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Review

Applications of mass spectrometry to food proteins and peptides

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Abstract

The application of mass spectrometry (MS) to large biomolecules has been revolutionized in the past decade with the development of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) techniques. ESI and MALDI permit solvent evaporation and sublimation of large biomolecules into the gaseous phase, respectively. The coupling of ESI or MALDI to an appropriate mass spectrometer has allowed the determination of accurate molecular mass and the detection of chemical modification at high sensitivity (picomole to femtomole). The interface of mass spectrometry hardware with computers and new extended mass spectrometric methods has resulted in the use of MS for protein sequencing, post-translational modifications, protein conformations (native, denatured, folding intermediates), protein folding/unfolding, and protein–protein or protein–ligand interactions. In this review, applications of MS, particularly ESI-MS and MALDI time-of-flight MS, to food proteins and peptides are described.

Keywords: Reviews; Mass spectrometry; Food analysis; Proteins; Peptides

Contents

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1. Introduction

Until the mid-1980s, the analysis of proteins and large polypeptides by mass spectrometry (MS) could not be accomplished because of their polarity and their non-volatile nature [1,2]. Initially, electron impact (EI) [3] and chemical ionization (CI) [4] were used for small molecules which could be vaporized without decomposition. In these ionization techniques, fragments and ions other than intact molecular ions, appeared in the mass spectra. The development of desorption techniques represented the first major breakthrough for the formation of gaseous protein or large peptide ions without fragmentation. These desorption techniques include field desorption (FD) [5], plasma desorption (PD) [6,7], laser desorption (LD) [8], secondary ion (SI) and fast atom bombardment (FAB) [9]. However, experimental difficulties and limited range of molecular masses (M_r), together with low sensitivity at high molecular mass, limited the application of these desorption techniques [10,11]. Subsequently, atmospheric pressure chemical ionization (APCI) [12] and thermospray ionization (TSP) [13] techniques were introduced primarily for on-line coupling of MS to separation techniques like high-performance liquid chromatography (HPLC). Although APCI and TSP advanced the analysis of proteins and peptides, experimental difficulties, such as low sensitivity and limited mass range due to non-volatility and polarity of the samples were still encountered [14]. Therefore, there was a need of new ionization methods with improved sensitivity and a wider range of applications.

In the early 1980s, two ionization techniques, electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) almost simultaneously emerged for the analysis of large biomolecules. Although the concepts associated with these ionization techniques dated back at least 2 decades [15], it was Yamashita and Fenn [16,17] and Aleksandrov and co-workers [18,19] who successfully coupled an electrospray ion source to a quadrupole and a magnetic sector mass analyzer, respectively. Karas and Hillenkamp developed the MALDI-TOF-MS technique in which relatively large numbers of intact protein ions were generated by laser desorption of a matrix containing protein molecules [20]. Both ESI-MS and MALDI-TOF-MS now extensively support research of proteins and peptides, each having unique capabilities, as well as some fundamental similarities [2]. Their common features include ionization without fragmentation, accurate mass determination, picomole-to-femtomole sensitivity, and broad applicability [1,2,21,22]; however, induced fragmentation during MS analysis is desirable for sequence analysis of peptides. They are the foundation of the new field of biological MS, a tool that can determine M_r [23], conformation changes [24,25], molecular interaction [26], sequence N-terminally blocked protein [27], define N and C terminal sequence heterogeneity [28], locate and correct errors in DNA [29], identify sites of deamination and isoasparty formation [2], of phosphorylation [30], of oxidation, of disulfide bone formation [21], and glycosylation [23,31].

While the principles of MS of proteins are broadly applicable, the focus of this review paper is primarily on applications to food proteins and peptides. During the past 2 decades, considerable effort has been devoted to structural characterization of food proteins and peptides. Although much useful information has been obtained through numerous spectroscopic methods, they give no information about specific regions undergoing structural or dynamic changes and require relatively high concentration and large quantities of purified protein and long analysis time. In contrast, MS can accurately and precisely probe the conformational changes of large protein M_r in a relatively short analysis time (10–90 min). Food proteins whose M_r have been determined by MS are

shown in Table 1. Comprehensive reviews of the recent theory, instruments and applications of ESI and MALDI have appeared in the literature [14,29,32–35]. This review address the contributions of MS to determination of the structure of proteins and peptides in some common protein foods such as milk and dairy products, meat and fish products, eggs, legumes, cereal and cereal products, although the principles applies to other proteins as well.

2. Animal proteins and peptides

2.1. Eggs

MS has established the heterogeneity of proteins from eggs and determined the M_r of the various egg protein components without purification. The principal proteins of egg, ovalbumin, conalbumin and lysozyme have all been characterized by ESI-MS; M_r values have been determined for ovalbumin (44 300), conalbumin (77 500) and lysozyme (14 305) [36]. ESI-MS has also shown that irradiation of egg white resulted in an increase in the M_r of ovalbumin to 44 630 and conalbumin to 77 790 while the M_r of lysozyme was not affected [36]. Lysozyme, in particular, has been the subject of numerous MS studies on protein conformational changes and changes in charge-state distribution. Loo et al. observed that the charge state of lysozyme was shifted from 12+ to 14+ by changing pH from 3.3 to 1.6, reflecting the conformational change from compact native state to disordered state [37]. A similar shift was observed in thermal denaturation of lysozyme [38,39]. A further shift from 15+ of denatured lysozyme to 20+ was observed by reducing four disulfide bridges [28]. Konermann and Douglas similarly observed more net negative charge upon unfolding of lysozyme in negative mode [40]. The results indicated that the protein was charged, positively or negatively depending on the positive or negative ionization mode, respectively, and the number of net charge predominantly reflected the protein conformation.

The ability of ESI-MS to resolve mass differences as small as a single proton was used by Katta and Chait to study conformational changes in egg lysozyme by hydrogen/deuterium ($H/^2H$) exchange ESI-MS as each deuteration increases the protein mass by 1 u [25,41]. The extent of $H/^2H$ exchange occurred in different protein conformers over defined time periods differed widely depending on the conformation [25]. Katta and Chait demonstrated, on the basis of both the changes in charge state distribution and $H/^2H$ exchange rates, that reduced lysozyme exists in an unfolded state [41]. The mechanism of folding in egg lysozyme was investigated using $H/^2H$ exchange technique both with MS and NMR [42].

2.2. Milk and dairy products

The proteins of milk and dairy products have been the subject of numerous MS investigations; these include identification of milk protein variants and glycoforms, fingerprinting, degree of glycoforms, detection of milk adulteration and identification of peptides in dairy products.

2.2.1. Whey proteins

ESI-MS studies with purified β-lactoglobulin (β-Lg) from bovine milk revealed the presence of multiple M_r species of β-Lg (A, B and C variants) [43]. In addition, the existence of multiple glycosylation of these bovine β-Lg variants has been identified by several ESI-MS studies [31,44–48]; the results indicated that nonenzymatic lactosylation of β-Lg occurs under mild heat treatment. ESI-MS has also been used to rapidly characterize the complex mixture of glycoforms of α-lactalbumin (α-Lac) without the need of further purification of these forms [47]. Alli et al. used ESI-MS to detect the presence of glycoforms in both β-Lg (Fig. 1A) and α-Lac (Fig. 1B), in whey protein concentrate (WPC) and in lyophilized whey; the presence of at least three glycoforms of β-Lg and one glycoform of α-Lac was detected [48]. MALDI-TOF-MS has also been used to detect nonenzymatic glycosylation of several peptides; these researchers concluded that MALDI-TOF-MS was helpful in conforming that amino acid residues, other than lysine, are glycosylated [49].

Two-dimensional electrophoresis coupled to MALDI-TOF-MS were used to detect the C-terminal of truncated forms of β-Lg in whey from Romagnola cow's milk [50]. The result clearly shows that two of minor components were related to the β-Lg A variant and two to the β-Lg B variant. Hu et al. used

Table 1
Report of some food proteins and peptides molecular mass by ESI-MS and MALDI-TOF-MS

Proteins/peptides	Note	M_r	Ref.
Animal proteins			
Egg proteins			
Lysozyme	Hen eggs	14 305	[10,11,28,36,37,39–42,114]
Ovotrasferrin	Hen eggs	77 500	[11]
Ovalbumin	Hen eggs	43 300–44 585	[114,116]
	Hen eggs	44 630	[36]
Conalbumin	Turkey eggs	77 500–77 650	[10,114]
	Hen eggs	77 790	[36]
Milk proteins			
Bovine serum albumin	Monomer	66 646	[11,52,114,118]
	Dimer	133 460	[52]
	Trimer	200 188	[52]
	Tetramer	266 329	[52]
	Pentamer	332 800	[52]
β-Lactoglobulin	B variant (bovine)	18 277	[31,43,44,114,117]
	A variant (bovine)	18 363	[31,43,44,114,117]
	Buffalo	18 306	[64]
	Ewe	18 102	[65]
α-Lactalbumin	Bovine	14 175	[11,48]
	Buffalo	14 270	[64]
	Ewe	14 139	[65]
Casein subunits bovine	α_{S1} phosphorylated	23 725–23 682	[64,67,117]
	α_{S1}dephosphorylated	23 005	[64,67,117]
	α_{S2}-CN	25 241	[64,67,117]
	β-CN	24 085	[64,67,117]
	κ-CN	19 125	[64,67,117]
	γ_1-CN	20 085	[64,67,117]
	γ_2-CN	11 856	[64,67,117]
	γ_3-CN	11 591	[64,67,117]
Casein subunits buffalo	α_{S1}-CN	23 406	[64]
	β-CN	24 066	[64]
	κ-CN	19 192	[64]
	γ_1-CN	20 090	[64]
	γ_2-CN	11 850	[64]
	γ_3-CN	11 588	[64]
Ovine (α_{S1})			
Variant A	9P (199 amino acid)	23 478	[54]
	9P (191 amino acid)	22 467	[54]
Variant C	8P (199 amino acid)	23 412	[54]
	8P (191 amino acid)	22 390	[54]
Variant D	4P (199 amino acid)	23 188	[54]
	4P (191 amino acid)	22 194	[54]
Caprine (α_{S1})			
Variant A	8P (199 amino acid)	23 362	[55]
	8P (198 amino acid)	23 239	[55]
	8P (191 amino acid)	22 351	[55]
Variant B	8P (199 amino acid)	23 345	[55]
	8P (198 amino acid)	23 215	[55]
	8P (191 amino acid)	22 333	[55]
Variant C	8P (199 amino acid)	23 267	[55]
	8P (198 amino acid)	23 141	[55]
	8P (191 amino acid)	22 252	[55]
	Dephosphorylated	21 770	[55]
Proteoso peptone p.p. 81	Bovine	9170	[64]
	Buffalo	8670	[64]

Table 1. Continued

Proteins/peptides	Note	M_r	Ref.
Meat proteins			
Pepsin	Porcine	34 584	[118]
Rennin	Bovine	35 646	[118]
Actin		42 000	[11]
Plant proteins			
Legume proteins			
Trypsin inhibitor	Soybean	20 097	[11,114,115,117]
	P. vulgaris	8406	[85]
		8957	[85]
α-Amylase	Bacterial source	54 700	[10]
	Kidney beans	54 857	[87]
Concanavalin A	Jack bean	25 573	[117]
	A1 fragment	12 937	[117]
	A2 fragment	12 653	[117]
Phaseolin polypeptides	*P. vulgaris*		
	Fraction 1	49 615	[86]
	Fraction 2	48 075	[86]
Phaseolin polypeptides	*P. lunatus*		
	Fraction 1	26 240	[23]
	Fraction 2	26 113	[23]
	Fraction 3	24 249	[23]
Soybean agglutinin (SBA)	Glycoprotein		
SBA I	β subunit	28 000	[83,84]
	α subunit	29 437	[83,84]
SBA II	β subunit	28 000	[83,84]
	γ subunit	28 327	[83,84]
	γ′ subunit	28 627	[83,84]
	α′ subunit	29 325	[83,84]
	α subunit	29 437	[83,84]
SBA III	β subunit	28 000	[83,84]
	γ subunit	28 327	[83,84]
	α′ subunit	29 325	[83,84]
	α subunit	29 437	[83,84]
Coconut proteins			
Coconut milk	Fraction 1	46 640	[90]
	Fraction 2	50 359–51 209	[90]
	Fraction 3	35 574 and 47 679	[90]
Insoluble solids	Fraction 1	46 640–46 861	[90]
	Fraction 2	50 376–51 100	[90]
	Fraction 3	49 040	[90]
Acid precipitate	Fraction 3	48 861–49 142	[90]
Cereal proteins			
Glutenin (high M_r subunits)			
Sicco isogene	1Ax1	87 500	[76]
Chinese spring	1Dx2	88 379	[76]
Cheyenne	1Dx5	88 930	[76]
Cheyenne	1Bx7	83 500	[76]
Cheyenne	1By9	72 500	[76]
Cheyenne	1Dy10	68 360	[76]
Chinese spring	1Dy12	69 520	[76]
Katepwa	Subunit 2	86 202 and 87 936	[77]
Katepwa	Subunit 7	82 279	[77]
Katepwa	Subunit 9	73 308	[77]
Katepwa	Subunit 10	67 280	[77]
Agglutinin	Wheat germ	17 090	[16]

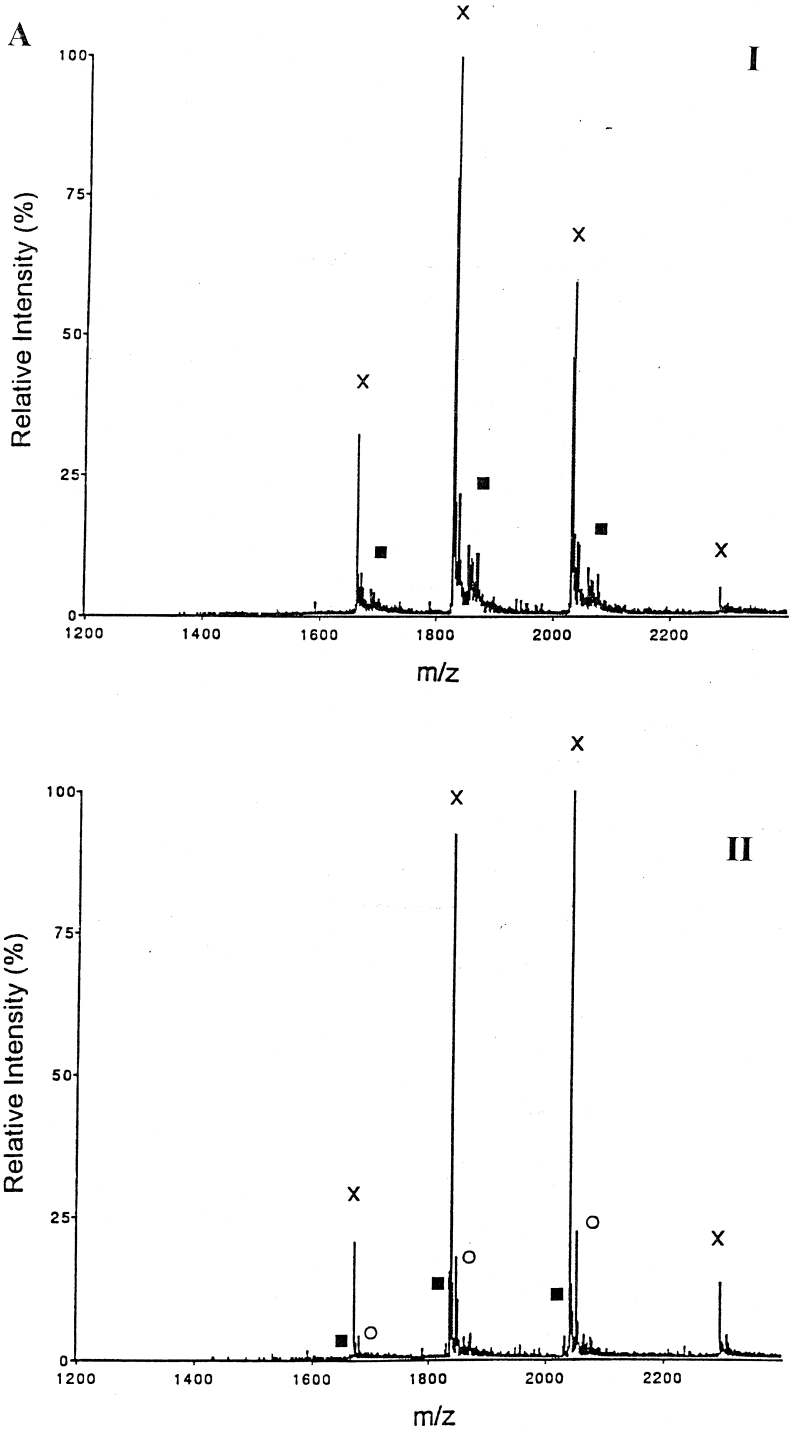

Fig. 1. (A) Interpreted mass spectra of (I) fraction β-F1 (×=18 279; ■=18 606) and (II) fraction β-F2 (×=18 366; ○=18 464; ■=18 692) from RP-HPLC of commercial β-Lg and (B) interpreted mass spectrum of fraction α-F from RP-HPLC of commercial α-Lac (×=14 181; ○=14 278; ■=14 504). Reprinted with permission from Ref. [48].

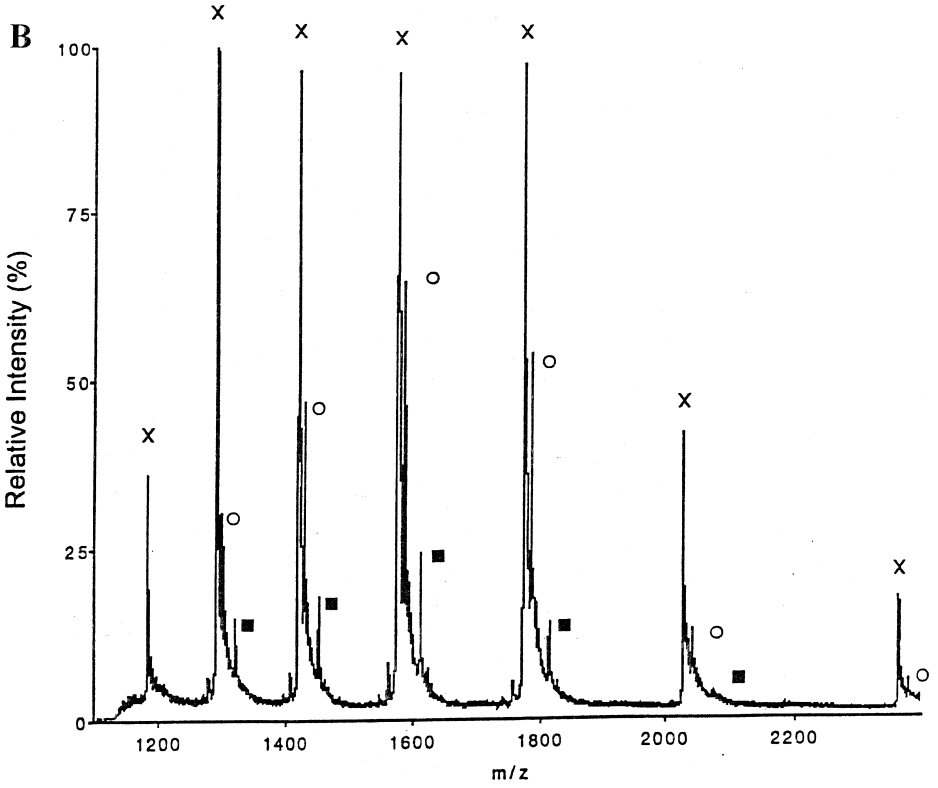

Fig. 1. (*continued*).

ESI-MS to determine calcium-binding stoichiometry for calcium-binding proteins [51]. They found that bovine α-Lac binds specifically to one Ca^{2+} ion and suggested that ESI-MS can be used to determine the number and type of metal ions that bind to protein.

MALDI-TOF-MS was used to study the effect of different chromatographic conditions on the elution of bovine serum albumin (BSA) in a reversed-phase high-performance liquid chromatography (RP-HPLC) system [52]; multiple peaks of BSA were observed when shallow gradients were used for elution. MALDI-TOF-MS revealed that these RP-HPLC multiple peaks were aggregated forms of BSA [52].

RP-HPLC of acid hydrolysates of WPC identified three fractions in the unhydrolyzed WPC and three other fractions after 18 h hydrolysis [53]. Each fraction was a mixture of a few to several peptides and their M_r values were determined by ESI-MS. The results confirmed that organic acid hydrolysis of WPC resulted in peptides that were smaller than those in unhydrolyzed WPC [53].

2.2.2. Casein proteins

MS has established the heterogeneity of proteins from casein (CN) and determined the M_r of the various casein protein components. Casein is made up of several components; the main molecular subunits are α_{S1}-, α_{S2}-, β-, κ- and γ-CN (Table 1).

Combined use of ESI-MS and FAB-MS confirmed the primary structure of mature ovine α_{S1}-CN as well as the amino acid substitutions in variants A, C and D, and identified the phosphorylation sites [54]. The mature protein of each variant was found to be a mixture of two subunits, both with multiple phosphorylated forms of the same protein; one subunit was full-length (199 amino acid residues), which accounted for about 80%, the other was the deleted form which lacked segment 141–148 of the mature protein and represented about 20% of the α_{S1}-CN

[54]. MS analysis also revealed that the differences among the three genetic variants (A, C, and D) were simple silent substitutions, which involved the degree to which the protein was phosphorylated [54]. Similarly, MS revealed that mature goat α_{S1}-CN exists as a mixture of at least four variants (A, B, C and D) which differ in peptide chain length and in the number of phosphorylated serine residues [55]. Variant A is the main component (48%) corresponds to the full-length of α_{S1}-CN (199 amino acid). The three short forms of the protein are variant B (198 amino acid, 29%) missing Gln 78, variant C (191 amino acid, 16%) missing residues 141–148 and subunit D (191 amino acid, 7%) missing residues 110–117 [55].

MS was used to resolve the heterogeneity of caseinomacropeptide (CMP), a polypeptide of 64 amino acids which is released from bovine κ-CN by the action of chymosin during the primary phase of milk clotting [56]. This study described the ability of RP-HPLC coupled with ESI-MS to characterize a complex mixture like CMP. MS was also used to identify the preferential cleavage sites of recombinant chymosin on purified κ-CN (A variant) over the pH range 6.6–2.6; the rate and extent of hydrolysis of κ-CN and its macropeptide moiety increased with decreasing the pH to a maximum at 3.6 [57].

2.2.3. Dairy products application

MALDI-TOF-MS was used to detect thermal degradation and to determine the protein content of milk samples obtained with different conditions of pasteurization (70–90°C for 10–30 s) and sterilization (140–150°C for 2–5 s) [58,59]. The capability of MALDI-TOF-MS to characterize protein profile from several cow milks was also studied [59]; it was found that the protein fingerprint of milk from four different breeds of cows at the same lactation stage and under the same feeding system were different in their MALDI spectra [59]. MALDI-TOF-MS was also used to evaluate the protein profile of cow's milk after different enzymatic and/or thermal treatments of 11 infant milk formulas; the results demonstrated the degree of hydrolysis of different protein hydrolysate formulas [60]. Similarly MALDI-TOF-MS was used to evaluate the effect of diet and pathological states on the protein profile of human milk [61]. MALDI-TOF-MS has also been used to determine the effects of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, on milk proteins during yogurt preparation [62]; the results indicated that fermentation with the former bacteria resulted in the breakdown of high-molecular-mass (high-M_r) casein with the production of a low-molecular-mass (low-M_r) peptide (3850) while the latter bacteria did not exhibit any proteolytic activity. Proteolysis was enhanced when milk is incubated with a mixture of the two bacteria [62]. Furthermore, MALDI mass spectra of different strains of the same bacteria species indicated differences in proteolytic activity [62,63].

Angeletti et al. examined the capabilities of MALDI-TOF-MS for the characterization of water-buffalo milk and mozzarella cheese to detect possible fraudulence in mozzarella cheese production [64]; results indicated that buffalo milk proteoso peptone p.p. 81 and α_{S1}-CN had lower M_r values (8670 and 23 406, respectively) than that of bovine milk (9170 and 23 682, respectively) (Fig. 2) [64]. Furthermore, it was determined that (a) the relative abundance of peaks due to α_{S2}-CN and β-Lg was lower in buffalo milk than in bovine milk (Fig. 2) and (b) a peak of M_r 15 790 (protein X), which is commonly found in buffalo milk, was still detected in water buffalo mozzarella cheese, indicating that this protein is resistant to thermal and enzymatic processes [64]. MALDI-TOF-MS was used to detect adulteration of ewe's cheese with bovine milk [65]; the mass spectrum of the ewe's milk indicated that β-Lg, γ_2-CN and α-Lac have M_r values of 18 102, 11 827 and 14 139, respectively, which are all lower than those of the same proteins in bovine milk. Furthermore, the relative abundance of (a) peak at m/z 11 325 (due to γ_3-CN) from ewe cheese and (b) peak at m/z 11 869 (due to γ_2-CN) from bovine cheese, can be used to determine the percentage of bovine milk fraudulently added to ewe's milk, in the production of ewe's cheese [65].

Both MALDI-TOF-MS and ESI-MS have been used to identify peptides in cheese [66,67]. MALDI-TOF-MS revealed that eight peptides in cheddar cheese were derived from α_{S1}-CN, seven from β-CN and one from α_{S2}-CN [66]. ESI-MS and electrospray ionization tandem mass spectrometry (ESI-MS–MS) identified a total of 25 peptides in mild, medium and old cheddar cheese and a commercial cheddar cheese

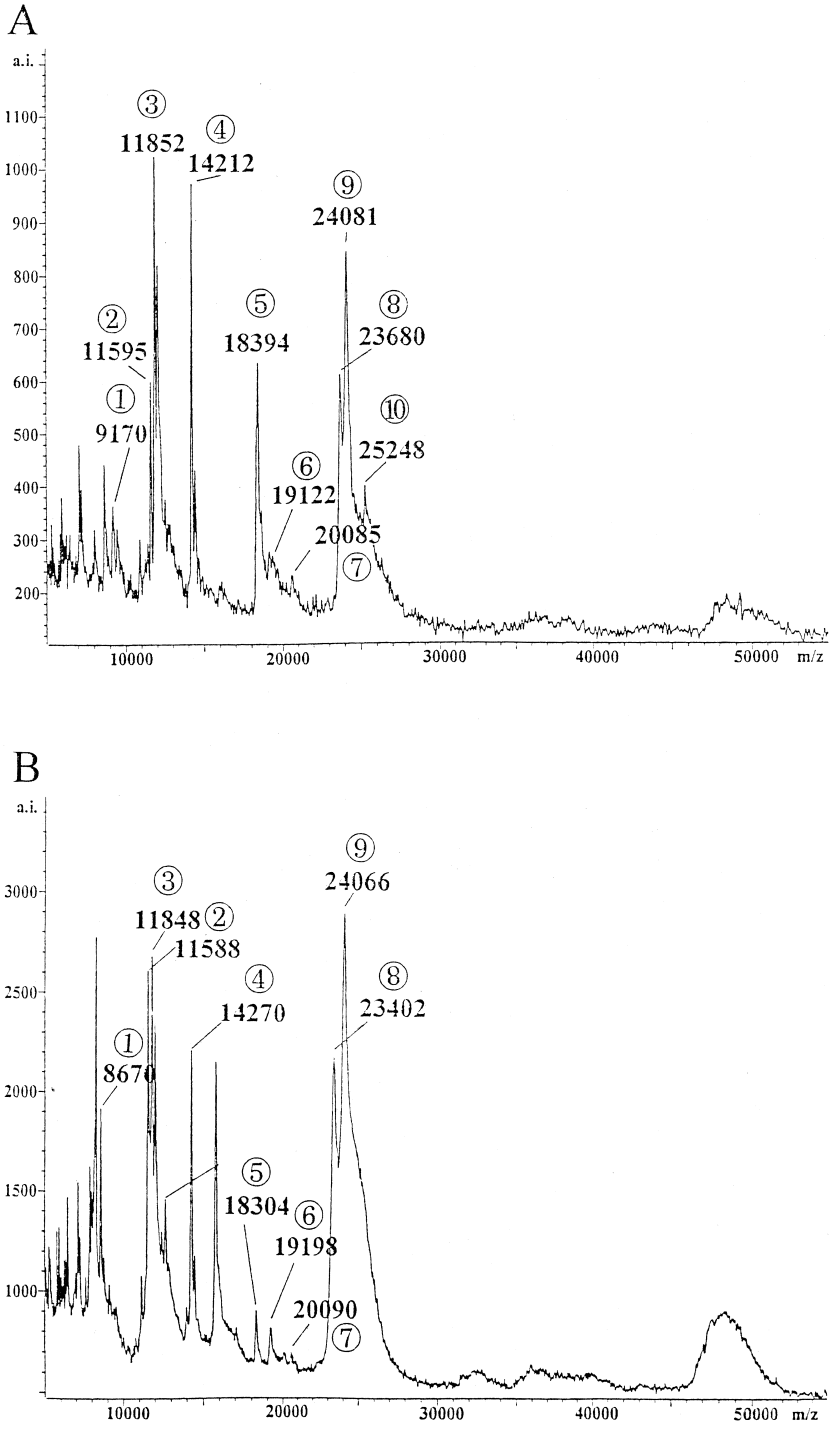

Fig. 2. MALDI mass spectrum of bulk bovine (A) and water buffalo (B) milk. Protonated molecules of (1) proteoso peptone p.p. 81, (2) γ_3-CN, (3) γ_2-CN, (4) α-Lac, (5) β-Lg, (6) κ-CN, (7) γ_1-CN, (8) α_{S1}-CN, (9) β-CN and (10) α_{S2}-CN. Reprinted with permission from Ref. [64].

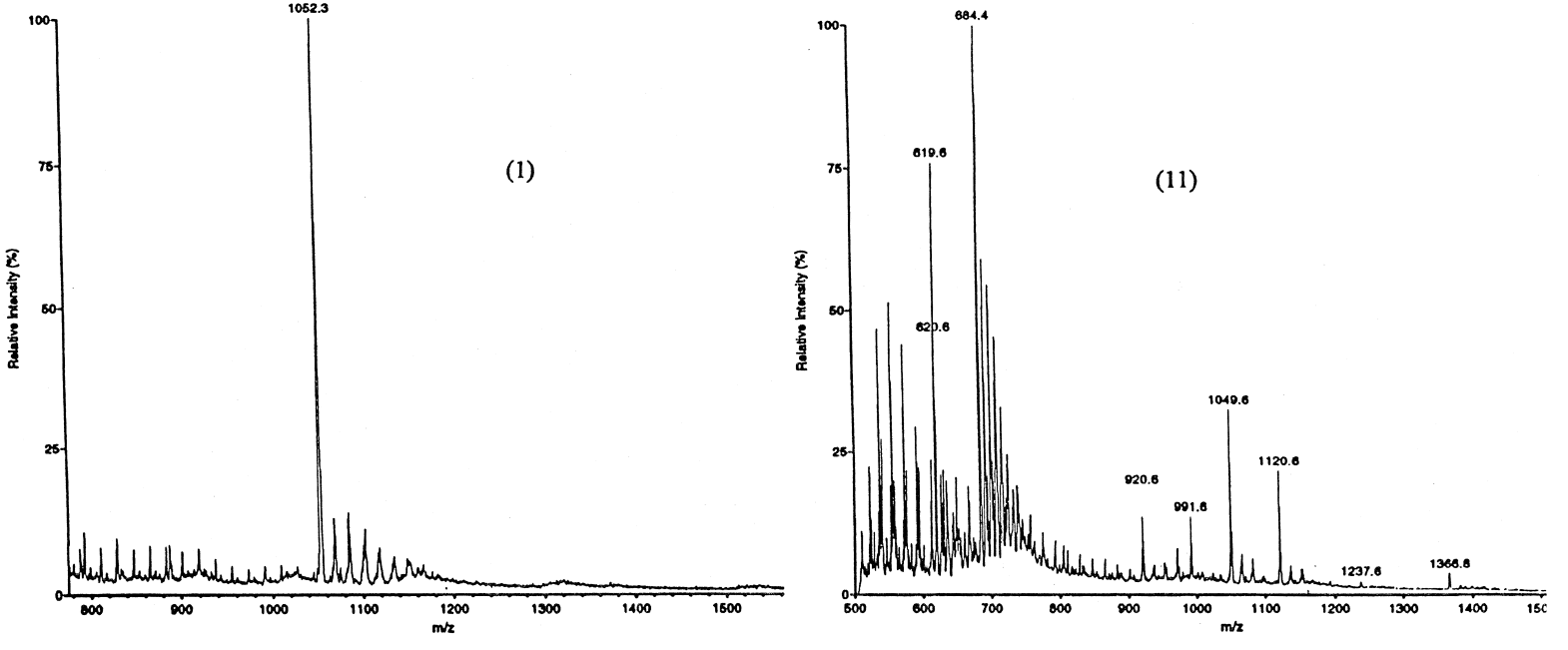

Fig. 3. Interpreted mass spectra of a fraction containing a single M_r species (I) and multiple species (II). Reprinted with permission from Ref. [67].

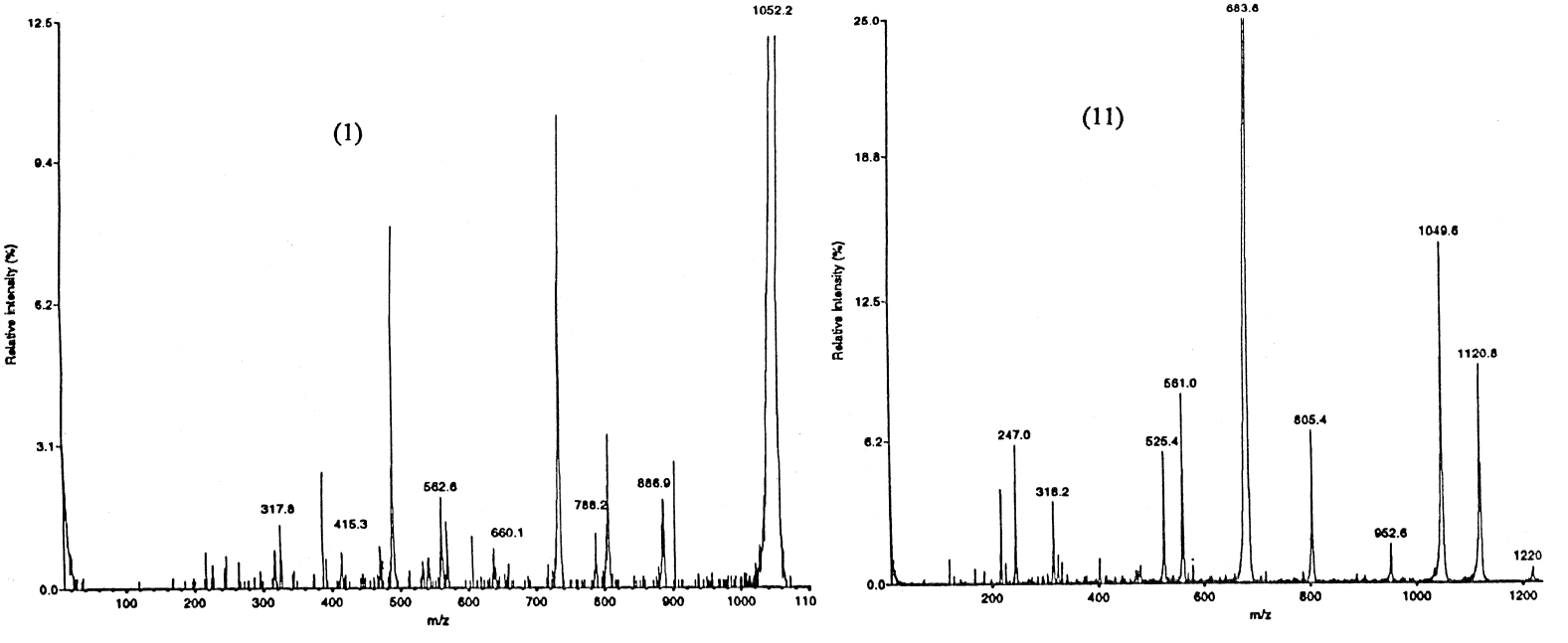

Fig. 4. ESI-MS–MS spectra of species (I) M_r=1052 (singly-charged) and (II) M_r=1366 (doubly-charged). Reprinted with permission from Ref. [67].

flavor [67]. Thirteen peptides were found to be derived from α_{S1}-CN, seven from β-CN and five from κ-CN. Fig. 3I and II show the mass spectra of a single M_r species (M_r 1052) and two or more molecular species, respectively, while Fig. 4I and II show the ESI-MS–MS of a singly charged species of M_r 1052 and a doubly charged species of M_r 1366, respectively [67]. MS results also revealed the presence of N-terminal segments of β-, α_{S1}- and α_{S2}-CN and α-Lac in water-soluble fractions of cheddar cheese; this was explained on the basis of known specificities of lactococcal cell envelope proteinases; such as chymosin, plasmin and phosphatase [68].

The relationship between the degrees of hydrolysis (DH) of CN using trypsin and pancreatin independently, and the release of casein phosphopeptide (CPP) has been studied by MALDI-TOF-MS [69]. Highest yields of CPP were obtained at casein DH of 17% and 19–23% for trypsin and pancreatin, respectively. The relationship between CPP production in Grana Padano cheese and ripening (4–38 months) was also studied using FAB-MS; 45 phosphopeptides (24 from β-CN, 16 from α_{S1}-CN and five α_{S2}-CN) were identified [70]. Moreover, CPP interaction with colloidal calcium phosphate (CCP) isolated by tryptic hydrolysis was characterized by RP-HPLC–ESI-MS [71]. It was shown that among the peptides produced, 14 phosphopeptides were identified (eight α_{S1}-CN and six β-CN) and half of SerP cluster from β-CN and all SerP from α_{S1}-CN can interact with CCP [71].

2.3. Meat and fish

In comparison with the proteins of milk and egg, there have been relatively fewer MS studies on proteins of meat and fish. FAB-MS has shown that the tryptric hydrolysate of calciprotein from crayfish contains at least eight peptides with M_r values ranging from 375 to 1455 with close agreement in sequence information obtained by FAB-MS and from

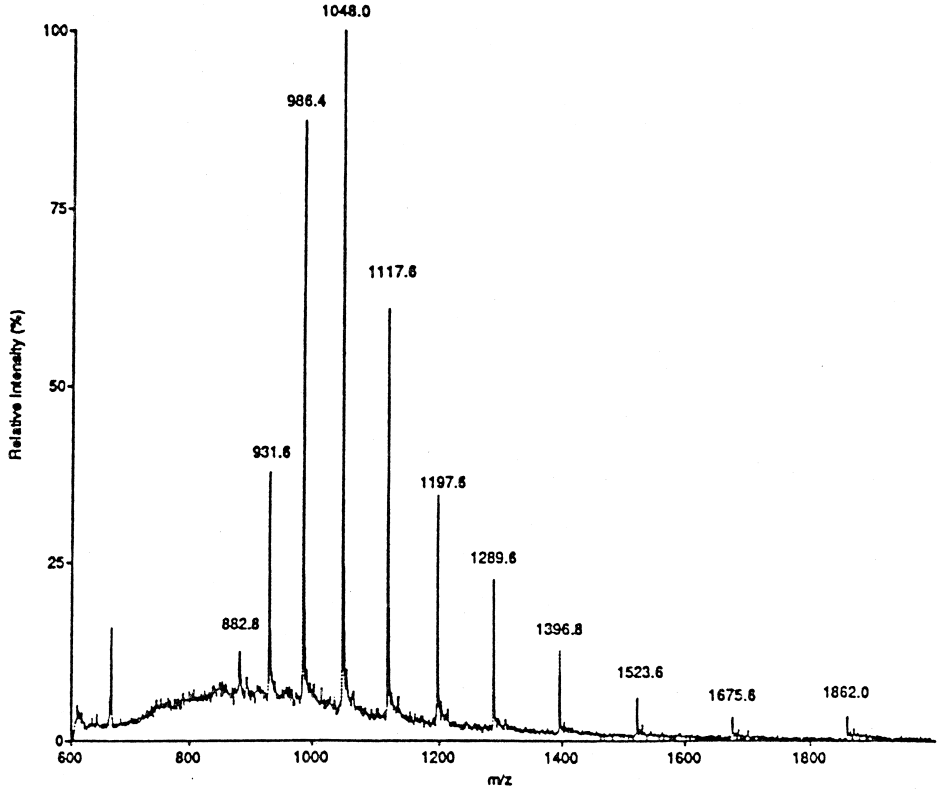

Fig. 5. A typical interpreted ESI-MS mass spectrum of carp fish polypeptide with M_r of 16 751. Reprinted with permission from Ref. [73].

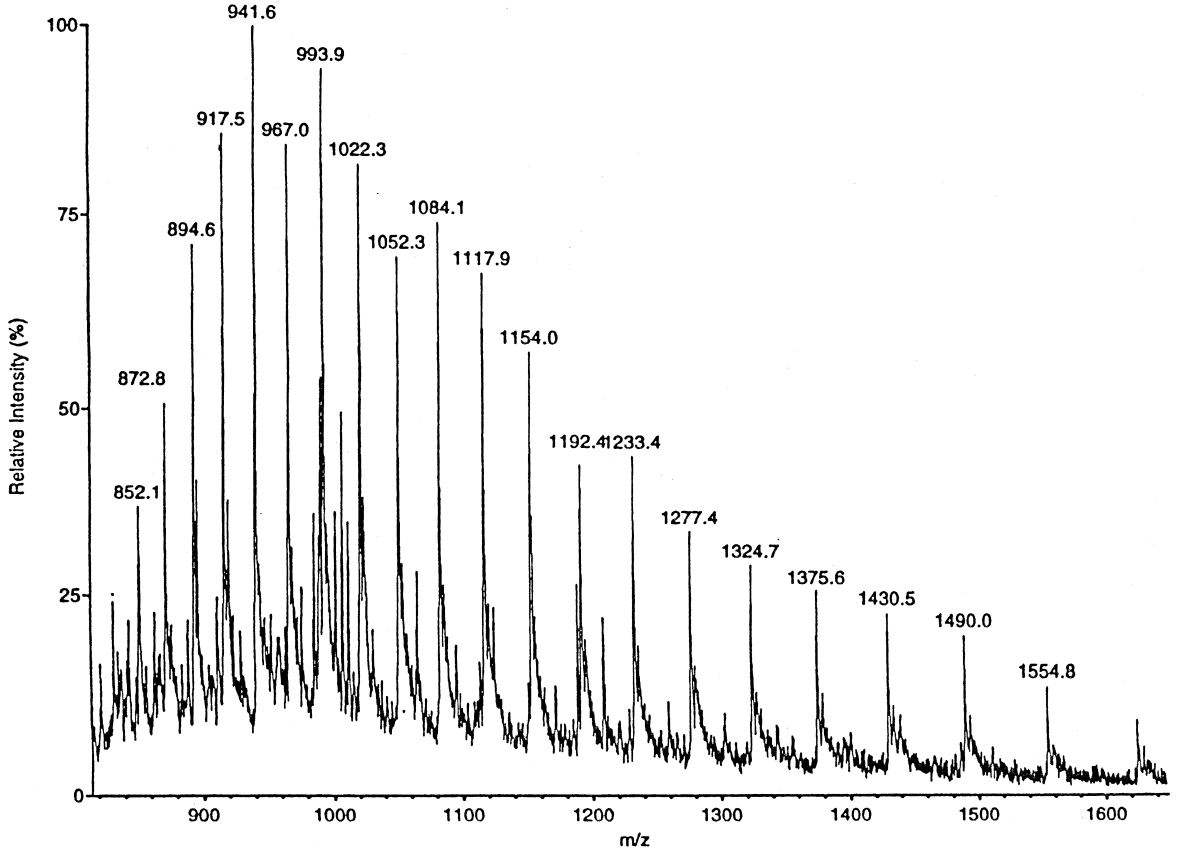

Fig. 6. Interpreted mass spectra of sarcoplasmic protein extracted from meat with M_r of 35 740. Reprinted with permission from Ref. [75].

amino acid analysis [72]. ESI-MS revealed the presence of at least 25 polypeptides with M_r values ranging from 2000 to 42 800 in the soluble nitrogen extract of fresh carp fish [73,74]; a typical interpreted ESI-MS mass spectrum of fish polypeptide with an M_r of 16 751 is illustrated in Fig. 5. RP-HPLC–ESI-MS demonstrated that the sarcoplasmic protein extracted from ground and whole meat contained at least 12 polypeptides with M_r values ranging from 11 000 to 42 000 [75]. The relative peak area of the M_r 35 700 protein shown in Fig. 6 decreased during storage of meat; this protein could be investigated as an indicator of freshness [75].

3. Plant proteins and peptides

Over the years plant storage proteins have become important functional ingredients in many prepared foods. Concurrently, there has been increased interest in understanding the structure–function relationships of these storage proteins to explain and even predict certain desired characteristics; this requires elucidation of the molecular characteristic of the proteins. To date, the use of MS for characterizing plant storage protein has been somewhat limited.

3.1. Cereals

Cereal grains contain complex mixtures of proteins with structurally different molecular characteristics. The classification of these proteins as globulins, albumins, glutenins and prolamins is widely accepted; each of these groups in turn, represent a complex mixture of proteins. MALDI-TOF-MS has been used in several studies to characterize the subunits of glutenin [76,77]. A total of seven high-M_r subunits of M_r 87 500, 88 379,

88 930, 83 500, 72 500, 68 360 and 69 520 have been identified [76]. In general, the M_r of these subunits are close to those calculated from gene sequence and within the range of analytical error; this study demonstrated that the high-M_r subunits are not extensively glycosylated, as previously reported [76]. Dworschak et al. reported the use of MALDI-TOF-MS for assessing the composition and mass distribution of crude and partially purified wheat gluten prolamins (gliadin) and reduced high-M_r and low-M_r glutenin subunit fractions from common and durum wheat varieties without prior separation by HPLC [77]. Gliadins and low-M_r glutenin subunits showed complex MALDI mass spectra with M_r ranging from 30 000 to 40 000, while the mass spectra of high-M_r glutenin subunits were fairly simple with M_r ranging from 87 936 to 71 520. The results indicated the feasibility of using MALDI-TOF-MS in wheat breeding programs for the rapid and routine identification of specific high-M_r subunits associated with superior quality [77].

MALDI-TOF-MS has also been used to quantify gluten gliadins in both processed and unprocessed foods [78,79]; the procedure is rapid and sensitive with good correlation with data from an immunological assay method. MALDI-TOF-MS can be used as a rapid screening technique for (a) the presence of gliadins in foods by monitoring the occurrence of the protonated gliadin mass pattern in the mass range from 25 000 to 40 000 and (b) the presence of other toxic gluten cereal prolamins fractions, such as barley hordeins, rye secalins and oat avenins [80,81].

The major wheat flour immunoreactive proteins that are responsible for baker's asthma has been identified as members of the α-amylase family using MS and the N-terminal amino acid sequence [82].

3.2. Legume seeds

ESI-MS has been used to characterize the quaternary structure of the three isolectins of soybean agglutinin (SBA), a tetrameric glycoprotein previously reported to consist of two subunits [83]. The ESI-MS results showed that the quaternary structure composition of the three isolectins of SBA (SBA I, SBA II, and SBA III) were approximately α2β2, α2βγ (and α2βγ′) and α2γ2, respectively. Similarly, ESI coupled with TOF has been used to investigate noncovalent protein–protein interactions in SBA [84].

A wide range of M_r values (8000–23 000) have been reported for trypsin inhibitors (TIs) of dry beans depending on the method used for estimating the M_r; i.e., ultracentrifugation, size-exclusion chromatography, sodium dodecyl sulfate–polyacrylamide gel electrophoresis and amino acid composition. Plasma desorption mass spectrometry (PD-MS), using ^{252}Cf as the ionizing source, was used in the partial characterization of TIs of great northern beans (*Phaseolus vulgaris*) [85]. The PD-MS of TIs revealed two peaks of M_r 8406 and 8957, corresponding to TI chain lengths of 76 and 81 amino acid residues, respectively [85].

ESI-MS and ESI-MS–MS were used to investigate the extent to which the polypeptide subunits in a crystalline protein isolated by citric acid from dried seeds of white kidney beans (*P. vulgaris*) were similar to the subunits of native phaseolin through the identification and characterization of the phaseolin polypeptides [86]; the isolated crystalline proteins were shown to contain polypeptides with average M_r values of 49 615 and 48 075 which were similar to those reported for α-type and β-type phaseolin precursors, respectively [86]. Using the same techniques (ESI-MS and ESI-MS–MS) for characterizing a crystalline protein isolated from large lima beans (*P. lunatus*), it was shown that a glycosylated phaseolin polypeptide fragment of M_r 26 240 was similar to a C-terminal segment of the phaseolin polypeptides of *P. vulgaris*, while a glycosylated subunit of M_r 26 113 and its non-glycosylated variant of M_r 24 249 were similar to an N-terminal segment of phaseolin polypeptides of *P. vulgaris* [23] (Fig. 7).

α-Amylase inhibitors which can effect the response of blood glucose insulin or general starch digestion and absorption in mammals has been studied by RP-HPLC–ESI-MS [87]. It was shown that in a crude extract prepared from white kidney beans (*P. vulgaris*), a high α-amylase inhibitor activity was associated with a glycoprotein whose deglycosylated M_r was estimated as 54 857 by ESI-MS. Fig. 8 shows the mass spectra of the purified deglycosylated (A) and glycosylated (B) inhibitors.

ESI-MS and ESI-MS–MS were used to investigate the differences in the tryptic digestion of crystalline

and amorphous (noncrystalline) proteins isolated from *Phaseolus* beans by comparing the peptides which resulted from the hydrolysis [88]; the results suggested that trypsin specific peptide bonds located in the β-structure region of β-type phaseolin was resistant to trypsin hydrolysis while the most accessible region to tryptic cleavage were located within α-helix structures and in regions of interconnecting secondary structure. MS has also been used to study the proteolytic changes associated with *Rhizopus oligosporus* fermentation of soybean to produce tempe from soybeans [89]. ESI-MS showed several tempe peptides with M_r ranging from 569 to 16 688.

3.3. Other plants

Proteins isolated from other plant sources such as coconut have been characterized by MS [90]. By use

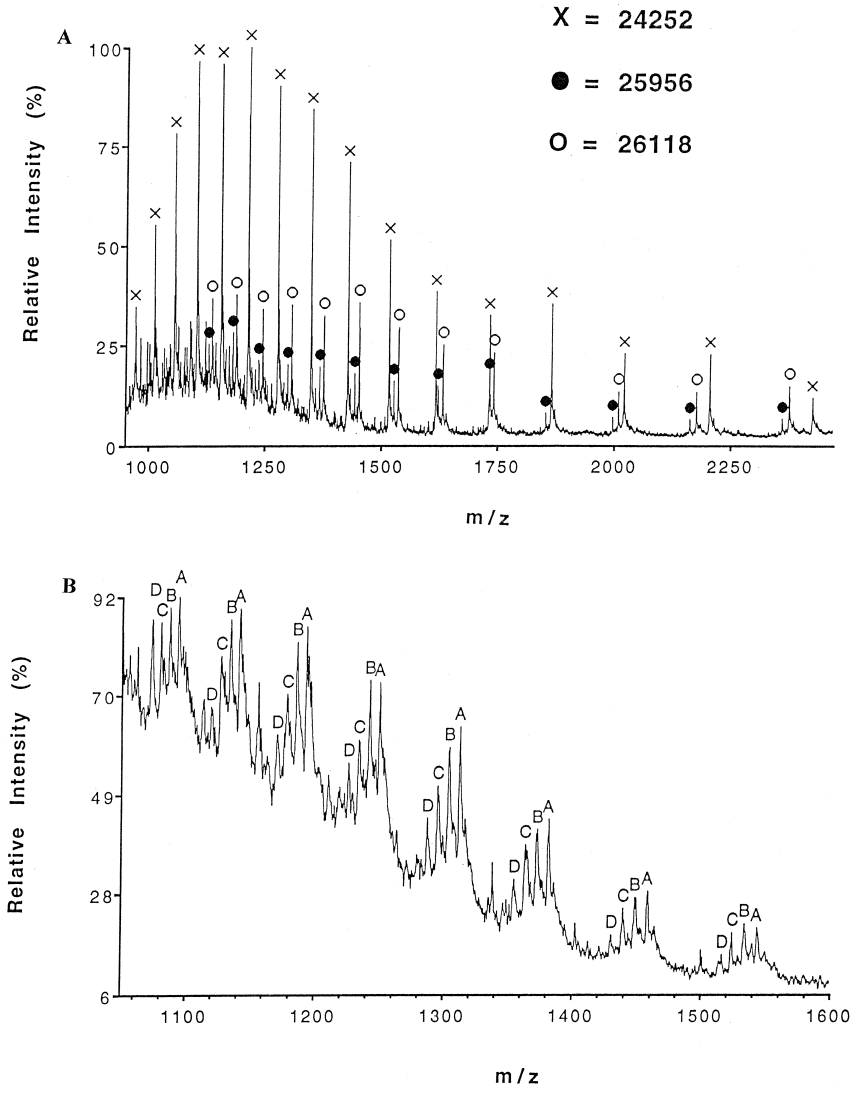

Fig. 7. (A) Interpreted mass spectra of unfractionated crystalline protein from large lima bean and interpreted mass spectra of fractions F1 (B), F2 (C), and F3 (D) obtained from crystalline protein of large lima beans. Values given for ×, ● and ○ are molecular masses. Reprinted with permission from Ref. [23].

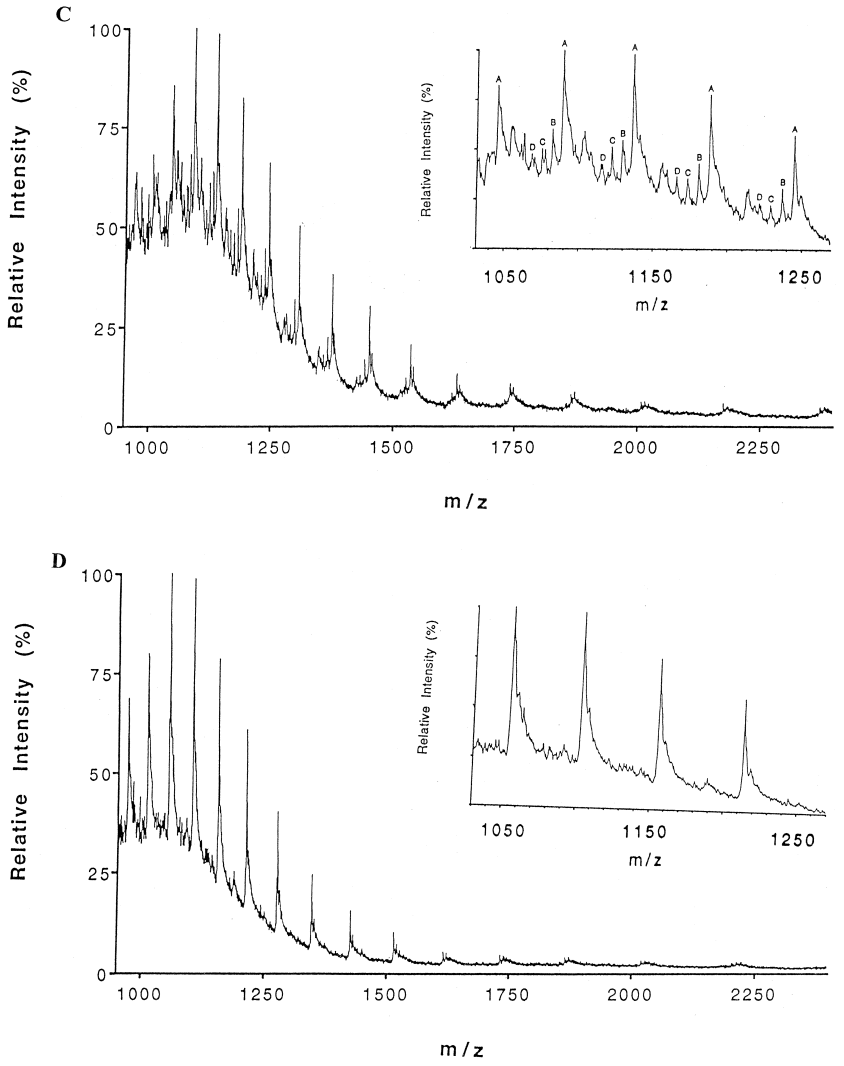

Fig. 7. (*continued*).

of RP-HPLC and ESI-MS proteins with M_r of 51 209, 50 359, 49 142, 49 040, 48 861, 47 679, 46 640 and 35 574 were separated and identified [90]. Fig. 9 shows the mass spectrum of the protein component with M_r 46 640.

4. Other proteins

In addition to study of food proteins, MS is used increasingly to identify and characterize other proteins which are not considered food proteins but which are of interest to food scientists. MALDI-TOF-MS has been used effectively to detect bacteriocins in the culture supernatant of producer organisms [91], while ESI-MS has been used to determine the M_r of a bacteriocin (Reutericin 6, M_r 2400) produced by *Lactobacillus reuteri* LA6 [92]. Both ESI-MS and MALDI-TOF-MS were used to assess the purity and stability of nisin and its degradation products [93]. In another study, nisin, its variants and degradation products were characterized and quantified using ESI Fourier transform ion cyclotron resonance (FT-ICR) MS [94]; it was

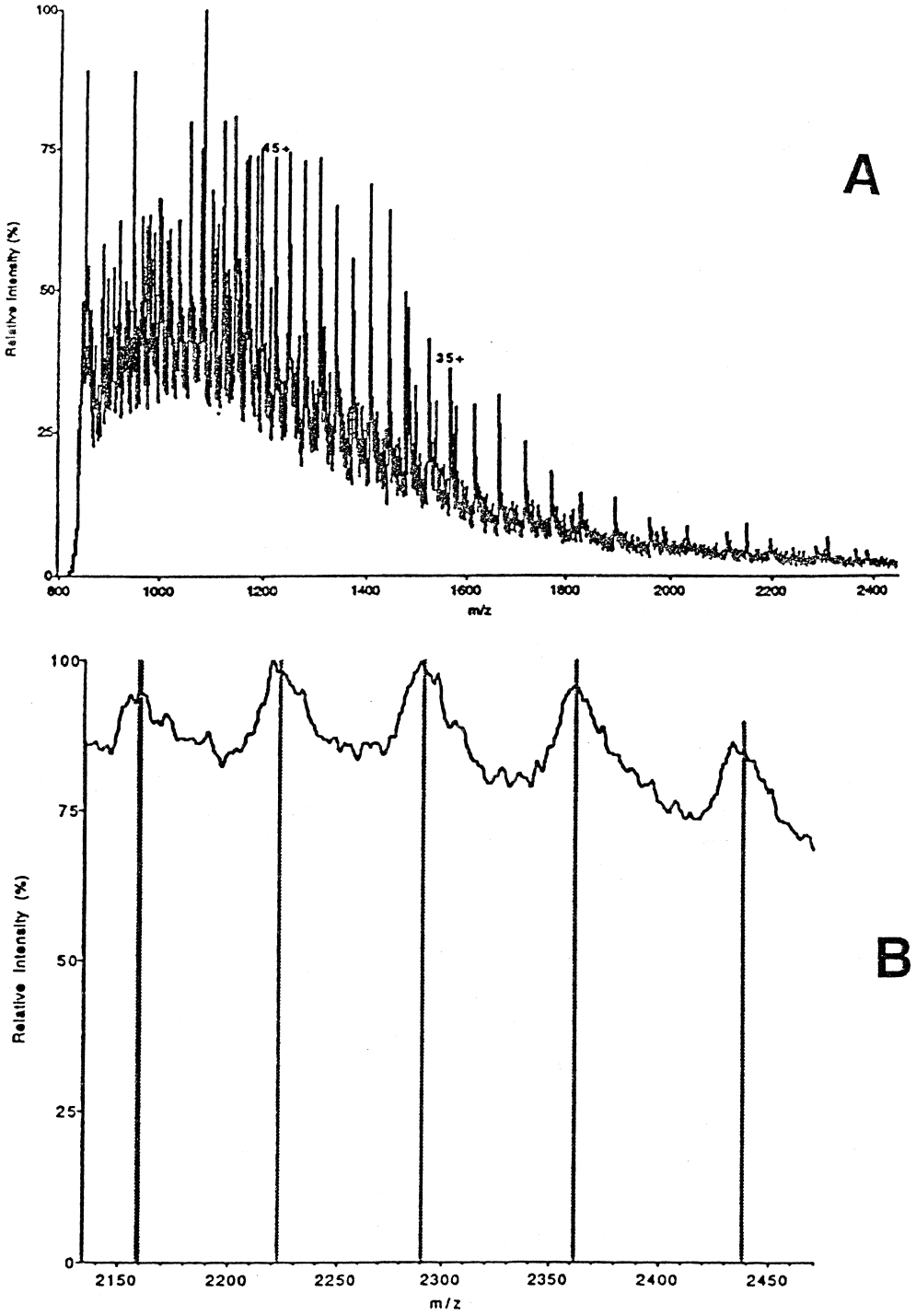

Fig. 8. Mass spectrum of deglycosylated (A; M_r=54 857±3) and glycosylated (B; M_r=57 071±10) α-amylase inhibitor from white kidney beans. Reprinted with permission from Ref. [87].

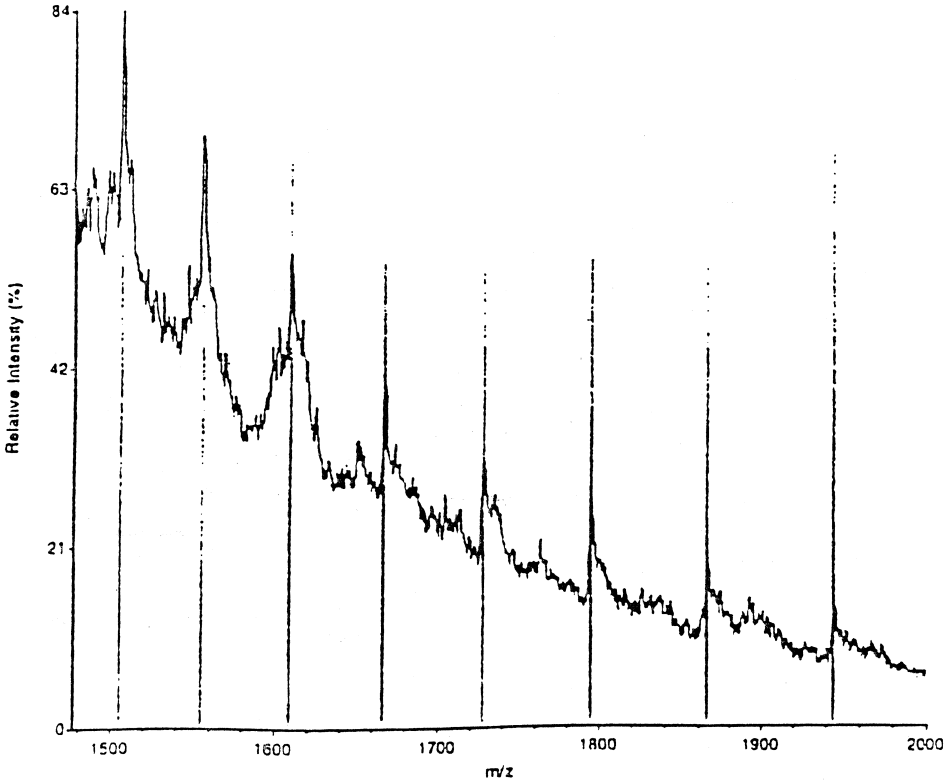

Fig. 9. Interpreted mass spectra of coconut milk protein with M_r 46 640. Reprinted with permission from Ref. [90].

shown that the [nisin+18 u] molecules present as a minor component in the mixture, was a species formed predominantly via hydration of nisin at position 33. In addition, the fate of nisin in meat products was monitored by MALDI-TOF-MS [95]. The results indicated that nisin was inactivated in raw meat, but not in cooked meat, due to enzymatic reaction with glutathione (307 u) present in raw meat [95].

MALDI-TOF-MS analysis of whole cells has been investigated as a technique for bacterial chemotaxonomy (classification based on biochemical composition) [96]; mass spectra of bacterial strains showed a few characteristic high-mass ions which are thought to be derived from specific bacterial proteins [96]. MALDI-TOF-MS has also been used to study cellular proteins as biomarkers from proteins isolated from the whole cells of bacteria; the observed biomarkers facilitate the distinction between pathogenic and nonpathogenic bacteria [97] and between gram positive and gram negative bacteria [98,99] and therefore allows for rapid chemotaxonomic classification of microorganisms. Furthermore, differentiation between gram positive and gram negative bacteria was also accomplished by RP-HPLC–ESI-MS [100]; the advantages of this technique over MALDI-TOF-MS technique include analysis of liquid samples, short analysis time, reproducibility and identification of the individual microorganism present in crude bacterial mixtures [100].

Recently, the spectra reproducibility of direct analysis of cellular proteins as biomarkers has been investigated by MALDI-TOF-MS [101]. It was demonstrated that although minor deviations in sample/matrix preparation procedures for MALDI resulted in significant changes in observed spectra, a number of peaks are conserved for the same bacteria and these conserved peaks are potentially biomarkers for bacterial identification [101]. MALDI-TOF-MS was used to locate five family specific biomarkers for the family *Enterobacteriaceae*; these biomarkers

have spectral peaks at m/z 4364, 5380, 6384, 6856 and 9540 while mass peaks at m/z 7324, 7724, 9136 and 9253 were assigned as genus-specific biomarkers for *Salmonella* [102]. MALDI-TOF-MS and ESI-MS have also facilitated the identification of biomarkers for specific bacteria including *Escherichia coli* spp. (*E. coli*) [99,101–109], *Bacillus* spp. [97,100,101,105,108,110], *Helicobacter pylori* [111], *Haemophilus* spp. [112], *Clostridium difficile* [113], *Brucella melitensis* [97,98,100], *Francisella tularensis* [98,100], *Yersinia pestis* [97,98,100], *Staphylococcus aureus* [99], *Klebsiella aerogenes* [99] and *Proteus mirabilis* [99].

5. Conclusions

This article reviews the rapidly increasing use of MS for the characterization and identification of food proteins and peptides. The published work demonstrate clear advantages of ESI-MS and MALDI-TOF-MS in terms of accuracy, sensitivity, reproducibility and short analysis time for obtaining structural information. MS now serves a central role in many applications including the determination of M_r, peptide sequencing, identification of post-translational modifications (phosphorylation and glycosylation), characterization of noncovalent protein–protein or –ligand complexes, identification of protein degradation products (enzymatically and chemically hydrolyzed proteins), investigation of protein folding.

6. Nomenclature

α-Lac	α-Lactalbumin
β-Lg	β-Lactoglobulin
APCI	Atmospheric pressure chemical ionization
BSA	Bovine serum albumin
CCP	Colloidal calcium phosphate
CI	Chemical ionization
CMP	Caseinomacropeptide
CN	Casein
CPP	Casein phosphopeptide
DH	Degrees of hydrolysis
E. coli	*Escherichia coli* spp.
EI	Electron impact
ESI	Electrospray ionization
ESI-MS	Electrospray ionization mass spectrometry
ESI-MS–MS	Electrospray ionization tandem mass spectrometry
FAB	Fast atom bombardment
FD	Field desorption
FT-ICR	Fourier transform ion cyclotron resonance
HPLC	High-performance liquid chromatography
H/^{2}H	Hydrogen/deuterium exchange
High-M_r	High-molecular-mass
LD	Laser desorption
Low-M_r	Low-molecular-mass
m/z	Mass-to-charge ratio
MALDI	Matrix-assisted laser desorption/ionization
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
M_r	Molecular mass
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PD	Plasma desorption
RP-HPLC	Reversed-phase high-performance liquid chromatography
SBA	Soybean agglutinin
SI	Secondary ion
TIs	Trypsin inhibitors
TOF	Time-of-flight
TSP	Thermospray ionization
u	Atomic mass units
WPC	Whey protein concentrate

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