Plant Foods for Human Nutrition (2020) 75:583–591
https://doi.org/10.1007/s11130-020-00850-y

ORIGINAL PAPER

Potential of Plant Proteins Digested *In Silico* by Gastrointestinal Enzymes as Nutritional Supplement for COVID-19 Patients

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Accepted: 18 August 2020 / Published online: 1 September 2020

Abstract

Currently, no specific drug and vaccine are available for the new coronavirus SARS-CoV-2, and nutritional supplementation should be helpful. This study tried to provide reference for protein supplementation. Specifically, *in silico* method was employed to simulate protein degradation by gastrointestinal enzymes and to produce a large number of active peptides, then, the binding ability of these peptides to SARS-CoV-2 spike protein receptor-binding domain (RBD) was evaluated. The results showed that wheat-derived alpha/beta-gliadin, oat-derived avenin, and ribulose bisphosphate carboxylase small chain of different origin could be good protein source in generating potent binders to SARS-CoV-2 spike RBD. In addition, some high-affinity oligopeptides (such as PISCR, VQVVN, PQQQF, etc.) were identified as potential binders of SARS-CoV-2 spike RBD. In summary, a number of plant proteins could be helpful for COVID-19 patients when supplemented with these proteins, the identified oligopeptides could be used as lead compound to design potential entry inhibitors against SARS-CoV-2.

Keywords COVID-19 · Spike · Protein supplementation · Oligopeptides · *In silico* evaluation

Introduction

Since the end of December 2019, a new coronavirus broke out in Wuhan of China, and then spread all over China and many countries in the world. This new virus, termed as SARS-CoV-2 by the World Health Organization (WHO), was found to cause disease named as COVID-19, including severe pneumonia and other symptoms like fever, dyspnea and asthenia [1]. According to the record of August 15, 2020, 89,695 and 21,179,695 persons were infected, and 4708 and 761,264 persons died, in China and other 200+ countries in the world, respectively. The genome analysis indicated that SARS-CoV-2 has as high as 79.5% of sequence identity with SARS-CoV, which breakout in Beijing 17 years ago [2]. It is well known that SARS-CoV infects human host cells by the interaction between its spike glycoprotein and the receptor ACE2 (angiotensin converting enzyme 2) on human cells [3]. Similar to SARS-CoV, SARS-CoV-2 was considered to use the same receptor ACE2 as virus entry into human [4].

To date, no specific drug and vaccine are available for this virus. In this situation, on the basis of drug treatment, it should be helpful to improve immunity through nutrition strengthening at the same time. Protein is the main "building material" of all parts of the body. Immunoglobulin, cytokines and other components needed for disease resistance need to be synthesized with protein. If there is a lack of protein, the disease resistance will be reduced. So, protein supplementation is usually a good option. It is noted that the protein, after ingestion, first denatured under the action of hydrochloric acid secreted by the gastric wall, the three-dimensional structure was destroyed, and the peptide bond was exposed. Then, under the action of pepsin, intestinal trypsin and chymotrypsin, the protein macromolecules were degraded into amino acids, oligopeptides or polypeptides [5, 6]. The purpose of this paper is to provide reference for protein supplementation. Briefly, *in silico* method was employed to simulate protein degradation by gastrointestinal enzymes and to produce a large number of active peptides, then, the binding ability of these peptides to SARS-CoV-2 spike protein receptor-binding domain (RBD) was evaluated. It is expected that proteins which release peptides with high binding ability can bring potential inhibition

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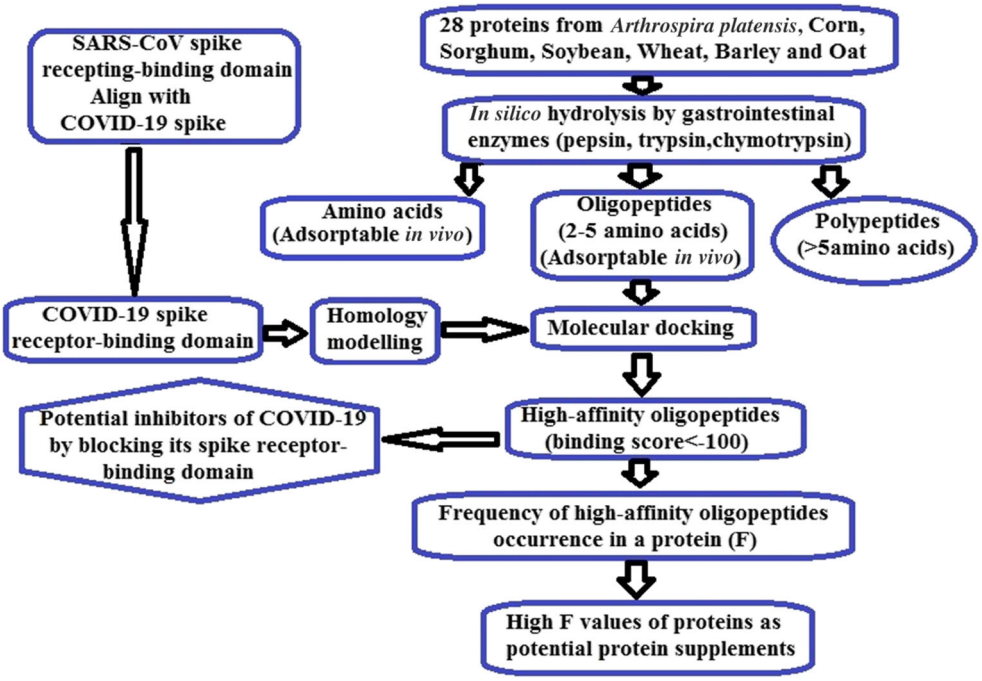

Fig. 1 Strategy for *in silico* evaluation

on SARS-CoV-2 while being used as supplements. Figure 1 displayed the strategy for this study.

Materials and Methods

Construction of 3D Model for SARS-CoV-2 Spike RBD

SARS-CoV-2 was considered to use the same receptor ACE2 as SARS coronavirus, *i.e.*, the coronavirus enters host by the binding of spike protein to ACE2 and infection happens. The amino acids sequence of SARS spike RBD was retrieved from PDB database (https://www.rcsb.org/structure/2AJF), and the sequence of SARS-CoV-2 spike was retrieved from NCBI database (https://www.ncbi.nlm.nih.gov/protein/QHO60594.1). The retrieved two sequences with 73.9% of identity were aligned (Fig. 2) to obtain the sequence of SARS-CoV-2 spike RBD. Then, the RBD was subjected to homology modeling using SWISS-MODEL (https://swissmodel.expasy.org), and the 3D model for SARS-CoV-2 spike RBD was constructed.

In Silico Hydrolysis of Plant Proteins by Gastrointestinal Enzymes

Twenty-eight proteins (Table 1) sequences from *Arthrospira platensis*, corn, sorghum, soybean, wheat, barley and oat (which are most widely used cereal grains or microalgae in functional foods) were obtained from NCBI database,

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1    CPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNV  60  SARS spike receptor-
                                                                       binding domain
336  CPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395 COVID-2019 spike
               * *    *              **         *       *             receptor-binding domain
61   YADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRY  120 SARS spike receptor-
                                                                       binding domain
396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRL  455 COVID-2019 spike
          ** *                    *   *   ** * ****    * *           receptor-binding domain
121  LRHGKLRPFERDISNVPFSPDGKPCTP-PALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFE  180 SARS spike receptor-
                                                                       binding domain
456  FRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFE  516 COVID-2019 spike
       *** *      *********  ******   * **   ** * *                   receptor-binding domain
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Fig. 2 Alignment between the receptor-binding domains of SARS and SARS-CoV-2 spike proteins

Table 1 Sequences and frequencies of high-affinity oligopeptides from plant proteins digested *in silico* by gastrointestinal enzymes

Protein names [amino acids length]	Accession number	Sequences of high-affinity oligopeptides (length < 6 and docking score < −100) binding to receptor-binding domain of SARS-CoV-2 spike RBD [Binding score]	Frequency of high-affinity oligopeptide in a protein (F)
Arthrospira platensis			
Phycocyanin alpha chain [162]	ABD64608.1	TTQM[−103.3],DISY[−123.9],SPSW[−131.2],IEAL[−100.4]	0.0247
Phycocyanin beta chain [172]	ABD64607.1	ITSN[−108.4]	0.0058
Phycobiliprotein lyase [174]	WP_006618130.1	DIVEF[−125],APDH[−109.5],PDQQR[−136.8],GSAVL[−107.8],TSIVN[−138.4],CSEIR[−115]	0.0345
Carotenoid biosynthesis protein [306]	WP_014274510.1	SSEL[−100.4],GSSW[−119.1],DPAM[−100.7], SQAPF[−131.2],PIVL[−119.2]	0.0163
Photosynthesis system II assembly factor Ycf48r [334]	WP_006616832.1	IAVAL[−121.1],PDVSY[−135],DIDF[−110],IVGSN[−127.3],TSVSF[−133.4],PGAPN[−124.6], SAAEM[−100.3],VGAIY[−135.4],GGEIR[−112.2],DPEEW[−130.3],SDPIK[−103.5],STSW[−131.9],TSASS[−103.8]	0.0389
Corn (*Zea mays*)			
Ribulose bisphosphate carboxylase large chain[476]	P00874.2	TPEY[−122.1],VTPQL[−124.5],TTVW[−138],VAY[−102.9], PPAY[−132.8],QGPPH[−125.1],GCTIK[−118.6],TGGF[−108.5],AVIDR[−125.4], SGGDH [−101.4],EGER[−107],EITL[−107.5],TQDW[−122.6],TEIF[−111.3],EIIK[−100]	0.0315
Ribulose bisphosphate carboxylase small chain[170]	P05348.1	APTVM[−120.7],STASL[−109.6],PVAR[−135.6],QVW[−133],QVDY[−135.8],IPCL[−108.7],QEAIK[−106.7],PDAF[−131.1],VIG-F[−111.7],IAY[−106.1],PPGSD[−127.8]	0.0647
Sorghum (*Sorghum vulgare*)			
Ribulose bisphosphate carboxylase large chain[476]	A1E9T2.1	ASVGF[−121.9],TPEY[−122.1],VTPQL[−124.5],TTVW[−138],VAY[−102.9],PPAY[−132.8],QGPPH[−125.1],GCTIK[−118.6],TGGF [−108.5],AVIDR[−125.4],SGGDH [−101.4],EGER[−107],EITL[−107.5],TQDW[−122.6],TEIF [−111.3],EIIK[−100]	0.0336
Ribulose biophosphate carboxylase small subunit[169]	BAJ40065.1	APTVM[−120.7],STATL[−104.9],PVAR[−135.6],STTSF[−126.6],QVW[−133],QVDY[−135.8],VPCL[−100.1],IAY[−106.1],PAGSE[−117]	0.0533
Wheat (*Triticum aestivum*)			
Alpha/beta-gliadin[286]	P02863.2	PVPQL[−133],QPQN[−114.9],P SQL[−102],QPF[−113.9],PQPQL[−133.1],PQQPY[−146.1],QQIL[−111.9],IPCM[−110.4],DVVL[−103.9],QQSTY[−134.8],CCQH[−119.8],V-VH[−106.8],AIIL[−117.5],QQQK[−105.4],QQQQ[−120.3],QQPL[−107.5],QQY[−111.9],GQGSF[−129.1],PSQQN [−134.8],EEIR[−111.1],IPPY [−133.1],GIF[−100.3]	0.0769
Glutenin, high molecular weight subunit[660]	P08488.1	QCER [−111.1],QESSL[−115.9],EACR [−103.4],CCQQL[−131.8],DVSAK [−101.3],GGSF[−117.1],QQGIF[−145.4],QGSY [−123],PTSL[−126.2],PTSL[−126.2],TGQR[−111.7],QQGY[−124.8],PASL[−119],PTSL[−126.2],PTSL[−126.2],PTSL[−126.2],PASL[−119],-PTSL[−126.2],DSPY[−118.2],PTVCR[−143]	0.0303
Glutenin, low molecular weight subunit[307]	P10386.1	CIPGL[−112.1],QQQPL[−120.1],PQQPL[−126],SQQQP[−115.7],PSIL[−118.7],EAIR[−110],AIIY[−127.1],SIIL[−108.3],AQGTF[−124.7],QI-AQL[−121.3],TSIAL[−112.4],CSVN [−107.8]	0.0391
Ribulose bisphosphate carboxylase large chain[477]	P11383.2	AGVGF[−116],TPEY[−122.1],TTVW[−138],VAY[−102.9],PPTY[−137.2],QGPPH [−125.1],GCTIK[−118.6],TGGF[−108.5],AVIDR[−125.4],SGGDH[−101.4],EGER[−107],TQDW[−122.6],TEIF[−111.3],EIIR[−109]	0.0294
Ribulose bisphosphate carboxylase small chain[175]	P26667.1	APAVM[−116],STAGL[−100.7],PISCR [−154],SSVSN [−117.7],QVW[−133],QVDY[−135.8],VPCL[−100.1],SSPGY[−126.3],PDAY[−131],VIGF[−111.7]	0.0571
Soybean (*Glycine hispida*)			
Basic 7S globulin[427]	P13917	PVQN [−118.6],QVPVL[−116.1],CEQQY[−132.2],QAPF[−122.3],PGCH[−119.4],APISL [−119.4],TTCL[−104.1],PTSK[−124],GAIIF[−132.4],QDIF[−128.7],TITL[−111.6],QGEY[−115.3],QQSVY[−135.9],TQVF[−131.5],AQQ-L[−104.4],QAQVK [−129.9],SVAPF[−125.7],PSVDL [−125.2],AEITL[−110.6],STSSL[−110.4]	0.0468

Table 1 (continued)

Protein names [amino acids length]	Accession number	Sequences of high-affinity oligopeptides (length < 6 and docking score < −100) binding to receptor-binding domain of SARS-CoV-2 spike RBD [Binding score]	Frequency of high-affinity oligopeptide in a protein (F)
Glycinin G5[516]	P04347	SSACF[−132.9],ECQL[−105.9],EPDH[−109.9],IETW[−132.5],GAIGF[−118.1],SQQQL [−123.5],QDSH[−106.9],EGDVL [−100.1],VIPL[−102.7],GVPY[−118.6],PDIEH [−125.8],PETM[−109.6],QGQH[−114.7],AQSF[−116.2],QEQED[−110.7],TPSY[−119.7],PPQR[−124.9],GCQTR[−121.1],ISTL[−102.7],SA-QY[−115.6],SPDW[−121.1],SVTM[−108.9],CQGN[−102.7],VVPQN [−129.4], AVSSY [−129.4]	0.0484
Beta-conglycinin beta subunit[439]	P25974	GTVF[−119.9],SPQL[−103.7],IVQF[−121.1],ADADF[−111.7],AIPVN[−117.4],ETSF[−121.5],EQIR[−109.3],SSSR[−105.4],AIVIL[−137.7],-EQQQK[−120.8],EVQR[−106],QIER[−114.7],QVQEL[−130.3],PSIL[−118.7]	0.0319
Beta-conglycinin alpha subunit[605]	P0DO16	GIAY [−109.4],QSCN[−107.2],QACH[−111.8],GEIPR[−111],PIPF[−137],PQPR[−124.2],QEEEH [−106.5],EEQEW[−137.7],SPQL[−103.7],ADADY[−106.1],GTAIL[−108.8],AIPVN [−117.4],EASY[−116.5],EQIR[−109.3],SSSR[−105.4],DPIY[−121.4],SIVDM[−114.3],AIVIL[−137.7],AIGIN[−110.1]	0.0314
Beta-conglycinin alpha' subunit[621]	P11827	GVVF[−116.4],GIAY[−109.4],QACH[−111.8],QEEEH[−106.5],QDER[−116.3],ADADY[−106.1, IVIL[−110.4], AIPVN[−117.4],EASY[−116.5],SSSR[−105.4],DPIY[−121.4],SVVDM[−124.1],AIVVL[−126.7],EQQQR[−142.7],PVVVN [−129.6],ATSDL[−102.1],PGSAK[−116.4]	0.0274
Barley (*Hordeum vulgare*)			
B hordein precursor[290]	Q40026	PQQPF[−147.4],PQQPF[−147.4],PQQP[−112.1],PQQPF[−147.4],QQPIL[−125.5],PQGQL[−126.8],QIPY[−128.9],PSIL[−118.7],EAI-R[−110],AIVY[−116.2],QIAQL[−121.3],PPDF[−131.5]	0.0414
D hordein precursor[707]	Q40054	TTAER[−114.5],QCER[−111.1],QESSL[−115.9],EACR[−103.4],VGQL[−100.8],PVAL[−119.7],SQVVR[−129.1],GGSF[−117.1],QQGG-W[−149.7],GTSVK[−102.2],QQSW[−144.4],PGSTF[−132.6],PSATF[−141],QPGQW[−146.5],PSATF[−141],QQGSY[−136.7],GQQ-L[−103.3],TSPH[−113.1],TQQK[−103.9],PGQGY[−138.8],QPF[−113.9],GQQSN[−112.7],GSPY[−115.6],AQQL[−104.4]	
Gamma-hordein-3[289]	P80198.1	PSGL[−105.7],PQQL[−129],PQQQF[−152.2],PQQM[−119.5],PQQM[−119.5],PQQK[−115.2],QQPL[−107.5],TQQPY[−141.1],QQCT-L[−120],ISQQN [−126.6],EQSR[−114.3],AIVM[−104],VQSQL[−119],PIQL[−115.9],VVGSL[−111.3],VIQTL [−125.9],VPPY[−138.5],CSPF[−120.1]	0.0623
Ribulose bisphosphate carboxylase large chain[479]	P05698.2	AGVGF[−116],QAGVK[−110.5],TPEY[−122.1],TTVW [−138],VAY[−102.9],PPTY[−137.2],QGPPH[−125.1],GCTIK[−118.6],TGGF[−108.5],AVIDR[−125.4],SGGDH[−101.4],EGER[−107],TQD-W [−122.6],TEIF[−111.3],EIIR[−109]	0.0313
Ribulose bisphosphate carboxylase small chain[174]	Q40004	APTVM[−120.7],STAGL [−100.7],PVSR[−128.8],QVW[−133],QVDY[−135.8],VPCL[−100.1],ASPGY[−122.1],IIGF[−122.5]	0.0460
Oat (*Avena sativa*)			
Avenin[214]	P27919.1	AQY[−117.3],CQQH[−121],DSCR[−101],VAER[−106.7],CTTM[−102.8],PITW[−143.5],ECCQL[−110.9],PSECR[−135],CDAIW[−130],SI-QR[−114.3],SAISP[−106.1],IAGK[−101.7],PVDVL[−115.2],[QEAR[−105.2],TQTSF[−137.2]	0.0701
11S globulin precursor[551]	Q38779	ATTSF[−122.3],QSSR[−124.6],QQQF[−130],QPF[−113.9],DQSQF [−142.2],QVGK[−108.3],STPY[−116.1],DQSF[−106],ADTY[−115],IVQM[−105.1],IQGH[−123.4],VQVVN[−152.4],GQTVF[−146.1],IVPQ-H[−139.1],EGCQY[−137],IAGK[−101.7],PIDVL[−116.4],QEAR[−105.2],GEEF[−100.4],TQTGF[−130.5]	0.0363
Ribulose bisphosphate carboxylase large chain[477]	P48684.1	ASVGF[−121.9],QAGVK[−110.5],TPEY[−122.1],TTVW[−138],VAY[−102.9],PPAY[−132.8],QGPPH[−125.1],GCTIK[−118.6],ITGG-F[−125.6],AVIDR[−125.4],SGGDH[−101.4],EGER[−107],TQDW[−122.6],TEIF[−111.3],EIIR[−109]	0.0314
Ribulose bisphosphate carboxylase small subunit[165]	Q9ZWG4	STAGL[−100.7],PVGR[−121],QVW[−133],QIDF[−128.3],VPCL[−100.1],PDAY[−131],IIGF[−122.5],PPGCE[−127.7]	0.0485

available at https://www.ncbi.nlm.nih.gov/protein/. Subsequently, these sequences were *in silico* digested by gastrointestinal enzymes (chymotrypsin, trypsin, pepsin) using the BIOPEP-UWM database [7]. The theoretical degree of hydrolysis (TDH) (proteins with larger TDH produce more oligopeptides) was calculated using the following equation: TDH = d/D*100%, where d is number of hydrolyzed peptide bonds and D is total number of peptide bonds in a protein chain.

Molecular Docking and Evaluation of Nutritional Supplement Potential

The peptides generated by *in silico* hydrolysis of 28 proteins were docked to SARS-CoV-2 spike RBD using HPEPDOCK [8]. The potential of various plant proteins to serve as nutritional supplement of COVID-19 patients was quantified based on the frequency of occurrence of oligopeptides (2 < =peptide length < =5) with high binding affinity (docking score < −100, the binding threshold (−100) just depends on the number of high-binding scores) relative to the length of the protein chain: F=N/L, where F is the occurrence frequency, N is the number of high-affinity oligopeptides within the protein chain, and L is the length of the protein chain (*i.e.*, number of amino acid residues). The binding residues of the selected high-affinity oligopeptides to SARS-CoV-2 spike RBD were determined by LigPlot [9].

Results and Discussion

In Silico Hydrolysis by Gastrointestinal Enzymes

After *in silico* proteolysis (chymotrypsin, trypsin and pepsin) of 28 proteins from various sources by BIOPEP tools, bioactive peptides were obtained (Fig. 3). For *Arthrospira platensis*, the theoretical degree of hydrolysis (TDH) was within the range of 30.9 to 39.3%, and 25–65 oligopeptides (with 2–5 amino acids) were generated from five selected proteins. For corn, ribulose bisphosphate carboxylase large

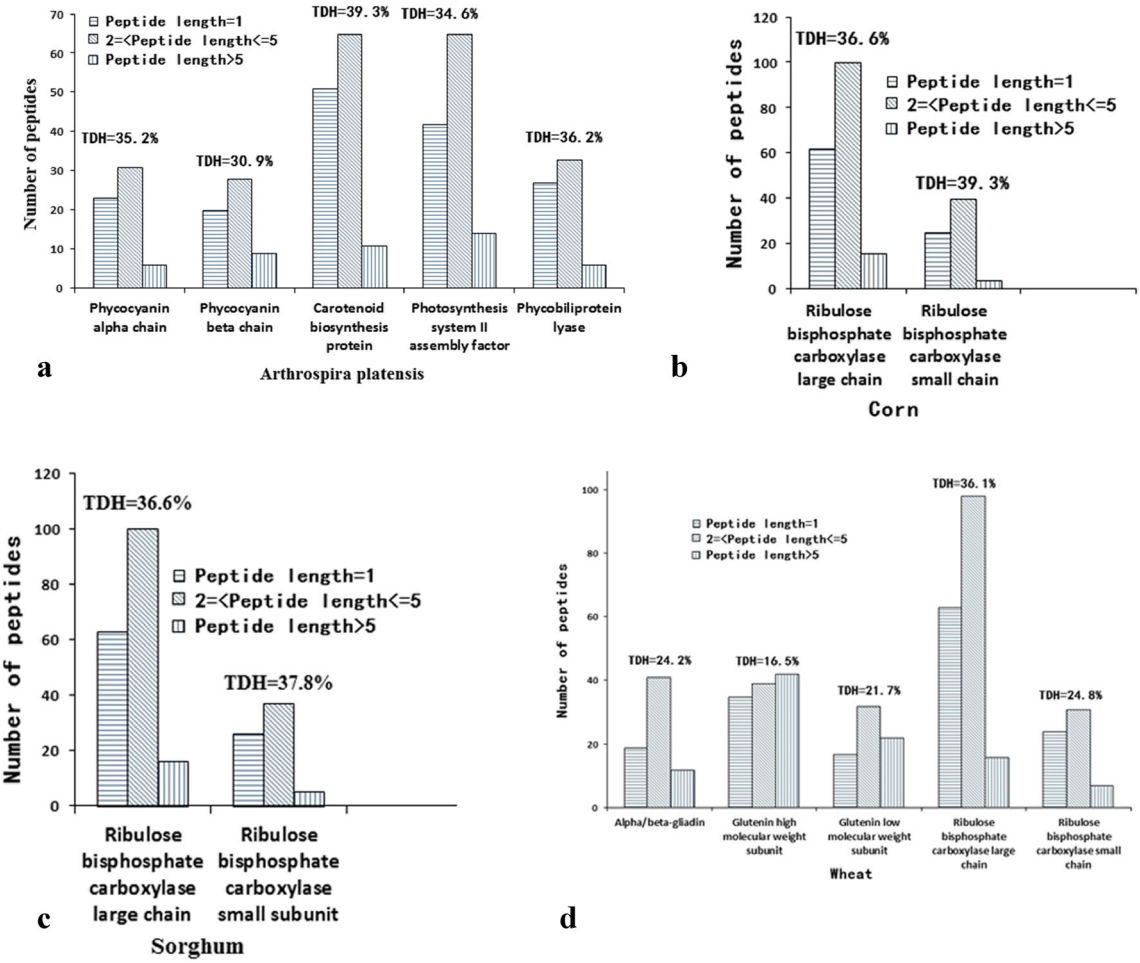

Fig. 3 *In silico* hydrolysis of *Arthrospira platensis* (**a**), corn (**b**), Sorghum (**c**), wheat (**d**), soybean (**e**), barley (**f**) and oak (**g**) proteins

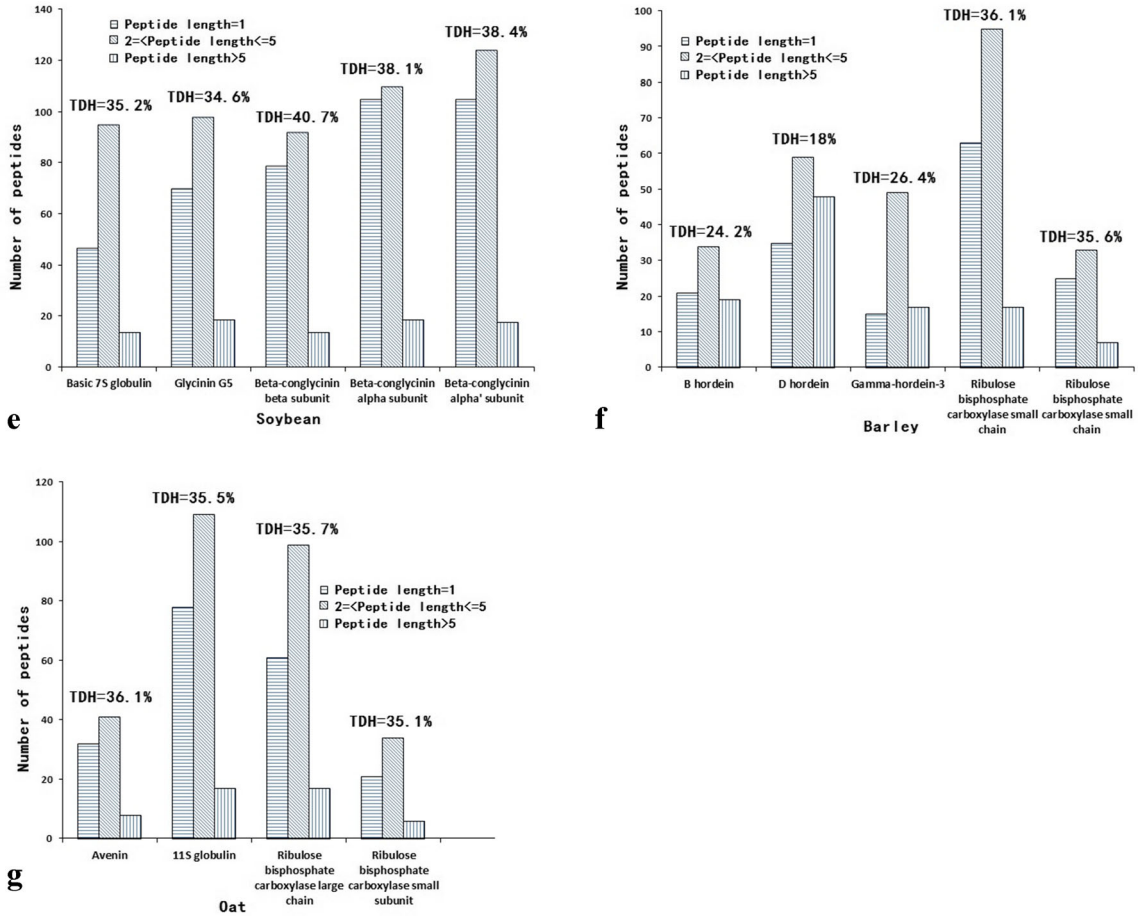

Fig. 3 (continued)

and small chains released 100 and 40 oligopeptides with 36.6 and 39.3% of TDH, respectively. Similarly, for sorghum, 100 and 37 oligopeptides were obtained from ribulose bisphosphate carboxylase large and small chains with 36.6 and 37.8% of TDH, respectively. For wheat, the TDH values were varied from 16.5 to 36.1%, and 31–98 oligopeptides

Table 2 Top high-affinity oligopeptides binding to SARS-CoV-2 spike RBD

Peptides	Source	Protein	Docking score
PQQQF	Barley	D hordein	−152.2
QQGGW	Barley	D hordein	−149.7
PQQPF	Barley	B hordein	−147.4
QPGQW	Barley	D hordein	−146.5
QQSW	Barley	D hordein	−144.4
TQQPY	Barley	D hordein	−141.1
PSATF	Barley	D hordein	−141
VQVVN	Oat	11S globulin	−152.4
GQTVF	Oat	11S globulin	−146.1
DQSQF	Oat	11S globulin	−142.2
PITW	Oat	Avenin	−143.5
PISCR	Wheat	Ribulose bisphosphate carboxylase small chain	−154
PQQPY	Wheat	Alpha/beta-gliadin	−146.1
EQQQR	Soybean	Beta-conglycinin alpha' subunit	−142.7

were generated from five selected proteins. Likewise, for soybean, the TDH values were within the scope of 34.6 to 40.7%, and 92–124 oligopeptides were generated from five selected proteins. For barley, the TDH values were within the scope of 18.6 to 36.1%, and 33–95 oligopeptides were released from five selected proteins. Finally, for oat, the TDH values were around 35%, and 34–109 oligopeptides were yielded from four selected proteins. In general, the majority of the released peptides were oligopeptides with length of 2–5 amino acids through *in silico* digest by gastrointestinal enzymes.

Potential Binding Ability of the Released Peptides to SARS-CoV-2 Spike RBD

In order to evaluate the binding ability of plant proteins *in silico* digested by gastrointestinal enzymes to SARS-CoV-2 spike RBD, molecular docking was performed and docking

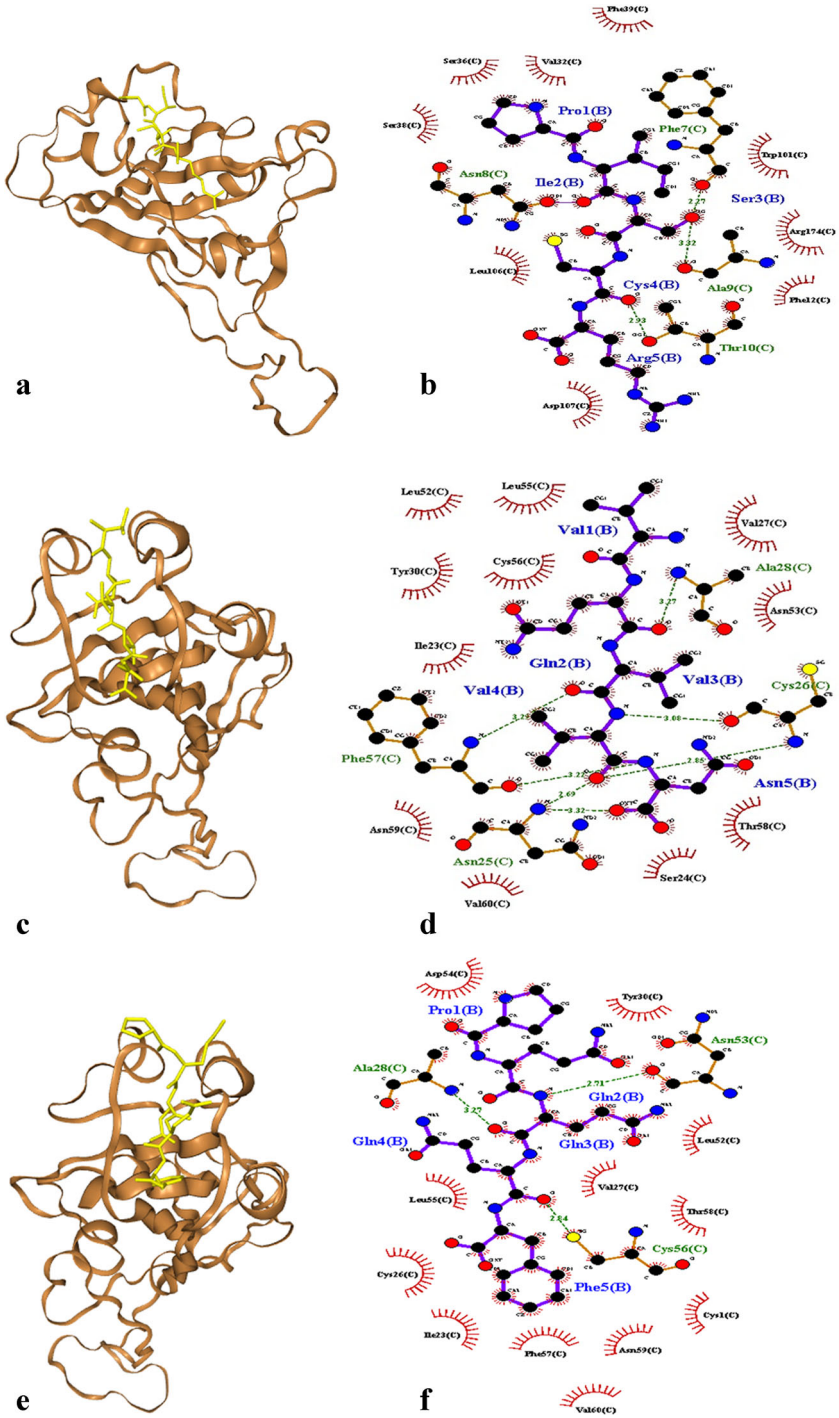

Fig. 4 Binding of SARS-CoV-2 Spike receptor-binding domain to the identified peptides and the interacting residues. PISCR (**a**, **b**), V QVVN (**c**, **d**), PQQQF (**e**, **f**)

scores were obtained. The potential of plant proteins as nutritional supplement was assessed by the frequency (F) of high-affinity (binding score < −100) oligopeptide (length 2–5 amino acids) in a protein. Table 1 showed the F values of high-affinity oligopeptides. The results indicated that the last two proteins with smaller F values were Phycocyanin beta chain (0.0058) and carotenoid biosynthesis protein (0.0163) from *Arthrospira platensis*; among the better binders were the four proteins ribulose bisphosphate carboxylase small chain (0.0647) from corn, gamma-hordein-3(0.0623) from barley, ribulose bisphosphate carboxylase small chain (0.0571) from wheat and ribulose biophosphate carboxylase small subunit (0.0533) from sorghum; and the top two proteins with larger F values were alpha/beta-gliadin (0.0769) from wheat and Avenin (0.0701) from oat.

The top high-affinity (binding score < −140) oligopeptides binding to SARS-CoV-2 spike RBD were presented in Table 2. There were seven peptides from barley, four peptides from oat and two peptides from wheat. The most potent binders were PISCR from wheat, VQVVN from oat and PQQQF from barley with binding scores −154, −152.4 and − 152.2, respectively. These peptides can properly bind to hydrophobic groove of SARS-CoV-2 spike RBD (Fig. 4a, c, e). For PISCR, three hydrogen bonds were formed in the following amino acids pairs (peptide *vs* spike RBD): Ser 3 *vs* Phe 7, Ser 3 *vs* Ala 9, and Cys 4 *vs* Thr 10 (Fig. 4b). For VQVVN, the formed seven hydrogen bonds included: Gln 2 *vs* Ala 28, Val 3 *vs* Cys 26, Val 4 *vs* Cys 26, Val 3 *vs* Phe 57, Asn 5 *vs* Phe 57, Val 4 *vs* Asn 25, and Asn 5 *vs* Asn 25 (Fig. 4d). For PQQQF, there also existed three hydrogen bonds: Gln 2 *vs* Asn 53, Gln 3 *vs* Ala 28, and Gln 4 *vs* Cys 56 (Fig. 4f).

In order to exert in vivo biological activities after oral administration, food derived bioactive peptides have to be absorbed into blood circulation and delivered to targets. It is widely accepted that di- and tri-peptides can be easily absorbed in the intestine by specific peptide transport systems via PepT1 or PepT2 [10], while tetra- and penta-peptides can also be transported through the paracellular tight-junction pathway [11]. Therefore, in this study, after in silico proteolysis by gastrointestinal enzymes, only oligopeptides with peptide length between two and five amino acids were investigated for their binding ability to SARS-CoV-2 spike RBD. The results demonstrated that wheat-derived alpha/beta-gliadin and oat-derived avenin generated highest frequency of high-affinity oligopeptides occurrence, with F values of 0.0769 and 0.0701, respectively; while ribulose bisphosphate carboxylase small chain possessed higher F values, whatever its source, with F values 0.0647 (corn), 0.0533 (sorghum), 0.0571 (wheat), 0.0460 (barley), 0.0485 (oat). It seems that barley and oat become the protein sources generating more potent binders, in which 7 and 4 high-affinity (binding score < −140) oligopeptides were generated respectively. However, it is not sure that barley and oat varieties are superior to other varieties, due to the fact that only limited number of proteins in each variety were evaluated. Anyway, the present data suggests that wheat-derived alpha/beta-gliadin, oat-derived avenin, and ribulose bisphosphate carboxylase small chain of different origin could be good protein source in generating potent binders to SARS-CoV-2 spike RBD. Thus, it could be helpful for COVID-19 patients when supplemented with these proteins.

Previous studies have shown that peptides can act as modulators in viral diseases. For example, the peptides with the positive WWIHS (Wimley-White interfacial hydrophobicity scale) values have been shown to inhibit various viruses, such as SARS-CoV and MERSCoV by interfering with fusion of host cellular and viral glycoprotein membranes [12, 13]. Struck et al. [14] demonstrated that the hexapeptide YKYRYL was specific against SARS-CoV by attaching to its ACE2 receptor. Zhao et al. [15] found that a peptide, NGAICWGPCPTAFRQIGNCGHFKVRCCKIR, exhibited potent antiviral effects against SARS-CoV and MERS-CoV. In this study, a number of high-affinity oligopeptides were identified as potential binders of SARS-CoV-2 spike RBD (Table 2), including PISCR, VQVVN, PQQQF, etc. These peptides should possess good safety due to their source of diet, and can be used as lead compound to design potential entry inhibitors against SARS-CoV-2.

Acknowledgements No funding is provided.

Compliance with Ethical Standards

Declarations of Interest The authors report no declarations of interest.

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