Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Ceria-based Nanocomposites in Enrichment and Identification of Phosphopeptides

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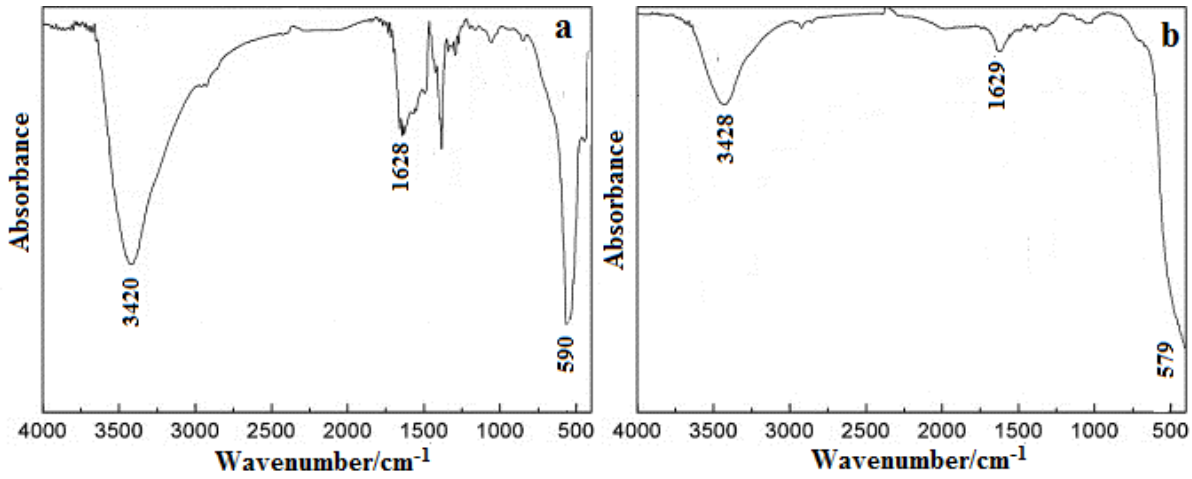

Figure 1S: FT-IR characterisation of ceria-nanocomposites (a) Ceria-iron nanocomposite. (b) Ceria-tin oxide nanocomposite.

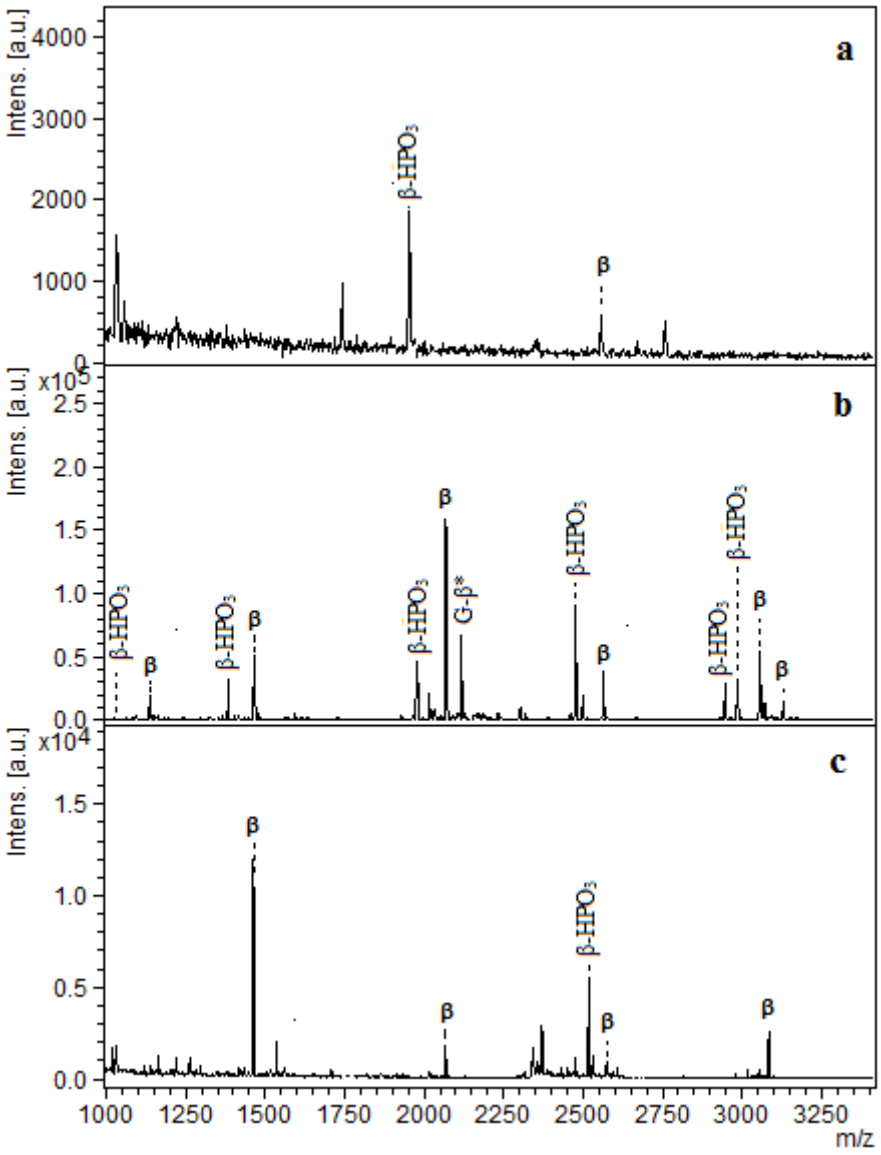

Figure 2S: MALDI-MS spectra of tryptic β-casein (1µg/mL) for phosphopeptide enrichment after elution for ceria-iron oxide nanocomposite in different ratios (a) Ce:Fe (1:2); (b) Ce:Fe (1:1); (c) Ce:Fe (2:1). The phosphopeptides, dephosphorylated and acidic residue derived from β-casein are marked with **β, β-HPO$_3$** and **G-β*** respectively.

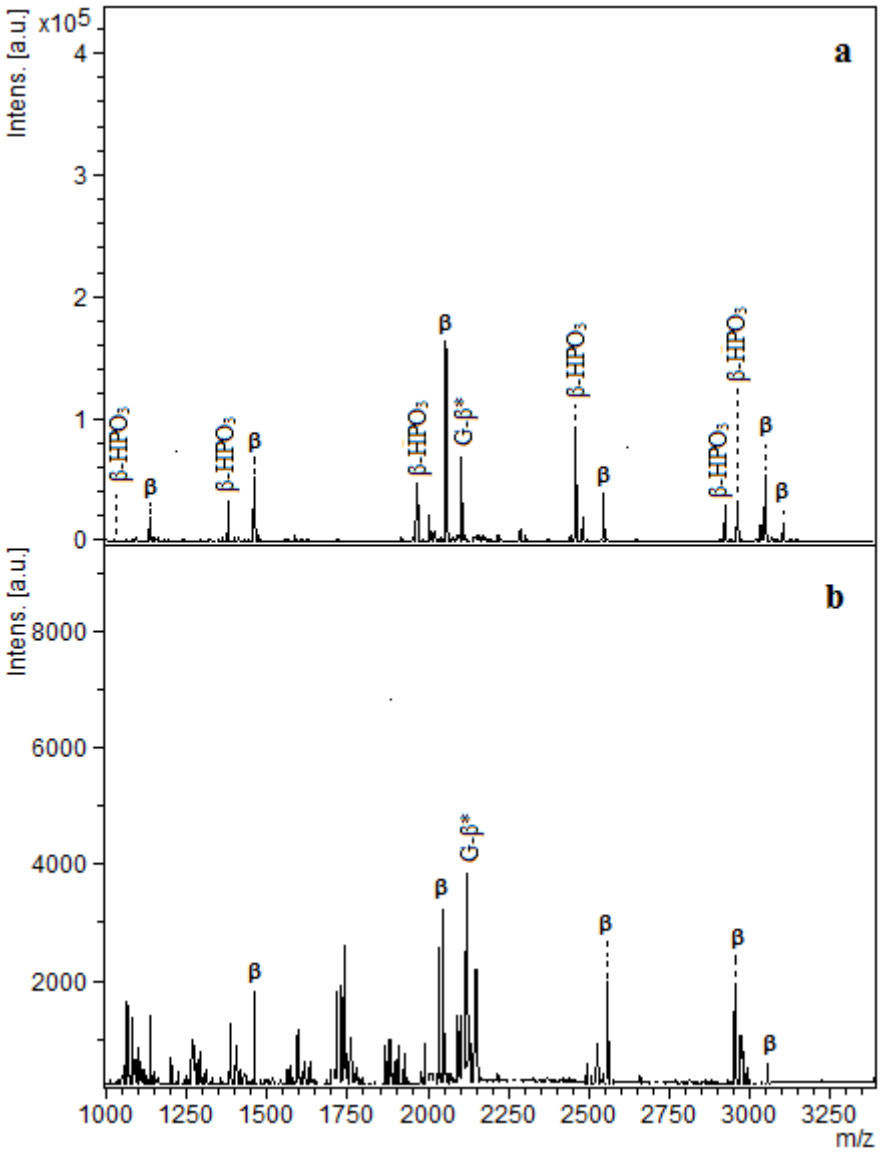

Figure 3S: MALDI-MS spectra of tryptic β-casein (1 µg/mL) for phosphopeptide enrichment after elution for (a) ceria-iron oxide nanocomposite, Ce:Fe (1:1). (b) Fe_3O_4 nanoparticles; The phosphopeptides, dephosphorylated and acidic residue derived from β-casein are marked with **β, β-HPO$_3$** and **G-β*** respectively.

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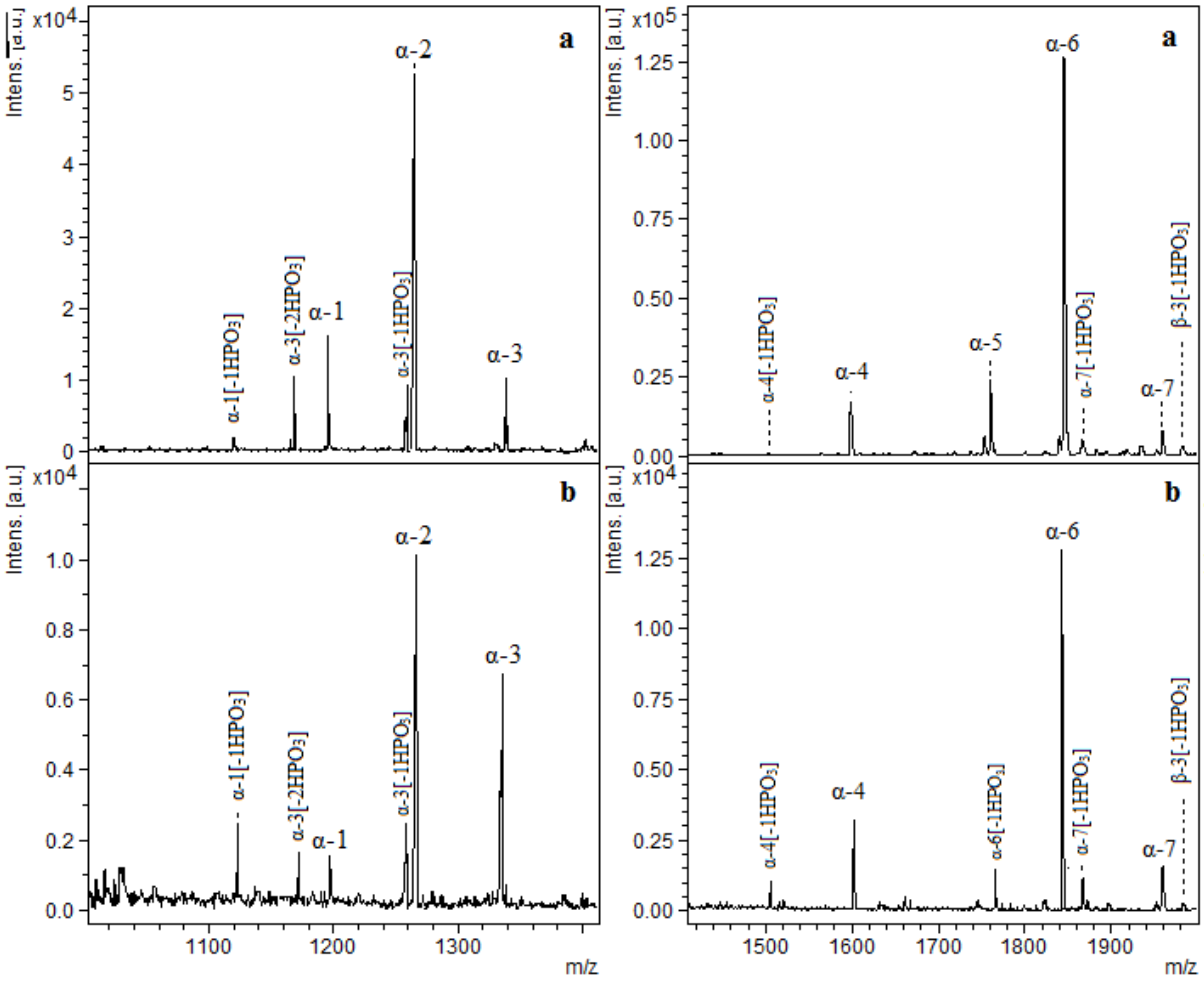

Figure S4A: MALDI-MS spectra of tryptic digest of non-fat milk for (a) after enrichment with ceria-iron oxide nanocomposite (b) after enrichment with ceria-tin oxide nanocomposite in two mass ranges for 1000-1500 Da and 1500 to 2000 Da. All identified peptide residues derived from non-fat milk are labelled as **α** and **β** for phosphopeptides derived from α- and β-casein and dephosphorylated fragments with -nHPO_3.

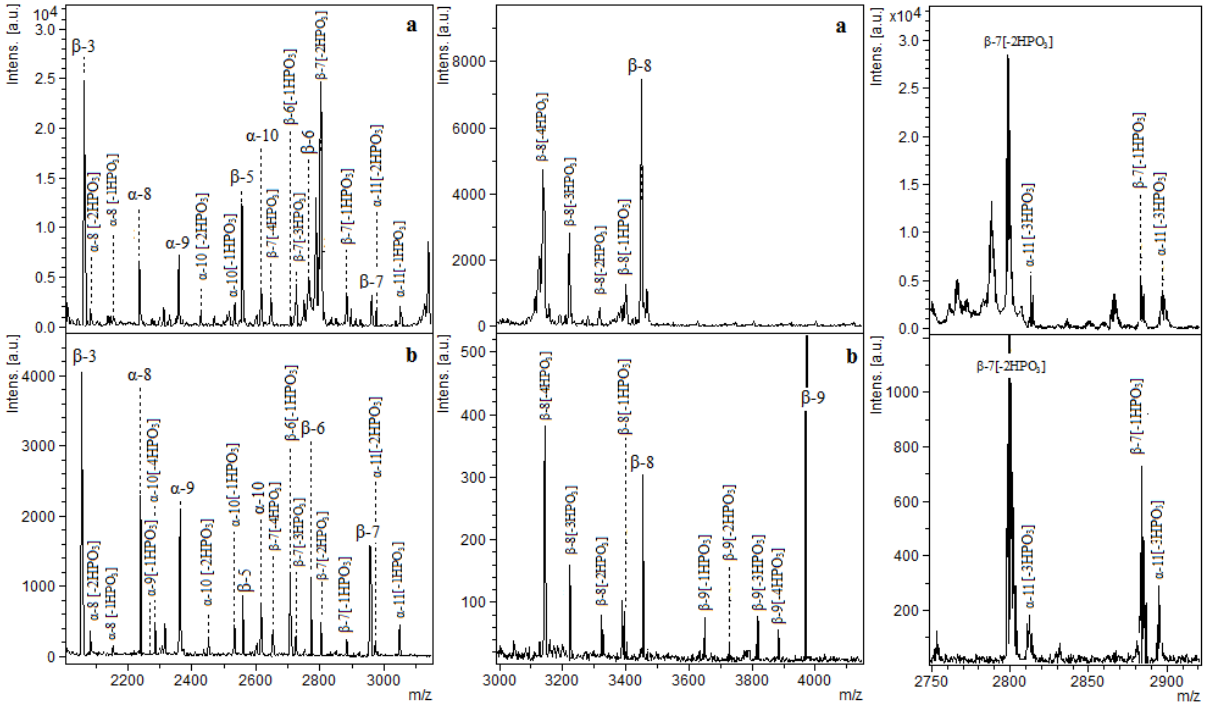

Figure S4B: MALDI-MS spectra of tryptic digest of non-fat milk in mass range for (a) after enrichment with ceria-iron oxide nanocomposite (b) after enrichment with ceria-tin oxide nanocomposite for 2000-3000 Da, 3000-4000 Da and excessive zoomed mass range for 2800-2900 Da. All identified peptide residues derived from non-fat milk are labelled as **α** and **β** for phosphopeptides derived from α- and β-casein and dephosphorylated fragments with -nHPO_3.

Table S1: Comparison of individual metal oxides and their synthesized nanocomposites in context to the literature.

Materials	Samples **Standards**	**Sample loading/ Concentration**	**Sensitivity**	Remarks	**Ref. no.**
CeO_2 NPs (26 nm)	β-casein	1 µmol	1.25 fmol	Dephosphorylation is highlighted. Serum analysis also present	27
Fe_3O_4	β-casein	1 pmol	100fmol	Comparative study for Fe(II) and Fe(III),	28
SnO_2	Standard peptides	1 µg	no results	Recovery studies, optimization and comparison to commercial materials	29
CeO_2-Fe_2O_3	β-casein	1 pmol	10-100 fmol	Presence of acidic residues, dephosphorylation, Fibrinogen detected from serum as in CeO_2	*Present study*
CeO_2-SnO_2	β-casein	1 pmol	10-100 fmol	No acidic residues. Reduce enrichment of high mass peptides with increase complexity.	*Present study*

Table S2: Overview of the identified peptides with dephosphorylated fragments for both ceria-nanocomposites by employing non-fat milk digest.

Peak No.	$[M+H]^+$	Amino Acid Sequence	Sequence No.	Dephosphorylated fragment[-nHPO$_3$]	CeO_2/ Fe_2O_3	CeO_2/ SnO_2
α-casein						
α-1	1197.6	KNMAINP***S*****KENL (α_S2)	39-50 (1P)	1117.7	♦	♦
α-2	1253.5	TVDMME***S*****TEVF (α_S2)	153-162(1P)	1173.9	♦	♦
α-3	1330.5	EQL***S***T***S*****EENSK (α_S2)	141-151(2P)	1249.9, 1170.3	♦	♦
α-4	1594.4	TVDME***S*****TEVFTKK (α_S2)	153-165(1P)	1514.9	♦	♦
α-5	1759.4	HQGLPQEVLNENLLR (α_S2)	23-37(N_p)	-	♦	○
α-6	1846.9	KDIGE***S***E***S*****TEDQAMEDIK (α_S1)	58-73 (1P)	1766.8	♦	♦
α-7	1951.4	KYKVPQLEIVPN***S*****AEERL (α_S1)	119-134(1P)	1871.9	♦	♦
α-8	2247.5	KEKVNEL***S***KDIGE***S***E***S*****TEDQA (α_S1)	35-52(3P)	2167.8, 2087.7	♦	♦
α-9	2362.2	PN***S***VEQKHIQKEDVPSERY (α_S1)	88-106(1P)	2282.9	♦	♦
α-10	2616.4	NTMEHV***S***S*S***EE***S***IISQETYK (α_S2)	17-36(4P)	2536.9,2456,7, 2376.3,2296.3	♦	♦
α-11	3132.6	KNTMEHV***S***S*S***EESII***S***QETYKQEK N (α_S2)	16-40(4P)	3052.1, 2975.3, 2892.3, 2812.1	♦	♦
ß-casein						
β-1	975.5	KFQ***S***EEQQQ	46-54 (1P)	895.4***	○	♦
β-2	1994.4	LLYQEPVLGPVRGPFPIIV	206-224 (N_p)	-	♦	○
β-3	2061.7	FQ***S***EEQQQTEDELQDK	47-62 (1P)	1981.7	○	♦
β-4	2186.7	DMPIQAFLLQEPVLGPVR	199-217(N_p)	-	♦	○
β-5	2556.9	FQ***S***EEQQQTEDELQDKIHPF	47-67(1P)	2476.9	♦	♦
β-6	2779.1	IEKFQ***S***EEQQQTEDELQDKIHPF	44-67(1P)	2699.8	○	♦
β-7	2965.1	RELEELNVPGEIVE***S***L***S***S*S***EESITR	15-39 (4P)	2885.2, 2805.3, 2725.9, 2646.9	♦	♦
β-8	3477.8	RELEELNVPGEIVE***S***L***S***S*S***EESITRI NK	15-43 (4P)	3397.2, 3317.3, 3237.8,3157.9	♦	♦
β-9	3975.8	RELEELNVPGEIVE***S***L***S***S*S***EESITRI NKKIEKF	15-47(4P)	3895.2,3815.2, 3735.7, 3655.4	○	♦

S* represents the phosphorylation site (phosphoserine)

♦ indicate the detected peptide in the mass spectra

○ indicate the absence of peptide in the mass spectra

*** Mass peak for dephosphorylated fragment not identified by both nanocomposites