{—O}^- \qquad (14)$$

All modified zeins had improved water-solubilities and emulsifying activities between pH 1 and 10, except for a slight decrease of emulsifying activity around pH 4 (Table 4). The nutritional quality of some modified zeins was determined using the *Tetrahymena thermophili* bioassay method of Matheis *et al.* (1983). The use of *Tetrahymena* sp. for protein quality evaluation relies on its similarity to mammals in the requirement for essential amino acids and in the presence of similar enzyme systems.

TABLE 6
Relative Growth Rate of *Tetrahymena thermophili* on Modified Zeins, Zein and Casein[a]

Sample	*Relative growth*[b]
Casein	100·0
Zein	4·5
Modified zeins[c]	
Z1	1·1
Z2	1·1
T1	22·2
L	8·8
M1	48·8
M2	24·4

[a] Chobert *et al.* (1987).
[b] Growth relative to that on casein.
[c] For sample description see Tables 3 and 5.

The relative growth rate of *T. thermophili* was very low on zein, compared to casein, and even lower on the samples phosphorylated in the absence of amino acids (Table 6). On zeins phosphorylated in the presence of amino acids, however, relative growth rates of *T. thermophili* were higher compared to zeins phosphorylated in the absence of amino acids (Table 6). This was due to the covalent attachment of limiting amino acids. Growth rate on sample M1 (containing 1·1% of tryptophan, 0·24% of lysine, and 1 mol of phosphorus per mol of zein) was nearly half of that on casein (Table 6), an 11-fold improvement over that of the original zein.

Soybean protein

Hirotsuka *et al.* (1984) covalently attached up to 1·5% of phosphorus to amino groups of soybean protein (Table 3). The modified protein had improved water-solubility and emulsifying and gel-forming properties (Table 4).

Phosphorylation did not affect the in-vitro digestibility of the protein, using pepsin, pancreatin, aminopeptidase, and prolydase (Hirotsuka *et al.*, 1984).

CONCLUSIONS

Phosphorylation with $POCl_3$ (in the absence and presence of essential amino acids) could be a promising tool for improving functional (Table 4) and nutritional properties (Tables 2, 5 and 6) of food proteins.

Several other methods have been used to reduce the nucleic acid content in yeast protein, e.g. the nucleic acid content was reduced to 1·0–2·0% by strong alkali, to 1·8–2·5% by succinylation, to 1·8–2·2% by reversible citraconylation–decitraconylation, and to 2·5–3·0% by the $NaClO_4$ method (Huang & Kinsella, 1986*a*; Kinsella, 1987). Hence, phosphorylation was as effective for reducing nucleic acid in yeast protein as the previous methods (compare Table 2). The residual nucleic acid is believed to be covalently bound to the protein and hence is not dissociated (Kinsella, 1987).

The amount of phosphorous covalently bound to proteins can reach up to 3·9%, but is usually in the order of 1–2% (Tables 1 and 3; Matheis & Whitaker, 1984*b*). Phosphoproteins are abundant in nature and some, e.g. milk casein, egg white albumin and egg yolk phosvitin, are part of the regular human diet.

In general, the in-vitro digestibility of food proteins phosphorylated with $POCl_3$ is not adversely affected (Matheis & Whitaker, 1984; Frank, 1987; this review). The in-vivo digestibility has been studied for casein (Matheis *et al.*, 1983) and zein (Table 6), using the *T. thermophili* bioassay. While the microorganism grew as well on phosphorylated casein as on untreated casein, this was not true for zein phosphorylated in the absence of limiting amino acids (Table 6). After phosphorylation in the presence of limiting amino acids, however, *T. thermophili* exhibited improved growth (Table 6).

Experiments with animals are necessary to determine the digestion, absorption and utilization of the amino acids of phosphorylated proteins by mammals, and to study the introduction of potentially toxic residues into the proteins that may prevent their direct utilization in foods.

REFERENCES

Chobert, J.-M., Sitohy, M. & Whitaker, J. R. (1987). Specific limited hydrolysis and phosphorylation of food proteins for improvement of functional and nutritional properties. *J. Amer. Oil Chem. Soc.*, **64**, 1704–11.

Damodaran, S. & Kinsella, J. E. (1984). Dissociation of yeast nucleoprotein complexes by chemical phosphorylation. *J. Agric. Food Chem.*, **32**, 1030–2.

Feeney, R. E. & Whitaker, J. R. (eds) (1977). Food proteins. Improvement through chemical and enzymatic modification. *Advances in Chemistry Series 160.* American Chemical Society, Washington, DC, 312 pp.

Feeney, R. E. & Whitaker, J. R. (eds) (1982). Modification of proteins: Food, nutritional and pharmacological aspects. *Advances in Chemistry Series 198.* American Chemical Society, Washington, DC, 402 pp.

Feeney, R. E. & Whitaker, J. R. (eds) (1985*a*). *Protein Tailoring for Food and Medical Uses.* Marcel Dekker, New York, 392 pp.

Feeney, R. E. & Whitaker, J. R. (1985*b*). Chemical and enzymatic modification of plant proteins. In *New Protein Foods. Vol. 5*, ed. Wilcke, H. L. & Altshul, A. M. Academic Press, New York, pp. 181–219.

Feeney, R. E. & Whitaker, J. R. (1988). Importance of cross-linking reactions in proteins. In *Advances in Cereal Science and Technology. Vol. 9*, ed. Y. Pomeranz. American Association of Cereal Chemists, St Paul, MA, pp. 21–46.

Fox, P. F., Morissey, P. A. & Mulvihill, D. M. (1982). Chemical and enzymatic modification of food proteins. In *Developments in Food Proteins. Vol. 1.* ed. B. J. F. Hudson. Applied Science Publishers, London, pp. 1–60.

Frank, A. W. (1984). Synthesis and properties of N-, O-, and S-phospho derivatives of amino acids, peptides, and proteins. *CRC Crit. Rev. Biochem.*, **16**, 51–101.

Frank, A. W. (1987). Non-enzymic methods for the phosphorylation of proteins. *Phosphorus and Sulfur*, **29**, 297–315.

Gill, B. P., O'Shaughnessey, A. J., Henderson, P. & Headon, D. R. (1985). An assessment of potential of peptidoglutaminases I and II in modifying the charge characteristics of casein and whey proteins. *Irish J. Food Sci. Technol.*, **9**, 33–41.

Gray, G. R. (1974). Direct coupling of oligosaccharides to proteins and derivatized gels. *Arch. Biochem. Biophys.*, **163**, 426–8.

Hamada, J. S., Shih, F. F., Frank, A. W. & Marshall, W. E. (1988). Deamidation of soy peptides and proteins by *Bacillus circulans* peptidoglutaminase. *J. Food Sci.*, **53**, 671–2.

Hirotsuka, M., Taniguchi, H., Narita, H. & Kito, M. (1984). Functionality and digestibility of a highly phosphorylated soybean protein. *Agric. Biol. Chem.*, **48**, 93–100.

Huang, Y.-T. & Kinsella, J. E. (1986*a*). Phosphorylation of yeast protein: Reduction of ribonucleic acid and isolation of yeast protein concentrate. *Biotechnol. Bioeng.*, **28**, 1690–8.

Huang, Y.-T. & Kinsella, J. E. (1986*b*). Functional properties of phosphorylated yeast protein: Solubility, water-holding capacity, and viscosity. *J. Agric. Food Chem.*, **34**, 670–4.

Huang, Y.-T. & Kinsella, J. E. (1987). Effects of phosphorylation on emulsifying and foaming properties and digestibility of yeast protein. *J. Food Sci.*, **52**, 1684–8.

Kikuchi, M., Hayashida, H., Nakano, E. & Sakaguchi, K. (1971). Peptidoglutaminase. Enzymes for selective deamidation of γ-amide of peptide-bound glutamine. *Biochemistry*, **10**, 1222–9.

Kinsella, J. E. (1987). Functional proteins from yeast nucleoprotein for food uses: Methods for isolation. In *Food Biotechnology*, ed. D. Knorr. Marcel Dekker, New York, pp. 363–91.

Krantz, M. J., Holtzman, N. A., Stowell, C. P., Lee, Y. C., Weiner, J. W. & Liu, H. H. (1976). Attachment of thioglycosides to proteins: Enhancement of liver membrane binding. *Biochemistry*, **15**, 3963–8.

Lee, H. S., Sen, L. C., Clifford, A. J., Whitaker, J. R. & Feeney, R. E. (1979). Preparation and nutritional properties of caseins covalently modified with sugars. Reductive alkylation of lysines with glucose, fructose, or lactose. *J. Agric. Food Chem.*, **27**, 1094–8.

Marshall, J. J. & Rabinowitz, M. L. (1975). Enzyme stabilization by covalent attachment of carbohydrate. *Arch. Biochem. Biophys.*, **167**, 777–9.

Matheis, G. & Whitaker, J. R. (1984*a*). Modification of proteins by polyphenol oxidase and peroxidase and their products. *J. Food Biochem.*, **8**, 137–62.

Matheis, G. & Whitaker, J. R. (1984*b*). Chemical phosphorylation of food proteins: An overview and a prospectus. *J. Agric. Food Chem.*, **32**, 699–705.

Matheis, G. & Whitaker, J. R. (1984*c*). ^{31}P NMR chemical shifts of phosphate covalently bound to proteins. *Int. J. Biochem.*, **16**, 867–73.

Matheis, G. & Whitaker, J. R. (1987). A Review: Enzymatic cross-linking of proteins applicable to foods. *J. Food Biochem.*, **11**, 309–27.

Matheis, G., Penner, M. H., Feeney, R. E. & Whitaker, J. R. (1983). Phosphorylation of casein and lysozyme by phosphorus oxychloride. *J. Agric. Food Chem.*, **31**, 379–87.

Puigserver, A. J., Sen, L. C., Gonzales-Flores, E., Feeney, R. E. & Whitaker, J. R. (1979). Covalent attachment of amino acids to casein. 1. Chemical modification and rates of in-vitro enzymatic hydrolysis of derivatives. *J. Agric. Food Chem.*, **27**, 1098–104.

Rimington, C. (1927). Phosphorylation of proteins. *Biochem. J.*, **21**, 272–81.

Sinskey, A. J. & Tannenbaum, S. R. (1975). Removal of nucleic acids in SCP. In *Single-cell protein II*, ed. S. R. Tannenbaum & D. I. C. Wang. MIT Press, Cambridge, MA, pp. 158–78.

Sung, H.-Y., Chen, H.-J., Liu, T.-Y. & Su, J.-C. (1983). Improvement of the functionalities of soy protein isolate through chemical phosphorylation. *J. Food Sci.*, **48**, 716–21.

Whitaker, J. R. & Puigserver, A. J. (1982). Fundamentals and applications of enzymatic modifications of proteins: An overview. *Adv. Chem. Ser.*, **198**, 57–87.

Willmitzer, L. & Wagner, K. G. (1975). Chemical synthesis of partially and fully phosphorylated protamines. *Eur. J. Biochem.*, **59**, 43–50.