BIOORGANIK KIMYO INSTITUTI HUZURIDAGI ILMIY DARAJALAR BERUVCHI DSc.02/30.12.2019.K/B.37.01 RAQAMLI ILMIY KENGASH

BIOORGANIK KIMYO INSTITUTI

WAILI AIHEMIDING

FUNKSIONAL OZIQ-OVQATLAR VA DORI VOSITALARINI ISHLAB CHIQISH UCHUN HAYVON VA O‘SIMLIK PEPTIDLARINING TUZILISHI VA BIOLOGIK FAOLLIGI

02.00.10 – Bioorganik kimyo

Xalqaro ilmiy ma‘lumotlar bazasiga kiritilgan va impakt faktori tegishli ravishda yuqori bo‘lgan ilmiy jurnallarda chop etilgan maqolalar asosida Kimyo Fanlari Doktori (DSc) ilmiy darajasini olish uchun (dissertatsiya himoyasisiz)
T A Q D I M N O M A

Toshkent– 2025

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Taqdimnoma mundarijasi

Contents of the presentation

Оглавление презентации

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Fan doktori (DSc) dissertatsiyasi mavzusi O‘zbekiston Respublikasi Oliy ta’lim, fan va innovatsiyalar vazirligi huzuridagi Oliy attestatsiya komissiyasida B2025.4.DSc/K240 raqam bilan ro‘yxatga olingan.

Dissertatsiya ishi O‘zR FA Bioorganik kimyo instituti va Xitoy Fanlar akademiyasi Shinjon fizika va kimyo texnika institutida bajarilgan.

Dissertatsiya avtoreferati uch tilda (o‘zbek, ingliz va rus (rezyume)) (www.biochem.uz) va «Ziyonet» axborot-ta’lim portalida (www.ziyonet.uz) joylashtirilgan.

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KIRISH (taqdimotnoma bayoni)

Tadqiqot mavzusining dolzarbligi va zarurligi. Dunyoda hayvon va o‘simlik peptidlarining tuzilishi va biologik faolligini o‘rganish funksional oziq-ovqatlar va dorilar ishlab chiqish nihoyatda dolzarbdir. Chunki bioaktiv peptidlar tabiiy, xavfsiz, yuqori selektivlikka ega va organizmga oson moslashuvchi biologik moddalar bo’lib, ular antioksidant, antigipertenziv, immunomodulyator, antimikrobiyal va metabolik jarayonlarni tartibga soluvchi xususiyatlari bilan zamonaviy nutrasevtiklar va farmatsevtik preparatlar yaratishda katta ahamiyatga ega. Peptidlarning biologik faolligi ularning tuzilishiga, aminokislota tarkibi va molekulyar xususiyatlariga bog‘liq bo‘lgani sababli, ularni chuqur o‘rganish yangi funksional oziq-ovqat qo‘shimchalari, tabiiy preparatlar va terapevtik vositalar ishlab chiqish uchun ilmiy asos yaratadi. Tabiiy manbali peptidlarga bo‘lgan global talabning ortib borishi, sintetik dori vositalarining nojo‘ya ta’sirlarini kamaytirish zarurati ushbu yo‘nalishni strategik ahamiyatga ega qiladi.

So’ngi yillarda dunyoning yetakchi ilmiy-tadqiqot markazlarida hayvon va o'simlik resurslaridan olingan bioaktiv makromolekulalar va peptidlarni ilmiy tadqiq qilishga qaratilgan tadqiqotlar olib borilmoqda. Bu borada, jumladan, tabiiy manbalardan biologik faol tabiiy oqsillar hamda peptidlar ajratib olish, ularning tuzilishini aniqlash hamda antioksidant, yallig'lanishga qarshi, gipoglikemik, mikroblarga qarshi va immunomodulyator faollikni tadqiq etishga katta e’tibor qaratilmoqda. Buning natijasida tabiiy manbalardan ajratib olingan biologik faol birikmalar asosida funktsional oziq-ovqat mahsulotlari, nutrasevtik vositalar va biotibbiy materiallarni ishlab chiqishdagi keng salohiyatini yanada kengaytirmoqda.

Mazkur yo’nalishda Respublikamizda ham xorijiy ilmiy markazlar bilan xamkorlikda tadqiqotlar olib borilib muayyan natijalarga erishilmoqda. Jumladan, Fanlar akademiyasi olimlari XXR olimlari bilan xamkorlikda tabiiy xom-ashyolardan (tuya suti, no'xat, chayon zahari, kavsh qaytaruvchi hayvonlarning oshqozon to'qimalari, zira urug'lari, ipak fibroini va mol suyak iligidan) biologik faol oqsil va peptid tabiatli birikmalarni ajratib olish, ularning tizilishi va biologik faolligini aniqlash borasida muayyan natijalarga erishimoqda. Bu kabi tadqiqotlar O‘zbekiston Respublikasini yanada rivojlantirish bo‘yicha Harakatlar strategiyasida “farmatsevtika sanoatini yanada rivojlantirish, aholini va tibbiyot muassasalarini arzon va sifatli dori vositalari bilan ta’minlashni yaxshilash” bo‘yicha belgilangan muhim vazifalarni bajarishga hizmat qilmoqda. Buning natijasida tabiiy manbalar asosida turli kasalliklarni davolash uchun samarali, arzon va noro’ya ta’siri kam bo’lgan yangi avlod dori vositalarini ishlab chiqish imkonini yaratmoqda.

Mazkur dissertatsiya O‘zbekiston Respublikasi Prezidentining 2017-yil 7-fevraldagi PF-4947-sonli “O‘zbekiston Respublikasini yanada rivojlantirish bo‘yicha Harakatlar strategiyasi to‘g‘risida” [1] va 2020-yil 29-oktabrdagi PF-6097-sonli “Ilm-fanni 2030-yilgacha rivojlantirish konsepsiyasini tasdiqlash to‘g‘risida”gi farmoni, O‘zbekiston Respublikasi Prezidentning 2018 yil 14 fevraldagi PQ-3532-son

[1] O‘zbekiston Respublikasi Prezidentining 07.02.2017-yildagi PF-4947-sonli “O‘zbekiston Respublikasini yanada rivojlantirish bo‘yicha Harakatlar strategiyasi to‘g‘risida” gi Farmoni.

“Farmasevtika tarmog‘ini jadal rivojlantirish bo‘yicha qo‘shimcha chora-tadbirlar to‘’g ‘risida” [2] gi Qarori va 2019-yil 6-maydagi PQ-4310-son “Tibbiyot va farmasevtika ta’limi va ilm-fani tizimini yanada rivojlantirish chora-tadbirlari to‘g‘risida” gi Qarori, shuningdek, yuqoridagi faoliyatga oid me’yoriy-huquqiy hujjatlarda nazarda tutilgan boshqa vazifalarni amalga oshirishga muayyan darajada xizmat qiladi.

Tadqiqotning respublika fan va texnologiyalari rivojlanishi ustuvor yo‘nalishlariga mosligi. Ushbu tadqiqot fan va texnologiyalarni rivojlantirishning VI "Tibbiyot va farmakologiya" ustuvor yo'nalishlariga muvofiq bajarilgan.

Dissertatsiyaning mavzusi bo‘yicha horijiy ilmiy-tadqiqotlar sharhi[3]. Tabiiy manbalardan biologik faol oqsillar va peptidlarni ajratib olish, ularning tuzilishi va faolligini o’rganish bo’yicha izlanishlar dunyoning yetakchi ilmiy tadqiqot markazlarida olib borilmoqda. Jumladan, peptid sintezining muqobil texnologiyalarini rivojlantirish bo'yicha Maastricht universitetida (Niderlandiya), murakkab peptidlarni sintez qilish usullarini takomillashtirish hamda yangi terapevtik moddalar, xususan, diabet va infeksiyalarni davolash uchun peptid dori vositalarini ishlab chiqish Florey institutida (Avstraliya), biokatalizatorlar va tabiiy va sintetik peptid dori vositalarini ishlab chiqaradi borasidar Peptid kimyosi tadqiqot instituti (Eron), o'simliklardan olingan biofaol peptidlarni kompleks ko'rib chiqish va ularni oziq-ovqat mahsulotlarida qo'llash bo'yicha tadqiqotlar Manitoba universiteti (Kanada) tabiiy manbalardan (tuya suti, chayon oqsillari, ipak seritsini, qo'zichoqlar va bug'ularning shirdoni) peptidlarni ajratib olish, strukturaviy tavsiflash, ularning funktsional oziq-ovqat, nutrasevtik vositalar va biotibbiy materiallarni ishlab chiqarish boyicha tadqiqotlar Bioorganik kimyo institutida (O’zbekiston) olib borilmoqda.

Tabiiy manbalardan biologik faol oqsillar va peptidlarni ajratib olish, ularning tuzilishi va faolligini o'rganish bo'yicha olib borilgan tadqiqotlar natijasida muhim natijalar qo'lga kiritilgan, jomladan, tadqiqotlar oziq-ovqat chiqindilari va innovatsion manbalardan biofaol peptidlarni olishga qaratilib, dengizdan olingan oqsillar va peptidlar, quruqlikdagi oqsillardan farqli o'laroq, noyob aminokislota tarkibi tufayli yuqori faollikni namoyish etishi aniqlangan (Oziq-ovqat, baliqchilik va akvakultura tadqiqot institute, Norvegiya), oziq-ovqatdan olingan ba'zi peptidlar (masalan, LILPKHSDAD, LTFPGSAED) gipoglikemik (qon shakarini tushiruvchi), gipolipidemik (lipidlarni kamaytiruvchi), antihipertenziv (qon bosimini pasaytiruvchi) va sitoprotektiv (hujayralarni himoya qiluvchi) kabi bir nechta xususiyatlarni namoyish etishi aniqlangan (Manitoba universiteti, Kanada), peptidlarning biologik faolligi ularning o'ziga xos tuzilishi, aminokislotalar ketma-ketligi va molekulyar og'irligiga bog'liqligi ilmiy jihatdan asoslash orqali tarkibida hidrofob (masalan, tirozin, leysin, triptofan, prolin) va aromatik aminokislotalar bo'lgan past molekulyar og'irlikli

[2] 2018-yil 14-fevraldagi PQ-3532-son “Farmasevtika tarmog‘ini jadal rivojlantirish bo‘yicha qo‘shimcha chora-tadbirlar to‘’g ‘risida” gi qarori.

[3] Dissertatsiya ishi mavzusi bo‘yicha xorijiy ilmiy tadqiqotlar sharhi https: // link.springer.com; https://onlinelibrary.wiley.com; https://pubmed.ncbi.nlm.nih.gov va boshqa manbalar asosida ishlab chiqilgan.

peptidlar yuqori antioksidant faollikka ega ekanligi aniqlangan (Oziq-ovqat fanlari va texnologiyalari milliy tadqiqot instituti, Ispaniya).

Dunyoda tabiiy birikmalar asosida istiqbolli dori vositalarini yaratish borasida qator, jumladan, quyidagi istiqbolli yo'nalishlarda tadqiqotlar olib borilmoqda: oqsil tabiatli birikmalardan peptidlarni ajratib olishning takomillashgan usullarini ishlab chiqish; biologik faollikka ega bo'lgan istiqbolli birikmalarning tuzilishi va fizik-kimyoviy xususiyatlarini aniqlsh; biologik faolliklari va ta'sir mehanizmlarini tadqiq etish orqali nojo'ya ta'siri kam va yuqori samaradorlikka ega bo'lgan dori vositalarini yaratish.

Muammoning o'rganilganlik darajasi. Tabiiy va barqaror sog'liqni saqlash yechimlariga global talab ortib borayotganini hisobga olsak, hayvonlar va o'simliklardan olingan peptidlar iste'molchilar ehtiyojlarini yaxshi qondira oladi. Ular keng xom ashyo manbalariga ega va qayta tiklanuvchandir. Ishlab chiqarish jarayonida odatda enzimatik gidroliz kabi usullardan foydalaniladi, bu esa energiyani tejash va chiqindilarni kamaytirish imkonini beradi. Qo'shimcha mahsulotlarni xom ashyo sifatida ishlatish iqtisodiy samaradorlikni oshirishi va ko'plab sohalarda sanoatning rivojlanishiga yordam beradi. Doimiy tadqiqotlar va innovatsiyalar bilan ushbu peptidlar kelajakdagi funktsional oziq-ovqatlar va terapevtik vositalarning ajralmas tarkibiy qismi bo'lib, umumiy salomatlik va farovonlikni qo'llab-quvvatlash imkoniyatiga ega.

Yangi Zelandiya olimi Klara S.F. Bah o'simliklar va zamburug'lar yordamida *Pteridium aquilinum* o'simligidan bioaktiv peptid saqlovchi mahsulotlarini ajratib olgan. Homayouni Tabrizi va boshqalar pepsin va pankreatin bilan fermentatsiya qilingan tuya sutidan ikkita antioksidant peptid ajratib olgan va bu ikki peptidning aminokislotalar ketma-ketligini aniqlagan. Dromedar (bir o'rkachli) tuya sutidan olingan atsetil xolin esteraza inhibitori bo'lgan peptidlarni Amin-Alhaj tomonidan aniqlangan. Yao R. fermentativ gidroliz orqali chayon oqsilidan yangi antikoagulyant peptidni ajratib olgan va aniqladi. Chayon tanasida bir nechta oqsillar mavjud, ammo hozirgacha chayon oqsilidan olingan antioksidant peptidlar haqida juda kam ma'lumot mavjud. No'xat niholi oqsili gidrolizatlarining antioksidant faolligi Torres-Fuentes tomonidan o'rganildi va ularning FVPH, ALEPDHR, TETWNPNHPEL va SAEHGSLH tadqiqotlari natijasida to'rtta no'xat peptidining antioksidant xususiyatlari o'rganib chiqilgan. W.F. Porto mikroblarga qarshi peptidlarni kompyuter simulyatsiyasi orqali optimallashtirish orqali peptid strukturasi dizaynini kombinatsion tadqiqotini amalga oshirgan.

Bioaktiv peptidlar va oqsillar bo'yicha ushbu tadqiqot landshafti dinamik va ko'p qirrali. Tadqiqotlar shuni ko'rsatdiki, enzimatik gidroliz tuya suti kabi manbalarda katta, faol bo'lmagan dastlabki oqsillaridan kuchli bioaktiv peptidlarni chiqarishning samarali usuli hisoblanadi. Bunday peptidlarning antioksidant xususiyatlari ko'pincha ularning molekulyar og'irligi va gidrofob aminokislotalarning mavjudligi bilan bog'liq.

Hayvon oqsillari sohasida olib borilgan tadqiqotlar shuni ko'rsatdiki, go'sht sanoatining ikkilamchi mahsulotlari, masalan, suyak iligi antimikrob va antioksidant potentsialga ega bo'lgan oqsillarning boy manbalari hisoblanadi. Ixtisoslashgan

hayvonot mahsulotlari, shu jumladan chayon zahari va qurbaqa terisi sekretsiyasi yuqori kuchli va o'ziga xos molekulalarning elita manbalari sifatida tan olingan. Tadqiqotlar ion kanallarini modulyatsiya qiluvchi neyrotoksinlarni ajratib olishga va dori-darmonlarga chidamli patogenlar bilan kurashish uchun tabiiy shablonlarga asoslangan yangi sintetik peptidlarni loyihalashga qaratilgan.

Bundan tashqari, oqsillarning funksionalligini oshirish uchun ilg'or strategiyalar ishlab chiqilmoqda. Ipak seritsini kabi oqsillarni kichik molekulali fenol birikmalar bilan kovalent konjugatsiya qilish ularning yallig'lanishga qarshi, antioksidant va emulsiyalash xususiyatlarini sezilarli darajada yaxshilash uchun innovatsion usul sifatida paydo bo'ldi. Shu bilan birga, an'anaviy tibbiyot manbalaridan glikoproteinlarni o'rganish, masalan, qo'zichoq va bug'u shirdoni, ular kuchli va o'ziga xos siklooksigenaza-2 ingibitori va gialuronidaza ingibitori kabi kuzatilgan terapevtik ta'sirlar uchun mas'ul bo'lgan asosiy faol komponentlar ekanligini ko'rsatmoqda. Ushbu jamoaviy ish nafaqat yangi molekulalarni kashf qilish, balki ularning ekstraktsiyasini optimallashtirish va ularning tabiiy funktsiyalarini yaxshilashga qaratilgan aniq tendentsiyani ta'kidlaydi.

Tadqiqot mavzusining ish bajarilgan ilmiy-tadqiqot muassasasining tadqiqot rejalari bilan bog'liqligi. Dissertatsiya tadqiqoti Xitoy Fan va texnologiyalar vazirligining "Xitoy-O'zbekiston yangi dori vositalari bo'yicha "Bir kamar, bir yo'l" qo'shma laboratoriyasini tashkil etish va innovatsion dori vositalarini tadqiq qilish" (2020YFE0205600, 2020-2023) nomli Milliy asosiy tadqiqot va ishlanmalar dasturi, Shanxay hamkorlik tashkiloti fan va texnologiyalar bo'yicha hamkorlik dasturi va "No'xat oqsili ozuqaviy kukunini ishlab chiqish va uni O'zbekistonda ro'yxatdan o'tkazish" (2022E01041, 2022-2025) nomli Xalqaro fan va texnologiyalar bo'yicha hamkorlik dasturi hamda "Ijtimoiy ahamiyatga ega kasalliklarni davolash uchun O'zbekistondagi sudralib yuruvchilar, o'rgimchaksimonlar va amfibiyalardan olingan yangi biologik faol moddalarning molekulyar ta'sir mexanizmlarini o'rganish" (FFA2021359, 2021-2026) mavzusidagi ilmiy loyiha doirasida bajarilgan.

Tadqiqotning maqsadi zira urug'lari, no'xat nihollari, suyak iligi (mol, tuya, ot va qo'y), tuya suti, chayon oqsillari, ipak seritsini, qo'zichoqlar va bug'ularning shirdoni oqsillaridan peptidlarni ajratib olish, tozalash va strukturaviy tavsiflash, ularning funktsional oziq-ovqat, nutrasevtik vositalar va biotibbiy materiallarni ishlab chiqarishdagi potentsial biologik faolligini aniqlashdan iborat.

Tadqiqotning vazifalari:

oqsillar, peptidlar, lipidlar va uglevodlarning kompozitsion ma'lumotlar bazasini yaratish uchun tuya suti, no'xat, chayon to'qimalari, kavsh qaytaruvchi hayvonlar oshqozoni, zira urug'i, ipak seritsini va qoramol suyak iligining tarkibiy tahlili va tizimli fizik-kimyoviy tavsifini o'tkazish;

sentrifugalash, mikrofiltratsiya, ultrafiltratsiya va radial xromatografiyani birlashtirgan yashil, ko'p bosqichli ajratish jarayonini ishlab chiqish va tozalash orqali peptidlarni ajratish va tozalash texnologiyalarini optimallashtirish;

tanlangan protein substratlaridan bioaktiv peptid fraksiyalarini hosil qilish uchun boshqariladigan enzimatik gidrolizni qo'llash, so'ngra yuqori toza funktsional peptidlarni olish uchun tozalash va fraksiyalash;

aminokislota tarkibi va ikkilamchi tuzilishini aniqlash uchun spektral va xromatografik usullar yordamida tozalangan peptidlarning strukturaviy va molekulyar tavsifi;

in vitro biokimyoviy va hujayrali tahlillar orqali ajratilgan peptidlarning antioksidant, diabetga, mikroblarga qarshi va immunomodullovchi faolligini tekshirish, molekulyar tuzilish va bioaktivlik munosabatlarini o'rganish.

mintaqaviy bioresurslardan barqaror foydalanish uchun nazariy va texnologik asos yaratish va kelajakda funktsional oziq-ovqat va biotibbiyot sanoatida qo'llanilishini qo'llab-quvvatlash maqsadida olingan ma'lumotlarni umumlashtirish.

Tadqiqot ob'ekti sifatida o'simlik materiallari - zira (*Cuminum cyminum L.*) urug'lari, no'xat o'simtalari (*Cicer arietinum L.*), hayvon to'qimalari va mahsulotlari: (qo'y, qoramol, ot va tuyadan olingan suyak iligi, Baqtriya tuyasining suti, butun tanasi, Manjuriya chayoni (*Buthus martensii* Karsch) butun tanasi va zahari, qo'zi va bug'udan (*Cervus elaphus*) olingan shirdon, hayvonlar sekretsiyasi va ikkilamchi mahsulotlari: qurbaqa terisi sekretsiyasi (sintez uchun shablon sifatida), ipak qurti pillasidan ipak seritsini olingan.

Tadqiqot predmeti tabiiy biologik faol oqsil va peptid tabiatli birikmalarni ajratib olish, tavsiflash va ularning mikroblarga qarshi, antioksidant, yallig'lanishga qarshi, o'smaga qarshi, fermentlarni ingibirlovchi, neyrotoksik xususiyatini aniqlash hamda yangi zamburuqqa qarshi sintetik peptidlar, flavonoidlar va fenol birikmalar bilan ipak seritsinning kovalent bog'langan konjugatlari sintesi hisoblanadi.

Tadqiqot usullari. Tadqiqotda ekstraksiya, filtrlash va konsentratsiyalash usullari, shuningdek, fizik-kimyoviy tahlil usullari (MS, NMR, LD, IR, DOV, GC-MS, LC-MS va CEM) va boshqa xromatografik uskunalar qo'llanildi. Birikmalarning biologik faolligi o'rganishda in vitro va in vivo tadqiqot usullari qo'llanildi.

Tadqiqotning ilmiy yangiligi quyidagilardan iborat:

birinchi marta turli manbalardan (*Cuminum cyminum* urug'lari, Bactrian tuya suti, chayon gidrolizatlari, *Cicer arietinum* L. o'simtalari) ajratib olingan yangi bioaktiv peptidlar asosida dorilarga chidamli *Candida albicans*ga zamburug'ga qatshi peptid (AKK8) topilgan va tavsiflangan;

to'rtta uy hayvonlari turida antibakterial/antioksidant faollikka ega suyak iligi oqsillari, qo'zi shirdonida COX-2 ni ingibirlovchi glikoproteinlar, *Cervus elaphus* shirdonidan gialuronidazani ingibirlovchi oqsil aniqlangan va ilk bor baqadan antioksidant hususiyatli oqsilni ekstraktsiyalash usuli takomillashtirilgan;

ipak seritsinni flavonoidlar/fenollar bilan kovalent kon'yugatsiya qilish orqali yuqori yaliglanishga qarshi va emulsifikatsiya qiluvchi xususiyatlarga ega kon'yugatlar olingan;

asosiy bioaktiv oqsillar uchun innovatsion ajratish metodologiyalari, yangi peptidlar/glikoproteinlar uchun tozalash protokollari ishlab chiqilgan va chayon (*Buthus martensii* Karsch) dan oqsil ajratib olish sxematik takomillashtirilgan;

ilk bor qo'zi va qizil bug’u (*Cervus elaphus*) shirdonning oqsil resurslari tizimli ravishda o'rganilgan va ajratilgan oqsillarning bioaktiv salohiyati aniqlangan;

xromatografik, spektroskopik, kompyuter tahlili va biokimyoviy yondashuvlarni birlashtirish orqali yallig'lanishga, o'simtaga qarshi va gialuronidazani ingibirlovchi faollikka ega yangi funktsional oqsillar ajratib olingan va ma'lumotlar bazalarida qayd etilmagan antioksidant peptidlar (masalan, SAPHP-A, SAPHP-B, LPTETLH, IEEDLER) aniqlangan;

ajratib olingan birikmalarning molekulyar strukturaviy ko’rsatgichlari (molekulyar og'irlik, gidrofoblik, aminokislotalar tarkibi) va antioksidantlik hamda metall xelatlash faolligi o'rtasidagi bog'liqlik aniqlangan;

hayvonlar/o‘simliklardan olingan oqsillarning struktura-funksiya munosabatlariga oid yangi tushunchalar hamda tabiiy resurslardan biologik faol oqsillar va peptidlardan samarali foydalanish uchun yangi nazariy va amaliy asoslar ishlab chiqilgan.

Tadqiqotning amaliy natijalari quyidagilardan iborat:

Tabiiy manbalardan biologik faol oqsillar va peptidlarni olish va tadqiq qilish uchun optimallashtirilgan ekstraksiya, gidroliz va tozalash usullari va protokollari ishlab chiqilgan;

Ikki o’rkachli (Baqtriya) tuya sutidan olingan TFI-b1, TFI-b2, TFI-b3 peptidlari va *Buthus martensii* Karsch chayonidan olingan P4-1 va P4-2 peptidlari funktsional oziq-ovqat mahsulotlarini ishlab chiqishda antioksidant sifatida foydali ekanligi isbotlangan;

No’xat (*Cicer arietinum* L.) unidan ajratilgan peptidlar funktsional oziq-ovqatlardagi erkin radikallarni neytrallashda foydali ekanligi va zira (*Cuminum cyminum* L.) urug'lari oziq-ovqat mahsulotlarida konservant sifatida istiqbolli ekanligi aniqlangan;

Ranacyclin AJ qurbaqa terisining sekretsiyasidan olingan peptid structurasi asosida ishlab chiqilgan ACC8 yangi peptidining mikroblarga qarshi va yallig'lanishga qarshi yuqori faollikni ko'rsatishi aniqlangan;

Qizil bug’u (*Cervus elaphus*) shirdonidan olingan RDA4-1 glikoproteini antioksidant va gialuronidazani inhibirlovchi ta'sir ko'rsatishi aniqlangan, bu uning nutrasevtik vositalarni ishlab chiqish uchun potentsial vosita sifatida tavsiya etilgan;

Seritsinning tripsinli gidrolizatidan ajratilgan peptid SHTCF2III mis ionini xelatlash xususiyatini namoyon qilishi aniqlangan;

Yuqori antioksidant faollikka ega ikkita yangi peptid SAPHP-A va SAPHP-B qo'y shirdoni oqsili SAPHdan ajratilgan va bu peptidlardan oziq-ovqat qo'shimchalari va farmatsevtika mahsulotlari sifatida foydalanish uchun tavsiya etilgan.

Tadqiqot natijalarining ishonchliligi tajribalarda qo'llaniladigan xalqaro miqyosda qabul qilingan va tasdiqlangan ilmiy usullar bilan tasdiqlangan. Barcha yangi birikmalarning strukturaviy o'ziga xosligi va tozaligi bir nechta, qo'shimcha yuqori aniqlikdagi analitik usullar (HPLC, SDS-PAGE, LC-MS, MALDI-TOF-MS/MS) yordamida qat'iy isbotlangan. Kuzatilgan ta'sirlar va munosabatlarning ahamiyatini tasdiqlash uchun ma'lumotlar ANOVA va regressiya modellashtirishni o'z

ichiga olgan qat'iy statistik tahlildan o'tkazilgan. Olingan natijalarning haqiqiyligi, shuningdek, natijalarning ekspertlar tomonidan ko'rib chiqilgan xorijiy ilmiy nashrlarda nashr etilishi, xalqaro konferensiyalarda muhokama qilinishi va patentlar olinishi bilan ham tasdiqlanadi.

Tadqiqot natijalarining ilmiy va amaliy ahamiyati. Tadqiqotning ilmiy ahamiyati bioaktiv peptidlar va oqsillarning manbai sezilarli darajada kengaytirilganligi, ilmiy adabiyotlarga o‘nlab yangi, to‘liq aniqlangan aminokislota ketma-ketligiga ega molekulalarni taqdim etilganligi, o‘rganilgan namunalar asosida turli biologik funksiyalarga ega peptidlarning tuzilishi va biologik faolligi o‘rtasidagi bog‘liqlik bo‘yicha yangi ilmiy tushunchalar shakllantirilganligi, tadqiqot natijasida aniqlangan yangi neyrotoksinlar va ferment ingibitorlari ion kanallari, hujayradan tashqari matritsa degradatsiyasi, signalizatsiya yo‘llari kabi murakkab biologik tizimlarni o‘rganish uchun qimmatli molekulyar vositalar sifatida katta ahamiyatga egaligi bilan izohlanadi.

Tadqiqot natijalarining amaliy ahamiyati, tadqiqot doirasida aniqlangan bioaktiv birikmalar antibiotiklarga chidamlilik (AKK8, zira peptidlari), yallig‘lanish jarayonlari (qizil bug‘u shirdoni glikoproteinlari) va qarish bilan bog‘liq teri kasalliklariga (bug‘u shirdoni oqsillari) qarshi samarali bo‘lishi mumkin bo‘lgan yangi dori vositalari uchun istiqbolli molekulalarni taklif etilganligi va olingan natijalar funksional oziq-ovqat sanoati uchun tabiiy manbali, kuchli antioksidant peptidlar va biologik faol moddalarning keng spektrini taqdim etilganligi va bu parhez mahsulotlar, nutrasevtiklar va sog‘lomlashtiruvchi qo‘shimchalar ishlab chiqishda amaliy imkoniyatlarni kengaytirishi, hamda tadqiqot qishloq xo‘jaligi va sanoatning qo‘shimcha mahsulotlari — masalan, suyak iligi, baliq terisi, ipak seritsin kabi xomashyolarni qayta ishlash orqali yuqori qo‘shilgan qiymatga ega bioaktiv moddalarni olish texnologiyalarini shakllantirishga xizmat qilishi bilan izohlanadi.

Tadqiqot natijalarning joriy qilinishi. Antibakterial, antioksidant va yallig'lanishga qarshi ta'sirga ega bo'lgan dorivor o'simliklar va hayvonlarning bioaktiv peptidlarining ilmiy natijalari va peptidlarning dizayni asosida:

no'xatdan peptidlarini ajratib olish va ularni qo'llash usuli ishlab chqilgan hamda ushbu usulga Xitoy Xalq Respublikasining ixtiro patenti olingan (ZL201510191380.6.). Natijada, ushbu usulda ajratib olingan peptidlardan funksional oziq-ovqatlardagi ozod radikallarni so‘ndirishda foydalanish imkonini bergan.

tuya sutidan antioksidant peptidlarni ajratib olish va ularni qo'llash usuli ishlab chqilib unga Xitoy Xalq Respublikasining ixtiro patenti olingan (ZL201811122673.9.) Natijada, ushbu peptidlar funktsional oziq-ovqat mahsulotlarini ishlab chiqishda antioksidant/erkin radikallarni yo'q qiluvchi vosita sifatida qollash imkonini bergan.

gialuronidaza ingibitori faolligiga ega Tyanshan qizil kiyik shirdoni glikoproteinlarini ajratib olish usuli ishlab chiqilib unga Xitoy Xalq Respublikasining ixtiro patenti olingan (ZL202111360384.4.) Natijada, qizil kiyik abomasum gastrit va shungi kabi boshqa xastaliklarni davolash imkonini bergan.

tadqiqot natijalari 100 ortiq yuqori impakt faktorli xalqaro ilmiy jurnallarda iqtibos qilingan: Journal of nanobiotechnology (IF 12.6), LWT-food science and

technology (6.6), Phytotherapy research (IF 6.3), Food bioscience (IF 5.9), Nutrients (IF 5.0), Molecules (IF 4.6), Scientific reports (IF 3.9), Journal of food science (IF 3.4), Journal of food science and technology-mysore (3.3), Journal of food measurement and characterization (IF 3.3), Frontiers in sustainable food systems (IF 3.1), Journal of food quality (IF 2.9), Journal of separation science (IF 2.8), Current microbiology (IF 2.6), International journal of peptide research and therapeutics (IF 2.4), Biotechnology letters (IF 2.1) va shu kabilar. Ushbu natijalar tabiiy manbalardan oqsil va peptid tabiatli birikmalarni ajratib olish, tuzilishini aniqlash va biologic faolliklarini aniqlash imkonini bergan.

Tadqiqot natijalarining aprobasiyasi. Ushbu tadqiqot natijalari, metodologiyasi va xulosalari 7 ta xalqaro konferentsiyada taqdim etilgan va muhokama qilingan.

Tadqiqot natijalarining e'lon qilinganligi. Ushbu mavzu bo'yicha 58 ta ilmiy ishlar nashr etilgan, jumladan, 32 tasi xalqaro ilmiy jurnallarda, 14 tasi xalqaro ilmiy konferensiyalarda ma’ruza matni, 12 ta Xitoy Xalq Respublikasi patentlari olingan.

Taqdimotning tuzilishi va hajmi. Ushbu taqdimot kirish, 10 ta asosiy tarkibiy qism, umumiy xulosa va foydalanilgan adabiyotlar ro'yxatidan iborat. Taqdimot hajmi 120 sahifadan iborat bo'lib, u xromatografik ajratish, mass-spektrlar, strukturaviy tahlillar va bioaktivlik ma'lumotlarini aks ettiruvchi ko'plab raqamlar, shuningdek unum, kimyoviy tarkib va miqdoriy natijalarni umumlashtiruvchi jadvallar bilan bayon qilingan.

TADQIQOTNING ASOSIY MAZMUNI

Biz no'xat, zira urug'lari, chayon (Buthus martensii), ipak qurti pillasi, qo'y shirdoni, Tyanshan qizil bug’usi shirdoni va qurbaqa terisi bezchalaridan ajratib olingan oqsillar va peptid birikmalarini o'rgandik. Ularning kimyoviy tuzilishi fizik-kimyoviy usullar yordamida aniqlandi va ajratib olingan birikmalarning biologik faolligi ham o'rganildi.

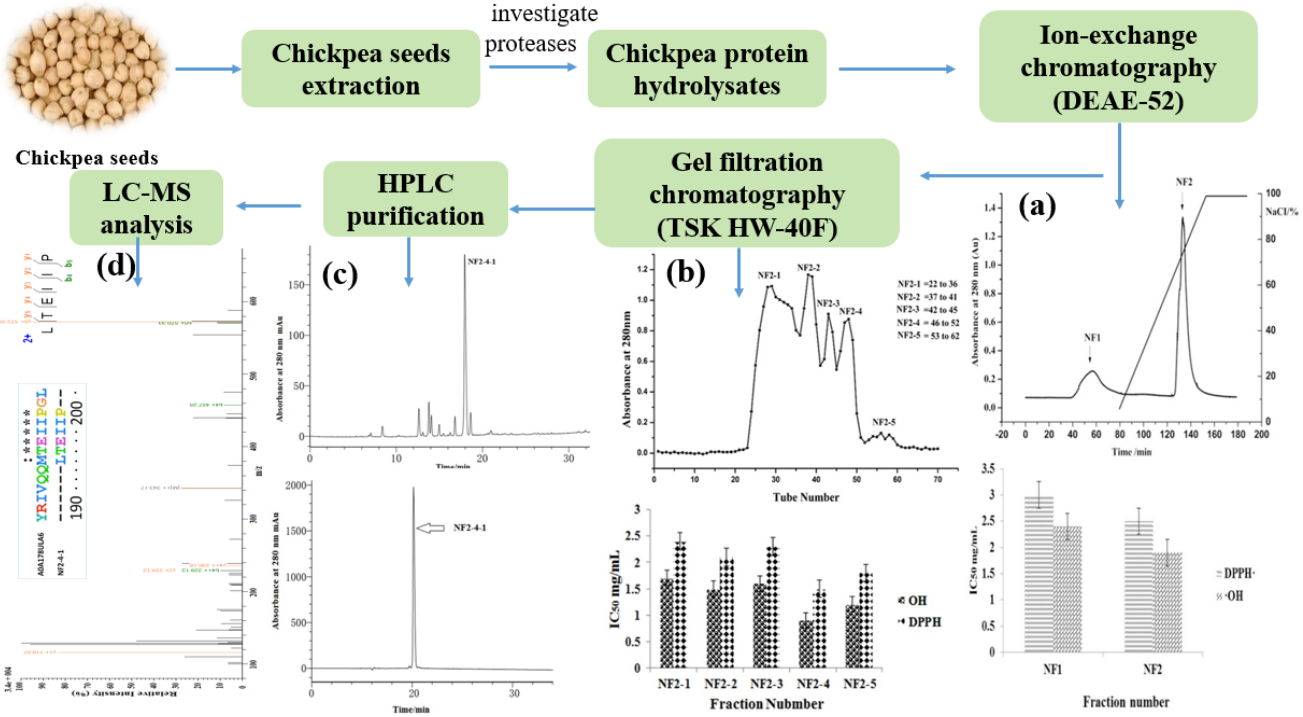
Nomi	**Peptid nomi**	**Aminokislota ketma-ketligi**	**Molekulyar Og'irligi (Da)**	**Biologik xossalari**
O'simlikning kelib chiqishi				
No'xat (*Cicer arietinum* L.)	NF2-4-1	LTEIIP	685.41	Antioksidant faolligi: DPPH: IC_{50} 0,24 mg/ml; •OH: IC_{50} 0,57 mg/ml
Zira (*Cuminum*	P1	EGGSFDECCR	1216.42	O'nta peptid *Escherichia coli, Candida albicans va Staphylococcus aureus ga qarshi yaxshi mikroblarga*
	P2	NVDEECRCDMLEEIAR	2054.82	
	P3	MSFLQLQQQAR	1365.69	
	P4	SQYEQLAEQNRK	1493.71	
	P5	SATQGKGEYTMEFSR	1707.74	
	P6	GGSGGSYGGGGSGGGYG	1791.71	

cyminum L.) urug‘lari		GGSGSR		*qarshi faollikka ega edi* .
	P7	GSYGSGGSSYGSGGGSYG SGGGGGGGSYGSGSSSGGYR	3312.26	
	P8	CAQKLDLPLDK	1243.64	
	P9	ACDQQGDSEER	1237.49	
	P10	DITAALAAERK	1158.61	
O’rganilgan hayvonlar				
Tuya suti	TFI-b1	RLDGQGRPRVWLGR	1665.94	DPPH: IC_{50} 1,9±0,04 mg/ml; ABTS: IC_{50} 2,4±0,07 mg/ml
	TFI-b2	TPDNIDIWLGGIAEPQVKR	2122.13	DPPH: IC_{50} 1,2±0,07 mg/ml; ABTS: IC_{50} 1,8±0,04 mg/ml
	TFI-b3	VAYSDDGENWTEYRDQGAVEG K	2489.09	DPPH: IC_{50} 0,6±0,02 mg/ml; ABTS: IC_{50} 0,9±0,02 mg/ml
Chayon (*Buthus martensii*)	P4-1	LPTETLH	810.43	Antioksidantlarni tozalash faolligi DPPH: 83,32% ABTS: 78,87% OH: 62,96%
	P4-2	IEEDLER	903.44	Antioksidantlarni tozalash faoliyati: DPPH: 81,22% ABTS: 78,75% OH: 58,69%
Baqa terisi	AKK-1	FRWTKSYSPKPLKR	1794.13	AKK8 to'rtta sinovdan o'tgan shtammga qarshi eng yuqori mikroblarga qarshi faollikni ko'rsatdi *E.coli* , *S.aureus* , *B.subtilis* va *C.albicans* . AKK8 ning *C.albicansga* nisbatan MIK 18,5 mkg/ ml ni tashkil qildi.
	AKK-2	FRWKYPKPLKR	1518.87	
	AKK-3	RWKYPKPLKR	1371.69	
	AKK-4	RWKQVKVKR	1227.52	
	AKK-5	RCVRWWKRVCK	1519.90	
	AKK-6	WRKQKVKK	1100.38	
	AKK-7	RWRFKWKK	1234.52	
	AKK-8	RWRFKWWKK	1420.73	
	AKK-9	RWRFKWAKK	1305.59	
	AKK-10	ARWRFKWAKK	1376,67	
Ipak qurti pillalari	1	SHHSGVNR	892.14	Ajratib olingan beshta peptid mis ionlarini xelatlash qobiliyatiga ega.
	2	TKDSIGGQAK	1004.40	
	3	DDSRADSSR	1007.24	
	4	SSNVQSDEK	1193.31	
	5	GGSVSSTGSSSNTDSSTK	1644.58	

Qo'y shirdoni	P3	LEDGLK	674.37	Antioksidant faolligi: DPPH: IC_{50} 0,63 mg/ml
	P7	IDDVLK	703.41	Antioksidant faolligi: DPPH: IC_{50} 0,58 mg/ml

1-qism. Cicer arietinum L ning antioksidant peptidlari

Tadqiqotning asosiy maqsadi funktsional oziq-ovqat mahsulotlari uchun potentsial ingredientlar bo'lib xizmat qilishi mumkin bo'lgan yangi tabiiy antioksidant peptidlarni tadqiq qilish hisoblanadi. Shu maqsadda no'xat urug'i oqsili (CSP) optimal sharoitlarda tripsin, alkalaza, neytraza proteazasi va papain bilan gidrolizlandi (1-jadval).



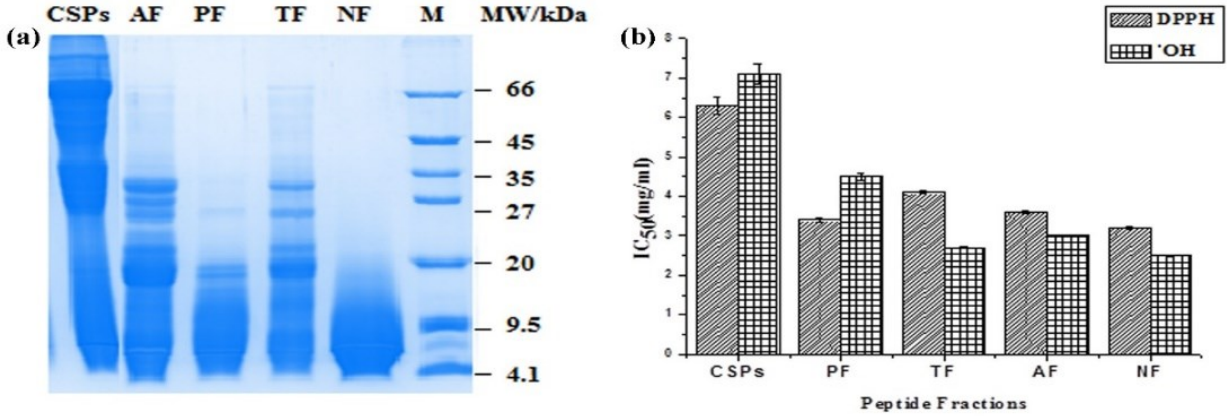
1-rasm. No'xat urug'larini qayta ishlash bosqichlari

CSP peptidlarining gidrolizlanish unumdorligi NF, TF, AF va PF uchun mos ravishda 80; 59; 78; va 66% ni tashkil etdi, NF peptidining gidrolizlanish unumdorligi boshqa fraktsiyalarga qaraganda ancha yuqori. SDS-PAGE dagi CSP gidroliz profillari Neutraza proteazasi tomonidan gidrolizdan keyin CSP boshqa proteolitik fermentlarga qaraganda ancha yaxshi ekanligini ko'rsatmoqda. Enzimatik gidrolizdan keyin NF peptidlarining MW qiymati asosan 20 kDa dan past edi. Natijalar shuni ko'rsatadiki, 4 soatlik gidroliz davomida Neutraza proteazasi boshqa proteazalarga qaraganda DH ning sezilarli darajada yuqori ko'rsatkichiga ega (2-rasm).

1-jadval. Har bir peptid fraksiyasining unumi va gidrolizlanish darajasi (DH)

Namuna	Unum, %	DH (%)
No'xot proteini	100	
Neytrasa fraktsiyasi (NF)	80 ± 0.09	19 ± 0.07
Tripsin fraktsiyasi (TF)	59 ± 0.05	14 ± 0.16
Alkalaza fraktsiyasi (AF)	78 ± 0.12	13 ± 0.15
Papain fraktsiyasi (PF)	66 ± 0.11	16 ± 0.08

Kim va boshqalar His, Pro, Ala, Gly, Glu va Leu kabi aminokislotalar antioksidant faollikni yaxshilashga hissa qo'shishini taxmin qilishdi. Tripsin, Neytraza, Alkalaza va papain bilan olingan gidrolizatlarning aminokislota miqdori mos ravishda 710,1, 982,7, 673,0 va 804,7 mg/g ni tashkil etdi (2-jadval). Bundan tashqari, NF tarkibidagi Ile, Leu, Val va Ala kabi aminokislotalarning miqdori eng yuqori (27,84%) bo'lib, bu peptid fraksiyalari orasida eng yuqori antioksidant faollikni ko'rsatadi. Bundan tashqari, NF fraksiyasidagi umumiy aminokislota miqdori 982,7 mg/g ni tashkil etdi, bu boshqa fraksiyalarga qaraganda yuqori. Uning kuchli antioksidant faollika egaligining mumkin bo'lish sababi NF komponentlarida Phe, His, Pro, Met, Ile va Cys ning yuqori miqdori bilan izohlanadi. Bu natijalar peptidlarning antioksidant faolligi aminokislotalarning gidrofob xususiyatlari bilan bog'liqligini ko'rsatadi.



2-rasm (a) SDS-PAAGE 15%: M - markerlar; CSP - No'xat oqsili; AF-Alkalaza, PF-Papain, TF-Tripsin, NF - Neytraza fraksiyalari. (b) CSP ekstraktlari va to'rt xil peptid fraksiyalarining DPPH˙ va •OH ga nisbatan IC50 qiymatlari.

2b-rasmda gidrolizatlarning DPPH˙ va •OH ga nisbatan faolligi ko'rsatilgan: NF, TF, AF va PF. Peptid fraktsiyalarining (5 mg/ml) DPPH˙ ga nisbatan faollik tartibi quyidagicha: NF > PF > AF > TF >> CSP. Peptid fraktsiyalarining (5 mg/ml) OH ga nisbatan faollik tartibi quyidagicha: NF > TF > AF > PF >> CSP. Past molekulyar peptid antioksidant faollikka ega edi. Shuning uchun, keyingi ajratish va faollikni tahlil qilish uchun neytraza gidrolizati tanlandi.

2-jadval. Turli CSP gidrolizat peptid fraktsiyalarining aminokislota tarkibi (mg/g)

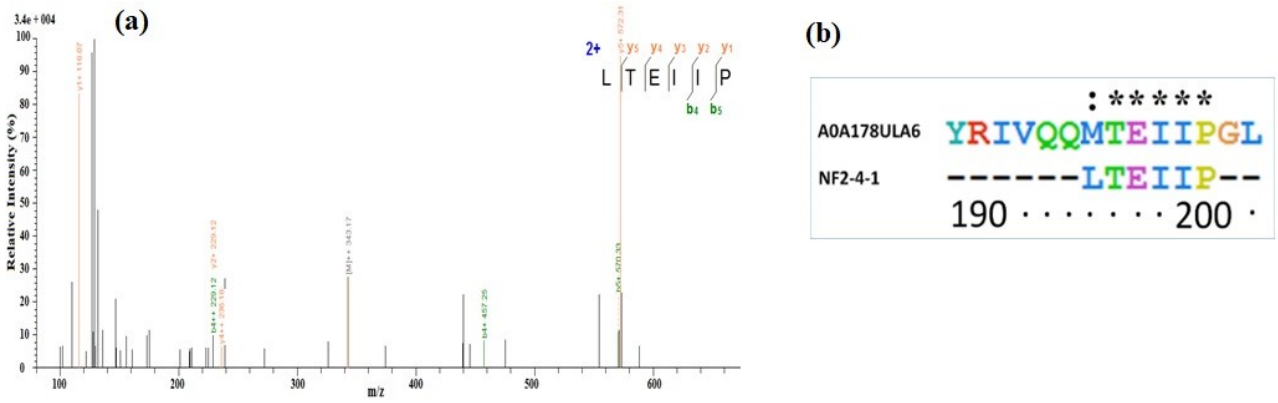
Amino kislotalar	CSP	NF	AF	TF	PF
Ala	15.3	96.1	28.1	33.8	26.9
Val	16.9	52.7	27.3	31.9	56.9
Phe	22.3	69.5	42.1	49.9	38.3
Pro	21.1	81.6	39.7	37.8	34.7
Met	9.1	9.9	6.7	9.1	0.51
Ile	19.5	59.2	29.9	33.3	23.9
Leu	42.0	65.6	53.2	44.7	55.1
Tyr	11.6	39.9	18.2	20.9	26.2
Gly	15.7	30.6	26.4	29.0	39.4
Ser	27.1	77.4	39.3	44.6	40.5
Thr	13.5	62.6	21.9	22.5	27.9
Asp	87.5	77.0	78.9	101.3	123.0
Glu	141.8	136.0	161.3	162.3	190.3
Lys	46.6	66.2	54.3	44.6	75.9
His	11.6	19.1	16.2	12.6	17.2
Arg	38.7	39.3	29.5	31.8	23.4
Ja'mi	**540.3**	**982.7**	**673.0**	**710.1**	**804.7**

Gidrolizat 20 mM natriy asetat buferida (pH 7.8) NF da eritilib, DEAE-52 kolonkali ion xromatografiya usulida ikkita: NF1 va NF2 fraksiyalari ajratib olindi (1a-rasm). NF2 fraksiyasi DPPH˙ ga qarshi antioksidant faolligi eng yuqori IC50 = 2.50 mg/ml va γ-gidroksifosfatga qarshi 1.90 mg/ml ni tashkil etdi. Shundan so'ng, NF2 fraksiya TSK HW-40F gel xromatografida 5 fraksiyaga ajratildi (1b-rasm). NF2-4 fraksiya (1b-rasm) DPPH˙ va •OH ga nisbatan yuqori faollikni ko'rsatdi (IC50 = DPPH uchun 1.50 mg/ml va •OH uchun 0.90 mg/ml). Keyin, NF2-4 fraksiya qo'shimcha ravishda Agilent C18 kolonkasida (9.4 x 250 mm) HPLC xromatografi yordamida tozalandi. Gidrofob peptidlar MeCN gradient (10% - 0-2 daqiqa, 10-40% - 2-20 daqiqa, 40-50% - 20-35 daqiqa, 50-60% - 35-40 daqiqa, 60-10% - 40-45 daqiqa, 0,1% TFA da) mobil faza yordamida ajratib olindi (1c-rasm). Natijada, NF2-4-1 antioksidant peptidi ajratib olindi (IC50 = DPPH˙ uchun 0,24 mg/ml, •OH uchun 0,57 mg/ml). Antioksidant peptidning tozaligi 92,8% ni tashkil etdi (1c-rasm, 3-jadval).

3-jadval. NF fraksiyasidan antioksidant peptidni toza holda ajratib olish

Fraksiyalar	bosqich	Antioxidant faolligi (IC50, mg/mL)	
		DPPH˙	·OH
CSP		6.30 ± 0.04	7.10 ± 0.08
Neutraza fraksiyasi (NF)	Gidroliz	3.20 ± 0.09	2.50 ± 0.05
NF2	DEAE-52	2.50 ± 0.05	1.90 ± 0.08
NF2-4	TSK HW-40F	1.50 ± 0.07	0.90 ± 0.03
NF2-4-1	RP-HPLC	0.24 ± 0.06	0.57 ± 0.04

NF2-4-1 peptidining tuzilishi MALDI-TOF-MS/MS usulida tahlil qilinib, tarkibi Leu-Thr-Glu-IIe-IIe-Pro (LTEIIIP) ekanligi aniqlandi va MW 685.41 Da ni tashkil qiladi (3-rasm). NF2-4-1 (LTEIIIP) aminokislota ketma-ketligining fragment tahlili Arabidopsis thaliana dan olingan oqsil A0A178ULA6-1 Uniprot kirish raqamiga ega (qoldiqlar 195–200) va boshqa bir qancha o'simlik oqsillari bilan past moslikni (E = 19.5) ko'rsatdi (3-rasm).



3-rasm (a) NF2-4-1 peptidining massa spektri va (b) uning mass fragmenti

Torres-Fuentes tomonidan CSP gidrolizatlarining antioksidant faolligi o'rganildi va ularning FVPH, ALEPDHR, TETWNPNHPEL va SAEHGSLH tadqiqotlari to'rtta no'xat urug'i peptidlarining antioksidant xususiyatlari o'rganib chiqildi. Olingan natijalar shuni ko'rsatdiki, CSP gidrolizatlari funktsional oziq-ovqat mahsulotlarining antioksidant xususiyatlarini oshirish va oziq-ovqat ishlab chiqarish jarayonida oksidlovchi reaksiyalarning oldini olish uchun tabiiy antioksidantlar sifatida ishlatilishi mumkin. Antioksidant peptidlarning inson salomatligidagi rolini aniqlash uchun qo'shimcha tadqiqotlar zarur. YLEELHRLNAGY aminokislotalar ketma-ketligiga ega bo'lgan izolyatsiya qilingan peptidlardan biri eng yuqori antioksidant faollikni (IC50 <0,01 mg/ml) ko'rsatdi va Glu va Leu aminokislotalarini o'z ichiga oldi.

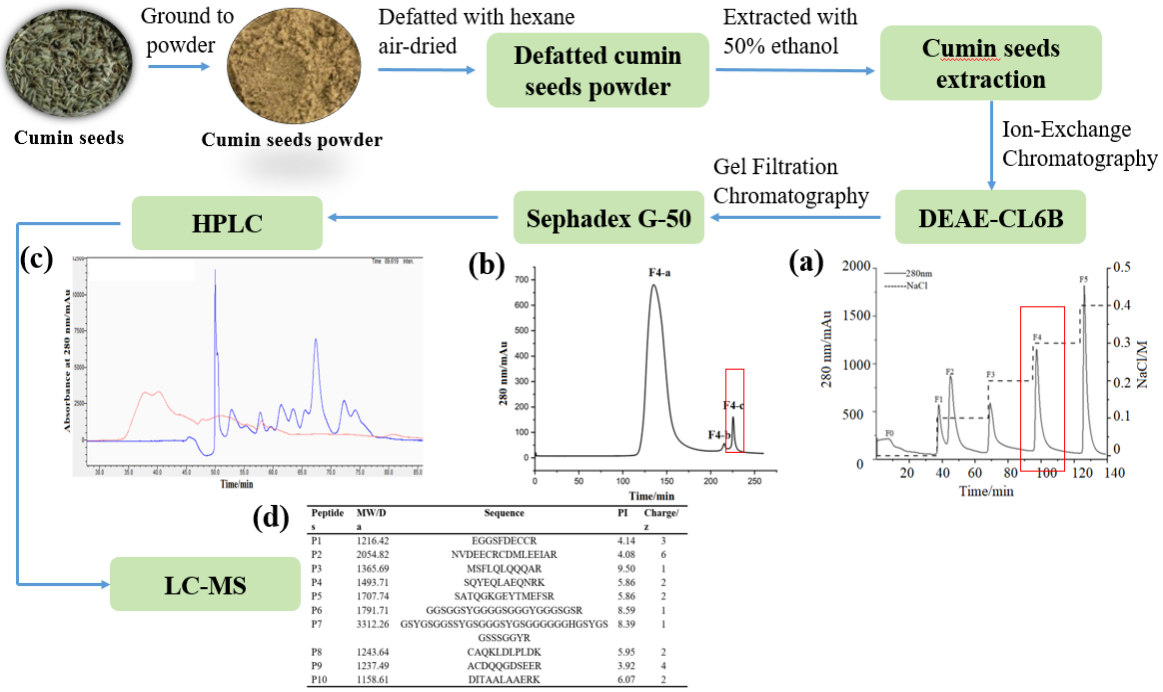
Bir nechta tadqiqotlar shuni ko'rsatdiki, Leu, Glu, His, Met va boshqa aminokislotalar peptidlarning turli xususiyatlarini yuzaga chiqishida muhim rol o'ynaydi. Biroq, o'rganilayotgan peptidlarda Pro va Leu mavjudligi sababli NF2-4-1 peptidi antioksidant faollikka ega bo'ldi. Gidrofob aminokislotalarning kislotali aminokislotalarga nisbati 83,3% ni tashkil etdi (LTEIIIP).

Natijalar shuni ko'rsatdiki, Leu-Glu va Izolat-izolat-protamat ketma-ketliklari, ehtimol, erkin radikallar bilan reaksiyaga kirishib, ularning barqarorroq mahsulotlarga aylanishiga olib keladi va ehtimol antioksidant faollikda muhim rol o'ynaydi.

Xulosa:

Cicer arietinum L. dan olingan no'xat uni oqsilining tripsin, papain, pepsin, alkalaza va neytraza-proteaza fermentlari bilan gidrolizlanish shart sharoitlari o'rganildi. Neytraza-proteaza fermenti bilan gidroliz eng samarali ekanligi aniqlandi, molekulyar og'irligi 20 kDa dan kam bo'lgan peptidlar 80% unum bilan ajratib olindi. Neytraza-peptid fraksiyasini 92,8% tozalikda neytraza peptid fraksiyasidan ajratish uchun ion almashinuvchi (DEAE-52 tsellyuloza), gel xromatografiyasi (HW-40F) va HPLC (Agilent C18 kolonka) dan iborat 3 bosqichli usul qo'llanildi. Uning molekulyar og'irligi 685,41 Da ekanligi va 6 ta LTEIIIP aminokislota ketma-ketligidan iborat ekanligi aniqlandi. NF2-4-1 peptidining antioksidant faolligi DPPH˙ bo'yicha IC50 = 0,24 mg/ml va •OH bo'yicha IC50 = 0,57 mg/ml ekanligi ko'rsatildi. Ushbu peptidlardan funktsional oziq-ovqatlar uchun antiradikal qo'shimcha sifatida foydalanish mumkin.

2-qism. Zira (cyminum L) urug'larining antimikrob peptidlari



Peptides	MW/Da	Sequence	PI	Charge/z
P1	1216.42	EGGSFDECCR	4.14	3
P2	2054.82	NVDEECRCDMLEEIAR	4.08	6
P3	1365.69	MSFLQLQQQAR	9.50	1
P4	1493.71	SQYEQLAEQNRK	5.86	2
P5	1707.74	SATQGKGEYTMEFSR	5.86	2
P6	1791.71	GGSGGSYGGGGSGGGYGGGSGSR	8.59	1
P7	3312.26	GSYGSGGSSYGSGGGSYGSGGGGGGHGSYGSGSSGGYR	8.39	1
P8	1243.64	CAQKLDLPLDK	5.95	2
P9	1237.49	ACDQQGDSEER	3.92	4
P10	1158.61	DITAALAAERK	6.07	2

4-rasm. Zira urug'larini qayta ishlash bosqichlari

Tadqiqotning maqsadi zira (Cuminum cyminum L.) urug'laridan olingan antimikrob peptidlarni o'rganish va ularning patogen mikroorganizmlarga qarshi tabiiy agent sifatidagi salohiyatini baholash edi. Buning uchun zira urug'i quyidagi ishlov berishdan o'tkazildi:

1000 g zira urug'i n-geksan yordamida kukunga aylantirildi va yog'sizlantirildi, natijada 756 g yog'sizlantirilgan zira urug'i kukuni olindi. Optimallashtirilgan ekstraksiya sharoitida kukun xona haroratida (25°C) har safar 3 soat davomida 50% etanol bilan uch marta ekstraksiya qilindi. Ekstraktlar birlashtirilib filtrlandi va keyin liofil quritgichda quritildi, natijada 239 g xom oqsil kukuni olindi. Zira oqsili ekstrakti DEAE CL-6B ustunli xromatogariyada ajratilganda (4a-rasm), oltita cho'qqi kuzatildi. Barcha fraksiyalar alohida to'plandi, tuzsizlantirilda, liofil usulda quritildi va tortildi (4-jadval).

4-jadval Cuminum cyminum L. urug‘ peptidlarini tozalash bosqichlari

Tozalash bosqichlari		Unumi (mg/10g)	Mikrobga qarshi faolligi		
			EC	CA	SA
Zira urug’i ekstraksiyasi		10000	+	+	+
DEAE CL-6B	F1	105.63	-	-	-
	F2	326.71	-	-	-
	F3	298.08	-	-	-
	F4	705.25	+	+	+
	F5	1085.93	+	-	-
Sephadex G-50	F4-a	267.53	-	-	+
	F4-b	112.84	-	+	-
	F4-c	115.50	+	+	+

Peptidlar fraksiyasi EC (Escherichia coli, ATCC11229), CA (Candida albicans, ATCC10231) va SA (Staphylococcus aureus, ATCC6538) ga qarshi antibakterial faollik uchun in vitro sezuvchanlik testi orqali sinovdan o'tkazildi (4-jadval). Natijalarni o’zaro taqqoslash shuni ko'rsatdiki, F4 fraktsiyasi Escherichia coli, Candida albicans va Staphylococcus aureus ga qarshi yaxshiroq antimikrob faollikka ega, F5 fraktsiyasi esa Escherichia coli ga qarshi yaxshiroq antimikrob faollikka egaligi aniqlandi. SDS-PAGE elektroforezi va dastlabki antibakterial faollik skrining tahlili asosida, DEAE CL-6B xromatografiya ustunidan ajratib olingan F4 fraksiyasi Sephadex G-50 xromatografiya ustunidan foydalanib keyingi tozalash uchun tanlandi. Elyuatsiya cho'qqilari 4-rasmda ko'rsatilgan. Uchta asosiy elyuatsiya cho'qqisi (F4-a, F4-b va F4-c) to'plandi, tuzsizlantirildi, liofillash usulida quritildi va keyingi tahlil va tozalash uchun massasi tortildi (4-jadval).

4-jadvaldagi mikroblarga qarshi natijalar shuni ko'rsatdiki, F4-a fraksiyasining Staphylococcus aureusga qarshi yaxshiroq antimikrob faolligi, F4-b fraksiyasining Candida albicansga qarshi yaxshiroq antimikrob faolligi va F4-c fraksiyasining Escherichia coli, Candida albicans va Staphylococcus aureusga qarshi yaxshiroq antimikrob faolligi bor. Tahlil natijalariga ko'ra, gel xromatografiyasidan olingan F4-c fraksiyasini suyuqlik xromatografi yordamida ajratish va tozalash amalga oshiriladi.

Suyuqlik xromatografiyasi yordamida F4-c fraksiyasidan 9 dan ortiq sifatli peptid ajratib olindi (4c-rasm). F4-c fraksiyasining peptid tuzilishini tasdiqlash uchun fraksiya LC/MS tahlilidan o'tkazildi. Namunalar (2 μL) Agilent Technologies Micro WPS asbobi yordamida Zorbax SB C18 ustuniga (75 μm × 43 mm, 5 μm) joylashtirildi. Gradient 1 daqiqa davomida A buferidan (suv + 0,1% TFA), so'ngra keyingi 60 daqiqa davomida 3 ml/min oqim tezligida 5 dan 45% gacha bo'lgan chiziqli gradient B buferidan (asetonitril + 0,1% TFA) iborat edi.

30% metanolli fraksiyalar natijalarining LC/MS tahlili shuni ko'rsatdiki, peptidlar fraksiyasida 10 dan ortiq kislotali peptidlar, 7 ta neytral peptidlar, 4 ta ishqoriy peptidlar mavjud. Ularning molekulyar og'irligi 2479,13 dan 9768,51 Da gacha. Kislotali peptidlar va neytral peptidlar suyuqlik xromatografida qayta ajratlidi va bunda olingan natijalar shuni ko'rsatdiki, peptidlarning aksariyati 8 dan 13 daqiqalarda ajralib chiqishi kuzatildi. 10.823, 12.014, 12.223 va 12.346 daqiqada ajratilgan peptidlar to'plandi va ularning molekulyar massasi mass-spektrometrda ESI-MS rejimida mos ravishda 3361.56, 3122.67, 3121.64, 2498.12, 2499.12 va 2498.10 Da ekanligi aniqlandi.

5-jadvalda F4-c fraksiyasidan o'nta monomerdian iborat peptidning aminokislota ketma-ketligi aniqlanganligi ko'rsatib o’tilgan. Aminokislota ketma-ketliklarini ma'lumotlar bazasi bilan taqqoslash shuni ko'rsatdiki, bu peptidlarning aksariyati albumin, transkripsiyani boshlash omili, o'zgartiruvchi o'sish omili, keratin, peptidil-tRNK gidrolaza va boshqa albumin bilan bog'liq boshqa peptidlar kabi biofaol peptidlar bilan yuqori gomologiyaga ega (5-jadval). Bu peptidlar immun tizimida va to'qimalarni tiklashda muhim rol o'ynaydi. Masalan, 2S albumin va TOPBP1 DNK ikki qatorli tuzilmalarni tiklashda ishtirok etishi ma'lum. Ular orasida dinamin-I to'rtta funktsional domenni o'z ichiga oladi, ularning har biri o'ziga xos funktsiyalarga va aniq tartibga solish mexanizmlariga ega bo'lib, sinaptik vezikula endotsitozida muhim rol

o'ynaydi. Shuning uchun dinamin-I ning to'rtta funktsional domenini, shuningdek, uning sinaptik vezikula endotsitozidagi tartibga solish mexanizmlari va joylarini chuqur o'rganish muhim nazariy va amaliy ahamiyatga ega..

5-jadval. MALDI TOF/TOF yordamida peptid molekulyar massasi va aminokislotalar ketma-ketligini aniqlashning batafsil natijalari

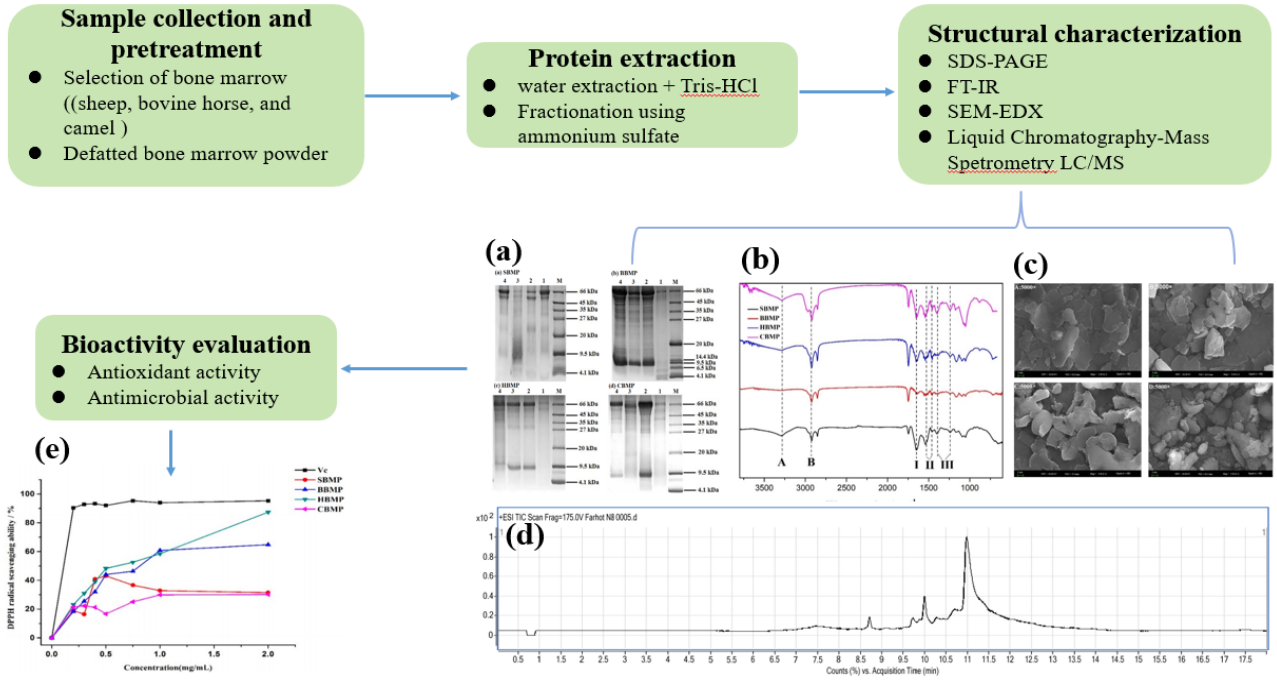
Peptidlar	MW/Da	Ketma ketlik	PI	Zaryad
P1	1216.42	EGGSFDECCR	4.14	3
P2	2054.82	NVDEECRCDMLEEIAR	4.08	6
P3	1365.69	MSFLQLQQQAR	9.50	1
P4	1493.71	SQYEQLAEQNRK	5.86	2
P5	1707.74	SATQGKGEYTMEFSR	5.86	2
P6	1791.71	GGSGGSYGGGGSGGGYGGGSGSR	8.59	1
P7	3312.26	GSYGSGGSSYGSGGGSYGSGGGGGGHGSYG GSSSGGYR	8.39	1
P8	1243.64	CAQKLDLPLDK	5.95	2
P9	1237.49	ACDQQGDSEER	3.92	4
P10	1158.61	DITAALAAERK	6.07	2

Xulosa:

Cuminum L. zira urug'laridan peptidlarni ajratish uchun ekstraksiya, ion almashinuv (DEAE-CL6B) va gel xromatografiyasidan (Sephadex G-50) iborat uch bosqichli ajratish usuli ishlab chiqildi. Gel xromatografiyasidan olingan F4-c fraksiyasining bir vaqtning o'zida *E. coli,* C. albicans va S. aureus bakteriyalari va zamburug'lariga qarshi samarali ekanligi ko'rsatildi. Molekulyar massa diapazoni 1158,61 – 3312,26 Da bo'lgan o'nta peptid HPLC yordamida F4-c fraksiyasidan ajratib olindi va ularning birlamchi tuzilmalari to'liq aniqlandi. Ushbu peptidlar tabiiy antibiotiklar hisoblanadi va kelajakda oziq-ovqat mahsulotlarida konservant sifatida foydalanish imkoniyatiga ega.

3-qism. Uy hayvonlarining suyak iligidan olingan bioaktiv oqsil va peptidlar

Ushbu tadqiqotning maqsadi to'rtta uy hayvonlari: qo'y (SBMP), mol (BBMP), ot (HBMP) va tuya (CBMP) suyak iligidan bioaktiv oqsillar va peptidlarni keng qamrovli ajratish, tavsiflash va baholashdan iborat edi. Go'sht sanoatining qo'shimcha mahsulotlaridan foydalanishning ekologik va iqtisodiy qiyinchiliklariga duch kelgan ushbu tadqiqot hayvonlarning suyak iligini - an'anaviy ravishda tan olingan, ammo ilmiy jihatdan kam o'rganilgan resursni - funktsional oziq-ovqat va farmatsevtikada qo'llash salohiyatiga ega ekanligini o'rganishga qaratilgan edi.



5-rasm. Suyak iligini qayta ishlash bosqichlari

Ushbu ishda to'rt xil hayvon suyak iligidan suv yordamida oqsillar va peptidlar ajratib olindi, shundan so'ng xom ekstraktlar turli xil eruvchanlikdagi oqsillar va peptidlarni cho'ktirish uchun turli konsentratsiyali ammoniy sulfat (30%, 50% va 70%) yordamida fraktsiyalandi. Oqsilning chiqish unumi va oqsil miqdori hayvon manbalari va ekstraksiya usullari orasida sezilarli darajada farq qildi. HBMP ning dastlabki suvda ekstraksiya qilishda ekstraksiya samaradorligi 90,47% ni tashkil etdi (6-jadval), ehtimol bu uning yumshoqroq tuzilishi va yuqori yog' miqdori bilan bog'liq. Natijalar shuni ko'rsatadiki (7-jadval), 50%li ammoniy sulfat eritmasida fraktsiyalangan BBMP va CBMP mos ravishda 52,3 mg/ml va 56,5 mg/ml ni tashkil etuvchi eng yuqori protein miqdoriga ega. Suvdan olingan BBMP eng yuqori protein miqdoriga (52,20 mg/ml) va boshqalarga qaraganda kuchliroq antimikrob faollikka ega, bu oqsil miqdori va antimikrob faolligi o'rtasida ma'lum dozaga bog'liq bog'liqlik borligini anglatadi (8-jadval).

6-jadval. Turli suyak iligidan suv bilan ajratib olingan oqsillarni taqqoslash.

No.	Protein miqdori (mg/mL)	Extraktsiya unumi (%)
SBMP	20.22	86.85
BBMP	52.20	68.43
HBMP	34.60	90.47
CBMP	25.60	72.07

7-jadval. Turli BMPlarni ammoniy sulfat fraksiyalari bo'yicha taqqoslash.

No.	Konsentratsiya (%)	Protein miqdori （mg/ml）	Extraktsiya unumi (%)
SBMP	30	40.66	7.95
	50	22.53	10.79
	70	25.37	7.38
BBMP	30	35.11	44.14
	50	52.35	52.41

	70	41.71	24.80
	30	13.6	55.70
HBMP	50	44.3	11.00
	70	25.1	5.5
	30	17.00	15.3
CBMP	50	56.5	7.50
	70	47.3	4.17

8-jadval. Turli BMPlarni antimikrob faolligi.

No.	Konsentratsiya (%)	CA (mm)	EC (mm)
suvdan olingan oqsillar			
SBMP		8	7
BBMP		8	9
HBMP		7	8
CBMP		8	8
ammoniy sulfat fraktsiyalari			
	30	9	9
SBMP	50	8	-
	70	9	-
	30	9	9
BBMP	50	10	9
	70	8	10
	30	8	-
HBMP	50	9	8
	70	7	-
	30	8	-
CBMP	50	9	9
	70	8	-

Izohlar: CA. Candida albicans; EC. Escherichia coli.

Suv bilan ekstraksiya qilingan oqsillarning ozuqaviy sifati ularning erkin aminokislota tarkibini tahlil qilish orqali aniqlandi va 17 xil aminokislotalarning mavjudligi aniqlandi (9-jadval). Erkin aminokislotalarning umumiy miqdori SBMPda 5,15 mg/g dan HBMPda 49,63 mg/g gacha keskin o'zgarib turdi. Umumiy erkin aminokislota miqdori bo'yicha umumiy reyting HBMP > BBMP > CBMP > SBMP edi. Har bir suyak iligi turida turli xil dominant aminokislota mavjud: SBMP uchun glisin (Gly), BBMP uchun leysin (Leu), HBMP uchun serin (Ser) va CBMP uchun alanin (Ala). HBMP va SBMP ning ozuqaviy profili ayniqsa diqqatga sazovor, chunki ularning muhim, muhim bo'lmagan aminokislotalarga (E/N) va muhim-umumiy aminokislotalarga (E/T) nisbati FAO/JSST tomonidan tavsiya etilgan ideal naqshga yaqin edi, bu yuqori ozuqaviy salohiyatni ko'rsatadi.

9-jadval. To'rt turdagi suv ekstrakti BMP tarkibidagi erkin aminokislotalarning tarkibi va miqdori.

Amino kislota	SBMP	BBMP	HBMP	CBMP
	tarkibi (mg/g)			
Asp	0.08	0.12	2.76	0.06
Thr	0.08	0.29	2.18	0.04
Glu	0.05	0.25	2.13	0.11

Ser	0.5	3.25	8.83	0.78
Gly	1.26	1.52	3.21	1.18
Ala	0.53	4.94	6.4	2.15
Val	0.44	5.97	3.61	1.92
Met	0.02	1.7	0.33	0.28
Ile	0.02	1.75	1.3	0.47
Leu	0.1	6.94	4.76	1.71
Tyr	0.31	3.1	2.39	1.17
Phe	0.18	4.61	3.5	1.14
Lys	1.13	4.21	4.26	1.39
His	0.09	2.43	4.07	0.67
Arg	0.01	0.12	1.96	0.07
Pro	0.36	1.87	1.54	0.85
Jami amino kislota (T)	5.15	43.05	49.63	13.98
Almashinadigan aminokislotalar (E)	1.97	25.45	19.94	6.95
Almashinmaydigan aminokislotalar (N)	3.19	17.6	29.7	7.04
Dori aminokislotalar (D)	3.15	22.57	25.31	7.1
N/T (%)	0.62	0.41	0.6	0.5
E/T (%)	0.38	0.59	0.4	0.5
E/N (%)	0.62	1.45	0.67	0.99
D/T (%)	0.61	0.52	0.51	0.51

Ajratib olingan fraksiyalarning tuzilish va molekulyar tavsifini aniqlash uchun bir qator analitik usullar qo'llanildi. SDS-PAGE tahlili (5a-rasm) shuni ko'rsatdiki, to'rtta hayvonning suyak iligi ekstraktlari ikkita asosiy tasmani o'z ichiga oladi: taxminan 66 kDa dagi yuqori molekulyar og'irlikdagi oqsil tasma va 4,1 kDa dan 9,5 kDa gacha bo'lgan past molekulyar og'irlikdagi polipeptid tasma. SBMP ning 70% fraktsiyasi va BBMP ning 30% fraktsiyasi asosan polipeptidlardan iborat bo'lib, ularni monomer peptidlarini keyingi ajratish uchun ideal nomzodlar sifatida aniqladi. Bu natijalar batafsilroq molekulyar profilni taqdim etgan LC/MS tahlili bilan tasdiqlandi. LC/MS ko'plab alohida peptidlar va oqsillarni aniqladi, masalan, SBMP suv ekstraktida, bu yerda 28 ta peptid (1,0-8,6 kDa) va to'qqizta oqsil (10,8–18,5 kDa) aniqlandi. Xuddi shunday, BBMP suv ekstraktida 25 ta peptid (1,0-9,9 kDa) topildi.

Oqsillarning ikkilamchi tuzilishini o'rganish uchun FT-IR tahlili qo'llanildi (5b-rasm). To'rtta BMP uchun spektrlar xarakterli amid A, B, I, II va III tasmalarini ko'rsatdi, amid I tasmasining joylashuvi (taxminan 1650 sm^{-1}) oqsillar asosan α-spiral tuzilmalaridan iboratligini ko'rsatadi. Sirt morfologiyasini kuzatish uchun SEM ishlatilgan. Tasvirlarda barcha oqsil fraktsiyalari kattalik, agregatsiya va g'ovaklikda sezilarli farqlarga ega bo'lishiga qaramay, parcha shaklidagi tuzilmalarni hosil qilganligi ko'rsatilgan, bu har bir hayvon manbai uchun alohida fizik xususiyatlarni ko'rsatadi.

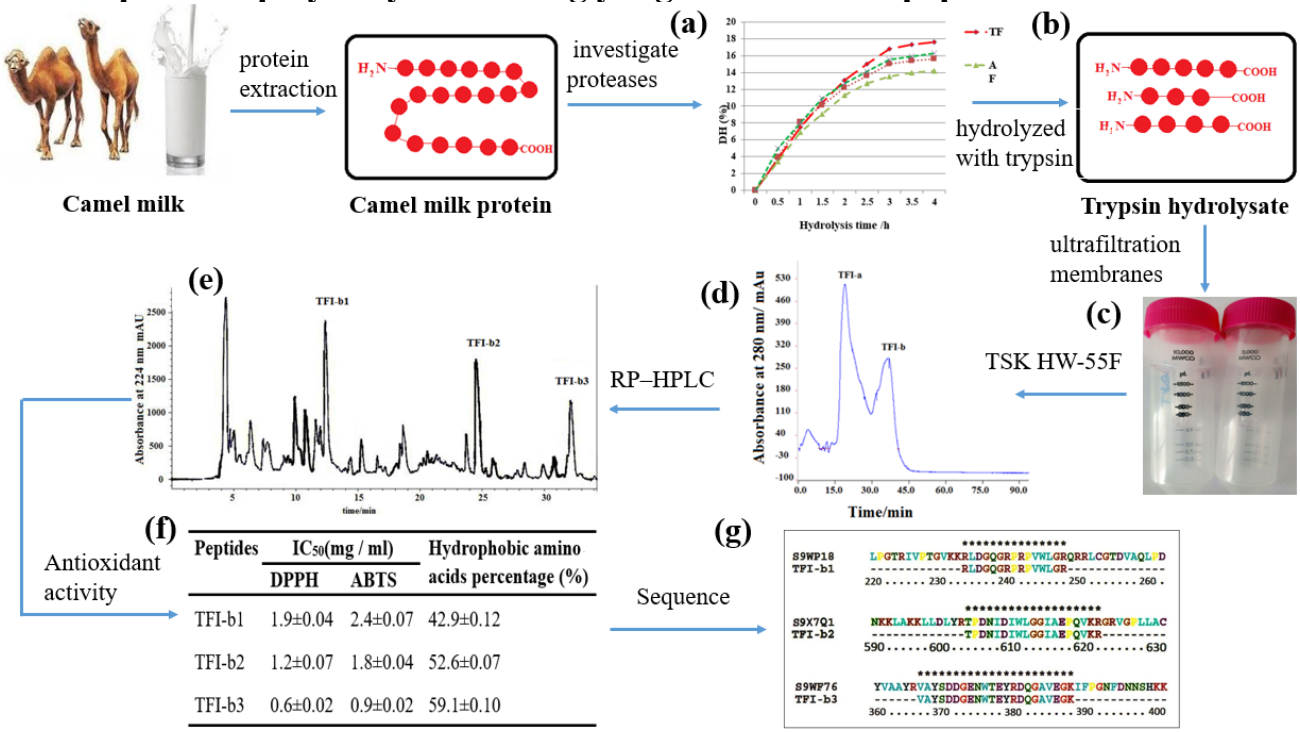
DPPH radikal tozalash tahlili antioksidant qobiliyatini o'lchash uchun ishlatilgan. Suv bilan olingan to'rtta oqsilning barchasi sinovdan o'tgan 0,025-2,0 mg/ml oralig'ida dozaga bog'liq tozalash faolligini namoyish etdi. Ular orasida ot suyagi iligi oqsili (HBMP) sezilarli darajada yuqori antioksidant faollikka ega bo'lib, 2 mg/ml da

maksimal 83,9% ga yetdi (5e-rasm). Uning IC50 qiymati 0,573 mg/ml ni tashkil etdi, bu BBMP dan (0,834 mg/ml) sezilarli darajada past edi. Bu yuqori faollik, ehtimol, HBMP ning umumiy erkin aminokislota miqdorining ko'pligi bilan bog'liq.

LC/MS tahlilida molekulyar og'irlik ma'lumotlari tekshirildi. Suvdan olingan SBMP tarkibida jami 28 ta peptid va to'qqizta oqsil topildi. Peptid Mw diapazoni 1053,4627–8673,46 Da, oqsillarniki esa 10,845,85–18,567,53 Da edi. Suvdan olingan BBMP tarkibida jami 25 ta peptid topildi. Peptidlarning Mw qiymati 1027,84–9916,32 Da oralig'ida edi. Peptidlarning maksimal zaryadi +9, mos keladigan Mw 4947.51 Da, minimal zaryadi +3, mos keladigan Mw esa 2757.38 Da. CBMP ning 50% ammoniy sulfat cho'kkan qismida jami to'qqizta peptid va ikkita oqsil topildi, bu yerda peptidlarning Mw 1040.35-3986.65 Da, oqsillarning Mw esa 11 430.35–15 178.48 Da edi. Peptidlarning maksimal zaryadi +8, mos keladigan Mw 3986.65 Da, minimal zaryad esa +4, mos keladigan Mw 2775.4276 Da.

Xulosa: Qo'y, qoramol, ot va tuya suyak iligidan oqsillar va peptidlarni 30, 50 va 70% $(NH_4)_2SO_4$ eritmalari bilan suv va tuzli suv bilan ekstraksiya qilish orqali ajratib olish usuli yaxshilandi. Ot suyak iligining suvli ekstraktidagi oqsillarning ajralish unumi 90,47% ga yetgani aniqlandi. $(NH_4)_2SO_4$ ning 50% eritmasida qoramol va tuya suyak iligidan ajratib olingan oqsillarning konsentratsiyasi mos ravishda 52,3 va 56,5 mg/ml ni tashkil etdi. Turli hayvonlarning suyak iligi tuzli ekstraktlarida suvli ekstraktlariga qaraganda oqsil miqdori yuqori ekanligi ko'rsatildi. Ot iligi oqsili yuqori antioksidant faollikka ega bo'lib, 2 mg/ml da 83,9% ga etadi va uning IC_{50} qiymati 0,573 mg/ml ni tashkil etdi. Yuqori antioksidant faollikni suyak iligida ko'p miqdordagi erkin aminokislotalar bo'lishi bilan bog'liqligi orqali izohlash mumkin.

4-qism. Baqtriya tuyasi sutining yangi antioksidant peptidlari



Peptides	IC_{50}(mg / ml)		Hydrophobic amino acids percentage (%)
	DPPH	ABTS	
TFI-b1	1.9±0.04	2.4±0.07	42.9±0.12
TFI-b2	1.2±0.07	1.8±0.04	52.6±0.07
TFI-b3	0.6±0.02	0.9±0.02	59.1±0.10

6-rasm. Tuya sutini qayta ishlash bosqichlari

Ushbu tadqiqot Baqtriya tuya suti (BCM) oqsil gidrolizatlaridan yangi antioksidant peptidlarni aniqlashga qaratilgan. Maqsad qaysi fermentativ gidroliz usuli eng kuchli antioksidant faollikka ega peptidlarni ishlab chiqarishini aniqlash va ularning tuzilishi va potentsial biofaolliklarini tavsiflash iborat**.**

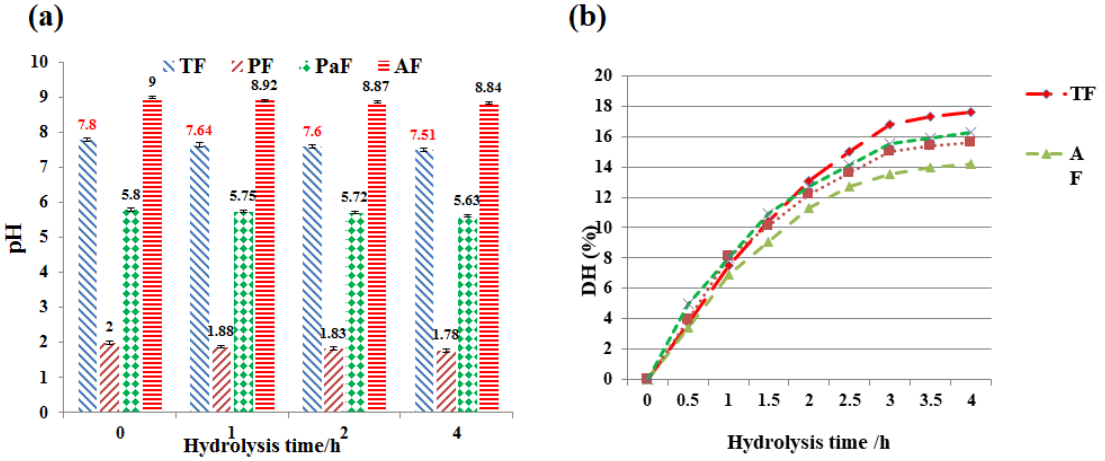
BCM oqsili optimal sharoitlarda to'rt xil turdagi fermentlar tomonidan gidrolizlandi. Peptinlarning unumi mos ravishda pepsin, alkalaza, papain va tripsin uchun 67, 59, 75 va 82% deb o'lchandi. Tripsin bilan gidrolizlangan peptidlarning chiqish unumi boshqa uchta proteazalarga qaraganda yuqori (10-jadval).

10-jadvalda eng yuqori DH – gidrolizlanish darajasi (17,6%) tripsin gidrolizat bilan olinganligi ko'rsatilgan. Tripsin gidrolizati DPPHda 72% va ABTSda 69% faollik namoyon qilishi keltirilgan. Ushbu tadqiqotga asoslanib, yuqori peptid unumi va DHga ega tripsin fraksiyasining (TF) yuqori antioksidant faollikka hissa qo'shishi taxmin qilingan.

9-jadval Peptid fraksiyalarining chiqish unumi va biologik faolligi

Namunalar	Unum (%)	DH (%)	ABTS faollik/ %	DPPH faollik/ %
BCM protein	100		43	39
TF	82	17.6	72	69
PF	67	16.3	64	55
AF	59	14.2	60	64
PaF	75	15.6	59	61

Tripsin, pepsin, papain va alkalaza uchun dastlabki pH qiymatlari 7,8, 2,0, 5,8 va 9,0 bo'lib, 4 soatlik gidrolizdan so'ng mos ravishda 7,52, 1,78, 5,65 va 8,84 gacha sezilarli darajada pasaydi (7a-rasm). Alkalaza, papain va pepsin fraktsiyalari bilan solishtirganda, tripsindagi pH qiymatining pasayish tezligi yuqoriroq bo'ldi, undan keyin pepsin fraktsiyalari pH qiymati pasayishi kuzatildi.



7-rasm (a) BCM oqsili gidrolizatlarining pH qiymatining o'zgarishi; (b) BCM oqsilining turli fermentlar (mos ravishda TF, PF, AF, PaF - tripsin, pepsin, alkalaza, papain gidrolizatlari) bilan DH (n=9).

BCM oqsilining gidroliz jarayoni 7b-rasmda ko'rsatilgan bo'lib, unda dastlab DH dastlabki 2 soatda oshganligi; keyinchalik DH sekinroq oshganligi va barqarorlashganligi ko'rsatilgan. Gidroliz tezligi reaksiya vaqti ortishi bilan pasayadi, bu mavjud peptid bog'lanishlari miqdorining pasayishiga mos keladi. Dastlabki 2 soat

davomida to'rtta ferment yuqori gidrolizlanish tezligiga ega bo'lib, bu asta-sekin pasayib ketdi.

DPPH va ABTSda faollikni aniqlash usulidan foydalangan holda to'rtta BCM peptidining antioksidant faolligi baholandi (9-jadval). 10 mg/ml konsentratsiyasida peptidlarning DPPH ga ta'siri quyidagi tartibda bo'lgan: TF > PF > AF > PaF, ABTS ga ta'siri TF > AF > PaF > PF bo'lgan.

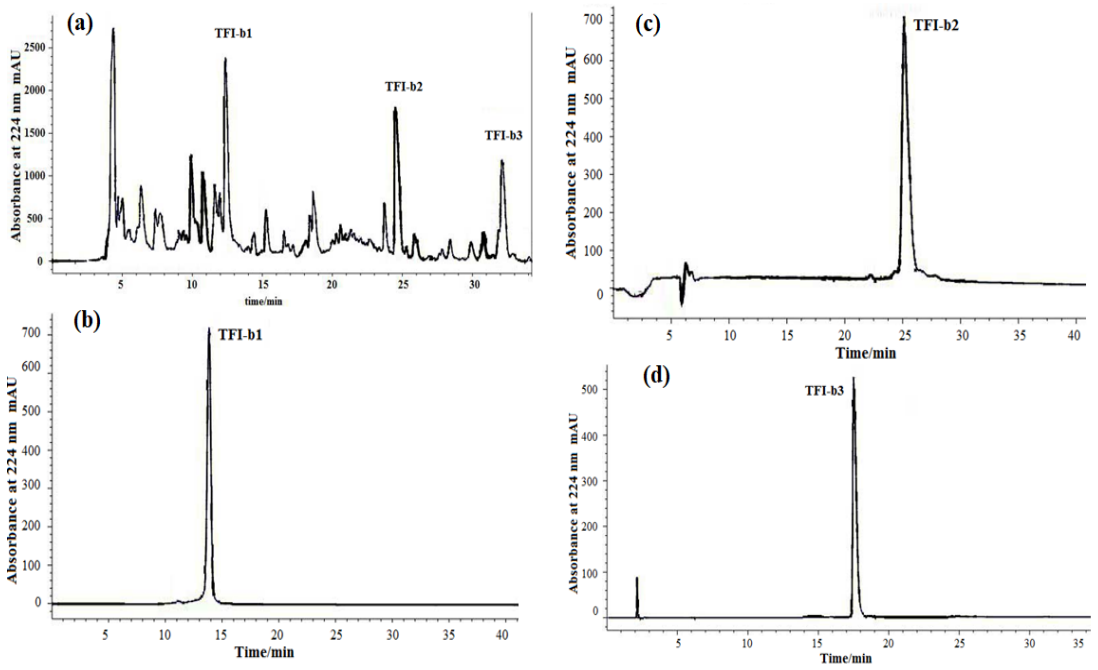
Peptidlarning aminokislota tarkibi va tuzilishi ularning antioksidant xususiyatini aniqlaydi; past molekulyar og'irlikdagi peptidlar kuchli antioksidant faollikka ega bo'lishga moyil bo'ladi. Antioksidant peptidlarni ajratish, tozalash va identifikatsiyalash uchun tripsin fraksiyalari tanlangan. Gidrolizdan so'ng, TF TFI (< 3 kDa) va TFII (> 3 kDa) deb belgilangan ikkita turli MW fraksiyalariga ajratildi. TFI TFII ga nisbatan DPPH va ABTS da eng yuqori antioksidant faollikni namoyish etdi (10-jadval).

10-jadval. Antioksidant peptidlarni tozalash bosqichlari, unumi va antioksidant faolligi (IC_{50})

Fraksiyalar	Bosqichlar	Unumi / (mg/g)	Antioksidant faolligi (IC_{50}) (mg/mL)	
			DPPH	ABTS
TF	Gidrolizdan so'ng	770	6.6±0.03	7.8±0.09
TFII (> 3kDa)	Ultrafiltrasiyadan so'ng	450	5.9±0.01	7.2±0.01
TFI (< 3kDa)	Ultrafiltrasiyadan so'ng	320	5.3±0.01	6.9±0.01
TFI-a	HW-55Fdan so'ng	175	5.1±0.05	6.6±0.03
TFI-b	HW-55Fdan so'ng	145	3.8±0.01	4.2±0.03
TFI-b1	RP-HPLCdan so'ng	7.8	1.9±0.04	2.4±0.07
TFI-b2	RP-HPLCdan so'ng	4.5	1.2±0.07	1.8±0.04
TFI-b3	RP-HPLCdan so'ng	3.2	0.6±0.02	0.9±0.02

TFI fraksiyasi yuqori antioksidant faollikni namoyish etdi va TSK HW-55F kolonkasi (2,5 x 100 sm, Whatman, Angliya) yordamida ikkita fraksiyaga (TFI-a va TFI-b) ajratildi (6d-rasm). Ikkala fraksiyaning faolligi DPPH va ABTS+ da o'lchandi va TFI-b fraksiya eng yuqori faollikni ko'rsatdi (IC50 = DPPH da 3,8 mg/ml, IC50 = ABTS+ da 4,2 mg/ml). Past molekulyar og'irlikdagi peptidlar ularning antioksidant faolligini sezilarli darajada oshiradi. Shuning uchun peptidning molekulyar og'irligi nafaqat antioksidant faollikda muhim rol o'ynaydi, balki peptid ketma-ketligi kabi boshqa omillarda ham katta ahamiyat kasb etadi.

Yuqori antioksidant faollikka ega TFI-b yarim preparativ xromatografida C18 RP-HPLC Agilent kolonkasi yordamida toza holda ajratib olindi. Uchta alohida peptidning (TFI-b1, TFI-b2 va TFI-b3) DPPH va ABTS+ yordamida faolligi (8a-rasm, 11-jadval) aniqlandi. DPPH ning IC50 qiymatlari TFI-b1 1.9, TFI-b2 1.2, TFI-b3 0.6 mg/ml va ABTS ning IC50 qiymatlari mos ravishda 2.4, 1.8 va 0.9 mg/ml ni tashkil etdi. TFI-b3 boshqa fraktsiyalarga nisbatan yuqori DPPH va ABTSda yuqori faollikni namoyish etdi. 10-jadvalda tozalash bosqichlari ham keltirilgan. Umuman olganda, antioksidant peptidlarning IC50 qiymati uch bosqichli tozalash orqali 5 dan 10 baravargacha oshirildi.



8-rasm. HPLC tahlili: (a) TFI-b; (b) TFI-b1; (c) TFI-b2; (d) TFI-b3 antioksidant peptidlari**.**

Uchta peptidning (TFI-b1, TFI-b2 va TFI-b3) aminokislotalar ketma-ketligi (11-jadval) MALDI-TOF-MS/MS yordamida aniqlandi. TFI-b1, TFI-b2 va TFI-b3 ning molekulyar massalari mos ravishda 1665.94, 2122.13 va 2489.09 Da ni tashkil etdi.

Tadqiqotlar shuni ko'rsatdiki, sut peptidlari odatda gidrofob (Tyr, Pro, Trp va His), kislotali aminokislotalar va uning amidlariga (Asn va Asp, Gln va Glu) ega. Sut oqsili gidrolizatlaridan oziq-ovqat mahsulotlarini qayta ishlashda oksidlanishning oldini olish uchun foydalanish mumkin.

11-jadval. Tuya sutidan tozalangan TFI-b1, TFI-b2 va TFI-b3 ning antioksidant faolligi

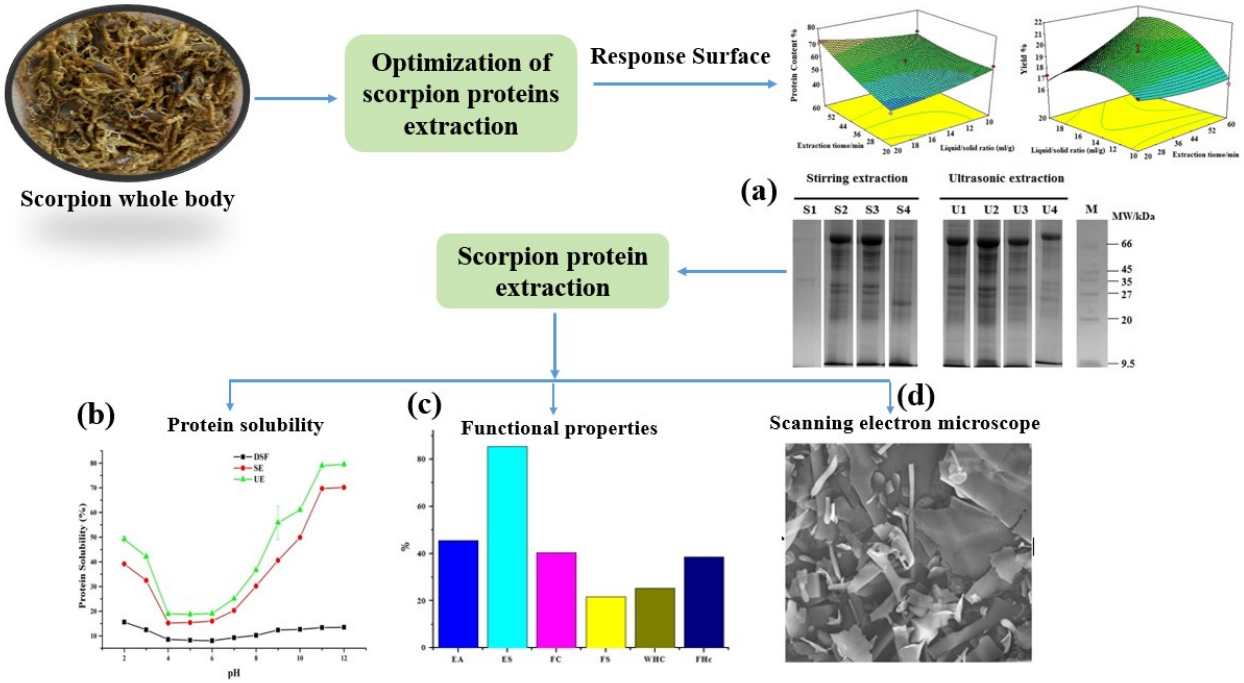
Peptid	**Ketma ketlik**	**IC_{50}(mg/ml)**		**Gidrofob aminokislotalar, (%)**
		DPPH	**ABTS**	
TFI-b1	RLDGQGRPRVWLGR	1.9	2.4	42.9
TFI-b2	TPDNIDIWLGGIAEPQVKR	1.2	1.8	52.6
TFI-b3	VAYSDDGENWTEYRDQGAVEGK	0.6	0.9	59.1

Xulosa:

BCM oqsillari optimal sharoitlarda 4 xil ferment: pepsin, alkalaza, papain, tripsin yordamida gidrolizlandi va hosil bo'lgan peptidlar unumi mos ravishda 67, 59, 75 va 82% ni tashkil etdi. TF (82% ni tashkil etdi) 3 bosqichli xromatografiya (ultrafiltratsiya, gel filtratsiyasi va HPLC) va 3 ta antioksidant faol peptid TFI-b1, TFI-b2 va TFI-b3 bilan ajratilgandan so'ng aniqlandi. DPPH ning IC50 qiymatlari TFI-b1 1.9, TFI-b2 1.2, TFI-b3 0.6 mg/ml va ABTS ning IC_{50} qiymatlari mos ravishda 2.4, 1.8 va 0.9 mg/ml ni tashkil etdi. TFI-b3 boshqa fraktsiyalarga nisbatan yuqori DPPH va ABTS tozalash faolligini namoyish etdi. MALDI TOF-MS/MS yordamida TFI-b1, TFI-b2 va TFI-b3 peptidlarining birlamchi tuzilmalari va molekulyar massasi mos ravishda

RLDGQGRPRVWLGR, TPDNIDIWLGGIAEPQVKR va VAYSDDGENWTEYRDQGAVEGK sifatida aniqlandi. O'rganilgan peptidlar funktsional oziq-ovqat mahsulotlarini ishlab chiqishda antioksidant/erkin radikallarni yo'q qiluvchi vosita sifatida qaralishi mumkin.

5-qism. Chayon oqsillarining tavsifi



9-rasm. Chayon oqsillarini qayta ishlash va o'rganish bosqichlari

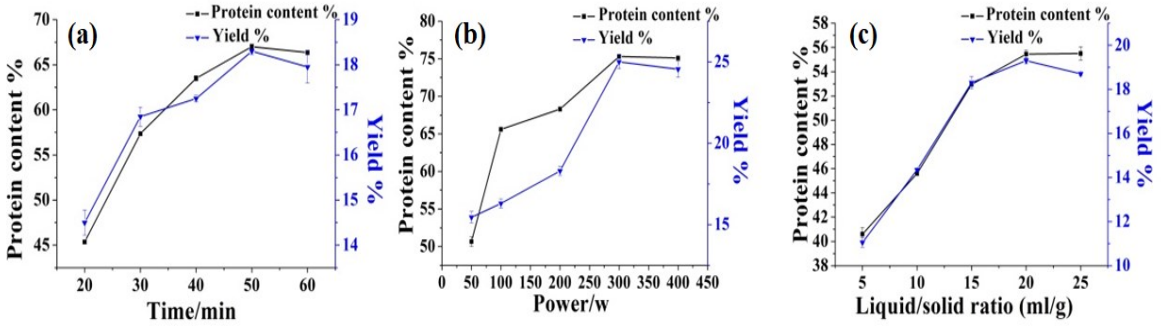
Tadqiqotning asosiy maqsadi chayonning butun tanasidan javob yuzasi metodologiyasi (RSM) yordamida optimallashtirilgan oqsil ajratib olish jarayonini ishlab chiqish, ajratib olingan oqsillarning aminokislota tarkibi va funktsional xususiyatlarini baholash hamda chayon oqsillarining sanoat va ozuqaviy qiymatga ega bo'lgan yangi bioaktiv ingredientlar manbai sifatidagi salohiyatini baholashdan iborat.

Ushbu tadqiqotda chayon oqsilini ajratib olish uchun turli xil bufer eritmalarining ta'siri o'rganildi. Shu bilan birga, ultratovush va aralashtirish ekstraksiyasining oqsil miqdori va unumiga ta'siri taqqoslandi. 9a-rasm va 12-jadvalda to'rtta bufer eritmasining hosil va oqsil miqdoriga ta'siri tartibi ko'rsatilgan: 0,5 M NaCl > 20 mM PBS > 0,02 M NaOH > suv. 0,5 M NaCl bufer eritmasi (unum 14,64 ± 0,08%, oqsil miqdori 79,06 ± 0,05%) ultratovush bilan chayon oqsilini ajratib olish uchun boshqa buferlarga qaraganda yaxshiroq bo'ldi, undan keyin 20 mM PBS (hosil 18,29 ± 0,05%, oqsil miqdori 60,98 ± 0,07%).

12-jadval. Chayon tanasidan umumiy oqsillarni ajratib olishga ultratovush va aralashtirish usullarining ta'siri.

Ekstraktsiya usuli	Ekstraksiya unumi (%)		Protein ekstraksiyasi (%)	
	Ultratovushli	Aralashtirish	Ultratovush	Aralashtirish
Suv	34.85 ± 0.06	34.15 ± 0.09	31.14 ± 0.04	18.08 ± 0.06
0.5 M NaCl	14.64 ± 0.08	11.80 ± 0.03	**79.06 ± 0.05**	35.26 ± 0.08
20 mM PBS	18.29 ± 0.05	13.45 ± 0.10	60.98 ± 0.07	37.25 ± 0.09
0.02 M NaOH	7.70 ± 0.11	7.57 ± 0.13	50.87 ± 0.07	51.91± 0.17

Ekstraksiyalash uchun ultratovush quvvati - 200 Vt, suyuqlik/qattiq modda nisbati - 15 va vaqt - 20-60 daqiqa (10a-rasm). Vaqt o'tishi bilan unum va oqsil miqdori tez o'sib, 50 daqiqada maksimal darajaga, ya’ni mos ravishda 17,95% va 66,35% ga yetdi. Vaqtning yanada oshishi unumga va oqsil miqdoriga ta'sir qilmadi.



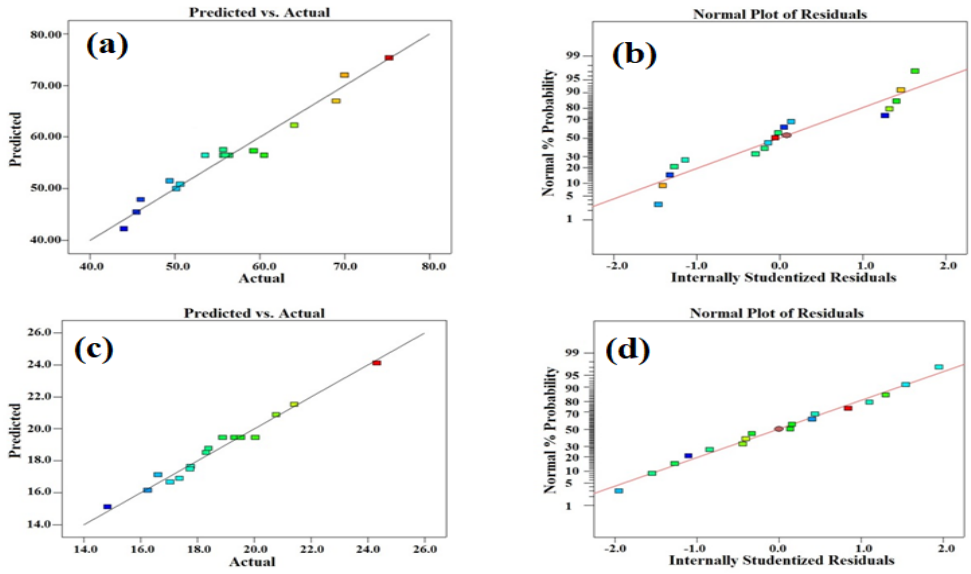
10-rasm. Ekstraksiya vaqtining (a), ultratovush quvvatining (b) va suyuqlik/qattiq nisbatining (c) chiqish unumiga va oqsil miqdoriga ta'siri.

Ultratovush kuchining chiqish unumi va oqsil miqdoriga ta'sirini baholash uchun ekstraktsiya vaqti 50 daqiqa, suyuqlik/qattiq modda nisbati (ml/g) 15 ga teng, ultratovush kuchi 50-400 vt ni tashkil etdi (10b-rasm). Olingan ma'lumotlar shuni ko'rsatadiki, quvvatning oshishi bilan unumi va oqsil miqdori tez o'sib, 300 vt da mos ravishda 23,60% va 75,30% gacha maksimal darajaga yetdi. Vaqtning yanada uzoqroq davom etishi oqsil miqdori va unumiga ta'sir qilmadi.

Suyuqlik/qattiq modda nisbatining unumi va oqsil miqdoriga ta'siri ultratovush quvvati - 200 Vt, ekstraktsiya vaqti - 50 daqiqa ostida o'rganildi va suyuqlik/qattiq modda nisbati 5-25 ml/g oralig'ida baholandi (10c-rasm). Suyuqlik/qattiq modda nisbati 15 ml/g gacha oshganda unumdorlik va oqsil miqdori tez ortadi va 20 ml/g ga yetganda, mos ravishda unumdorlik ortishini sekinlashganini va maksimal 19,21% va 55,32% ga yetganini ko'rish mumkin. Vaqtning yanada ortishi oqsil unumi va miqdoriga ta'sir qilmadi.

RSM yordamida ekstraksiya parametrlarini optimallashtirish

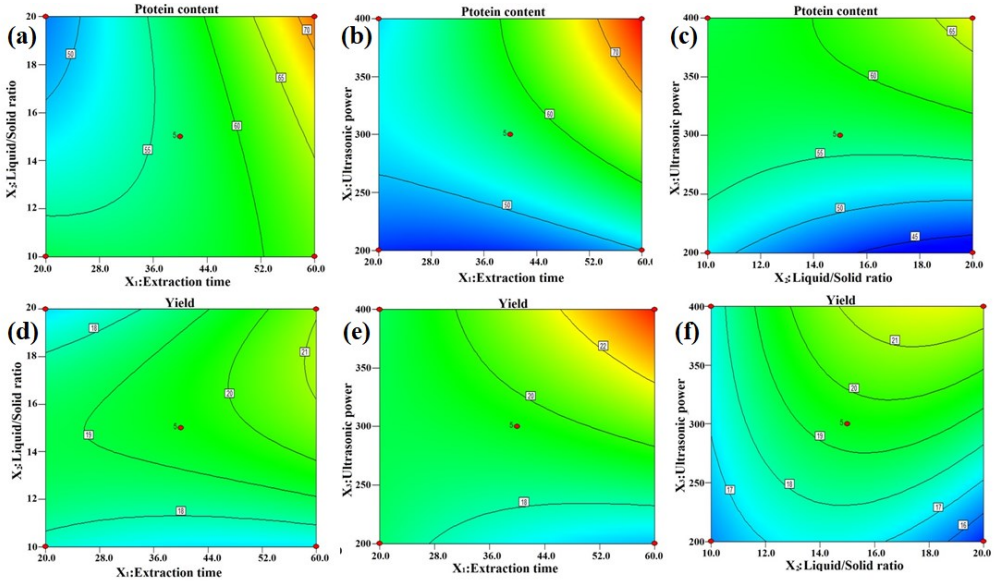
Omil belgisi musbat bo'lganda, bu javob o'zgaruvchisining miqdori uning qiymatining oshishi bilan ortishini va aksincha ekanligini ko'rsatadi. 11-rasm (a, c) oldindan bashorat qilingan model va eksperimental natijalarni taqqoslash uchun ham ishlatilishi mumkin. 11-rasm (b, d) nuqtalar chiziqli diagrammaga amal qilishini ko'rsatuvchi normal ehtimollik diagrammasi. Regressiya modelining oqsil miqdori va unumdorligi bo'yicha F - qiymati mos ravishda 17.49, 34.12 va P-qiymati mos ravishda 0.0005, < 0.0001 ni tashkil etdi. Bu qiymatlar olingan modelning ahamiyatli ekanligini ko'rsatdi. Oqsil miqdori va unumi bo'yicha mos kelmaslikning F-qiymati va P-qiymati mos ravishda 1.54, 2.04 va 0.3354, 0.2506 ni tashkil etdi, bu tenglamaning yaxshi moslik darajasi va yuqori ishonchlilikka ega ekanligini ko'rsatadi. Bundan tashqari, modelning qaror qabul qilish koeffitsienti (R2) mos ravishda 0,9574, 0,9777 va sozlangan aniqlash koeffitsienti (Adj-R2) mos ravishda 0,9027, 0,9491 ni tashkil etdi.



11-rasm. Modelni tekshirish uchun standart statistik diagrammalar. (a,c) model tomonidan bashorat qilingan qiymatlar haqiqiy ma'lumotlarga nisbatan (b,d) qoldiqlarning normal ehtimollik grafigi.

Har bir moslashtirilgan model uchun uchta o'zgaruvchining ta'sirini ko'rsatadigan kontur grafiklari (uchta omilning oqsil miqdori va unumdorlikka birgalikdagi ta'sirini vizualizatsiya qilish uchun) yaratildi. 12-rasmda kontur grafiklari orqali o'zgaruvchilarning oqsil miqdori va unumiga ikkilik o'zaro ta'sirining 2D grafiklari ko'rsatilgan.

Ekstraksiya vaqti va suyuqlik/qattiq nisbati o'rtasidagi o'zaro ta'sir 12-rasmda (a, d) ko'rsatilgan. Ushbu grafik oqsil miqdori va cjiqish unumi X2 ga qaraganda X1 ga ko'proq bog'liqligini ko'rsatadi. 12-rasmda (a, d) X1 ning past qiymatlarida maksimal oqsil miqdori va chiqish unumi X2 ning yuqori qiymatlarida sodir bo'lishi ko'rsatilgan. Biroq, X1 ning yuqori qiymatlarida maksimal oqsil miqdori va unum X2 ning past qiymatlarida sodir bo'ladi. 12-rasmda (a, d) ko'rinib turganidek, ikki omil o'rtasidagi o'zaro ta'sir kuchsiz. Ekstraksiya vaqti va ultratovush kuchi o'rtasidagi o'zaro ta'sir 12-rasmda (b, e) ko'rsatilgan. Ushbu grafik oqsil miqdori va unumi X1 ga qaraganda X3 ga ko'proq bog'liqligini ko'rsatadi. 12-rasmda (c, f) suyuqlik/qattiq nisbati va ultratovush kuchi o'rtasidagi o'zaro ta'sirning ta'siri tasvirlangan. 12-rasmda (c, f) turli ultratovush kuchida suyuqlik/qattiq nisbatini oshirish oqsil miqdori va hosildorlikka muhim ta'sir ko'rsatmasligi ko'rsatilgan, shuning uchun grafik oqsil miqdori va unum X2 ga qaraganda X3 ga ko'proq bog'liqligini ko'rsatadi. Shuning uchun, dasturiy ta'minot tomonidan aniqlangan X1, X2 va X3 ning optimal qiymatlari mos ravishda 50 daqiqa, 400 w va 18 ml/g ni tashkil qiladi.



12-rasm. Operatsion o'zgaruvchilarning oqsil miqdori va chiqish unumiga ta'siri Quyidagilar orasidagi o'zaro ta'sir: (a) suyuqlik/qattiq modda nisbati va ekstraksiya vaqti; (b) ultratovush kuchi va ekstraksiya vaqti; (c) ultratovush kuchi va suyuqlik/qattiq moddaning oqsil miqdoriga nisbati; (d) suyuqlik/qattiq modda nisbati va ekstraksiya vaqti; (e) ultratovush kuchi va ekstraksiya vaqti; (f) ultratovush kuchi va suyuqlik/qattiq moddaning unumiga nisbati.

Optimal ekstraksiya sharoitlarini aniqlash va tasdiqlash

Design-Expert V8.0.6 dasturi tomonidan olingan optimal sharoitlar quyidagicha: ekstraksiya vaqti - 47,68 daqiqa; ultratovush kuchi - 395,84 w; va qattiq/suyuq nisbatlar - 18,01 ml/g. Tajribaning amalga oshirilishini hisobga olgan holda, oqsillar ekstraksiyasi optimal sharoitlari quyidagicha tanlab olindi: ekstraksiya vaqti 50,00 daqiqa, ultratovush quvvati 400 Vt va qattiq/suyuqlik nisbati 18,00 ml/g. Bir nechta sinovlardan so'ng (n > 3), oqsil miqdori va unumi mos ravishda 78,94% va 24,80% ni tashkil etdi. Regressiya tenglamasi va javob yuzasi usuli bilan olingan optimal sharoitlar ishonchli ekanligi tasdiqlandi.

SP ning funktsional xususiyatlari

9b-rasmda turli pH oralig'ida (2,0-12,0) yog'sizlantirilgan chayon unining (DSF) suv yordamida ultratovushli ekstraksiya (UE) va mexanik aralashtirish orqali ekstraksiyasining (SE) oqsil eruvchanlik profillari ko'rsatilgan. DSF, UE va SE ning PSlari sezilarli darajada farq qildi va 10a-rasmda bir xil U shaklidagi egri chiziqlar ko'rsatilgan. pH qiymati 2-4 oralig'ida bo'lganda, DSF, UE va SE ning eruvchanligi pasaygan, ammo 6-10 oralig'ida bo'lganda, DSF, UE va SE ning eruvchanligi sezilarli darajada oshgan. DSF, UE va SE ning minimal oqsil eruvchanligi pH 4.0 da mos ravishda 8.05%, 15.25% va 18.75% qiymatlari bilan ko'rsatilgan. Maksimal oqsil eruvchanligi esa pH 12 da mos ravishda 13.5%, 70.15% va 79.5% qiymatlari bilan ko'rsatilgan. Shunday qilib, ultratovush usuli bilan ajratib olingan chayon oqsili ishqoriy muhitda da ajoyib eruvchanlikni ko'rsatdi. Ultratovush va mexanik aralashtirish orqali ajratib olingan oqsillarning suvni yutish qobiliyati (WAC) va moyni yutish qobiliyati (OAC) bo'yicha olingan natijalar 13-jadvalda keltirilgan. Ikkala

usulning WAC va OAC qiymati sezilarli darajada farq qilgan, pH 7.0 da WAC (33.45) va OAC (18.80) bo'lgan UE SE ga qaraganda eng yuqori WAC (40.3) va OAC (27.70) ga ega bo'lgan.

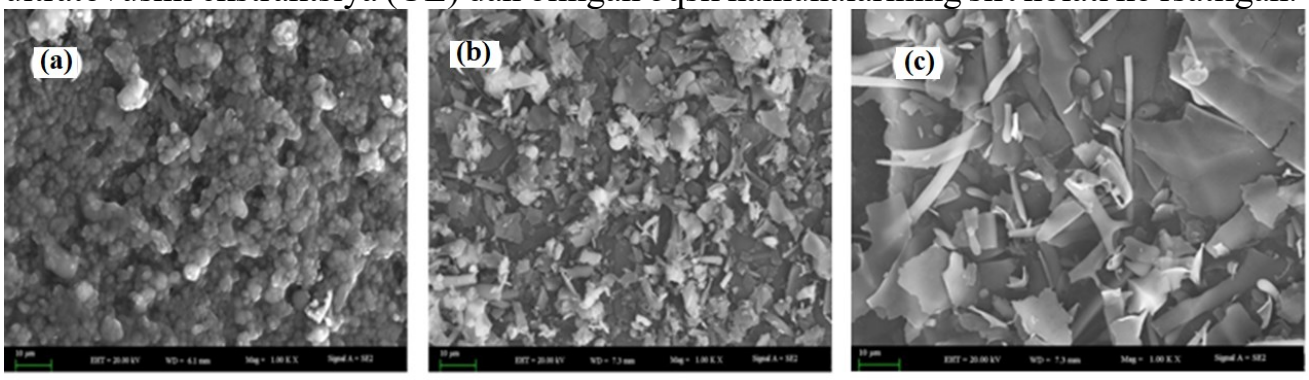
UE va SE ning emulsiya hosil qilish xususiyatlari natijalari 13-jadvalda keltirilgan. pH 7.0 da UE va SE ning emulsiya hosil qilish xususiyatlari mos ravishda 45.55%, 40.25% qiymatlari bilan bir-biridan sezilarli darajada farq qilgan. Biroq, UE ning emulsiya hosil qilish xususiyati sezilarli darajada farq qilgan va SE ga (69.45%) nisbatan yuqori (85.50%) bo'lgan. UE emulsiya hosil qilish xususiyatiga sezilarli ta'sir ko'rsatgan, chunki u suv va moy chegarasiga ko'proq gidrofob guruhlarni ta'sir qilishi mumkin, bu esa emulsiya hosil qilish qobiliyatining oshishiga va barqaror emulsiyaga olib kelgan.

13-jadval. Chayon oqsillarining funktsional xususiyatlari

Xususiyatlari	Ultratovushli ekstraksiya	Mexanik aralashtirish orqali ekstraksiya
Suvni tutib qolish sig'imi (g/g)	25.25 ± 0.21	20.45 ± 0.07
Moyni tutib qolish sig'imi (g/g)	38.50 ± 0.14	30.65 ± 0.64
Emulsiya hosil qilish faolligi (%)	45.55 ± 0.64	40.25 ± 0.07
Emulsiya stabilligi (%)	85.50 ± 0.28	69.45 ± 0.35
Ko'pik hosil qilishi (%)	40.30 ± 0.28	33.45 ± 0.07
Ko'pik stabilligi (%)	21.70 ± 0.14	18.80 ± 0.15

Ko'pik hosil bo'lishi emulsiya hosil bo'lishiga o'xshaydi. Ko'pik hosil bo'lishida suv molekulalari havo tomchilarini o'rab oladi, bu esa qutbsiz fazadir. pH 7.0 da UE ning ko'pik sig'imi (FC) SE ga qaraganda ancha yuqori bo'lib, mos ravishda 40.30% va 33.45% qiymatlarga ega. UE ning ko'pik barqarorligi (FS) mos ravishda 21.70% va 18.80% qiymatlarga ega bo'lib, SE ga qaraganda ancha yuqori edi (13-jadval). Olingan natijalar shuni ko'rsatadiki, SE ni taqqoslash uchun UE suvli eritmada ko'proq moslashuvchan oqsil tuzilishiga ega va havo-suv chegarasida o'zaro ta'sir qilish uchun yanada barqaror ko'piklar hosil qiladi. Yuqori oqsil konsentratsiyasi ko'pik sig'imini, barqarorligini yaxshilaydi, yopishqoqlikni oshiradi va ko'p qatlamli membrananing hosil bo'lishiga yordam beradi.

Oqsil namunalari ham SEM usuli bilan o'rganildi. Quyidagi 13-rasmda butun tanadan yog'sizlantirilgan oqsil (DSF) kukuni, aralashtirilgan ekstraktsiya (SE) va ultratovushli ekstraktsiya (UE) dan olingan oqsil namunalarining sirt holati ko'rsatilgan.



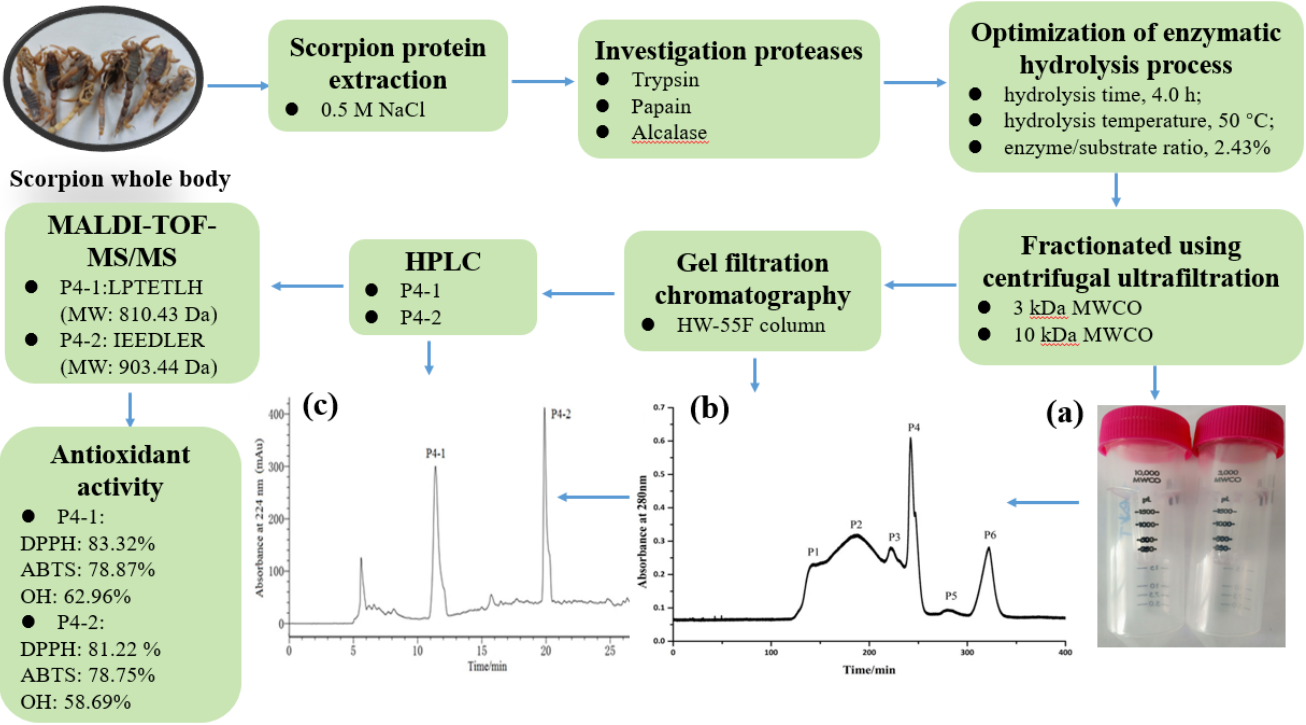
13-rasm: (a) DSF; (b) SE; (c) UE ning skanerlovchi elektron mikroskopi**.**

13a-rasmda oqsil molekulalarining yopishqoq sirt qatlamlarini ko'rish mumkin. Bu yuqori kristallik darajasini ko'rsatadigan kollaps shaklidir. 13b-rasmda quritilgan SE oqsil qatlamlarida "yog'och qirindilari"ga o'xshash bir nechta elementlarni ko'rish mumkin. 13a-rasmdan farqli o'laroq, bu elementlar oqsilni sirtdan ajralish natijasida hosil bo'lgan. Quritish jarayonida oqsil molekulalari 19b-rasmda ko'rsatilganidek, kristall holatdan amorf holatga o'tadi. Shuningdek, 13c-rasmda uning UE dan keyin olingan oqsil molekulalarining quritish natijalarini ko'rish mumkin.

Xulosa:

0,5M NaCl, 20mM PBS, 0,02M NaOH va H2O erituvchilarining chayon oqsilini ajratib olishga ta'siri o'rganildi. 0,5M NaCl bilan ultratovush tekshiruvi boshqalarga qaraganda yaxshiroq natijalarni ko'rsatdi (unum 14,64%, oqsil miqdori 79,06%). RSM usuli uchun optimal ekstraksiya qiymatlari X1, X2 va X3 uchun 50 daqiqa, 400 Vt va 18 ml/g ni tashkil etdi, oqsil miqdori va unum mos ravishda 78,94% va 24,80% ni tashkil etdi. Yog'sizlantirilgan chayon oqsili (DSF), ultratovushli ekstraksiya (UE) va aralashtiruvchi ekstraksiya (SE) ning pH 4.0 da minimal oqsil eruvchanligi mos ravishda 8,05%, 15,25%, 18,75% ni, pH 12 da maksimal eruvchanligi esa mos ravishda 13,5%, 70,15% va 79,5% ni tashkil etdi. UE va SE ning pH 7.0 da emulsiya hosil qilish xususiyatlari mos ravishda 45,55% va 40,25% ni, UE ning pH 7.0 da ko'piklanish xususiyatlari esa mos ravishda SE ga nisbatan 40,30% va 33,45% yuqori bo'ldi. SEM namunalarning "yog'och qipiqlari" ga o'xshash yuqori kristalik tuzilishini ko'rsatdi. Quritish jarayonida oqsil molekulalari kristall holatdan amorf holatga o'tishi ko'rsatildi.

6-qism. Buthus martensii oqsil gidrolizatlarining antioksidant peptidlari



14-rasm. Chayon oqsili gidrolizatlarini qayta ishlash va o'rganish bosqichlari

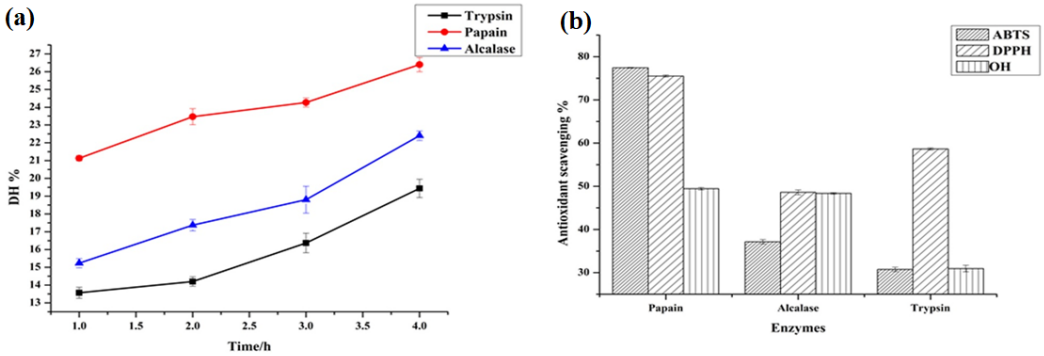
Ushbu tadqiqotning vazifasi fermentativ gidrolizlangan chayon oqsilidan (*Buthus martensii* Karsch) olingan antioksidant peptidlarni ajratish, tozalash va tavsiflashdan

iborat. Tadqiqot kuchli antioksidant faollikka ega bo'lgan maxsus peptidlarni aniqlash va maksimal peptid chiqish unumiga erishish va faolligi uchun fermentativ gidroliz sharoitlarini optimallashtirishga qaratilgan edi.

Chayon oqsili (SP) optimal sharoitlarda tripsin, papain va alkalaza tomonidan gidrolizlandi. SPH ning SDS-PAGE profillari shuni ko'rsatdiki, SP gidroliz vaqtida peptidlarga asta-sekin gidrolizlangan. 20 kDa dan yuqori SP asosan papain tomonidan 2 soat ichida gidrolizlangan va asosiy oqsillarning to'liq hazm bo'lishiga 3 soat ichida erishilgan.

Gidrolizlanish DH ni baholash orqali kuzatildi va DH borishi 15a-rasmda ko'rsatilgandek vaqtga bog'liq holda amalga oshirildi. Bitta ferment tomonidan gidrolizlanish DH tartibi quyidagicha: Papain > Alkalaza > Tripsin. 15a-rasmda ko'rsatilgandek, uchta ferment tomonidan gidrolizlangan peptidlar turli xil gidrolitik jarayonlarni ko'rsatdi va SPH ning DH si gidroliz vaqti bilan oshdi. Papain boshqa fermentlarga nisbatan ancha yuqori gidroliz ta'siriga ega bo'lib, 4 soatlik gidroliz vaqtidan keyin 26,46% ga yetdi. Uchta fermentning turli bo'linish joylari DH qiymatining farqiga olib keladi deb taxmin qilingan.

Uchta chayon oqsili peptidlarining antioksidant faolligi 15b-rasmda ko'rsatilgandek. OH, ABTS·+ va DPPH· radikal tozalash tahlillari yordamida o'lchandi. 15b-rasmda ko'rsatilgandek, 5,0 mg/ml konsentratsiyada peptidlarning ABTS•+ ga tozalash ta'siri quyidagi tartibda edi; papain (77,45%) >> alkalaza (37,15%) > tripsin (30,75%), DPPH• da papain (75,54%) > tripsin (58,65%) > alkalaza (48,60%) va .OH da papain (49,44%) ≈ alkalaza (48,35%) > tripsin (30,95%). Papain fraksiyasining boshqa peptid fraksiyalari orasida eng yuqori antioksidant faolligi mavjud. DH va antioksidant faollik shuni ko'rsatadiki, SP ning papain bilan gidrolizi boshqa fermentlardan sezilarli darajada farq qiladi va papain SPH ni tayyorlash uchun samarali ishlatilishi mumkin. Shu bilan birga, tripsin va alkalaza fermenti bilan ishlov berish uchun SP ning peptidlarga gidrolizi ko'proq vaqt talab qildi.

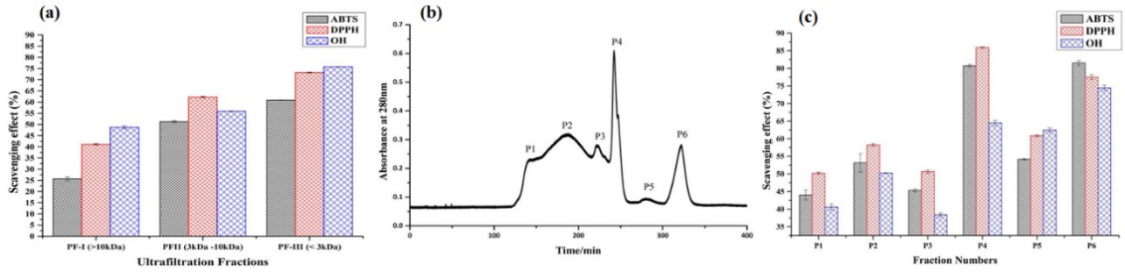


15-rasm (a) SP ning gidrolizi; (b) DPPH, OH va ABTS antiradikal faolligi.

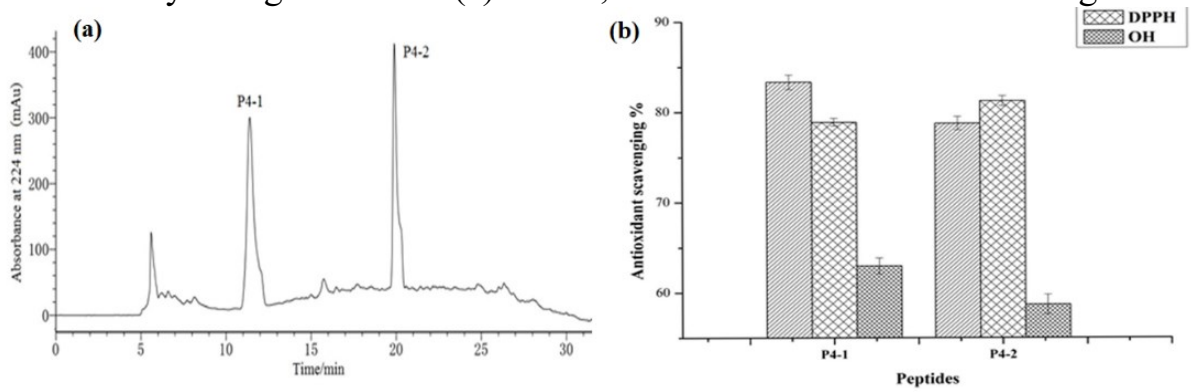
Antioksidant peptidlarni ajratish va tozalash

ABTS·+ tozalash faolligiga, .OH tozalash faolligiga va DPPH tozalash faolligiga tozalash ta'sirini ko'rib chiqishda, antioksidant peptidni ajratish va tozalash uchun papain fraksiyasini tanladik. 16a-rasmda ko'rsatilganidek, PF-III (< 3 kDa) 5,0 mg/ml konsentratsiyada PF-II (3-10 kDa) va PF-I (> 10 kDa) fraktsiyalariga nisbatan kuchliroq ABTS·+ tozalash faolligini (77,45 ± 0,08%), .OH tozalash faolligini (49,44

± 0,23%) va DPPH· tozalash faolligini (75,54 ± 0,30%) namoyish etdi. Oun Ki va boshqalar, Bing va boshqalar va Liu va boshqalar. past molekulyar og'irlikdagi peptidlar yuqori antioksidant faollikka ega ekanligi haqida xabar berilgan, bu odatda antioksidant peptidlar 2-20 aminokislotalarni o'z ichiga olishini ko'rsatuvchi ilgari xabar qilingan ma'lumotlarga mos keladi. Shu sababli PF-III fraksiya boshqa ikkita fraksiyaga qaraganda yuqori antioksidant faollikni ko'rsatgan bo'lishi mumkin. Eng yaxshi antioksidant faollikka ega peptid fraksiya (PF-III) HW-55F gel filtrlash xromatografiyasi kolonkasi (1,5 sm × 100 sm) bilan qo'shimcha ravishda fraksiyalandi va oltita fraksiyalar, P1, P2, P3, P4, P5 va P6 to'plandi (16b-rasm). P4 fraksiyasi qolgan boshqa beshta fraksiya bilan taqqoslaganda, eng yaxshi antioksidant faollikni ko'rsatdi, ABTS·+, DPPH· va .OH fraksiyalarida mos ravishda 2,0 mg/ml konsentratsiyada 80,75 ± 0,35, 85,90 ± 0,14% va 64,50 ± 0,71% ni tozalash faolligi bilan, undan keyin P6 fraksiyasi kuzatildi (16c-rasm). Shuning uchun, kuchliroq antioksidant tozalash faolligiga ega P4 fraksiya liofil quritgichda quritib olindi va RP-HPLC preparativ xromatografiyasiusuli bilan qo'shimcha tozalandi.



16-rasm (a) SP dan olingan turli MWCO da papain gidrolizatining fraksiyalarining tozalash effektlari (%). (b) TSK HW-55F (1.5x100 sm) kolonkasi orqali papain fraksiyasini gel filtrlash. (c) DPPH, OH va ABTS antiradikal faolligi.



17-rasm (a) P4 fraksiyasining HPLC profili; (b) P4-1 va P4-2 ning antioksidant faolligi.

17a-rasmda ikkita - P4-1 va P4-2 peptidlari to'plab olindi va antioksidant faollikni aniqlash uchun qo'llanildi. 17b-rasmda P4-1 ning ABTS·+, DPPH· va .OH dagi antioksidantlarni tozalash faolligi mos ravishda 83,32, 78,87 va 62,96% ni tashkil etganligi, P4-2 ning ABTS·+, DPPH va .OH dagi faolligi esa mos ravishda 78,75, 81,22 va 58,69% ni tashkil etganligi ko'rsatilgan. P4-1 va P4-2 ning har bir tozalash

bosqichlarining unumi 14-jadvalda keltirilgan. P4-1 ning yakuniy unumi 1,04% ni, P4-2 esa 1,39% ni tashkil qiladi.

SP, SPH va har bir tozalash jarayonida fraksiyalarining aminokislota tarkibi HPLC yordamida aniqlandi. 15-jadvalda ko'rsatilganidek, 16 ta aminokislota aniqlandi va umumiy aminokislota miqdori mos ravishda 544,24, 346,24, 301,99 va 273,56 mg/g ni tashkil etdi. Tozalash jarayonining ortishi bilan gidrofob aminokislotalarning (Ala, Val, Leu, Ile, Pro va Phe) miqdori oshdi, ayniqsa PF-III (72,96%) va P4 (75,49%) uchun. Gidrofob aromatik aminokislotalar, Tyr, Trp, His va Phe ham oshdi. Ala va Metning ulushi PF-III dan P4 gacha oshirildi. Shuning uchun SPHs peptidlaridagi bu gidrofob aminokislotalar asosan antioksidant faollik uchun javobgar edi. Peptid ketma-ketligidagi Tyr va Pro ning boy miqdori yuqori antioksidant faollikka erishishi mumkinligi haqida xabar berilgan.

14-jadval. P4-1 va P4-2 ning har bir tozalash jarayonining samaradorligi

Fraksiyalar	Jarayon	Unum (mg/g protein)	Unum (%)
SP	Protein ekstraksiyasidan so'ng	796.30	100
SPHs	Fermentativ gidrolizdan so'ng	414.24	52.02
PF-III	Ultrafiltrasiyadan so'ng (UF)	293.75	36.89
P4	HW-55F da tozalashdan so'ng	91.48	11.49
P4-1	RP-HPLCda tozalashdan so'ng	8.27	1.04
P4-2	RP-HPLCda tozalashdan so'ng	11.04	1.39

P4-1 va P4-2 peptidlarining aminokislotalar ketma-ketligi MALDI-TOF-MS/MS yordamida aniqlandi. P4-1 ketma-ketligi LPTETLH, P4-2 ketma-ketligi IEEDLER, bu peptidlarning molekulyar massasi mos ravishda 810.43 Da va 903.44 Da edi. Ikkala peptidning ketma-ketliklari BLAST dan foydalanib qidirildi (http://blast.ncbi.nlm.nih.gov/Blast.cgi). LPTETLH peptidi eng yuqori ABTS·+ tozalash faolligini 83.32% qiymati bilan, IEEDLER peptidi esa eng yuqori DPPH· tozalash faolligini 81.22% qiymati bilan ko'rsatdi. Bundan tashqari, ushbu peptidlarning .OH yutilish faolligi mos ravishda 62,96% va 58,69% ni tashkil etdi, bu ABTS·+ va DPPH· ga qaraganda pastroq.

15-jadval. Har bir tozalash jarayoni fraksiyalarining aminokislota tarkibi (mg/g)

Amino acids	SP	SPHs	PF-III	P4
Ala	36.47	29.68	2.40	39.3
Val	20.40	7.56	34.66	27.29
Phe	50.89	17.86	61.35	48.15
Pro	27.38	22	2.98	46
Met	12.59	3.32	13.15	35.85
Ile	22.83	7.5	33.11	17.37
Leu	58.06	9.92	72.69	60.26
Tyr	15.75	8.76	18.10	16.39
Gly	20.32	17.06	2.28	3.38
Ser	27.28	20.1	2.49	3.42
Thr	28.47	20.94	1.76	2.60
Asp	82.39	53.72	4.44	9.35
Glu	102.48	83.18	9.12	17.30
Lys	38.73	1.36	39.51	30.73

His	19.02	10.08	0.53	1.05
Arg	44.66	33.2	3.42	4.80
Total	544.24	346.24	301.99	363.24

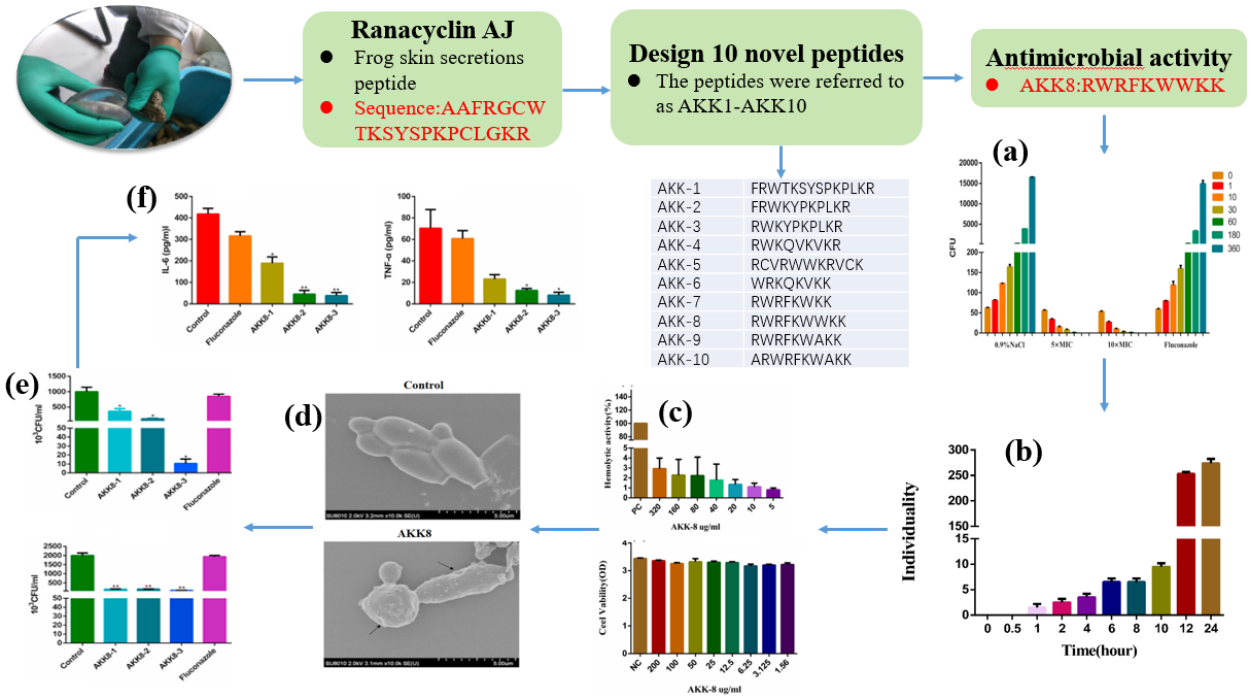
Xulosa:

Buthus martensii Karsch chayon oqsili papain, alkalaza va tripsin fermentlari bilan gidrolizlandi. Papain gidrolizati sezilarli darajada yuqori gidroliz ta'siriga ega bo'lib, 26,46% ga yetdi. 5,0 mg/ml konsentratsiyadagi papain fraktsiyasi yuqori antioksidant faollikni ko'rsatdi (ABTS•+ - 77,45%; DPPH• - 75,54% va •OH - 49,44%). Papain fraksiyasini ultrafiltratsiya, gel filtratsiyasi va HPLC usullari bilan ajratish natijasida P4-1 va P4-2 peptidlari olindi. P4-1 va P4-2 peptidlarining aminokislotalar ketma-ketligi MALDI-TOF-MS/MS yordamida mos ravishda LPTETLH va IEEDLER ekanligi aniqlandi. P4-1 peptidining ABTS faolligi 83,22% va P4-2 peptidining DPPH faolligi 81,22% ekanligi aniqlandi. P4-1 va P4-2 peptidlarining OH radikallariga nisbatan faolligi mos ravishda 62,96% va 58,69% ni tashkil etdi, bu ABTS•+ va DPPH• faolligiga qaraganda pastroq qiymatlarni ko'rsatdi.

7-qism. Candida albicansga qarshi antifungal peptidni o'rganish

Candida albicans ning doriga chidamliligi ortib borayotgani sababli, yallig'lanish kasalliklarini davolashning yangi terapevtik vositalarini ishlab chiqish zaruriyati paydo bo'ldi. Antimikrob peptidlar (AMP) eng istiqbolli antifungal dorilardan biri hisoblanadi. Biroq, ishlab chiqilgan AMPlarning aksariyatida nojo'ya ta'sirlar mavjud.

Ushbu tadqiqotda qurbaqa terisi sekretsiyasi peptidi (Ranacyclin AJ) ketma-ketligiga asoslanib 10 ta yangi peptid ajratib olindi.



18-rasm. Baqa terisi sekretsiya suyuqligini qayta ishlash va o'rganish bosqichlari

Loyihalashtirilgan peptidlarning funktsional skriningi

Baqa terisi sekretsiyasidan olingan 20 ta aminokislota qoldig'idan tashkil topgan Ranacyclin AJ peptidining aminokislotalar ketma-ketligi asosida o'nta yangi peptid ishlab chiqildi. Peptidlar AKK1, AKK2 va boshqalar deb nomlandi, AKK10 gacha.

Loyihalashtirilgan 10 ta peptidning aminokislotalar ketma-ketligi, fizik-kimyoviy xususiyatlari va MIClari 16 va 17-jadvallarda keltirilgan.

10 ta peptid orasida AKK8 sinovdan o'tgan to'rtta shtammga - E.coli, S.aureus, B.subtilis va C.albicansga qarshi eng yuqori antimikrob faollikni namoyish etdi. AKK8 ning C.albicans ga qarshi MIC qiymati 18,5 μg/ml ni tashkil etdi, bu AKK8 ning E.coli, S.aureus va B.subtilis ga qarshi ko'rsatkichidan (37,5 μg/ml) pastroq, bu AKK8 ning boshqa 3 ta shtammga nisbatan C.albicans ga nisbatan yuqori ingibirlovchi ta'sirga egaligini ko'rsatadi. AKK8 ning yuqori darajada o'ldirish ta'siri tufayli uning faolligini klinik jihatdan dori-darmonlarga chidamli ATCC2002, 08032815 va 08030401 C.albicans shtammlariga qarshi sinovdan o'tkazildi. 17-jadvalda ko'rsatilganidek, ampitsillin yuqorida aytib o'tilgan 3 ta shtammga qarshi antimikrob faollikni ko'rsatmadi; Shu bilan birga, flukonazol C.albicans ATCC2002 ga qarshi 0,8 μg/ml MIC bilan antimikrob faollikni namoyish etdi, ammo C.albicans 08032815 va 08030401 ga qarshi faollik namoyon etmadi. Natijalar shuni ko'rsatdiki, C.albicans 08032815 va 08030401 flukonazolga chidamli. Shunisi e'tiborga loyiqki, AKK8 nafaqat C.albicans ATCC2002 ning, balki boshqa 2 shtammning ham o'sishini ingibirladi va standart shtammlar bilan bir xil MIC ni namoyish etdi (16 va 17-jadvallar). Bu kuzatishlar AKK8 klinik dorilarga chidamli patogenlarga nisbatan sezilarli antibakterial ta'sir ko'rsatishini ko'rsatadi.

16-jadval. Loyihalashtirilgan peptidlarning fizik-kimyoviy xususiyatlari

Peptid	Ketma ketlik	MW	NC	PR/n%	NPR/n%
Ranacyclin AJ	AAFRGCWTKSYSPK PCLGKR	2256.67	+5	11/55	9/45
AKK-1	FRWTKSYSPKPLKR	1794.13	+5	9/64.28	5/35.71
AKK-2	FRWKYPKPLKR	1518.87	+5	6/54.54	5/45.45
AKK-3	RWKYPKPLKR	1371.69	+5	6/60	4/40
AKK-4	RWKQVKVKR	1227.52	+5	6/66.66	3/33.33
AKK-5	RCVRWWKRVCK	1519.90	+5	7/63.63	4/36.36
AKK-6	WRKQKVKK	1100.38	+5	6/75	2/25
AKK-7	RWRFKWKK	1234.52	+5	5/62.5	3/37.5
AKK-8	RWRFKWWKK	1420.73	+5	5/55.55	4/44.44
AKK-9	RWRFKWAKK	1305.59	+5	5/55.55	4/44.44
AKK-10	ARWRFKWAKK	1376.67	+5	5/50	5/40

17-jadval. Peptidlarning bir nechta mikroorganizm shtammlariga qarshi faolligi MIC (mkg/ml)

Peptid	*C.albicans* ATCC10231	*B.Subtilis* ATCC6633	*E.coli* ATCC25922	*S.aureus* ATCC25923
Ranacyclin-AJ	-	-	-	-
AKK-1	-	-	-	-
AKK-2	>100	>100	>100	>100
AKK-3	75	37.5	75	>100
AKK-4	37.5	75	75	>100
AKK-5	100	75	100	100
AKK-6	-	-	-	-
AKK-7	>100	>100	>100	>100
AKK-8	18.5	37.5	37.5	37.5

AKK-9	>100	>100	>100	>100
AKK-10	75	75	75	75

AKK8 ning o'ldirish kinetikasi

An'anaviy antifungal dorilar kabi bakteriya o'sishini kechiktirish o'rniga AKK8 C. albicans 08032815 ni tezda o'ldirishi mumkin. Flukonazol bilan davolangandan so'ng, C. albicans 08032815 0,5 soat ichida 52 dan 160 gacha va undan keyin 6 soat davomida inkubatsiyadan keyin 14887 gacha sezilarli darajada oshdi (18a-rasm). Flukonazoldan farqli o'laroq, AKK8 C. albicans 08032815 ni 30 daqiqa ichida 5 x va 10 x MIC konsentratsiyalarida o'ldirdi. AKK8 ning antifungal ta'siri uzoq davom etdi; 24 soat davomida C. albicans 08032815 koloniyasi kuzatilmadi (18a-rasm).

Odam plazmasi va tuz ionining AKK8 ning antifungal faolligiga ta'siri

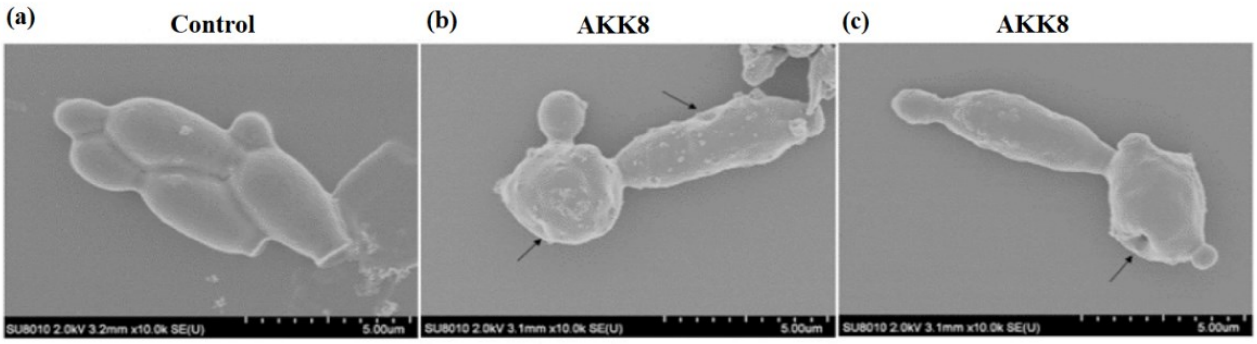
Plazma va tuz ioni MIC ga ta'sir qiluvchi muhim omillar sifatida qaraldi. Shunday qilib, biz AKK8 ning inson plazmasidagi va tuz ionidagi barqarorligini antifungal tahlil yordamida aniqladik. Shunisi e'tiborga loyiqki, AKK8 ning antifungal faolligiga inson plazmasi sezilarli darajada ta'sir ko'rsatmadi. Qon plazmasi bilan 10 soat inkubatsiya qilinganidan keyin ham AKK8 sinovdan o'tgan C. albicans ga qarshi antimikrob faollikni namoyon etdi, bu esa AKK8 ning plazmadagi barqarorligini ko'rsatadi (18b-rasm). Tuz ionining AKK8 ning antibakterial faolligiga ta'sirini baholash uchun peptid ketma-ket ddH2O, PBS va 150 mM NaCl da eritildi. Natija shuni ko'rsatdiki, 18,5 μg/ml MIC bilan AKK8 C.albicans faolligini ingibirladi. Tuz ionlari AKK8 ning C.albicans ga qarshi antimikrob faolligini o'zgartirmadi, bu uning klinik qo'llanilish uchun yaroqliligini ko'rsatadi.

Gemolitik va sitotoksik tahlillar

AKK8 ning inson qizil qon hujayralariga qarshi gemolitik faolligi yangi inson eritrotsitlaridan gemoglobinning ajralib chiqishi asosida baholandi. Natijalar shuni ko'rsatdiki, AKK8 320 μg/ml konsentratsiyasida, ya'ni C.albicans 08032815 ga nisbatan MIC dan 17 baravar yuqori bo'lgan holda, inson qizil qon hujayralarida atigi 3% gemolitik faollikni namoyon etdi (18c-rasm). AKK8 ning xavfsizligini tekshirish uchun uning potentsial toksikligi in vitro baholandi. Natijalar AKK8 bilan 1,56-200 μg/ml oralig'ida ishlov berilgandan so'ng RAW267.4 va L6 hujayralariga hech qanday aniq toksiklik ko'rsatmadi (18c-rasm). Birgalikda, AKK8 preparatni ishlab chiqish uchun ideal nomzod bo'lishi mumkin.

C. albicans ning membrana morfologiyasi

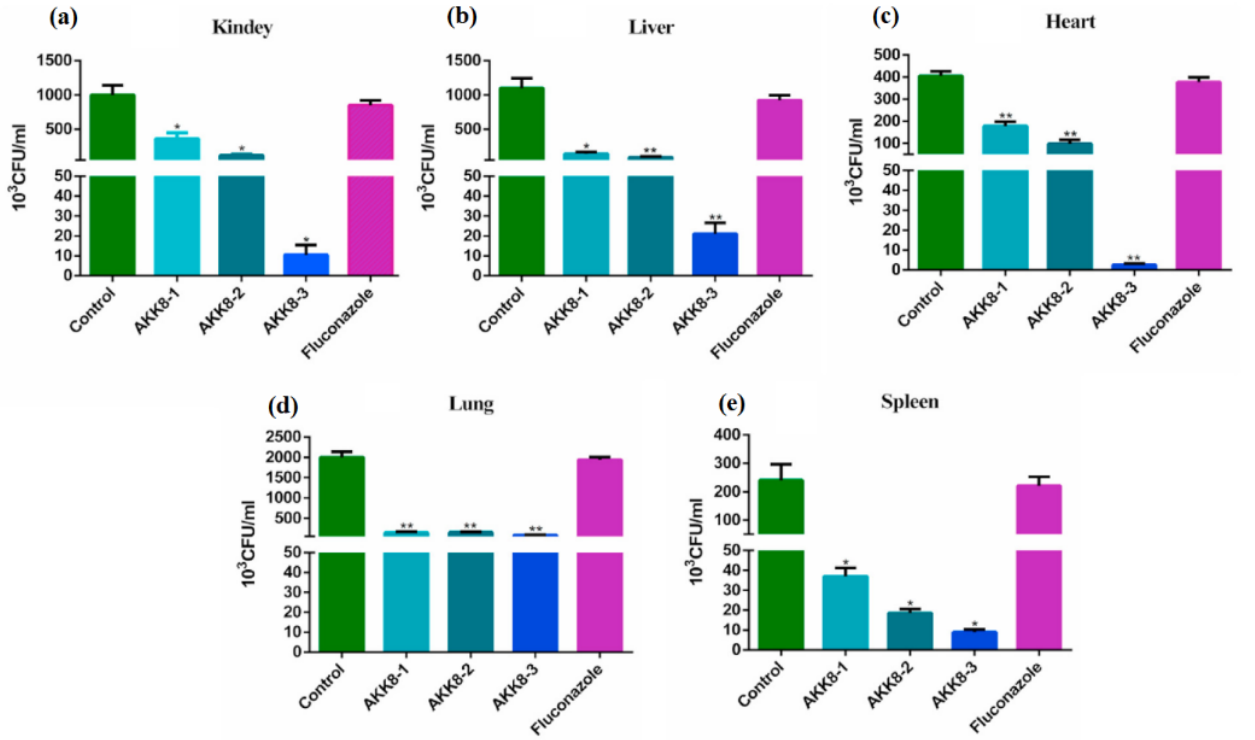
AKK8 ning C.albicans ga qarshi o'ldirish faolligining asosiy mexanizmini yanada chuqurroq o'rganish uchun AKK8 bilan ishlov berilgan va davolanmagan C.albicans (08032815) o'rtasidagi morfologik farq SEM yordamida tekshirildi. Davolanmagan C.albicans hujayra devori, sitoplazmatik membrana va silliq tashqi membranalarni ko'rsatdi. Aksincha, AKK8 bilan ishlov berilgan C.albicans psevdogifa va gifalarda teshilishlarni ko'rsatdi (19-rasm). Bu kuzatishlar shuni ko'rsatadiki, AKK8 hujayra devoriga bog'langan va keyin C.albicansga zarar yetkazgan bo'lishi mumkin.



19-rasm. AKK8 peptidining C.albicans morfologiyasiga ta'siri. (a) nazorat; (b) 2,5 x MIC peptidi AKK8 psevdogifalarda hujayra devorining yorilishiga olib keldi; (c) gifalarda. Strelkalar C.albicans plazmasiga odatiy zarar yetkazilishini ko'rsatadi.

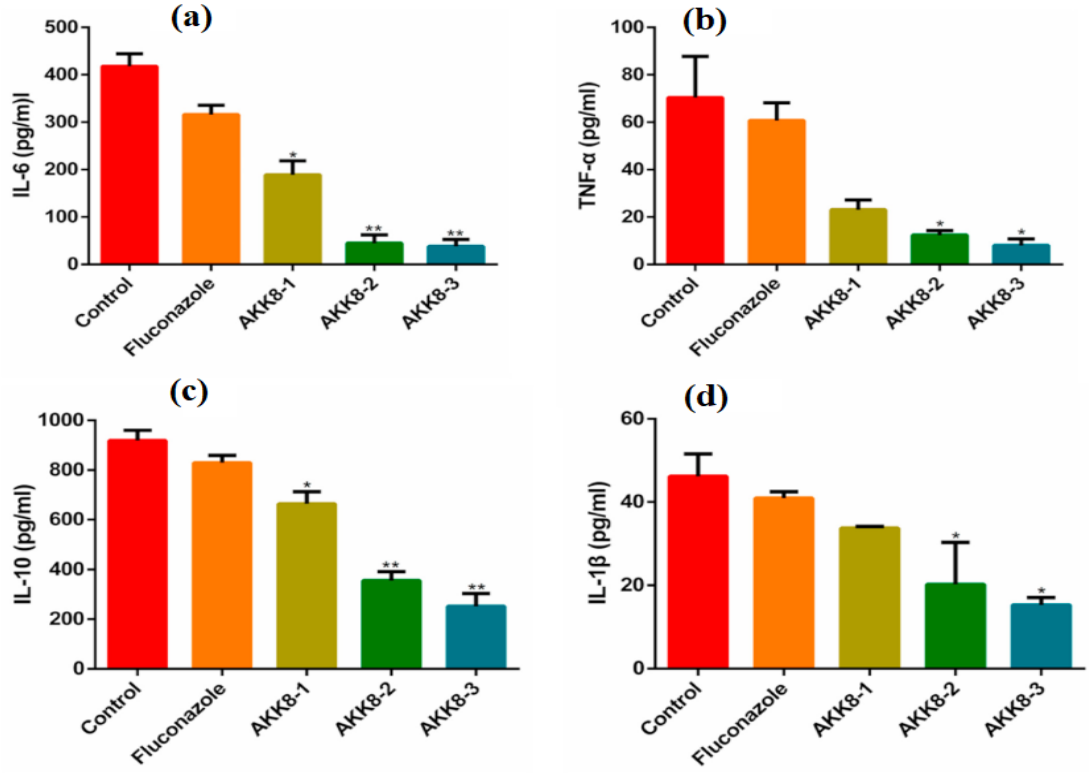
AKK8 ning sichqonlarga C.albicans modeliga ta'siri

AKK8 ning klinik xavfsizligi va terapevtik ta'sirini tasdiqlash uchun uning in vivo antifungal faolligi tizimli zamburug' infektsiyasi uchun sichqon modeli yordamida baholandi. Sichqonlarga C.albicans (08032815) 5,0 x 107 CFU/sichqoncha dozasida vena ichiga in'ektsiya yo'li bilan yuborildi va keyin 2, 4 va 8 mg/kg AKK8, flukonazol va 0,9% fiziologik eritma bilan davolandi. 2 kundan so'ng plazmadagi yallig'lanish sitokinlari darajasi va asosiy organ to'qimalarida C. albicans koloniyalari soni tekshirildi. Natijalar shuni ko'rsatdiki, 0,9% fiziologik eritma bilan kasallangan sichqonlarda buyrak, jigar, yurak, o'pka va taloqda C.albicans (08032815) ning invaziv o'sishi kuzatildi.



20-rasm AKK8 ning *C.albicans* soniga ta'siri. AKK8 bilan davolashdan keyin sichqonlarning asosiy organlarida: (a) buyrakda; (b) jigarda; (c) yurakda; (d) o'pkada; (e) taloqda kolonizatsiya qilingan C.albicans soni (*p < 0,05, **p < 0,001) (AKK8-1:2 mg/kg, AKK8-2: 4 mg/kg va AKK8-3: 8 mg/kg).

Flukonazol bilan davolash ham kasallangan sichqonlarga sezilarli terapevtik ta'sir ko'rsatmadi. Shunisi e'tiborga loyiqki, 2, 4 va 8 mg/kg AKK8 bilan davolangan 3 guruh, 0,9% fiziologik eritma va flukonazol bilan davolangan guruhlarga nisbatan sinovdan o'tgan organlar to'qimalarida *C.albicans* o'sishini sezilarli darajada ingibirlagan (18e-rasm). IL-6, TNF-a, IL-10 va IL-1b kabi yallig'lanish sitokinlari tizimli yallig'lanishning eng muhim ko'rsatkichlaridan biridir. Bizning natijalarimiz shuni ko'rsatdiki, nazorat sichqonlarining plazmasidagi IL-6, TNF-a, IL-10 va IL-1b konsentratsiyasi normal darajadan sezilarli darajada yuqori edi (mos ravishda 85,4, 15,3, 218,5 va 20,2 pg/ml) (20-rasm), bu tizimli yallig'lanish C. albicans (08032815) in'ektsiyasi orqali muvaffaqiyatli qo'zg'atilganligini ko'rsatadi. 2, 4 va 8 mg/kg AKK8 bilan davolash infeksiyalangan sichqonlarning plazmasidagi IL-6, TNF-a, IL-10 va IL-1b miqdorini sezilarli darajada kamaytirdi. Xususan, 4 mg/kg AKK8 bilan davolash infeksiyalangan sichqonlarda yallig'lanish sitokinlari konsentratsiyasini normal darajaga tushirdi (21-rasm).



21-rasm (a) AKK8 ning zardobdagi IL-6 ga ta'siri; (b) TNF-a; (c) IL-10; (d) C.albicans tomonidan qo'zg'atilgan IL-1b ishlab chiqarilishi. 0,9% sho'r suv bilan ishlov berilgan qiymatlardan sezilarli farq (*p < 0,05, **p < 0,001) (Nazorat: Flukonazol: 2 mg/kg, AKK8-1: 2 mg/kg, AKK8-2: 4 mg/kg va AKK8-3: 8 mg/kg).

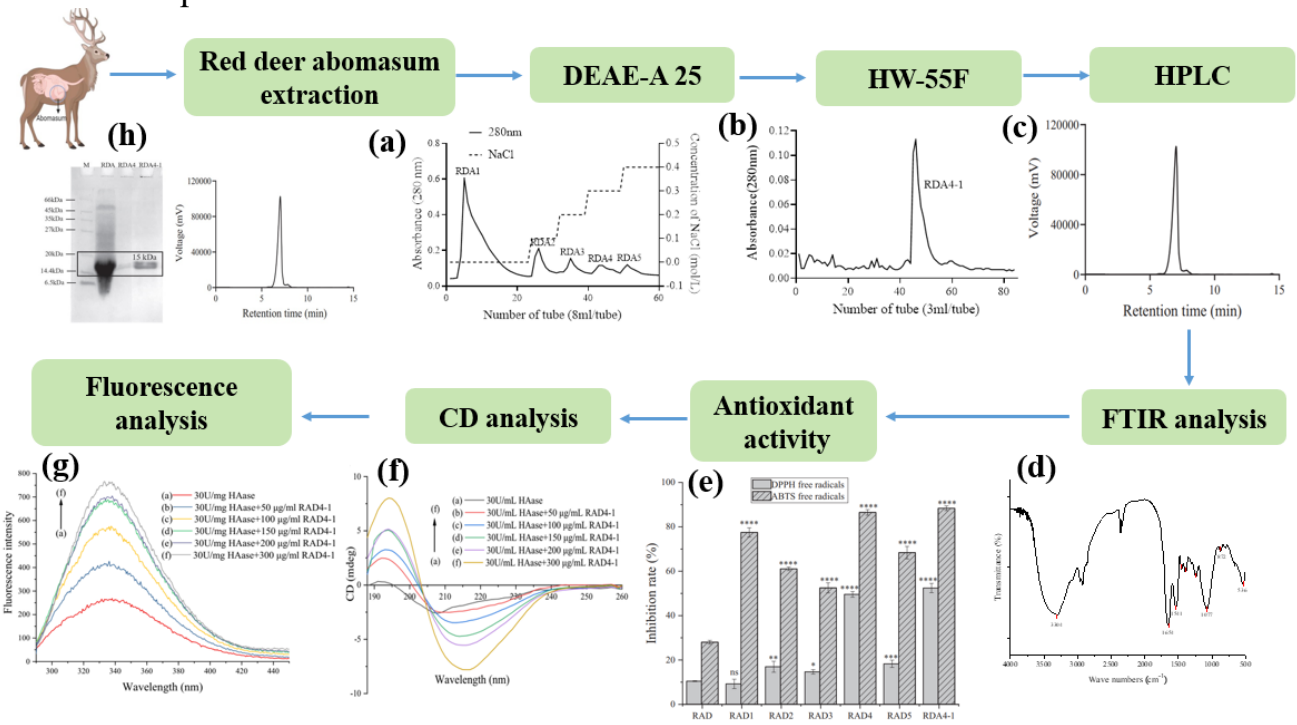
Xulosa:

Baqa terisi sekretsiyasidan olingan 20 ta aminokislota qoldiqli Ranacyclin AJ peptidining aminokislotalar ketma-ketligi asosida o'nta yangi peptid ishlab chiqildi. Peptidlar AKK1 - AKK10 deb nomlandi. 10 ta peptid orasida AKK8 *E.coli*, *S.aureus*, *B.subtilis* va *C.albicans*ga qarshi eng yuqori antimikrob faollikni namoyish etdi. AKK8 ning *C.albicans*ga qarshi faolligi boshqalarga qaraganda 18,5 µg/ml yuqori edi. Ushbu kuzatishlar shuni ko'rsatadiki, AKK8 klinik dorilarga chidamli patogenlarga qarshi

sezilarli antibakterial ta'sir ko'rsatadi. Tuz ionlari AKK8 ning *C.albicans*ga qarshi antimikrob faolligini o'zgartirmadi, bu uning klinik qo'llanilish uchun yaroqliligini ko'rsatadi. AKK8 peptidining (4 mg/kg) in'ektsiyasi infektsiyalangan sichqonlarda yallig'lanish sitokinlarini normal darajaga tushirdi. Oddiy tuzilishi, kichik gemolitik faolligi, past sitotoksikligi va fiziologik muhitda yuqori barqarorligi hisobga olinsa, AKK8 dori-darmonlarga chidamli *C.albicans*ni davolash uchun terapevtik vositalarni ishlab chiqish uchun ajoyib namuna bo'lishi mumkin..

8-qism. Cervus elaphus shirdonidan olingan oqsilni o'rganish

Tadqiqotning maqsadi bug'ularni oshqozoninig qo'shimcha qismi bo'lgan qizil bug'u shirdonidan (RDA) potentsial antioksidant va gialuronidaza ingibirlovchi gomogenlangan oqsilni aniqlash va tavsiflash, tozalangan oqsil va gialuronidaza o'rtasidagi o'zaro ta'sir mexanizmini o'rganish va uning nutrasevtik va qarishga qarshi vositalarda qo'llanilishini baholashdan iborat.



22-rasm. Cervus elaphus shirdon oqsilini qayta ishlash va o'rganish bosqichlari

Gomogenlangan oqsilni tayyorlash

DEAE-Sephadex A-25 anion almashinuv xromatografiyasining elyutatsiya profilida beshta asosiy cho'qqi aniqlandi: RDA1, RDA2, RDA3, RDA4 va RDA5 (22a-rasm). RDA4 HW-55F gel xromatografiyasi yordamida RDA4-1 ni olish uchun qo'shimcha tozalandi (22b-rasm).

SDS-PAGE tahlili (22h-rasm) tozalash jarayonida RDA4-1 ning sofligi oshganini ko'rsatdi. Tozalashning ikki bosqichidan so'ng, gomogenlangan RDA4-1 oqsili olindi va uning molekulyar og'irligi 15 kDa ni tashkil etdi. HPLC tahliliga ko'ra (22h-rasm) RDA4-1 ning yutilish cho'qqisi 95% dan katta maydonga ega bo'lgan bitta cho'qqi bo'lib, tozalash jarayonining samaradorligini namoyish etdi.

Kimyoviy tarkib tahlili

RDA4-1 ning oqsil miqdori 91,63% ni, polisaxarid miqdori esa 3,09% ni tashkil etdi. RDA4-1 glikozillangan oqsil bo'lishi mumkinligi haqidagi gipoteza ilgari surildi, bu aminokislotalar va monosaxarid tahlillari natijalari bilan ham tasdiqlandi. Glikozillangan oqsillar glikozillanmagan oqsillarga qaraganda barqarorroq va biologik faolroqdir. Ular hujayralarga himoya, stabillovchi, tashkil etuvchi va to'siq ta'sirini orttiruvchi faollikni namoyon qiladilar.

Aminokislotalar tarkibini tahlil qilish

Aminokislotalarning tarkibi 18-jadvalda ko'rsatilgan. RDA4-1 ning peptid zanjiri asosan alanin, glitsin, lizin, tirozin va valindan iborat. Bu aminokislotalar oqsilning tuzilishi va funktsiyasini aniqlashda hal qiluvchi rol o'ynaydi. Masalan, alanin peptid zanjirining tuzilishini saqlab qolish uchun javobgardir. Tirozin va valin esa peptid zanjirining gidrofob yadrosini tashkil etuvchi gidrofob aminokislotalardir. Shu bilan birga, muhim aminokislotalar sifatida lizin va valin miqdori 40% dan oshadi. Bu RD4-1 organizm tomonidan osonroq so'riladigan va ishlatiladigan yuqori sifatli oqsil ta'rifiga javob berishini ko'rsatadi.

18-jadval. RDA4-1 ning aminokislota tarkibi (%)

Aminokislota	**Asp**	**Glu**	**Ser**	**Gly**	**His**	**Thr***	**Ala**	**Pro**
Nisbiy molyarlik	0.33	0.64	0.42	11.1	2.75	0.61	10.5	1.76
Aminokislota	**Tyr**	**Val***	**Cys**	**Ile***	**Leu***	**Phe***	**Lys***	
Nisbiy molyarlik	15.1	20.0	13.7	0.26	0.16	0.21	22.2	

Monosaxarid tarkibini tahlil qilish

Monosaxarid tahlil natijalari 19-jadvalda ko'rsatilgan. RDA4-1 asosan glyukuron kislotasi, glyukoza va aminogalaktozadan iborat. Aminosaxaridlar odatda polisaxarid zanjiri glikozillangan oqsillarda peptid zanjiriga ulanadigan joyda joylashgan bo'lib, bu RDA4-1 glikozillangan oqsil ekanligini ko'rsatadi. Bundan tashqari, RDA4-1 tarkibida L-fukoza va mannoza mavjud. L-fukozaning mavjudligi RDA4-1 antibakterial va antiviral faollikka ega bo'lishi mumkinligini ko'rsatadi, mannoza esa RDA4-1 immunomodulyator xususiyatlariga ega ekanligini ko'rsatadi.

19-jadval. RDA4-1 ning monosaxarid tarkibi (%)

Monosaxaridlar	**Man**	**Glu**	**GalUA**	**GluN**	**L-Fuc**
Nibiy molyarlik	8.76	29.86	38.55	20.13	2.71

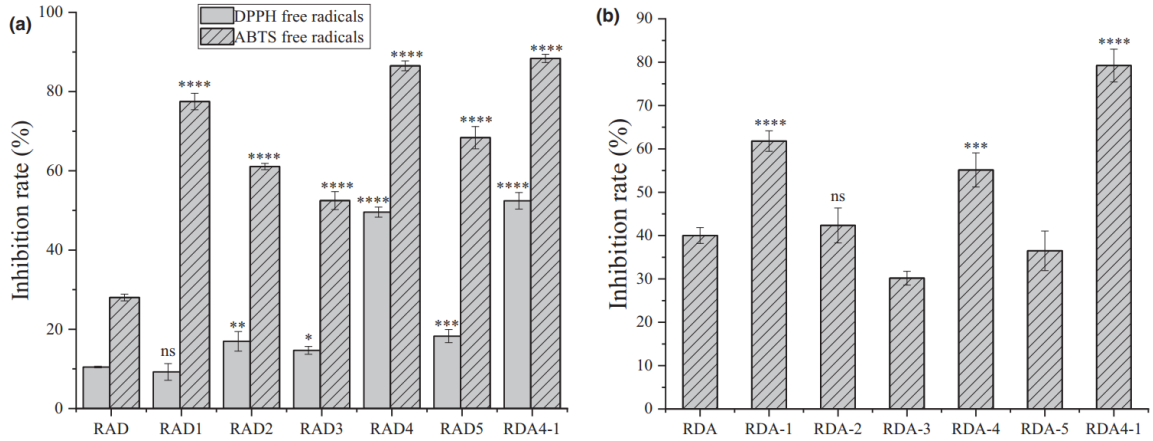
FT-IR tahlili

FT-IR yutilish cho'qqilaridan birikmaning funktsional guruhlarini tahlil qilish va uning tuzilishini tavsiflash uchun foydalanish mumkin. 22d-rasmda ko'rsatilganidek, 3304 sm-1 da aniq keng cho'qqi kuzatiladi, bu kislorod-vodorod (O-H) bog'lanishlarining ustma-ust tushgan cho'zilish tebranishini ifodalaydi. 1654 sm-1 da yutilish azot-vodorod (N-H) ning deformatsiya tebranishi bo'lib, namunada oqsil tuzilishining mavjudligini ko'rsatadi. 1541 sm-1 da yutilish polosa uglerod-azot (C-N) cho'zilish tebranishiga mos keladi. Uglerod-kislorod (C-O) cho'zilish tebranishi 1077 sm-1 da yutilish cho'qqilariga ega. α-tipdagi glikozid bog'lanishlarning mavjudligi 872 sm-1 da yutilish polosa bilan ko'rsatilgan. RDA4-1 ning ikkilamchi tuzilishi amid I

polosasini (1700 sm-1 dan 1600 sm-1 gacha) tahlil qilish orqali aniqlandi. RDA4-1 65,22% β-yassi va 34,78% β-burilishdan iborat. β-burilish tuzilishi oqsil barqarorligini saqlash uchun juda muhimdir. β-yassi tuzilishning mavjudligi oqsil tuzilmalarining barqarorligini oshiradi va denaturatsiyaga qarshi turishga yordam beradi.

Antioksidant faollik

Oksidlanish stressi qarish kabi muammolarga olib kelishi mumkin. Xususan, terining qarishi muammolari gialuronidaza tomonidan kollagen va elastinning parchalanishi bilan bog'liq. Shuning uchun, antioksidant va gialuronidaza ingibirlash faolligiga ega birikmalarni izlash qarishni bartaraf etishda juda muhimdir. Antioksidant faollik tahlili shuni ko'rsatdiki, abomacum oqsili DPPH• va ABTS• + tozalash faolligini namoyish etdi. Kuchaytirilgan tozalash yuqori antioksidant faollik bilan bog'liq edi (23a-rasm). 1,5 mg/ml konsentratsiyada gomogenlangan RDA4-1 oqsili maksimal tozalash qobiliyatini 50,97% va 88,37% ga ko'rsatdi. Avvalgi tadqiqotlar shuni ko'rsatdiki, qo'y shirdonidan olingan antioksidant peptidlarning yarim maksimal ingibirlash konsentratsiyasi (IC_{50}) 6,92 mg/ml ni tashkil etdi, bu RDA4-1 ga nisbatan past antioksidant faollikni ko'rsatadi. Bu farq glikan zanjirlarining mavjudligi bilan bog'liq bo'lishi mumkin, ular peptidlarga nisbatan oqsil kimyoviy tuzilmalarining barqarorligini oshiradi va ularga erkin radikallarni samaraliroq yo'q qilishga imkon beradi. Antioksidant tajribalar shuni ko'rsatdiki, RDA4-1 oziq ovqatlarga faol qo'shimcha sifatida qo'llanilishi mumkin bo'lgan istiqbolli antioksidant xom ashyo hisoblanadi.



23-rasm (a) Namunalarning antioksidlanish faolligi va (b) HAase ingibirlashi; (RDA xom ekstrakt, RDA1-RDA5 RDA dan DEAE-Sephadex A-25 bilan tozalangan fraktsiyalar, RDA4-1 RDA4 dan HW-55F bilan tozalangan gomogenlangan oqsili; '*' xom ekstrakt RDA ga nisbatan ahamiyatni anglatadi, 'ns' ahamiyatsiz, '*' P ⩽ 0,05 ni anglatadi, '**' P ⩽ 0,01 ni anglatadi, '***' P ⩽ 0,001 ni anglatadi va '****' P ⩽ 0,0001 ni anglatadi va yorliqlanmagan xom ekstrakt RDA ga qaraganda pastroq faollikni anglatadi).

HAase ingibirlash faolligi

1) HAase ga ingibirlovchi ta'sir

Namunaning HAase ga qarshi ingibirlash faolligi 23b-rasmda ko'rsatilgan. HAase ingibirlash faolligi uning tozaligi ortishi bilan bilan oshdi. 1 mg/ml konsentratsiyada

RDA4-1 tomonidan HAase ning ingibirlash darajasi 79,23% ni tashkil etdi. Marlin teri kollagenidan olingan peptidlar HAase ni 39,83% ga ingibirladi. RDA4-1 HAase ni Marlin teri kollageni gidrolizlangan peptidga qaraganda samaraliroq ingibirladi.

2) Ingibirlash reaksiya jarayoni CD tahlili

HAase bilan aralashtirilgan RDA4-1 ning turli konsentratsiyalarining CD tahlili 22f-rasmda ko'rsatilgan. RDA4-1 bo'lmaganda, 208 nm va 222 nm da o'xshash intensivlikka ega ikkita salbiy cho'qqilar kuzatildi. 207 nm da kuchli salbiy yutilish keng cho'qqisi paydo bo'ldi, bu HAase asosan α-spiraldan iboratligini ko'rsatadi. RDA4-1 qo'shilganda, salbiy cho'qqining paydo bo'lgan to'lqin uzunligi asta-sekin oshib bordi va musbat cho'qqining yutilishi ham oshdi. Bu tizimning ikkilamchi tuzilishi β-varaq tuzilishiga o'tganligini va konsentratsiyaga bog'liqligini ko'rsatayotganini ko'rsatadi. RDA4-1 ning yuqori konsentratsiyasi ko'proq β-varaq tuzilmalari va kamroq α-spiral tuzilmalarining shakllanishiga olib keldi. Bu shuni ko'rsatadiki, RDA4-1 HAase inhibitori sifatida ishlaganda, u oqsilni birlashtiruvchi tananing ikkilamchi tuzilishini o'zgartiradi va shu bilan fermentning gialuron kislotasiga bog'lanishiga to'sqinlik qiladi.

3) Flyurotsent spektroskopiyasi tahlili

Flyuorotsen spektroskopiyasi past dozani va yuqori sezgirlikni talab qiladi. Flyuoretsen spektrlari ferment oqsil molekulalarining flyuoretsen tuzilishidagi o'zgarishlarni aks ettiradi. Triptofanni o'z ichiga olgan ferment oqsil molekulalarining flyuoretsen spektrida odatda 348 nm ga yaqin musbat emissiya cho'qqisi kuzatiladi. RDA4-1 ning turli konsentratsiyalari bilan aralashtirilgan HAase ning flyuoresen spektri 22g-rasmda ko'rsatilgan. HAase lyuminestsentsiya spektri fermentlar va oqsillarning lyuminestsentsiya spektral xususiyatlariga mos keladigan 340 nm atrofida keng musbat emissiya cho'qqisini namoyish etdi. RDA4-1 qo'shilganda lyuminestsentsiya spektrida yangi emissiya cho'qqisi paydo bo'lmadi va cho'qqi pozitsiyasi o'zgarishsiz qoldi. 340 nmdagi emissiya cho'qqisining lyuminestsentsiya intensivligi qo'shilgan RDA4-1 konsentratsiyasining ortishi bilan oshdi, bu intensivlik va RDA4-1 konsentratsiyasi o'rtasidagi bog'liqlikni ko'rsatadi. Emissiya cho'qqi pozitsiyasi o'zgarishsiz qoldi, bu RDA4-1 HAase ning triptofan qoldig'iga ta'sir qilmaganligini ko'rsatadi, bu RDA4-1 ning HAase ga kovalent bo'lmagan bog'lanishini ko'rsatadi.

Konsentratsiyadagi izchil tendentsiyalarga ega lyuminestsentsiya intensivligi bog'lanishning takrorlanishini anglatadi. Ushbu tajribalar shirdon oqsilining HAase faolligini ingibirlash potentsialini tasdiqladi, va bu o’z navbatida shirdon oqsili HAase ingibitori sifatida yangi nomzod bo'la olishi mumkinligini ko'rsatadi.

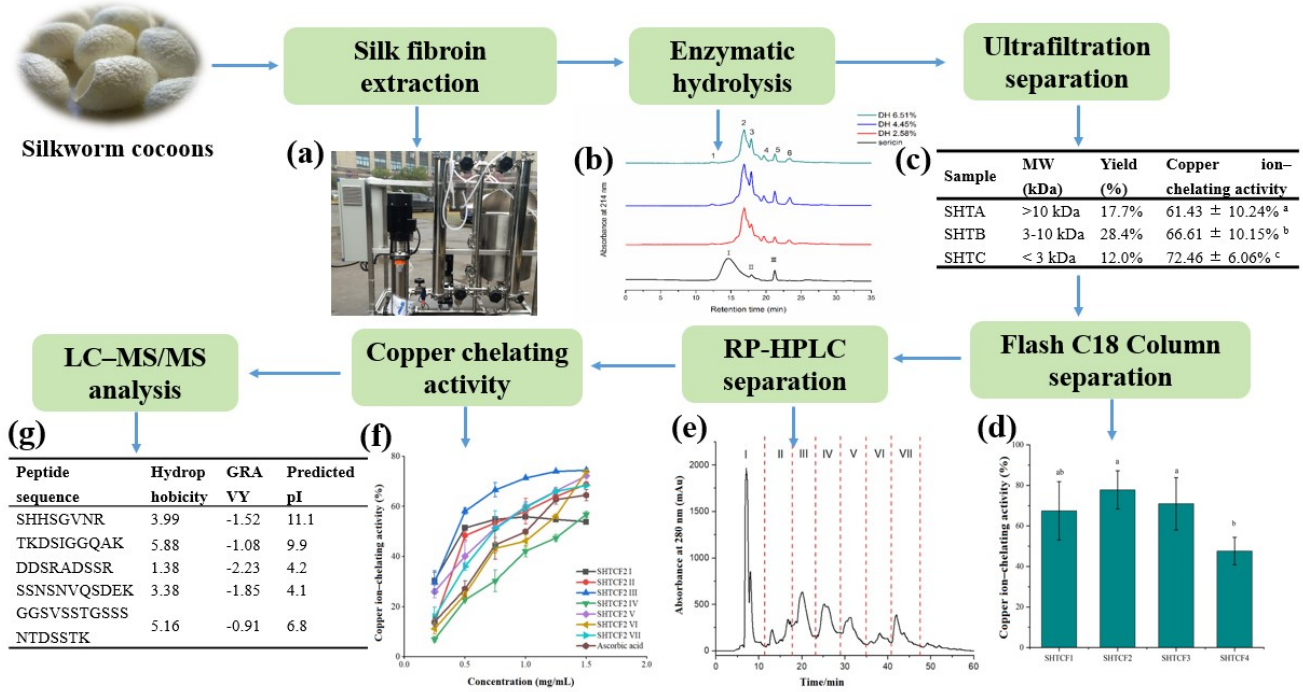
Xulosa:

DEAE-Sephadex A-25 anion almashinuvi xromatografiyasi yordamida qizil bug’u shirdoni (RDA) oqsillaridan RDA1, RDA2, RDA3, RDA4 va RDA5 deb belgilangan 5 ta fraksiya olindi. RDA4 HW-55F gel xromatografiyasi yordamida qo'shimcha tozalandi va Mm 15 kDa bo'lgan RDA4-1 peptidi olindi. RDA4-1 ning oqsil miqdori 91,63% ni, polisaxarid miqdori esa 3,09% ni tashkil etdi. RDA4-1 ning peptid zanjiri asosan Ala, Gly, Lys, Tyr, Val va mos ravishda 8,76, 29,86, 38,55, 20,13

va 2,71 konsentratsiyadagi Man, Glu, GalUA, GluN, L-Fuc kabi monosaxaridlardan iborat. FT-IR tahlili sm^{-1} da yutilishni ko'rsatdi: 1654 (N-H), 1541 (C-N), 1077 (C-O). α-tipdagi glikozidik bog'lanishlarning mavjudligi 872 sm^{-1} da yutilish diapazoni bilan ko'rsatildi. RDA4-1 ning ikkilamchi tuzilishi amid I diapazonini (1700 sm^{-1} dan 1600 sm^{-1} gacha) tahlil qilish orqali aniqlandi. RDA4-1 65,22% β-varaq va 34,78% β-burilishdan iborat. Antioksidant faollik tahlili shuni ko'rsatdiki, shirdon oqsili DPPH• va ABTS• + tozalash faolligini namoyish etdi. RDA4-1 (1,5 mg/ml) maksimal tozalash qobiliyati 50,97% va 88,37% ni tashkil etdi. RDA4-1 (1 mg/ml) tomonidan HAase ning ingibirlash darajasi 79,23% ni tashkil etdi. Marlin terisi kollagenidan olingan peptidlar HAase ni 39,83% ga ingibirlaydi. Ushbu tadqiqot natijalari shuni ko'rsatadiki, qizil bug'u shirdoni gastritni davolovchi boshqa dorilarni ishlab chiqishda qo'llaniluvchi fiziologik faollikka ega birikma bo'la oladi.

9-qism. Modifikatsiyalangan seritsinning biologik faolligi va funktsiyasi

Seritsin ipak sanoatining arzon qo'shimcha mahsuloti bo'lib, har yili sanoat chiqindi suvlari bilan taxminan 50 000 tonna ishlatilmagan seritsin chiqariladi. Seritsin oziq-ovqat, kosmetika va tibbiyot sohalarida keng qo'llanilish istiqboliga ega. Ushbu ish seritsinning strukturaviy modifikatsiyasi orqali uning fizik, kimyoviy va funktsional xususiyatlarini yaxshilashga qaratilgan.



24-rasm. Seritsinni qayta ishlash va o'rganish bosqichlari

Seritsin va uning gidrolizatlarini tayyorlash

10 g ipak qurti pillasi 500 ml suvga (1:50, w/v) botirildi va avtoklavda 121°C da 60 daqiqa davomida qizdirildi. Sovutgandan so'ng, ekstrakt 10000 g da 10 daqiqa davomida santrifuga qilindi. Supernatant 37°C da 5 daqiqa davomida inkubatsiya qilindi va pH 0,5 M NaOH eritmasi yordamida pH qiymati 8,0 ga qadar keltirildi. Enzimatik gidroliz reaksiyasi ferment-substrat nisbati (E/S) 1:50 da tripsin qo'shish orqali boshlandi. Gidroliz paytida pH 0,1 M NaOH eritmasini doimiy ravishda qo'shish

orqali pH qiymati 8,0 da saqlandi va aralashma 37°C da 1000 rpm tezlikda aralashtirildi. Reaksiya aralashmani 80°C da suv hammomida 15 daqiqa davomida qizdirildi va gidrolizatlar liofil quritish orqali quritildi. Olingan kukunlar −20°C da saqlandi.

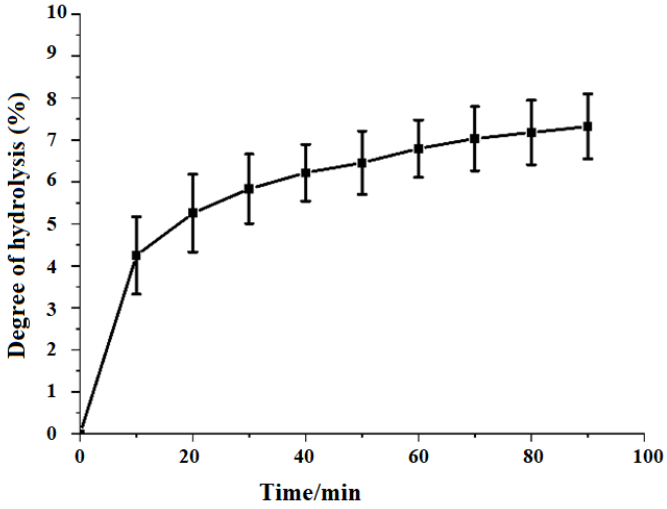
Seritsin (fermentativ ishlov bermasdan) bir xil sharoitlarda tayyorlandi. Oqsil hosil bo'lishi 95,15% ni tashkil etdi.

Seritsinning tripsin bilan gidrolizi paytida gidroliz darajasi (DH) pH-stat usuli yordamida 90 daqiqa davomida kuzatildi. 25-rasmda ko'rsatilganidek, DH dastlabki 10 daqiqada tez oshdi, bu esa kuchli gidrolitik reaksiyani ko'rsatadi, keyin esa keyingi 80 daqiqada nisbatan sekin o'sish kuzatildi. DH qiymatlari 2,58%, 4,45% va 6,51% bo'lgan gidrolizatlar tripsin bilan qo'zg'atilgan gidrolizning seritsinning strukturaviy va funktsional xususiyatlariga ta'sirini qo'shimcha o'rganish uchun tayyorlandi.

Mis ion-xelatlovchi peptidlarni ultrafiltratsiyalash orqali ajratish

Ultrafiltratsiya usuli yordamida seritsin tripsin gidrolizati (SHT) uchta fraksiyaga ajratildi: SHTA (>10 kDa), SHTB (3–10 kDa) va SHTC (<3 kDa), unumi mos ravishda 17,7%, 28,4% va 12,0% ni tashkil etdi. 2 mg/ml konsentratsiyada SHTA, SHTB va SHTC ning mis ion-xelatlash qobiliyati mos ravishda 61,43 ± 10,24%, 66,61 ± 10,15% va 72,46 ± 6,06% ni tashkil etdi (24c-rasm). Molekulyar massasi 3 kDa dan past bo'lgan fraksiya (SHTC) >10 kDa fraksiyasiga (SHTA) va 3-10 kDa fraksiyasiga (SHTB) qaraganda ancha yuqori mis ion-xelatlash faolligini namoyish etdi.

Molekulyar og'irligi 3 kDa dan past bo'lgan peptidlar antioksidant, ACE ingibitori, yallig'lanishga qarshi va mikroblarga qarshi ta'sir ko'rsatishi haqida xabar berilgan. Shuning uchun, qo'shimcha tadqiqotlar uchun SHTC fraksiyasini tanlab oldik.



25-rasm. Seritsinning 90 daqiqa davomida gidrolizlanish darajasi

Flash C18 kolonkasi yordamida mis ion-xelatlovchi peptidlarni ajratish

SHTC SePaFlash C18 teskari fazali kolonkali xromatografiya yordamida qo'shimcha ravishda ajratildi. Natijada to'rtta fraksiya elyuati yig’ib olindi. Har bir fraksiya liofilizatsiya qilindi va keyin mis ion-xelatlovchi faolligini aniqlash uchun ultra toza suvda 2 mg/ml yakuniy konsentratsiyaga eritildi.

24d-rasmda ko'rsatilganidek, 2 mg/ml konsentratsiyada 30% etanol (SHTCF2) bilan eluatlangan fraksiya eng yuqori mis ion-xelatlovchi faollikni (77,80 ± 9,42%), 10%, 50% va 70% etanol bilan eluatlangan fraksiyalar esa mos ravishda 67,43 ±

14,39%, 70,91 ± 12,91% va 47,61 ± 6,75% xelatlovchi faollikni ko'rsatdi. Bu natijalar shuni ko'rsatadiki, ko'proq gidrofil peptidlar mis ionlarini xelatlash qobiliyatini eng yuqori darajada namoyon qiladi. SHTCF2 fraktsiyasi keyingi tozalash va tahlil qilish uchun yig'iladi.

Yuqori samarali suyuqlik xromatografiyasi (HPLC) yordamida tozalash

SHTCF2 fraktsiyasi RP-HPLC yordamida qo'shimcha ravishda tozalandi (24e-rasm). Buning natijasida jami yettits fraksiya olindi. Har bir fraksiya yig'ildi, liofilizatsiya qilindi va keyin faollikni baholash uchun tayyorlandi.

RP-HPLC ajratilgan SHTCF2I, SHTCF2II, SHTCF2III, SHTCF2IV, SHTCF2V, SHTCF2VI va SHTCF2VII fraksiyalari liofilizatsiya qilindi va mis ionlarini xelatlash faolligi tekshirildi, askorbin kislotasi esa musbat nazorat sifatida ishlatildi (24f-rasm). Mis ionlari bilan xelatlash faolligi uchun IC_{50} qiymatlari mos ravishda 0,7839, 0,7028, 0,4013, 1,280, 0,6499, 0,9664, 0,7723 va 0,9406 mg/ml ni tashkil etdi. Ular orasida SHTCF2III eng ko'p mis ionlari bilan xelatlash qobiliyatini namoyish etdi. Shuning uchun SHTCF2III aminokislotalar ketma-ketligini aniqlash uchun LC-MS/MS tahlili uchun tanlandi.

SHTCF2III fraksiyasining LC-MS/MS tahlili

SHTCF2III fraksiya LC-MS/MS yordamida tahlil qilindi va olingan spektrlar Mascot dasturi yordamida Swiss-Prot ma'lumotlar bazasiga nisbatan qidirildi. Peptidlarni identifikatsiyalash standart ball berish algoritmlari asosida amalga oshirildi. Eng yuqori mos keladigan ballarga ega bo'lgan beshta peptid massa-zaryad nisbatlarini (m/z) 298.3860, 503.2090, 504.6280, 597.6640 va 823.2990 ga tenglashtirdi, bu mos ravishda 892.1362, 1004.4034, 1007.2414, 1193.3134 va 1644.5834 Da molekulyar massasiga to'g'ri keladi. Aniqlangan peptid ketma-ketliklari quyidagicha edi: SHHSGVNR, TKDSIGGQAK, DDSRADSSR, SSNSNVQSDEK, GGSVSSTGSSNSNTDSSTK.

20-jadval. Aniqlangan peptidlarning massa-zaryad nisbati va molekulyar og'irliklari

Peptid ketma ketligi	Massa/zaryad (m/z)	Molekulyar og'irlik (Da)
SHHSGVNR	298.3860	892.1362
TKDSIGGQAK	503.2090	1004.4034
DDSRADSSR	504.6280	1007.2414
SSNSNVQSDEK	597.6640	1193.3134
GGSVSSTGSSSNTDSSTK	823.2990	1644.5834

Ushbu natijalar SHTCF2III fraksiyasining molekulyar ma'lumotlarini taqdim etadi va uning biologik funktsiyalari va potentsial farmakologik faolligi bo'yicha keyingi tadqiqotlarga o’z hissasini qo'shadi.

Peptid ketma-ketliklarining gidrofobligi, gidropatiklikning umumiy o'rtacha qiymati (GRAVY) va nazariy izoelektrik nuqtasi (pI) Thermo Fisher Peptid Analysis Tool (24g-rasm) yordamida hisoblab chiqildi.

Gidrofillik/gidrofoblik xossa peptidlar va oqsillarning funktsiyasi va faolligiga ta'sir qiluvchi eng muhim omillardan biridir. Umuman olganda, ko'pgina peptidlar yoki oqsillarning GRAVY qiymatlari -2 dan +2 gacha. Salbiy GRAVY qiymati gidrofillikni ko'rsatadi, pastroq qiymatlar esa kuchliroq gidrofil xususiyatni ifodalaydi. Aksincha, musbat GRAVY qiymati gidrofobiklikni, yuqori qiymatlar esa kuchliroq gidrofobik xususiyatlarni ifodalaydi. Hisoblash natijalari shuni ko'rsatdiki, beshta peptidning barchasi gidrofildir. Eng yuqoridan eng pastgacha bo'lgan gidrofilligiga asoslanib, tartib quyidagicha: DDSRADSSR > SSNSNVQSDEK >SHHSGVNR > TKDSIGGQAK > GGSVSSTGSSSNTDSSTK.

Peptidlarning metall ionlarining xelatlanish qobiliyati asosan ularning aminokislotalar ketma-ketligiga bog'liq. Metall kationlari va peptidlar orasidagi xelatlanish mexanizmi metall ionlarining N-terminal amino guruhi, C-terminal karboksil guruhi, aminokislotalarning yon zanjirlari, shuningdek, peptid zanjiridagi karbonil va imino guruhlari kabi turli funktsional guruhlar bilan koordinatsiya va kovalent bog'lanishni o'z ichiga oladi va halqasimon tuzilmalarni hosil qiladi. Mis ionlari asosan turli funktsional guruhlarning azot atomlariga, masalan, arginindagi guanidino guruhi (Arg), gistidindagi imidazol guruhi (His) va lizindagi ε-amino guruhi (Lys) bilan bog'lanadi. Mis ionlari Arg, Lys va Hisga kuchli yaqinlikka ega. Bundan tashqari, tadqiqotlar shuni ko'rsatdiki, aspartat kislota (Asp) va glutamin kislota (Glu) metall ionlarini ham xelatlashi mumkin.

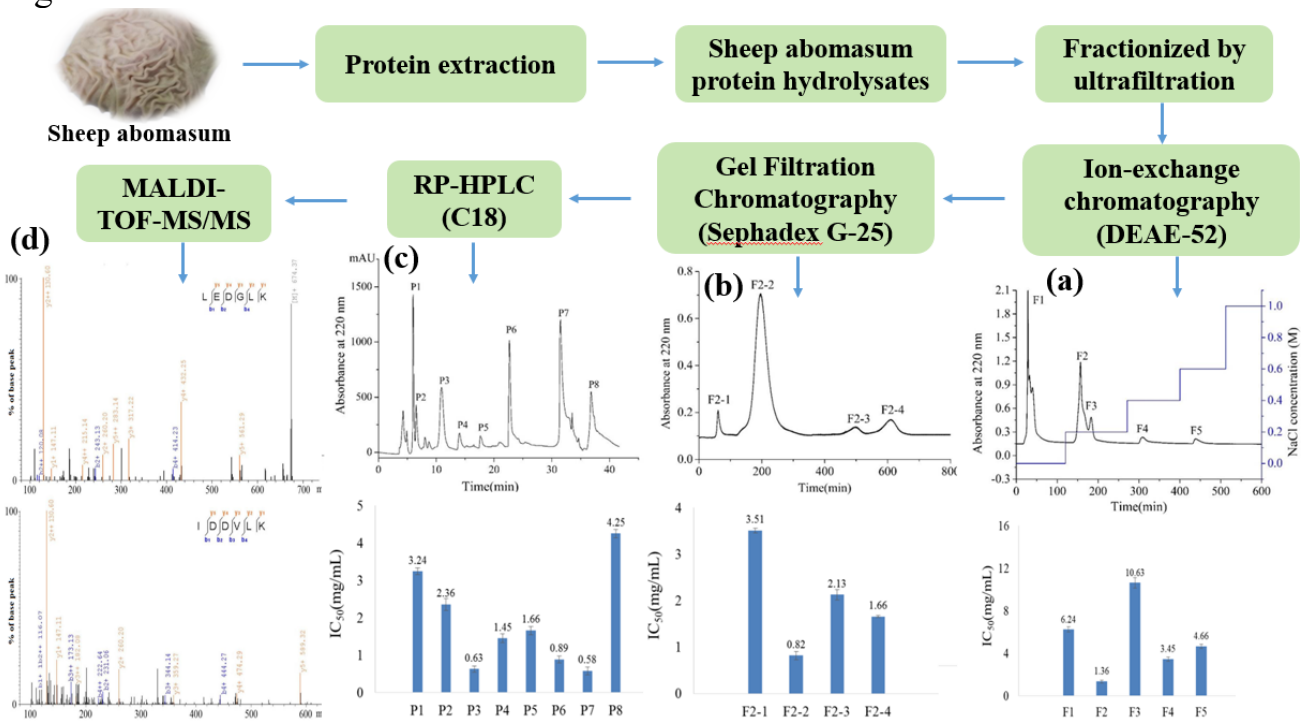
20-jadvalda ko'rsatilganidek, aniqlangan beshta peptidning nisbiy molekulyar og'irliklari 2000 Da dan past. Ular orasida TKDSIGGQAK, SSNSNVQSDEK va GGSVSSTGSSSNTDSSTK peptidlari C-terminalida lizin qoldiqlariga ega, SHHSGVNR va DDSRADSSR esa C-terminalida arginin qoldiqlariga ega. Shunisi e'tiborga loyiqki, SHHSGVNR tarkibida ikkita gistidin qoldig'i mavjud bo'lib, ular oraliq metallari uchun ajoyib xelatlovchi moddalar bo'lib, mis ionlariga bog'lanishni kuchaytiradi. DDSRADSSR uchta aspartat kislota qoldig'ini, SSNSNVQSDEK esa bitta aspartat kislota va bitta glutamat kislota qoldig'ini o'z ichiga oladi.

Xulosa:

Ultrafiltratsiya usuli yordamida seritsin tripsin gidrolizati (SHT) uchta fraksiyaga ajratildi: SHTA (>10 kDa), SHTB (3–10 kDa) va SHTC (<3 kDa), mos ravishda 17,7%, 28,4% va 12,0% hosil bilan. 2 mg/ml konsentratsiyada SHTA, SHTB va SHTC ning mis ionlarini xelatlash qobiliyati mos ravishda 61,43%, 66,61% va 72,46% ni tashkil etdi. Mm 3 kDa ga ega SHTC fraksiyasi boshqalarga qaraganda yuqori mis ionlarini xelatlash faolligini namoyish etdi. HPLC bilan SHTC fraksiyasini ajratish natijasida 4 ta fraksiya olindi. 2 mg/ml konsentratsiyada 30% etanol (SHTCF2) bilan eluatsiya qilingan fraksiya boshqalarga qaraganda eng yuqori mis ionlarini xelatlash faolligini (77,80%) namoyish etdi. SHTCF2 fraksiya RP-HPLC yordamida 7 fraksiyaga ajratildi. SHTCF2III mis ion-xelatlash faolligi uchun IC_{50} qiymatlari faolroq bo'lib, mos ravishda 0,4013 mg/ml ga teng edi. LC-MS/MS tomonidan aniqlangan SHTCF2III peptidining aminokislotalar ketma-ketligi DDSRADSSR edi.

10-qism. Qo'y shirdoni oqsili gidrolizatlarining antioksidant peptidlari

Ushbu tadqiqotning maqsadi qo'y shirdoni oqsili gidrolizatlaridan (SAPH) olingan antioksidant peptidlarni ajratib olish, tozalash va aniqlash, shuningdek, ularning molekulyar tuzilishi va antioksidant faolligini tavsiflash, ularning oziq-ovqat va farmatsevtika qo'llanmalari uchun tabiiy antioksidant agentlar sifatidagi salohiyatini o'rganishdir.



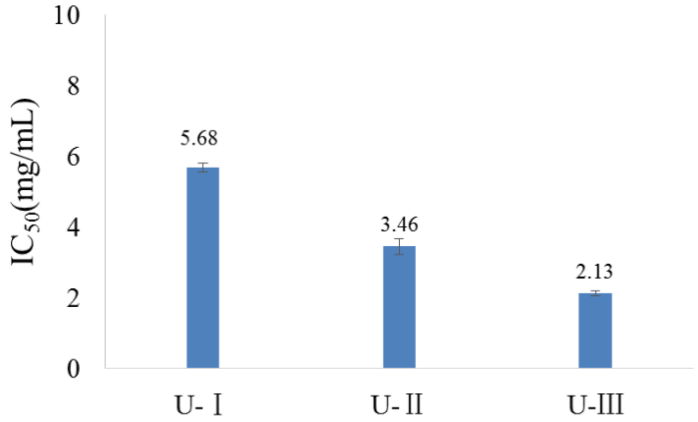
26-rasm. Qo'y shirdon oqsili gidrolizatlarini qayta ishlash va o'rganish bosqichlari

Qo'y shirdoni oqsili gidrolizatlarini (SAPH) tayyorlash

Qo'y shirdoni oqsili konsentratsiyasi (SAPC) distillangan suvda eritildi va pH qiymati 6,0 ga sozlandi. Eritma 30 daqiqa davomida oldindan qizdirildi, so'ngra papain qo'shilib, 46°C da ferment-substrat nisbati 1,5% (w/w) bo'lgan fermentativ gidroliz amalga oshirildi. Reaksiya davomida pH qiymati 0,1 mol/L HCl yoki 0,1 mol/L NaOH bilan sozlash orqali pH-stat usuli yordamida ushlab turildi. 4 soatlik gidrolizdan so'ng, reaksiya aralashmani 95°C suv hammomida 5 daqiqa davomida qizdirish orqali to'xtatildi. Olingan gidrolizat 10000 aylanish tezligida 10 daqiqa davomida santrifuga qilindi va supernatant SAPH olish uchun liofilizatsiya qilindi.

Antioksidant peptidlarni ultrafiltratsiya orqali ajratish

SAPH 3 kDa va 10 kDa molekulyar massaga ega ultrafiltratsiya membranalari yordamida ajratildi, natijada UF-I (MW > 10 kDa), UF-II (3 kDa < MW < 10 kDa) va UF-III (MW < 3 kDa) deb belgilangan uchta fraksiya hosil bo'ldi. 27-rasmda ko'rsatilganidek, UF-III ning DPPH radikal tozalash faolligi (2,13 mg/ml) UF-I (5,68 mg/ml) va UF-II (3,46 mg/ml) ga qaraganda ancha yuqori edi.



27-rasm. Har bir fraksiyaning ultrafiltratsiya orqali DPPH radikal tozalash faolligi

UF-III ning DPPH erkin radikaliga qarshi faolligi asl SAPH ga nisbatan 2,99 baravar yuqori edi (21-jadval). Bu natijalar past molekulyar og'irlikdagi UF-III fraksiyasining eng yuqori antioksidant faollikni namoyish etganligini ko'rsatadi.

21-jadval. Fraksiyalarning tozalash darajasi va antioksidant faolligi

Namunalar	Unum (%)	IC_{50} (mg/mL) DPPH	Tozalik qatlami
SAPH	100	6.36	-
Ultrafiltratsiya (U-III)	25.82	2.13	2.99
DEAE-52 (F2)	4.13	1.36	4.68
Sephadex G-25 (F2-2)	2.32	0.82	7.76
RP-HPLC (P3)	0.43	0.63	10.10
RP-HPLC (P7)	0.66	0.58	10.97

Qo'y shirdoni oqsili gidrolizatlarining (SAPH) antioksidant peptidlarini bir qator tozalashdan keyingi chiqish unumi (%) va DPPH erkin radikalga qarshi faolligi (IC50, mg/ml) va tozaligi haqida qisqacha ma'lumot.

Shunga o'xshash izlanishlar avvalgi tadqiqotlarda ham qayd etilgan, ularda yog'sizlantirilgan yeryong’oq uni, tuxum oqi, ko'k midiya va ovomutsindan olingan gidrolizatlarning past molekulyar og'irlikdagi fraksiyalari (3 kDa dan past) eng kuchli antioksidant faollikni ko'rsatgan. Shuning uchun, quyi molekulyar og'irlikdagi peptidlarni o'z ichiga olgan UF-III keyingi tozalash va tahlil qilish uchun tanlangan.

Ion almashinuvi xromatografiyasi (DEAE-52)

26a-rasmda ko'rsatilganidek, UF-III DEAE-52 anion almashinuvi xromatografik kolonkasi yordamida qo'shimcha ravishda ajratildi va tozalandi, natijada beshta fraksiya (F1 – F5) hosil bo'ldi. F1 fraksiya deionizatsiyalangan suv bilan, F2 va F3 0,2 M NaCl bilan, F4 0,4 M NaCl bilan va F5 0,6 M NaCl bilan eluatsiya qilindi. 26a-rasmda ko'rsatilganidek, bu fraksiyalarning DPPH erkin radikallarni yo'q qilish faolligi mos ravishda 6,24, 1,36, 10,63, 3,45 va 4,66 mg/ml ni tashkil etdi. Ular orasida F2 fraksiya eng kuchli DPPH antiradikal faolligini namoyish etdi, IC_{50} qiymati SAPH ga qaraganda 4,68 baravar past (21-jadval).

Antioksidant faollikning ortishi kislotali aminokislota qoldiqlarining mavjudligi bilan bog'liq bo'lishi mumkin, chunki anion almashinuv xromatografiyasi odatda manfiy zaryadlangan molekulalarni neytral va asosli molekulalardan ajratadi. Shuning

uchun, anion almashinuv qatronida adsorbsiyalangan peptidlar kuchli antioksidant faolligi uchun mas'ul bo'lgan bir yoki bir nechta kislotali guruhlarni o'z ichiga olishi mumkin. Xulosa qilib aytganda, keyingi tozalash uchun F2 fraksiya tanlandi.

Gel filtrlash xromatografiyasi (Sephadex G-25)

26b-rasmda ko'rsatilganidek, sezilarli antioksidant faollikni namoyish etgan F2 fraksiya Sephadex G-25 gel filtrlash xromatografik kolonka yordamida qo'shimcha ravishda tozalandi va natijada to'rtta fraksiya (F2-1 dan F2-4 gacha) hosil bo'ldi. Bu fraksiyalarning DPPH radikalga qarshi faolligi mos ravishda 3,51, 0,82, 2,13 va 1,66 mg/ml ni tashkil etdi (26b-rasm). Ular orasida F2-2 eng yuqori DPPH radikalga qarshi faolligini ko'rsatdi, IC_{50} qiymati SAPH ga qaraganda 7,76 baravar past (21-jadval).

Umuman olganda, past molekulyar og'irlikdagi peptidlar yuqori molekulyar og'irlikdagi peptidlarga qaraganda kuchliroq antioksidant faollikni namoyon etadi. Biroq, ushbu tadqiqotda boshqa fraksiyalarga qaraganda nisbatan yuqori molekulyar og'irlikka ega bo'lgan F2-2 kuchliroq antioksidant faollikni namoyish etdi. Shuning uchun antioksidant faollik nafaqat molekulyar og'irlikka bog'liq, balki peptid ketma-ketligi va tarkibi kabi boshqa omillarga ham ta'sir qiladi.

RP-HPLC yordamida tozalash

Eng yuqori antioksidant faollikni namoyish etgan F2-2 fraksiyasi yarim tayyor RP-HPLC bilan yanada tozalandi. Takroriy in'ektsiyalar amalga oshirildi va eluatsiyalangan fraksiyalar ularning DPPH radikalga qarshi faolligini baholash uchun to'plandi. 26c-rasmda ko'rsatilganidek, 220 nm da kuzatilgan F2-2 xromatogrammasi P1 dan P8 gacha bo'lgan sakkizta aniq cho'qqini aniqladi. Ushbu fraksiyalarning DPPH radikal tozalash faolligi mos ravishda 3,24, 2,36, 0,63, 1,45, 1,66, 0,89, 0,58 va 4,25 mg/ml ni tashkil etdi (26c-rasm). Ular orasida P3 va P7 fraksiyalari eng yuqori DPPH erkin radikalga qarshi faolligini namoyish etdi.

Ushbu tadqiqotda qo'llanilgan to'rt bosqichli tozalash jarayoniga asoslanib, P3 va P7 antioksidant peptidlarining IC_{50} qiymatlari dastlabki SAPH bilan solishtirganda mos ravishda 10 va 11 baravar oshirildi (21-jadval). Bu natijalar RP-HPLC kuchli antioksidant salohiyatga ega peptidlarni samarali ajratib olganligini ko'rsatadi.

Aminokislotalar ketma-ketligi va molekulyar massalarni aniqlash

Ushbu tadqiqotda P3 va P7 antioksidant peptid fraksiyalarining molekulyar og'irliklari va aminokislotalar ketma-ketligini aniqlash uchun MALDI-TOF-MS va TOF-MS/MS qo'llanildi. P3 va P7 ning MALDI-TOF-MS spektrlari peptidlarning birlamchi massa spektrlarini ifodalaydi. Ko'rsatilganidek, x o'qi massa-zaryad nisbatini (m/z), y o'qi esa ion intensivligini bildiradi. m/z 674.37 dagi ko'zga ko'ringan cho'qqi P3 peptidining protonlangan molekulyar ioniga $[M+H]^+$ mos keladi, bu P3 ning nisbiy molekulyar og'irligi 674.37 Da ekanligini ko'rsatadi. Xuddi shunday, 48-rasmda m/z 703.41 dagi cho'qqi P7 peptidining protonlangan ioniga $[M+H]^+$ mos keladi, bu nisbiy molekulyar og'irligi 703.41 Da ekanligini ko'rsatadi.

MALDI-TOF-MS/MS tandem mass-spektrometriyasi MS/MS rejimida keyingi parchalanish uchun MS spektridan eng yuqori signal intensivligiga ega bo'lgan prekursor ionlarini tanlash imkonini beradi. Prekursor ionlari inert gaz molekulalari bilan to'qnashadi, natijada peptid bog'lanishi uziladi va parcha ionlari hosil bo'ladi.

Olingan spektrlardan peptidlarning aminokislotalar ketma-ketligini aniqlash uchun dastur yordamida avtomatik ravishda tahlil qilish mumkin. Ushbu tadqiqotda MALDI-TOF-MS/MS antioksidant peptidlari P3 va P7 ning aminokislotalar ketma-ketligini aniqlash uchun qo'llanildi. 28d-rasmda ko'rsatilganidek, P3 ketma-ketligi Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK) sifatida aniqlangan, 49b-rasmda esa P7 Ile-Asp-Asp-Val-Leu-Lys (IDDVLK) sifatida aniqlangani ko'rsatilgan. SWISS-PROT va BIOPEP ma'lumotlar bazasidagi qidiruvlar ikkala peptid uchun ham mos kelmasligini aniqladi, bu esa ushbu tadqiqotda aniqlangan ikkita peptid yangi antioksidant peptidlarni ifodalashi mumkinligini ko'rsatadi.

P3 va P7 antioksidant peptidlarining dastlabki tahlili

P3 va P7 ning nisbiy molekulyar og'irliklari mos ravishda 674,37 va 704,31 Da ni tashkil etdi, bu ularni past molekulyar og'irlikdagi peptidlar deb tasniflaydi. Ko'plab tadqiqotlar shuni ko'rsatdiki, past molekulyar og'irlikdagi peptidlar odatda yuqori antioksidant faollikni namoyon qiladi. Aminokislotalar ketma-ketligi tahlili ikkala P3 va P7 peptidlar Ile, Asp, Val, Leu va Glu kabi kislotali va gidrofob aminokislota qoldiqlariga boyligini ko’rsatdi. Avvalgi tadqiqotlardan ma’lumki, kislotali yoki gidrofob qoldiqlar bilan boyitilgan peptidlar kuchli radikallarni yo'q qilish qobiliyatiga ega bo’ladi. Bundan tashqari, N- yoki C-terminallarida gidrofob qoldiqlarning (Val, Leu, Ile, Ala, Phe va Lys) mavjudligi antioksidant faollikning kuchayishi bilan bog'liq. Shuning uchun, P3 va P7 ning antioksidant faolligi ularning Ile, Lys va Leu kabi gidrofob terminal qoldiqlari bilan bog'liq bo'lishi mumkin.

P3 tarkibidagi -Gly-Leu- (GL) ketma ketligi uning kuchli antioksidant faolligida muhim rol o'ynashi mumkin. Rajapakse va boshqalar ulkan kalmar mushaklaridan ajratilgan Asn-Gly-Leu-Glu-Gly-Leu-Lys peptidi, asosan GL ketma-ketligi mavjudligi sababli yuqori antioksidant xususiyatga ega ekanligini tasdiqlashdi. Gidrofob dipeptid ketma ketliklari erkin radikallar bilan o'zaro ta'sirni osonlashtirishi va shu bilan antioksidant faollikni oshirishi ma'lum.

Xuddi shunday, P7 tarkibidagi takroriy dipeptid ketma-ketligi -Asp-Asp- (DD) ning mavjudligi uning kuchli radikallarni yo'q qilish potentsialiga hissa qo'shishi mumkin. Jin va boshqalar shuningdek, peptidlardagi takroriy di- yoki tri-qoldiq motivlari ko'pincha yuqori antioksidant faollikni ta'minlashini ko'rsatdilar. Birgalikda, P3 va P7 ning kuchli antioksidant faolligi, ehtimol, ularning past molekulyar og'irliklari, gidrofob terminal qoldiqlari va sinergik ta'sir ko'rsatadigan xarakterli aminokislotalar ketma ketliklari bilan bog'liq.

Xulosa:

Ultrafiltratsiya, ion almashinuvi xromatografiyasi, gel filtrlash xromatografiyasi va teskari fazali yuqori samarali suyuqlik xromatografiyasi orqali yuqori antioksidant faollikka ega ikkita yangi peptid (P3 va P7) SAPH dan tozalandi. Peptid ketma-ketliklari mos ravishda 674,37 va 703,41 Da molekulyar og'irliklarga ega Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK, SAPHP-A) va Ile-Asp-Asp-Val-Leu-Lys (IDDVLK, SAPHP-B) sifatida aniqlandi. SAPH peptidlari oziq-ovqat qo'shimchalari va farmatsevtika mahsulotlari sifatida ishlatilishi mumkin.

XULOSALAR

1. No’xat *(Cicer arietinum L.)* oqsillarining gidrolizi orasida eng samarali usul neytraza-proteaza fermenti bo‘lib, 20 kDa dan kichik peptidlar 80% unum bilan olindi. Uch bosqichli xromatografik tozalash orqali 92,8% tozalikdagi, Mm 685,41 Da va LTEIIP ketma-ketligiga ega NF2-4-1 peptidi ajratildi. Peptidning DPPH˙ va •OH radikallariga nisbatan IC_{50} qiymatlari mos ravishda 0,24 va 0,57 mg/ml bo‘lib, u yuqori antioksidant faollikka ega ekanini tasdiqladi. Ushbu peptid funksional oziq-ovqatlarda erkin radikallarni neytrallash uchun istiqbolli bioaktiv modda sifatida tavsiya etildi.

2. *Cuminum cyminum L.* (zira) urug‘idan peptidlarni ajratish uchun ekstraksiyalash, ion-almashinish va gel-xromatografiyadan iborat uch bosqichli samarali usul ishlab chiqildi. Gel-xromatografiyadan olingan F4-c fraksiyasi E. coli, C. albicans va S. aureus kabi patogenlarga qarshi kuchli antimikrob faollik ko‘rsatishi aniqlandi. Ushbu fraksiyadan Mm 1158,61 – 3312,26 Da diapazondagi 10 ta gomogen peptid YuSSX yordamida ajratilib, ularning birlamchi tuzilishlari to‘liq aniqlandi. Olingan peptidlar tabiiy antibiotik bo‘lib, kelajakda oziq-ovqat mahsulotlari uchun xavfsiz konservant sifatida qo‘llash mumkinligi aniqlandi.

3. Qo‘y, qoramol, ot va tuya suyak iliklaridan oqsillar va peptidlarni ajratish texnologiyasi suvli ekstraksiya va $(NH_4)_2SO_4$ning 30–70% to‘yingan eritmalarida fraksiyalash orqali takomillashtirildi. Ot suyagi iligidan oqsil ajralishi eng yuqori unumga ega bo‘lib, uning suvli ekstraktida chiqish unumi 90,47% ga yetdi. $(NH_4)_2SO_4$ning 50% to‘yinishida qoramol va tuya suyak iliklari oqsillari mos ravishda 52,3 va 56,5 mg/ml bo‘ldi. Tuzli ekstraktlar suvli ekstraktlarga nisbatan oqsilga boy ekani aniqlandi. Ot suyagi iligi oqsili kuchli antioksidant xususiyat ko‘rsatib, 83,9% radikal neytrallanishi va IC_{50} = 0,573 mg/ml ekanligi aniqlandi.

4. Baqtriya tuyasi suti oqsillari pepsin, alkalaza, papain va tripsin yordamida gidroliz qilinib, peptidlar mos ravishda 67%, 59%, 75% va 82% unum bilan olindi. Eng yuqori unumli TF (82%) fraksiyani uch bosqichda (ultrafiltratsiya, gel-filtratsiya va YuSSX) tozalash natijasida TFI-b1, TFI-b2 va TFI-b3 peptidlari ajratib olindi. Ularning antioksidant faolligi DPPH bo‘yicha IC_{50} = 1,9; 1,2; 0,6 mg/ml, ABTS bo‘yicha esa IC_{50} = 2,4; 1,8; 0,9 mg/ml ni tashkil etdi. TFI-b3 peptidi eng yuqori radikal-so‘ndirish faolligini ko‘rsatdi. MALDI TOF-MS/MS orqali ularning birlamchi strukturalari aniqlanib, mos ravishda RLDGQGRPRVWLDR, TPDNIDIWLGGIAEPQVKR va VAYSDDGENWTEYRDQGAVEGK ketma-ketliklari qayd etildi. Ushbu peptidlar funksional oziq-ovqatlarda kuchli antioksidant sifatida qo‘llanishga tavsiya etildi.

5. Chayon oqsilining ekstraksiyasi sharoitlari o‘rganilib, ultratovush + 0,5 M NaCl eritmasi eng yaxshi natija ko‘rsatdi (unum 14,64%, oqsil 79,06%). RSM optimizatsiyasi bo‘yicha ekstraksiya uchun optimal parametrlar 50 min, 400 Vt va 18 ml/g bo‘lib, oqsilning olinishi 78,94% va unumdorlik 24,80% ni tashkil etdi. DSF, UE va SE usullarida oqsilning rN 4,0 dagi minimal eruvchanligi mos ravishda 8,05%, 15,25%, 18,75%, rN 12 dagi maksimal eruvchanligi esa 13,5%, 70,15%, 79,5% bo‘ldi. rN 7,0 da UE emulsiyalanish (45,55%) va ko‘piklanish (40,30%) xususiyatlari SE ga nisbatan yuqori ekanligi aniqlandi. SEM tasvirlari oqsilning quritish jarayonida kristall

holatdan amorf tuzilishga o‘tishini va yuqori kristallik "yog‘och payraxalari"ga o‘xshash struktura hosil bo‘lishini ko‘rsatdi.

6. Buthus martensii Karsch chayon oqsili papain, alkalaza va tripsin yordamida gidroliz qilinib, eng yuqori gidroliz unumiga papain bilan erishildi (26,46%). Papain fraksiyasi 5,0 mg/ml konsentratsiyada kuchli antioksidant faollik namoyon etdi (ABTS – 77,45%, DPPH – 75,54%, •OH – 49,44%). Ushbu fraksiyani uch bosqichli tozalash natijasida R4-1 va R4-2 peptidlari ajratildi. MALDI-TOF-MS/MS orqali ularning tuzilishi mos ravishda LPTETLH va IEEDLER ketma-ketliklariga teng ekani aniqlandi. R4-1 peptidi ABTS bo‘yicha 83,22%, R4-2 esa DPPH bo‘yicha 81,22% faollik ko‘rsatdi. ON radikaliga nisbatan faollik esa mos ravishda 62,96% va 58,69% bo‘lib, boshqa radikal turlariga nisbatan pastroq ekanligi aniqlandi.

7. Ranacyclin AJ qurbaqa terisi sekresiyasidan ilhomlanib yaratilgan 10 ta yangi peptid ichida AKK8 eng yuqori antimikrob faollik ko‘rsatdi va *E. coli*, *S. aureus*, *B. subtilis* hamda ayniqsa C. albicans ga nisbatan kuchli ta’sirga ega bo‘ldi (MIC = 18,5 μg/ml). Tuz ionlari uning faolligini kamaytirmadi, bu AKK8 peptidning klinik sharoitda barqarorligini tasdiqlaydi. Infeksiyalangan sichqonlarda 4 mg/kg dozada qo‘llanganda u yallig‘lanish sitokinlarini normallashtirdi. Oddiy tuzilishi, past gemolitik va sitotoksik ta’siri, fiziologik muhitda barqarorligi AKK8 ni dorilarga chidamli C. albicans infeksiyalariga qarshi istiqbolli terapevtik shablon sifatida qo‘llash imkonini beradi.

8. DEAE-sefadeks A-25 yordamida qizil kiyik shirdon oqsilidan 5 ta fraksiya ajratilib, gel-xromatografiya orqali Mm 15 kDa li RDA4-1 peptidi olingan. RDA4-1 yuqori tozalikdagi oqsil (91,63%) bo‘lib, tarkibida Ala, Gly, Leu, Tyr, Val va Man, Glu, GalUA, GluN, L-Fuc kabi monosaxaridlar mavjud. IQ spekrtoskopiya tahlili uning oqsil–polisaxarid kompleks tuzilishini tasdiqladi, ikkilamchi tuzilishi esa 65,22% β-tahlangan va 34,78% β-bukilishdan iborat ekanini ko‘rsatdi. RDA4-1 ning antioksidant faolligi (1,5 mg/ml) DPPH bo‘yicha 50,97%, ABTS bo‘yicha 88,37%, NAaza ingibitsiyasi esa 79,23% ni tashkil etdi. Bu ko‘rsatkich marlin kollagen peptidlaridan (39,83%) ancha yuqori bo‘lib, qizil kiyik shirdonidan olingan peptidlar gastrit va boshqa yallig‘lanish kasalliklarini davolashda istiqbolli ekanini ko’rsatdi.

9. Seritsin tripsin gidrolizati ultrafiltratsiya orqali SHTA (>10 kDa), SHTB (3–10 kDa) va SHTC (<3 kDa) fraksiyalarga ajratilib, ularning mis ionini xelatlash faolligi 2 mg/ml konsentratsiyada mos ravishda 61,43%, 66,61% va 72,46% ni tashkil etdi. Eng yuqori xelatlovchi faollikka ega SHTC fraksiyasi YuSSX bilan tozalanib, SHTCF2 fraksiyasi 30% etanolda elyusiya qilinganda 77,80% xelatlash xususiyati bilan ajralib chiqdi. Keyingi fraksionlash natijasida SHTCF2III peptidi olingan bo‘lib, uning mis ionini xelatlash bo‘yicha IC_{50} = 0,4013 mg/ml bilan barcha fraksiyalar orasida eng yuqori faollikka ega ekani aniqlandi. LC-MS/MS tahlili ushbu peptidning aminokislota ketma-ketligi DDSRADSSR ekanini tasdiqlandi.

10. Ultrafiltratsiya, ion almashinish, gel xromatografiya va teskari fazali yuqori samarali suyuqlik xromatografiyasi orqali qo’y shirdonidan yuqori antioksidant faollikka ega ikkita yangi peptid (R3 va R7) tozalandi. Peptidlarning aminokislota ketma-ketligi mos ravishda 674,37 va 703,41 Da molekulyar og‘irliklarga ega bo‘lgan

Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK, SAPHP-A) va Ile-Asp-Asp-Val-Leu-Lys (IDDVLK, SAPHP-B) sifatida aniqlandi. SAPH peptidlari oziq-ovqat qo‘shimchalari va farmasevtika mahsulotlari sifatida tavsiya etildi.

SCIENTIFIC COUNCIL ON AWARDING SCIENTIFIC DEGREES DSc. 02/30.12.2019.K/B.37.01 AT THE INSTITUTE OF BIOORGANIC CHEMISTRY

INSTITUTE OF BIOORGANIC CHEMISTRY

WAILI AIHEMIDING

STRUCTURE AND BIOLOGICAL ACTIVITY OF PEPTIDES OF ANIMAL AND PLANT ORIGIN FOR THE DEVELOPMENT OF FUNCTIONAL FOODS AND DRUGS

02.00.10 – Bioorganic chemistry

PRESENTATION
To obtaining the degree of Doctor of Chemical Sciences (DSs) based on articles published in scientific journals having a correspondingly high impact factor and included in the international scientific database (without defending dissertation)

Tashkent– 2025

The title of the research (DSc) has been registered by the Supreme Attestation Commission of the Cabinet of Ministers of the Republic of Uzbekistan with registration number of B2025.4.DSc/K240.

The work was carried out at the Xinjiang Technical Institute of Physics and Chemistry, Academy of Sciences of the People's Republic of China and Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan.

The research work of the presentation is posted in three (Uzbek, English, Russian (resume) languages on the website of the Scientific Counsil (www.biochem.uz) and on the website of «Ziyonet» information and educational portal (www.ziyonet.uz).

Scientific consultant:

Shavkat Ismailovich Salikhov
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The presentation will take place on 24.12.2025 year at 10^{00} at the meeting of the Scientific Council No. 02/30.12.2019.K/B.37.01 of the Institute of Bioorganic Chemistry. Address: 100125, Tashkent, 83 M. Ulugbek street. Phone: 262-35-40, Fax: (99871) 262-70-63), e-mail: info@biochem.uz.

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INTRODUCTION (description of the presentation)

The relevance and necessity of the research topic. The study of the structure and biological activity of animal and plant-derived peptides is highly relevant to the development of functional foods and modern medicines. Owing to their natural origin, safety, high selectivity, and excellent biocompatibility, bioactive peptides play an important role in creating advanced nutritional and pharmaceutical products with antioxidant, antihypertensive, immunomodulatory, antimicrobial, and metabolism-regulating properties. Since the biological activity of peptides is determined by their structure, amino acid composition, and molecular characteristics, comprehensive investigations into these parameters provide a scientific foundation for the development of new functional food supplements, natural health products, and therapeutic agents. The increasing global demand for peptides of natural origin, coupled with the need to minimize the side effects associated with synthetic drugs, further underscores the strategic importance of this research direction.

In recent years, leading research centers around the world have intensified studies focused on bioactive macromolecules and peptides derived from animal and plant sources. Particular attention has been directed toward the isolation of natural proteins and peptides, the elucidation of their structural characteristics, and the evaluation of their antioxidant, anti-inflammatory, hypoglycemic, antimicrobial, and immunomodulatory activities. These efforts have significantly broadened the potential for developing functional food products, nutritional supplements, and biomedical materials based on biologically active compounds obtained from natural resources.

In this field, research is also being actively carried out in our Republic in collaboration with international scientific centers, yielding notable results. In particular, scientists of the Academy of Sciences, together with researchers from the People’s Republic of China, have made progress in isolating biologically active natural proteins and peptides from various raw materials-including camel milk, Chickpea, scorpion, ruminant stomach tissues, cumin seeds, silk fibroin, and bovine bone marrow-followed by the characterization of their structure and biological activity. Such research supports the implementation of key priorities outlined in the Strategy of Action for the Further Development of the Republic of Uzbekistan, specifically the advancement of the pharmaceutical industry and the improvement of access to affordable, high-quality medicines for the population and medical institutions. Ultimately, these efforts create opportunities for developing new-generation natural therapeutic agents that are effective, affordable, and associated with fewer side effects in the treatment of various

This dissertation research, to a certain extent, serves the fulfillment of the tasks stipulated in the Decrees of the President of the Republic of Uzbekistan No. UP-4947 of February 7, 2017 "On the Action Strategy for the Further Development of the Republic of Uzbekistan" and No. UP-6097 of October 29, 2020 "On Approving the Concept for the Development of Science until 2030," in the Resolutions of the President of the Republic of Uzbekistan No. PP-3532 of February 14, 2018 "On Additional Measures for the Accelerated Development of the Pharmaceutical Industry" and No. PP-4310 of May 6, 2019 "On Measures for the Further Development of the

System of Medical and Pharmaceutical Education and Science," as well as in other regulatory legal documents adopted in this area.

Compliance of the research with the priority directions of the development of science and technology in the country. This study was carried out in accordance with the priority direction of the development of science and technology VI "Medicine and pharmacology".

International context of the research. Research on the isolation of biologically active proteins and peptides from natural sources, their structure and activity is being conducted at leading scientific research centers in the world. In particular, research on the development of alternative technologies for peptide synthesis is being conducted at Maastricht University (Netherlands), on improving methods for the synthesis of complex peptides and the development of new therapeutic agents, in particular, peptide drugs for the treatment of diabetes and infections at the Florey Institute (Australia), on the production of biocatalysts and natural and synthetic peptide drugs at the Research Institute of Peptide Chemistry (Iran), on the comprehensive review of bioactive peptides obtained from plants and their use in food products at the University of Manitoba (Canada), on the isolation of peptides from natural sources (camel milk, scorpion proteins, silk sericin, lamb and deer liver), on their structural characterization, and on the production of functional foods, nutraceuticals and biomedical materials at the Institute of Bioorganic Chemistry (Uzbekistan).

Significant results have been achieved as a result of studies on the isolation of biologically active proteins and peptides from natural sources, their structure and activity, including studies on the extraction of bioactive peptides from food waste and innovative sources, and it has been found that proteins and peptides from marine sources, unlike terrestrial proteins, exhibit high activity due to their unique amino acid composition (Food, Fisheries and Aquaculture Research Institute, Norway). It has been found that some peptides from food (e.g. LILPKHSDAD, LTFPGSAED) exhibit several properties, such as hypoglycemic (lowering blood sugar), hypolipidemic (lowering lipids), antihypertensive (lowering blood pressure) and cytoprotective (protecting cells) (University of Manitoba, Canada). It has been scientifically proven that the biological activity of peptides depends on their specific structure, amino acid sequence and molecular weight, and that they contain hydrophobic (e.g. tyrosine, leucine, Low molecular weight peptides containing tryptophan, proline) and aromatic amino acids have been found to have high antioxidant activity (National Research Institute of Food Sciences and Technology, Spain).

In the world, research is being conducted in a number of promising areas to create promising drugs based on natural compounds, including the following promising areas: developing improved methods for isolating peptides from proteinaceous natural compounds; determining the structure and physicochemical properties of promising compounds with biological activity; creating drugs with low side effects and high efficacy by studying their biological activity and mechanisms of action.

Current state of the research on the topic. Given the growing global demand for natural and sustainable health solutions, animal and plant - derived peptides can

well meet consumers' needs. They have a wide range of sources and are renewable. The production process commonly uses methods such as enzymatic hydrolysis, which can save energy and reduce emissions. Using by-products as raw materials can improve economic efficiency and promote the development of industries in multiple fields. With continued research and innovation, these peptides have the potential to become integral components of future functional foods and therapeutic interventions, fostering overall health and well-being.

New Zealand scientist Clara S.F. Bah extracted bioactive peptide storage products from cow ferns using plants and fungi. HomayouniTabrizi et al. isolated two antioxidant peptides from pepsin and pancreatin digested camel milk, and determined the amino acids sequences of these two peptides. ACE-inhibitory peptides from dromedary camel milk were identified by Amin-Alhaj. Yao R have isolated and identified a novel anticoagulant peptide from scorpion protein by enzymatic hydrolysis. The body of scorpion contains several proteins, but until now there is little information about antioxidant peptides from scorpion protein. The antioxidant activity of CSP hydrolysates was studied by Torres-Fuentes and their study of FVPH, ALEPDHR, TETWNPNHPEL, and SAEHGSLH examined the antioxidant properties of four chickpea peptides. W.F. Porto achieved combinatorial exploration of peptide structure design by optimizing antimicrobial peptides through computer simulation.

The current research landscape in bioactive peptides and proteins is dynamic and multifaceted. Studies have established that enzymatic hydrolysis is an effective method for releasing potent bioactive peptides from larger, inactive parent proteins in sources like camel milk. The antioxidant properties of such peptides are often linked to their molecular weight and the presence of hydrophobic amino acids.

In the realm of animal proteins, research has demonstrated that by-products of the meat industry, such as bone marrow, are rich sources of proteins with both antimicrobial and antioxidant potential. Specialized animal products, including scorpion venom and frog skin secretions, are recognized as elite sources of highly potent and specific molecules. Research has focused on isolating neurotoxins that modulate ion channels, and designing novel synthetic peptides based on natural templates to combat drug-resistant pathogens.

Furthermore, advanced strategies are being developed to enhance the functionality of proteins. Covalent conjugation of proteins, such as silk sericin, with small-molecule phenolic compounds has emerged as an innovative method to significantly improve their anti-inflammatory, antioxidant, and emulsifying properties. Concurrently, the study of glycoproteins from traditional medicinal sources, like lamb and deer abomasum, is revealing them to be key active components responsible for observed therapeutic effects, such as potent and specific COX-2 inhibition and hyaluronidase inhibition. This collective body of work underscores a clear trend towards not only discovering new molecules but also optimizing their extraction and enhancing their natural functionalities.

Connection of the research topic with the research plans of the research institution where the work was carried out. The dissertation research was carried

out within the framework of the National Program of Basic Research and Development of the Ministry of Science and Technology of China "Creation of a joint Chinese-Uzbek laboratory for new medicines "One Belt, One Road" and research of innovative medicines" (2020YFE0205600, 2020-2023), the Cooperation Program of the Shanghai Cooperation Organization for Science and Technology and the International Cooperation Program for Science and Technology "Development and registration of chickpea protein nutritional powder in Uzbekistan" (2022E01041, 2022-2025), as well as the scientific project "Study of the molecular mechanisms of action of new biologically active substances obtained from reptiles, arachnids, and amphibians in Uzbekistan for the treatment of socially significant diseases" (FFA2021359, 2021-2026).

The purpose of the research study is the isolation, purification, and structural characterization of peptides from cumin seeds, chickpea sprouts, bone marrow (bovine, camel, horse, and sheep), camel milk, scorpion proteins, silk fibroin, lamb and red deer abomasum proteins, revelation of their potential biological activities for developing functional foods, nutraceuticals, and biomedical materials.

The tasks of the research:

Conducting a structural analysis and systematic physicochemical characterization of camel milk, chickpeas, scorpion tissues, ruminants' stomachs, cumin seeds, silk sericin, and cattle bone marrow to create a composite database of proteins, peptides, lipids, and carbohydrates;

Optimization of peptide separation and purification technologies by developing and purifying a green, multi-stage separation process that combines centrifugation, microfiltration, ultrafiltration, and radial chromatography;

Application of controlled enzymatic hydrolysis to obtain bioactive peptide fractions from selected protein substrates, followed by purification and fractionation to obtain high-purity functional peptides;

Structural and molecular characteristics of peptides purified by spectral and chromatographic methods for determining the amino acid composition and secondary structure;

Investigation of the antioxidant, antidiabetic, antimicrobial, and immunomodulatory activity of peptides isolated through biochemical and cellular analyses in vitro, study of the relationship between molecular structure and bioactivity.

Generalization of the obtained data in order to create a theoretical and technological basis for the sustainable use of regional bioresources and support their further application in the functional food and biomedical industries.

The objects of the research are plant materials - cumin seeds (*Cuminum cyminum* L.), pea sprouts (*Cicer arietinum* L.), animal tissues and products: bone marrow from sheep, cattle, horses and camels, milk and whole body of Bactrian camels, whole body and venom of Manchurian scorpion (*Buthus martensii* Karsch), rennet from lambs and deer (*Cervus elaphus*), animal secretions and secondary products: frog skin secretions (as a template for synthesis), silk sericin from silkworm cocoons.

The subject of the research is the isolation, characterization of natural biologically active protein and peptide compounds and the determination of their antimicrobial, antioxidant, anti-inflammatory, anti-tumor, enzyme-inhibiting, neurotoxic properties, as well as the synthesis of covalently bound conjugates of silk sericin with new synthetic antifungal peptides, flavonoids and phenolic compounds.

Methods of the research. The study used extraction, filtration and concentration methods, as well as physicochemical analysis methods (HPLC, NMR, UV, FT-IR, CD, GC-MS, LC-MS and SEM) and other chromatographic equipment. In vitro and in vivo research methods were used to study the biological activity of the compounds.

The scientific novelty of the study is as follows:

For the first time, a drug-resistant anti-fungal peptide (ACP8) against Candida albicans was found and described based on new bioactive peptides isolated from various sources (*Cuminum cyminum* seeds, Bactrian camel milk, scorpion hydrolysates, *Cicer arietinum* L. sprouts);

Bone marrow proteins with antibacterial/antioxidant activity were identified in four types of domestic animals, glycoproteins inhibiting COX-2 in lamb rennet, a protein inhibiting hyaluronidase from Cervus elaphus rennet, and for the first time, a method for extracting antioxidant protein from frogs was improved;

By covalent conjugation of silk sericin with flavonoids/phenols, conjugates with high anti-inflammatory and emulsifying properties were obtained;

Innovative isolation methodologies for basic bioactive proteins, purification protocols for new peptides/glycoproteins have been developed, and schematic isolation of protein from scorpion (*Buthus martensii* Karsch) has been improved;

For the first time, the protein resources of the rennet of a lamb and a red deer (*Cervus elaphus*) were systematically studied, and the bioactive potential of the isolated proteins was determined;

By combining chromatographic, spectroscopic, computer analysis, and biochemical approaches, new functional proteins with anti-inflammatory, antitumor, and hyaluronidase-inhibiting activity have been isolated and antioxidant peptides (for example, SAPHP-A, SAPHP-B, LPTETLH, IEEDLER) not registered in databases have been identified;

The relationship between the molecular structural indicators (molecular weight, hydrophobicity, amino acid composition) and antioxidant and metal-chelating activity of the isolated compounds was determined;

new concepts of structural-functional relationships of animal/plant proteins and new theoretical and practical foundations for the effective use of biologically active proteins and peptides from natural resources have been developed.

The practical results of the research are as follows:

Optimized methods and protocols of extraction, hydrolysis and purification have been developed for obtaining and studying biologically active proteins and peptides from natural sources;

It has been proven that the peptide TFI-b1, TFI-b2, TFI-b3, obtained from Bactrian camel milk, and the peptide P4-1 and P4-2, obtained from the *Buthus*

martensii Karsch scorpion, are useful as antioxidants in the development of functional food products;

It has been established that peptides isolated from chickpea (*Cicer arietinum* L.) flour are useful in neutralizing free radicals in functional foods, and cumin (*Cuminum cyminum* L.) seeds are promising as preservatives in food products;

It was found that the new peptide ACC8, developed on the basis of the peptide structure obtained from the secretion of Ranacyclin JSC frog skin, exhibits high antimicrobial and anti-inflammatory activity;

It has been established that the glycoprotein RDA4-1 obtained from the rumen of the red deer (*Cervus elaphus*) has an antioxidant and hyaluronidase-inhibiting effect, which suggests its potential for the development of nutrative agents;

It was found that the peptide SHTCF2III isolated from the trypsin hydrolysate of sericin exhibits the property of chelating the copper ion;

Two new peptides, SAPHP-A and SAPHP-B, with high antioxidant activity, were isolated from sheep rennet protein SAPH and recommended for use as food additives and pharmaceutical products.

Reliability of research results is confirmed by the internationally accepted and validated scientific methods applied in the experiments. The structural identity and purity of all novel compounds were rigorously confirmed using multiple, complementary high-precision analytical techniques (HPLC, SDS-PAGE, LC-MS, MALDI-TOF-MS/MS). The data were subjected to rigorous statistical analysis, including ANOVA and regression modeling, to confirm the significance of the observed effects and relationships. The validity of the results obtained is also confirmed by the publication of the results in peer-reviewed foreign scientific publications, discussion at international conferences and the receipt of patents.

Scientific and practical significance of research results. The scientific significance of the research is explained by the fact that the source of bioactive peptides and proteins has been significantly expanded, dozens of new molecules with fully defined amino acid sequences have been introduced into the scientific literature, new scientific concepts have been formed on the relationship between the structure and biological activity of peptides with different biological functions based on the studied samples, new neurotoxins and enzyme inhibitors identified as a result of the research are of great importance as valuable molecular means for studying complex biological systems, such as ion channels, extracellular matrix degradation, signaling pathways.

The practical significance of the research results lies in the fact that the bioactive compounds identified within the framework of the study have been proposed as promising molecules for new drugs that can be effective against antibiotic resistance (ACK8, cumin peptides), inflammatory processes (reindeer rennet glycoproteins), and skin diseases associated with aging (reindeer rennet proteins), and the results obtained provide a wide range of natural sources, powerful antioxidant peptides, and biologically active substances for the functional food industry, which expands practical opportunities in the development of dietary products, nutritional substances, and health-improving supplements, and the research serves to form technologies for

obtaining bioactive substances with high added value through the processing of agricultural and industrial by-products - such as bone marrow, fish skin, silk sericin.

Implementation of research results. Based on the scientific results and design of bioactive peptides of medicinal plants and animals with antibacterial, antioxidant and anti-inflammatory effects:

A method for isolating and using peptides from chickpeas was developed, and an invention patent of the People's Republic of China was obtained for this method (ZL201510191380.6.). As a result, the peptides isolated by this method can be used to quench free radicals in functional foods.

A method for isolating and using antioxidant peptides from camel milk was developed, and an invention patent of the People's Republic of China was obtained for this method (ZL201811122673.9.). As a result, these peptides can be used as antioxidant/free radical scavengers in the development of functional foods.

A method for isolating glycoproteins from Tianshan red deer abomasum with hyaluronidase inhibitory activity was developed, and an invention patent of the People's Republic of China was obtained for this method (ZL202111360384.4.). As a result, red deer abomasum can be used to treat gastritis and other diseases.

The results of the study are cited in more than 100 international scientific journals with a high impact factor: Journal of nanobiotechnology (IF 12.6), LWT-food science and technology (6.6), Phytotherapy research (IF 6.3), Food bioscience (IF 5.9), Nutrients (IF 5.0), Molecules (IF 4.6), Scientific reports (IF 3.9), Journal of food science (IF 3.4), Journal of food science and technology-mysore (3.3), Journal of food measurement and characterization (IF 3.3), Frontiers in sustainable food systems (IF 3.1), Journal of food quality (IF 2.9), Journal of separation science (IF 2.8), Current microbiology (IF 2.6), International journal of peptide research and therapeutics (IF 2.4), Biotechnology letters (IF 2.1) and others. These results made it possible to isolate protein and peptide compounds from natural sources, determine their structure, and determine their biological activity.

Approval of research results. The results, methodologies, and conclusions of this research have presented and discussed at 7 international conferences, in particular: 12th International Symposium on the Chemistry of Natural Compounds (Tashkant, Uzbekestan 2017), 13th International Symposium on the Chemistry of Natural Compounds (Shanghai, China 2019), 7th International Symposium on Edible & Medical Plant Resources and the Bioactive Ingredients (Urumqi, China 2022), 15th International Symposium on the Chemistry of Natural Compounds (Antalya, Turkiye 2023), International Symposium on the Current issues of development of bioorganic chemistry (Tashkent, Uzbekistan 2023), 8th International Symposium on Edible & Medical Plant Resources and the Bioactive Ingredients (Samarkand, Uzbekistan 2024), The First Silk Road Conference on Food Science and Foodomics (Bukhara, Uzbekistan 2025) .

Publication of research results. On this topic, 58 scientific works have

been published, including 32 in international scientific journals, 14 reports at international scientific conferences, and 12 patents of the Republic of China have been received.

The structure and size of the presentation. This presentation consists of an introduction, 10 main content parts, a general conclusion, and a list of references. The presentation volume is 120 pages, supported by numerous figures illustrating chromatographic separations, mass spectra, structural analyses, and bioactivity data, as well as tables summarizing yields, chemical compositions, and quantitative results.

MAIN CONTENT OF THE RESEARCH

We have studied the proteins and peptide compounds isolated from chickpeas, cumin seeds, scorpion (*Buthus martensii*), silkworm cocoons, lamb abomasum, Tianshan red deer abomasum, and frog skin secretions. Their chemical structures were determined using physicochemical methods, and the biological activities of the isolated compounds were also investigated.

The results of these scientific studies are presented below.

Name	**Pepties name**	**Sequence**	**Molecular Weight (Da)**	**Biological activities**
Plant Origin				
Chickpea (*Cicer arietinum* L.)	NF2-4-1	LTEIIP	685.41	Antioxidant scavenging activity: DPPH: IC_{50} 0.24 mg/ml; •OH: IC_{50} 0.57 mg/ml
Cumin (*Cuminum cyminum* L.) seeds	P1	EGGSFDECCR	1216.42	The ten peptides had better antimicrobial activity against *Escherichia coli, Candida albicans and Staphylococcus aureus*.
	P2	NVDEECRCDMLEEIAR	2054.82	
	P3	MSFLQLQQQAR	1365.69	
	P4	SQYEQLAEQNRK	1493.71	
	P5	SATQGKGEYTMEFSR	1707.74	
	P6	GGSGGSYGGGGSGGGYG GGSGSR	1791.71	
	P7	GSYGSGGSSYGSGGGSYG SGGGGGGHGSYGSGSSSGGYR	3312.26	
	P8	CAQKLDLPLDK	1243.64	
	P9	ACDQQGDSEER	1237.49	
	P10	DITAALAAERK	1158.61	
Animal Origin				
	TFI-b1	RLDGQGRPRVWLGR	1665.94	DPPH: IC_{50}1.9±0.04 mg/ml; ABTS: IC_{50}2.4±0.07 mg/ml

Camel milk	TFI-b2	TPDNIDIWLGGIAEPQVKR	2122.13	DPPH: IC_{50}1.2±0.07 mg/ml; ABTS: IC_{50}1.8±0.04 mg/ml
	TFI-b3	VAYSDDGENWTEYRDQGAVEGK	2489.09	DPPH: IC_{50}0.6±0.02 mg/ml; ABTS: IC_{50}0.9±0.02 mg/ml
Scorpion (*Buthus martensii*)	P4-1	LPTETLH	810.43	Antioxidant scavenging activity DPPH: 83.32% ABTS: 78.87% OH: 62.96%
	P4-2	IEEDLER	903.44	Antioxidant scavenging activity: DPPH: 81.22 % ABTS: 78.75% OH: 58.69%
Frog skin	AKK-1	FRWTKSYSPKPLKR	1794.13	AKK8 exhibited the highest antimicrobial activity against the four tested strainsd *E.coli*, *S.aureus*, *B.subtilis*, and *C.albicans*. The MIC of AKK8 against *C.albicans* was 18.5 μg/mL.
	AKK-2	FRWKYPKPLKR	1518.87	
	AKK-3	RWKYPKPLKR	1371.69	
	AKK-4	RWKQVKVKR	1227.52	
	AKK-5	RCVRWWKRVCK	1519.90	
	AKK-6	WRKQKVKK	1100.38	
	AKK-7	RWRFKWKK	1234.52	
	AKK-8	RWRFKWWKK	1420.73	
	AKK-9	RWRFKWAKK	1305.59	
	AKK-10	ARWRFKWAKK	1376.67	
Silkworm cocoons	1	SHHSGVNR	892.14	The five peptides have better copper ion–chelating abilities.
	2	TKDSIGGQAK	1004.40	
	3	DDSRADSSR	1007.24	
	4	SSNSNVQSDEK	1193.31	
	5	GGSVSSTGSSSNTDSSTK	1644.58	
Sheep abomasum	P3	LEDGLK	674.37	Antioxidant scavenging activity: DPPH: IC_{50} 0.63 mg/mL
	P7	IDDVLK	703.41	Antioxidant scavenging activity: DPPH: IC_{50} 0.58 mg/mL

Part 1. Antioxidant Peptide of the *Cicer arietinum* L

Aim of the task was to investigate of novel natural antioxidant peptides that could serve as potential ingredients for functional foods. For this purpose, Chickpea Sprout Protein (CSP) was hydrolyzed with trypsin, Alcalase, Neutraz protease, and papain, under optimal conditions (Table 1).

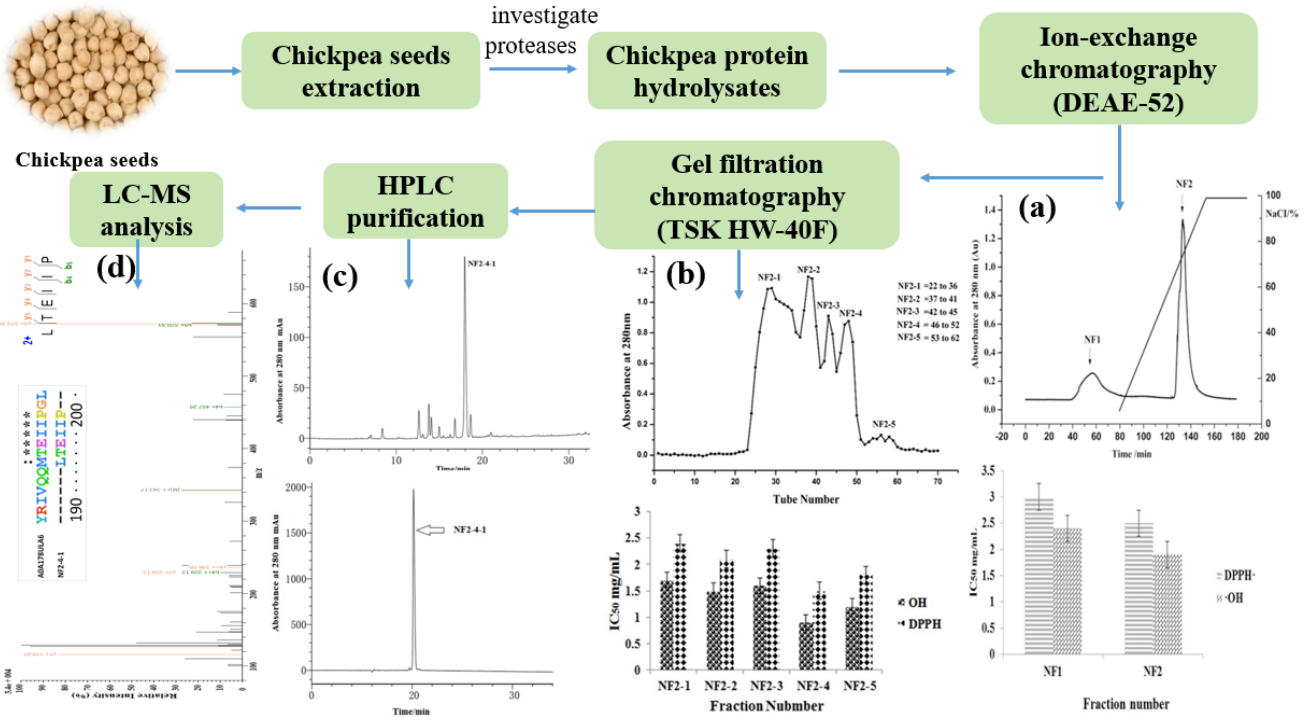


Fig. 1 Processing steps of Chickpea seeds

The yields of CSP peptides were 80; 59; 78; and 66% for NF, TF, AF, and PF, respectively, with the yield of NF peptide being significantly higher than that of the other fractions. The CSP hydrolysis profiles in SDS-PAGE is showing that CSP after hydrolysis by Neutraz protease was significantly better than other proteolytic enzymes. The MW of NF peptides after enzymatic hydrolysis was mainly below 20 kDa. The result shows that Neutrase protease has a signify (Fig. 2) cantly higher DH than other proteases during 4 hours of hydrolysis.

Table 1 Yields and degree of hydrolysis (DH) of each peptide fractions

Samples	Yield, %	DH (%)
Chickpea protein	100	
Neutrase fractions (NF)	80 ± 0.09	19 ± 0.07
Trypsin fractions (TF)	59 ± 0.05	14 ± 0.16
Alcalase fractions (AF)	78 ± 0.12	13 ± 0.15
Papain fractions (PF)	66 ± 0.11	16 ± 0.08

Kim et al. estimated that amino acids such as: His, Pro, Ala, Gly, Glu, and Leu contribute to improved antioxidant activity. The results of amino acid contents of hydrolysates digested with trypsin, Neutraze, Alcalase, and papain (Table 2) were 710.1, 982.7, 673.0, and 804.7 mg/g, respectively. Moreover, the content of amino acids such as Ile, Leu, Val, and Ala in NF was the highest (27.84%), indicating the

highest antioxidant activity among the peptide fractions. Furthermore, the total amino acid content in the NF fraction was 982.7 mg/g, which is higher than in the other fractions. A possible reason for its strong antioxidant activity was the high content of Phe, His, Pro, Met, Ile, and Cys in the NF components. These results suggest that the antioxidant activity of the peptides is related to the hydrophobic properties of amino acids.

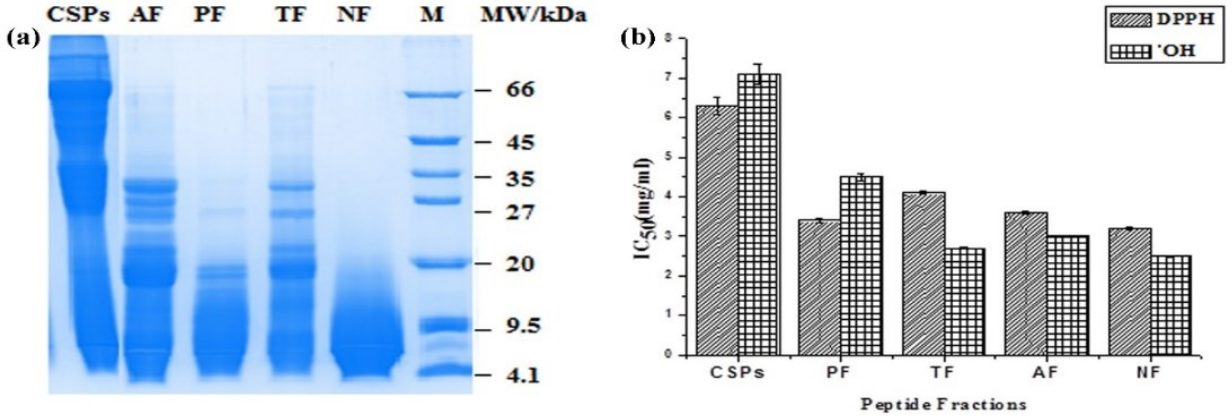


Fig. 2 (a) SDS-PAAGE 15%: M - markers; CSP - Chickpea protein; AF-Alcalase, PF-Papain, TF-Trypsin, NF - Neutrase fractions. (b) IC50 values of the CSP extracts and four different peptide fractions against DPPH˙ and •OH.

Fig. 2b shows activity of hydrolysates toward DPPH˙ and •OH: NF, TF, AF, and PF. The order of activity of the peptide fractions (5 mg/ml) toward DPPH˙ is: NF > PF > AF > TF >> CSP. The order of activity of the peptide fractions (5 mg/ml) toward OH is: NF > TF > AF > PF >> CSP. The low molecular peptide had antioxidant activity. Therefore, neutrase hydrolysate was chosen for further separation and activity analysis.

Table 2 Amino acid composition (mg/g) of different CSP hydrolysate peptide fractions

Amino acids	CSP	NF	AF	TF	PF
Ala	15.3	96.1	28.1	33.8	26.9
Val	16.9	52.7	27.3	31.9	56.9
Phe	22.3	69.5	42.1	49.9	38.3
Pro	21.1	81.6	39.7	37.8	34.7
Met	9.1	9.9	6.7	9.1	0.51
Ile	19.5	59.2	29.9	33.3	23.9
Leu	42.0	65.6	53.2	44.7	55.1
Tyr	11.6	39.9	18.2	20.9	26.2
Gly	15.7	30.6	26.4	29.0	39.4
Ser	27.1	77.4	39.3	44.6	40.5
Thr	13.5	62.6	21.9	22.5	27.9
Asp	87.5	77.0	78.9	101.3	123.0
Glu	141.8	136.0	161.3	162.3	190.3
Lys	46.6	66.2	54.3	44.6	75.9
His	11.6	19.1	16.2	12.6	17.2
Arg	38.7	39.3	29.5	31.8	23.4
Total	**540.3**	**982.7**	**673.0**	**710.1**	**804.7**

Dissolved in 20 mM sodium acetate buffer (pH 7.8) NF, applied to a DE-52 column and in a result, two: NF1 and NF2 fractions were collected (Fig. 1a). Also antioxidant activity of NF2 against to DPPH˙ was highest IC_{50} = 2.50 mg/mL and 1.90 mg/mL against γ-hydroxyphosphate. After that, NF2 fraction was separated to 5 fractions on a TSK HW-40F column (Fig. 1b). Fraction NF2-4 (Fig. 1b) showed high activity towards DPPH˙ and •OH (IC_{50} = 1.50 mg/mL for DPPH and 0.90 mg/mL for •OH). Then, fraction NF2-4 was additionally purified by HPLC on an Agilent C18 column (9.4 x 250 mm). Hydrophobic peptides were eluted using MeCN gradient (10% - 0-2 min, 10-40% - 2-20 min, 40-50% - 20-35 min, 50-60% - 35-40 min, 60-10% - 40-45 min, in 0.1% TFA) (Fig. 1c). As a result, the antioxidant peptide NF2-4-1 was obtained (IC_{50} = 0.24 mg/ml for DPPH˙, 0.57 mg/ml for •OH). The purity of the antioxidant peptide was 92.8% (Fig. 1c, Table 3).

Table 3 Purification of antioxidant peptide from the fraction NF

Fractions	Steps	Antioxidant activities (IC_{50}, mg/mL)	
		DPPH˙	·OH
CSP		6.30 ± 0.04	7.10 ± 0.08
Neutrase fractions (NF)	Hydrolysis	3.20 ± 0.09	2.50 ± 0.05
NF2	DEAE-52	2.50 ± 0.05	1.90 ± 0.08
NF2-4	TSK HW-40F	1.50 ± 0.07	0.90 ± 0.03
NF2-4-1	RP-HPLC	0.24 ± 0.06	0.57 ± 0.04

The structure of the NF2-4-1 peptide was determined by MALDI-TOF-MS/MS to be Leu-Thr-Glu-IIe-IIe-Pro (LTEIIP), and the MW is 685.41 Da (Fig. 4). Blast analysis of the NF2-4-1 (LTEIIP) amino acid sequence reveals a low match (E = 19.5) with an uncharacterized protein from Arabidopsis thaliana with Uniprot accession number A0A178ULA6-1 (residues 195–200) and several other plant proteins (Fig. 3).

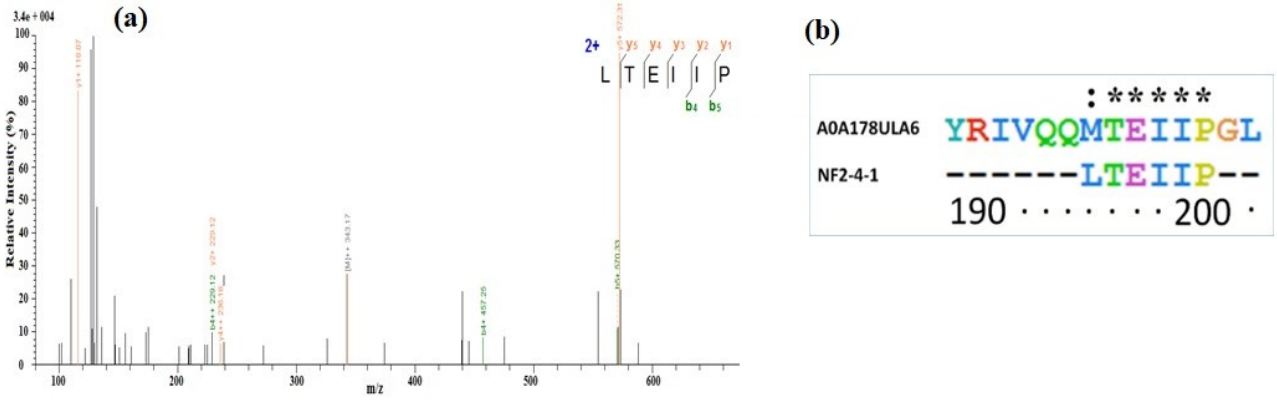


Fig 3 (a) Mass spectra and (b) blast pattern of the NF2-4-1 peptide.

The antioxidant activity of CSP hydrolysates was studied by Torres-Fuentes and their study of FVPH, ALEPDHR, TETWNPNHPEL, and SAEHGSLH examined the antioxidant properties of four chickpea peptides. The results showed that CSP hydrolysates can be used as natural antioxidants to enhance the antioxidant properties of functional foods and prevent oxidative reactions during food production. Further research is needed to elucidate the role of antioxidant peptides in human health. One of the isolated peptides, with the amino acid sequence YLEELHRLNAGY, exhibited

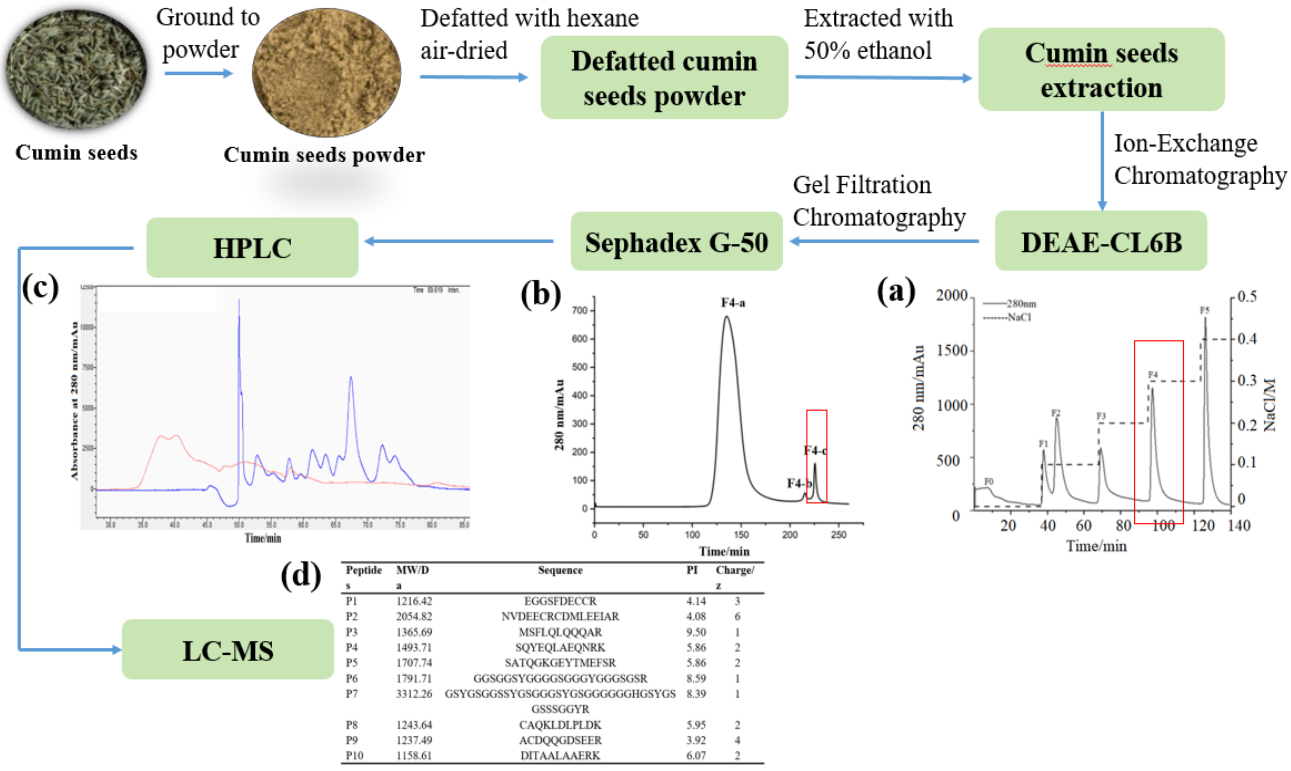
the highest antioxidant activity (IC_{50} <0.01 mg/ml) and contained the amino acids Glu and Leu.

Several studies have shown that amino acids, such as Leu, Glu, His, Met, and others, play a significant role in the various properties of peptides. However, the NF2-4-1 peptide, due to the presence of Pro and Leu had antioxidant activity. The ratio of hydrophobic to acidic amino acids was 83.3% (LTEIIP). The results showed that the Leu-Glu and Isolate-isolate-protamate sequences presumably react with free radicals, causing their conversion into more stable products and possibly playing a significant role in antioxidant activity.

Conclusion:

The conditions for the hydrolysis of chickpea flour protein from *Cicer arietinum* L. with trypsin, papain, pepsin, alcalase, and neutrase-protease enzymes were studied. Hydrolysis with neutrase-protease enzyme was the most efficient, with peptides with a molecular weight of less than 20 kDa being produced in 80% yield. A 3-step method consisting of ion exchange (DE-52 cellulose), gel chromatography (HW-40F), and HPLC (Agilent C18 column) was used to isolate the neutrase-peptide fraction from the neutrase peptide fraction with a purity of 92.8%. It was found that its molecular weight was 685.41 Da and it consisted of 6 LTEIIP amino acid sequences. The antioxidant activity of NF2-4-1 peptide was shown to be IC_{50} = 0.24 mg/ml by DPPH˙ and IC_{50} = 0.57 mg/ml by •OH. These peptides can be used to quench free radicals in functional foods.

Part 2. Antimicrobial Peptides of the *Cuminum cyminum* L Seeds



Peptides	MW/Da	Sequence	PI	Charge/z
P1	1216.42	EGGSFDECCR	4.14	3
P2	2054.82	NVDEECRCDMLEEIAR	4.08	6
P3	1365.69	MSFLQLQQQAR	9.50	1
P4	1493.71	SQYEQLAEQNRK	5.86	2
P5	1707.74	SATQGKGEYTMEFSR	5.86	2
P6	1791.71	GGSGGSYGGGGSGGGYGGGSGSR	8.59	1
P7	3312.26	GSYGSGGSSYGSGGGSYGSGGGGGGHGSYGSGSSSGGYR	8.39	1
P8	1243.64	CAQKLDLPLDK	5.95	2
P9	1237.49	ACDQQGDSEER	3.92	4
P10	1158.61	DITAALAAERK	6.07	2

Fig. 4 Processing steps of Cumin seeds

Aim of the task was to investigate of antimicrobial peptides from cumin (*Cuminum cyminum* L.) seeds and assess their potential as natural agents against pathogenic microorganisms. For that cumin seed subjected to following processing:

A 1000 g of cumin seeds was powdered and defatted using n-hexane, yielding 756g of defatted cumin seed powder. Under the optimized extraction conditions, powder was extracted three times with 50% ethanol at room temperature (25°C) for 3 hours each time. The combined extracts were filtered and then freeze-dried, resulting in 239g of crude protein powder. After loading the cumin protein extract onto a DEAE CL-6B column (Fig 4a), six peaks were observed. All five fractions were collected, desalted, freeze-dried, and weighed (Table 4).

Table 4 *Cuminum cyminum* L. seed peptides purification steps

Purification step		**Yield**	**Antimicrobial activity**		
		(mg/10g)	**EC**	**CA**	**SA**
Cumin extraction		10000	+	+	+
DEAE CL-6B	F1	105.63	-	-	-
	F2	326.71	-	-	-
	F3	298.08	-	-	-
	F4	705.25	+	+	+
	F5	1085.93	+	-	-
Sephadex G-50	F4-a	267.53	-	-	+
	F4-b	112.84	-	+	-
	F4-c	115.50	+	+	+

The peptides fraction were tested by in vitro susceptibility testing for antibacterial activity against EC (*Escherichia coli*, ATCC11229), CA (*Candida albicans*, ATCC10231) and SA (*Staphylococcus aureus*, ATCC6538) (Table 4). A comparison to the results in Table 3 showed that F4 fraction had better antimicrobial activity against *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*, and F5 fraction had better antimicrobial activity against *Escherichia coli*.

Based on the SDS-PAGE electrophoresis and preliminary antibacterial activity screening analysis, the F4 fraction eluted from the DEAE CL-6B chromatography column was selected for further purification using a Sephadex G-50 chromatography column. The the elution peaks are shown in Fig. 4. Three major elution peaks (F4-a, F4-b, and F4-c) were collected, desalted, freeze-dried, and weighed (Table 4) for subsequent analysis and purification.

The antimicrobial results in Table 4 showed that F4-a fraction had better antimicrobial activity against *Staphylococcus aureus*, F4-b fraction had better antimicrobial activity against *Candida albicans*, and F4-c fraction had better antimicrobial activity against *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*. Based on the results of the analysis, the F4-c fraction obtained from gel chromatography will be further separation and purification using HPLC.

HPLC condition has good resolution that there are more than 9 peptides were found from F4-c fraction (Fig. 4c). In order to confirm peptides constitutions of F4-c fraction, the fraction was subject to the LC/MS analysis. Samples (2 μL) were placed

onto a Zorbax SB C18 column (75 μm × 43 mm, 5 μm) using an Agilent Technologies Micro WPS instrument. The gradient consisted of buffer A (water + 0.1% TFA) for 1 min followed by a linear gradient from 5 to 45% buffer B (acetonitrile + 0.1% TFA) over the next 60 min at a flow rate of 3 mL/min.

The LC/MS analysis of 30% methanol eluted fraction results indicated that peptides fraction contain more than 10 acidic peptides, 7 neutral peptides, 4 alkalesence peptides. They have molecular weight from 2479.13 to 9768.51 Da. Acidic peptides and Neutral peptides were further subjected to reversed-phase HPLC, and results have showed that most of the peptides were appeared at 8 to 13 min. The peptides that appeared at 10.823, 12.014, 12.223 and 12.346 min were collected and their molecular mass determined by ESI-MS apparatus as 3361.56, 3122.67, 3121.64, 2498.12, 2499.12 and 2498.10 Da respectively.

Table 5 shows that ten monomeric peptide amino acid sequences were identified from the F4-c fraction. Comparison with database sequences revealed that most of these peptides share high homology with bioactive peptides such as albumin, transcription initiation factor, transforming growth factor, keratin, peptidyl-tRNA hydrolase, and other albumin-related peptides (Table 5). These peptides play important roles in the immune system and tissue repair. For example, 2S albumin and TOPBP1 are known to be involved in DNA double-strand break repair. Among them, dynamin-I contains four functional domains, each with specific functions and distinct regulatory mechanisms, playing a crucial role in synaptic vesicle endocytosis. Therefore, an in-depth investigation of the four functional domains of dynamin-I, as well as its regulatory mechanisms and sites in synaptic vesicle endocytosis, holds significant theoretical and practical importance.

Table 5. Detailed results of spot molecular mass and amino acid sequence identification by MALDI TOF/TOF

Peptides	MW/Da	Sequence	PI	Charge/z
P1	1216.42	EGGSFDECCR	4.14	3
P2	2054.82	NVDEECRCDMLEEIAR	4.08	6
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P6	1791.71	GGSGGSYGGGGSGGGYGGGSGSR	8.59	1
P7	3312.26	GSYGSGGSSYGSGGGSYGSGGGGGGHGSYG GSSSGGYR	8.39	1
P8	1243.64	CAQKLDLPLDK	5.95	2
P9	1237.49	ACDQQGDSEER	3.92	4
P10	1158.61	DITAALAAERK	6.07	2

Conclusion:

For the isolation of peptides from *Cuminum cyminum* L. seeds, a three-step separation method consisted from extraction, ion-exchange (DEAE-CL6B) and gel chromatography (Sephadex G-50) was developed. The F4-c fraction from gel chromatography was shown to be effective against E. coli, C. albicans and S. aureus

bacteria and fungi at the same time. Ten peptides with a molecular mass range of 1158.61 – 3312.26 Da were homogeneously isolated from the F4-c fraction by HPLC, and their primary structures were fully determined. These peptides are considered as a natural antibiotics and have the potential to be used as preservatives in food products in the future.

Part 3. Bioactive Protein and Peptides from Domestic Animals' Bone Marrow

The aim of this research was to conduct a comprehensive isolation, characterization, and evaluation of bioactive proteins and peptides from the bone marrow of four domestic animals: sheep (SBMP), bovine (BBMP), horse (HBMP), and camel (CBMP). Faced with the environmental and economic challenge of utilizing by-products from the meat industry, this study sought to explore animal bone marrow-a traditionally recognized but scientifically under-studied resource for its potential application in functional foods and pharmaceuticals.

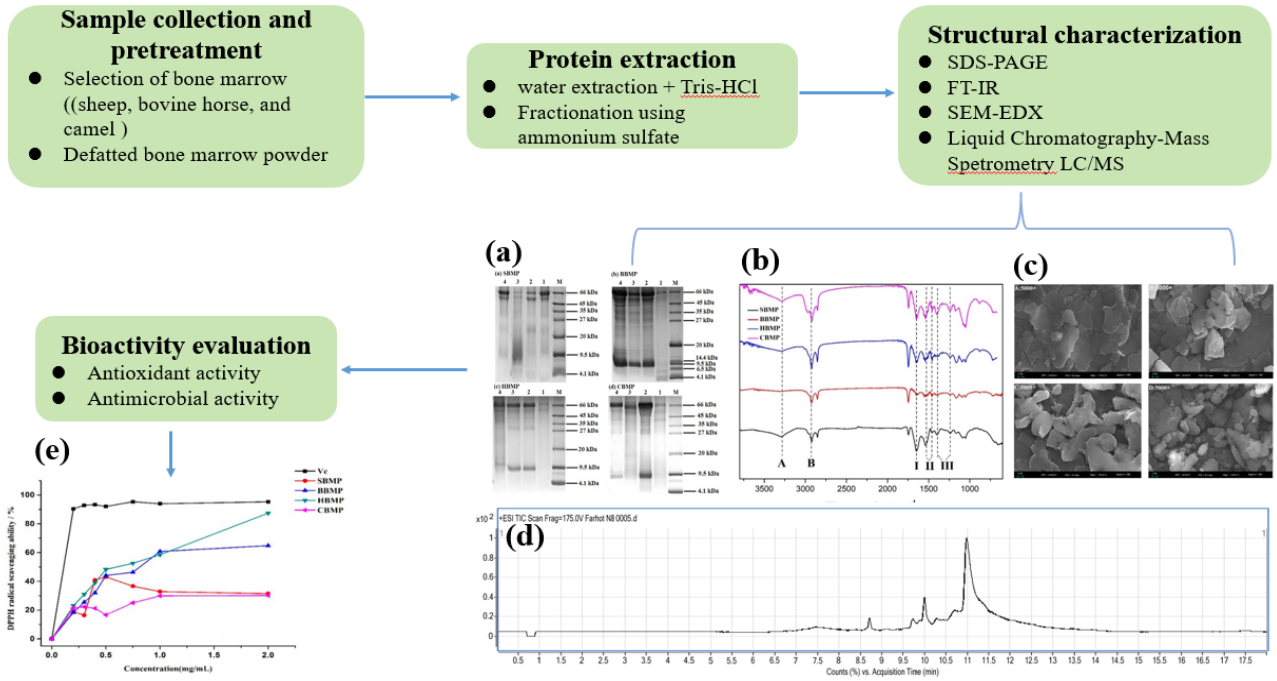

Fig. 5 Processing steps of bone marrow

In this work, proteins and peptides were extracted from four kinds of animal bone marrow using water, after which the crude extracts were fractionated using different concentrations of ammonium sulfate (30%, 50%, and 70%) to precipitate proteins and peptides of varying solubility. The yields and protein content varied significantly among the animal sources and extraction methods. In the initial water extraction of HBMP showed extraction yield at 90.47% (Table 6), likely due to its softer texture and higher oil content. The results shows that (Table 7), 50% ammonium sulfate fractionated BBMP and CBMP have the highest protein contents of 52.3 mg/mL and 56.5 mg/mL, respectively. Water extracted BBMP has the highest protein content (52.20 mg/mL) and stronger antimicrobial activity than others, which implies that there has certain dose-dependent relationship between the protein content and antimicrobial activity (Table 8).

Table 6. Comparison of water extracted proteins from different bone marrow.

No.	Protein Content (mg/mL)	Extraction Yield (%)
SBMP	20.22	86.85
BBMP	52.20	68.43
HBMP	34.60	90.47
CBMP	25.60	72.07

Table 7. Comparison of different BMP by ammonium sulfate fractions.

No.	Concentration (%)	Protein content （mg/ml）	Extraction Yield (%)
	30	40.66	7.95
SBMP	50	22.53	10.79
	70	25.37	7.38
	30	35.11	44.14
BBMP	50	52.35	52.41
	70	41.71	24.80
	30	13.6	55.70
HBMP	50	44.3	11.00
	70	25.1	5.5
	30	17.00	15.3
CBMP	50	56.5	7.50
	70	47.3	4.17

Table 8. Comparison of different BMP by ammonium sulfate fractions.

No.	Concentration (%)	CA (mm)	EC (mm)
water extracted proteins			
SBMP		8	7
BBMP		8	9
HBMP		7	8
CBMP		8	8
ammonium sulfate fractions			
	30	9	9
SBMP	50	8	-
	70	9	-
	30	9	9
BBMP	50	10	9
	70	8	10
	30	8	-
HBMP	50	9	8
	70	7	-
	30	8	-
CBMP	50	9	9
	70	8	-

Notes: CA. *Candida albicans*; EC. *Escherichia coli*.

The nutritional quality of the water-extracted proteins was determined by analyzing their free amino acid composition, revealing the presence of 17 different amino acids (Table 9). The total content of free amino acids varied dramatically, from 5.15 mg/g in SBMP to 49.63 mg/g in HBMP. The overall ranking for total free amino acid content was HBMP > BBMP > CBMP > SBMP. Each type of marrow had a different predominant amino acid: Glycine (Gly) for SBMP, Leucine (Leu) for BBMP,

Serine (Ser) for HBMP, and Alanine (Ala) for CBMP. The nutritional profile of HBMP and SBMP was particularly noteworthy, as their ratios of essential-to-non-essential amino acids (E/N) and essential-to-total amino acids (E/T) were close to the ideal pattern recommended by the FAO/WHO, indicating high nutritional potential.

Table 9. The composition and contents of free amino acids in four kinds of water extract BMP.

Amino Acid	SBMP	BBMP	HBMP	CBMP
	Content (mg/g)			
Asp	0.08	0.12	2.76	0.06
Thr	0.08	0.29	2.18	0.04
Glu	0.05	0.25	2.13	0.11
Ser	0.5	3.25	8.83	0.78
Gly	1.26	1.52	3.21	1.18
Ala	0.53	4.94	6.4	2.15
Val	0.44	5.97	3.61	1.92
Met	0.02	1.7	0.33	0.28
Ile	0.02	1.75	1.3	0.47
Leu	0.1	6.94	4.76	1.71
Tyr	0.31	3.1	2.39	1.17
Phe	0.18	4.61	3.5	1.14
Lys	1.13	4.21	4.26	1.39
His	0.09	2.43	4.07	0.67
Arg	0.01	0.12	1.96	0.07
Pro	0.36	1.87	1.54	0.85
Total amino acid (T)	5.15	43.05	49.63	13.98
Essential amino acids (E)	1.97	25.45	19.94	6.95
Non-essential amino acids (N)	3.19	17.6	29.7	7.04
Drug amino acid (D)	3.15	22.57	25.31	7.1
N/T (%)	0.62	0.41	0.6	0.5
E/T (%)	0.38	0.59	0.4	0.5
E/N (%)	0.62	1.45	0.67	0.99
D/T (%)	0.61	0.52	0.51	0.51

A suite of analytical methods was employed for the structural and molecular characterization of the isolated fractions. SDS-PAGE analysis (Fig. 5a) showed that the bone marrow extracts from all four animals contained two principal bands: a high-molecular-weight protein band at approximately 66 kDa and a low-molecular-weight polypeptide band between 4.1 kDa and 9.5 kDa. The 70% fraction of SBMP and the 30% fraction of BBMP were composed mainly of polypeptides, identifying them as ideal candidates for the further isolation of monomer peptides. These results were corroborated by LC/MS analysis, which provided a more detailed molecular profile. LC/MS identified numerous distinct peptides and proteins, such as in the SBMP water extract, where 28 peptides (1.0-8.6 kDa) and nine proteins (10.8–18.5 kDa) were detected. Similarly, 25 peptides (1.0-9.9 kDa) were found in the water extract of BBMP.

FT-IR analysis was used to investigate the secondary structure of the proteins (Fig. 5b). The spectra for all four BMPs displayed characteristic amide A, B, I, II, and III

bands, with the position of the amide I band (around 1650 cm^{-1}) indicating that the proteins are predominantly composed of α-helix structures. SEM was used to observe the surface morphology. The images showed that all protein fractions formed flake-like structures, though with noticeable differences in size, aggregation, and porosity, suggesting distinct physical properties for each animal source.

The DPPH radical scavenging assay was used to measure antioxidant capacity. All four water-extracted proteins demonstrated dose-dependent scavenging activity in the tested range of 0.025-2.0 mg/mL. Among them, Horse Bone Marrow Protein (HBMP) showed markedly superior antioxidant potential, reaching a maximum scavenging ability of 83.9% at 2 mg/mL (Fig. 5e). Its IC50 value was 0.573 mg/mL, which was significantly lower than that of BBMP (0.834 mg/mL). This higher activity is likely attributable to HBMP's greater total free amino acid content.

In the LC/MS analysis, molecular weight information was examined. A total of 28 peptides and nine proteins were found in the water extracted SBMP. The peptide Mw range was 1053.4627–8673.46 Da, and that of the proteins was 10,845.85–18,567.53 Da. A total of 25 peptides were found in the water extracted BBMP. The Mw of peptides was in the range of 1027.84–9916.32 Da. The maximum charge of peptides is +9, and the corresponding Mw is 4947.51 Da, while the minimum charge is +3, and the corresponding Mw is 2757.38 Da. A total of nine peptides and two proteins were found in the 50% ammonium sulfate precipitated part of CBMP, where the Mw of the peptides was 1040.35-3986.65 Da, and the Mw of proteins was 11,430.35–15,178.48 Da. The maximum charge of the peptides is +8, and the corresponding Mw 3986.65 Da, while the minimum charge is +4, with a corresponding Mw of 2775.4276 Da.

Conclusion:

A method for the isolation of proteins and peptides from bone marrow of sheep, bovine, horse and camel by water extraction and salting with 30, 50 and 70% (NH4)2SO4 solutions was improved. It was found that the yield of proteins in water extract of horse bone marrow reached 90.47%. At 50% saturation of (NH4)2SO4, concentration of proteins extracted from bone marrow of bovine and camel was 52.3 and 56.5 mg/ml, respectively. It was shown that salt extracts of bone marrow of various animals had a higher protein content than water extracts. Horse marrow protein has a high antioxidant activity, reaching 83.9% at 2 mg/ml, and its IC50 value was 0.573 mg/ml. The high activity can be explained by the association of large amount of free amino acids.

Part 4. Novel antioxidant peptides of the Bactrian camel milk

This study focused to identify novel antioxidant peptides from Bactrian camel milk (BCM) protein hydrolysates. The goal was to determine which enzymatic hydrolysis method produces peptides with the strongest antioxidant activity and to characterize their structures and potential bioactivities.

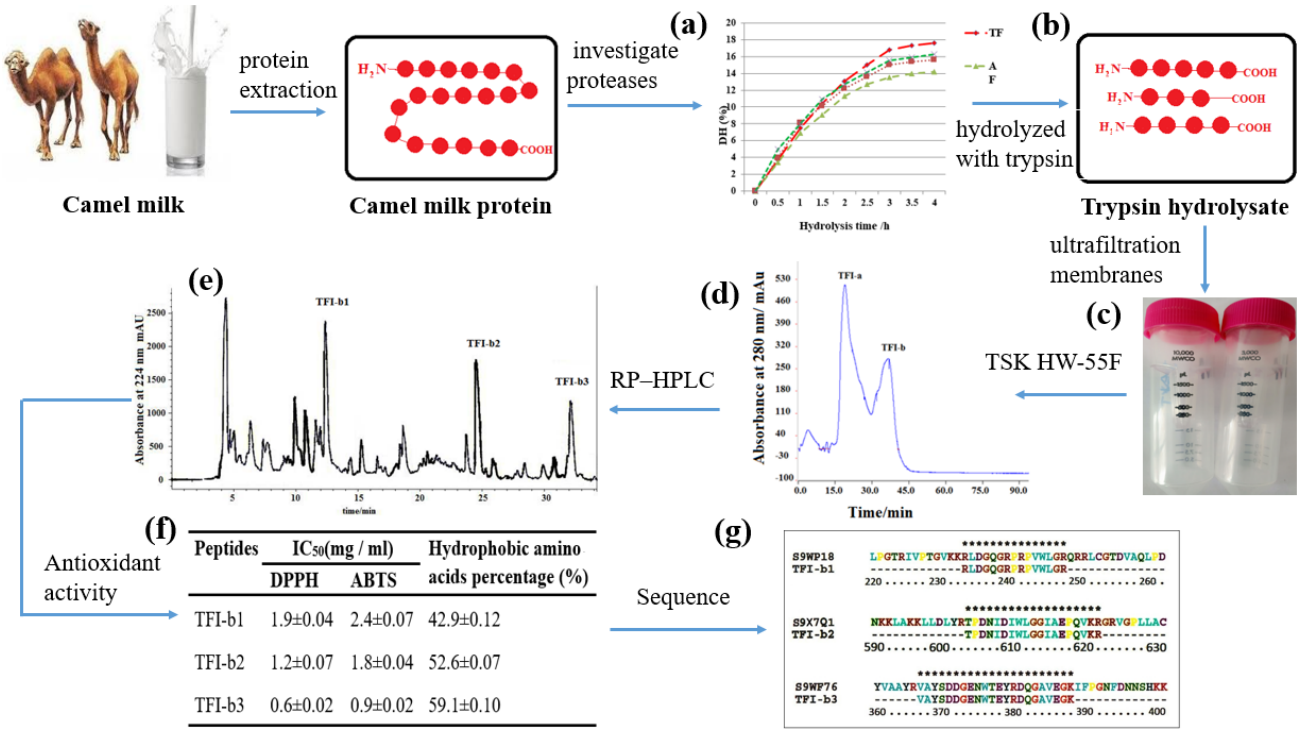

Fig.6 Processing steps of camel milk

BCM protein was hydrolyzed by four different types of enzymes at optimal conditions. The yields of peptides were measured as 67, 59, 75 and 82% for pepsin, alcalase, papain and trypsin, respectively. The yield of peptides hydrolyzed with trypsin is higher than that by other three proteases (Table 10).

Table 9 Yield and biological activities of peptide fractions

Samples	Yield (%)	DH (%)	ABTS activity/ %	DPPH activity/ %
BCM protein	100		43	39
TF	82	17.6	72	69
PF	67	16.3	64	55
AF	59	14.2	60	64
PaF	75	15.6	59	61

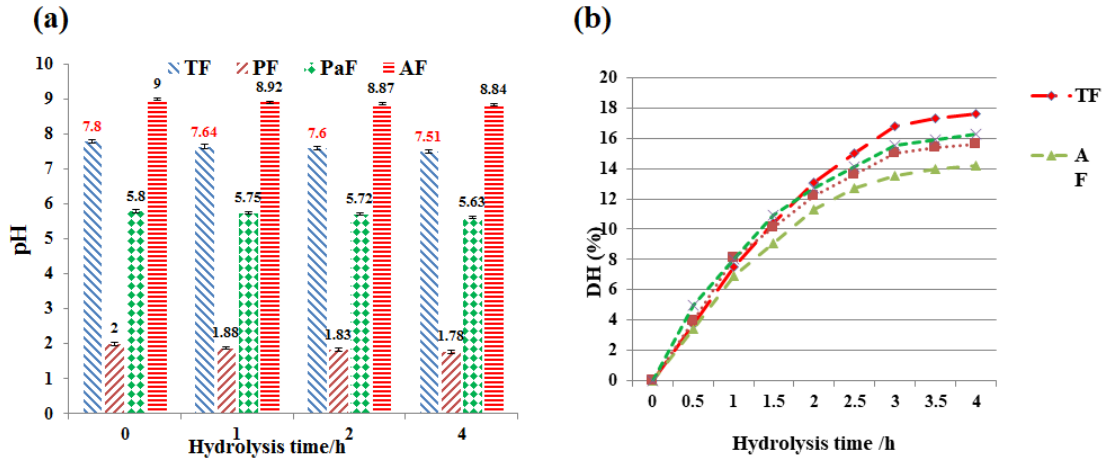

Fig. 7 (a) Change in pH of BCM protein hydrolysates; (b) DH of BCM protein with different enzymes (TF, PF, AF, PaF - trypsin, pepsin, alcalase, papain hydrolysates, respectively) (n=9).

Table 10 shows that the highest DH (17.6 %) was obtained with trypsin hydrolysate. Trypsin hydrolysate had 72% inhibition on DPPH and 69% on ABTS. Based on the present study, the trypsin fraction (TF) with high peptide yield and DH has been predicted to contribute higher antioxidant activity.

The initial pH values 7.8, 2.0, 5.8 and 9.0, for trypsin, pepsin, papain and alcalase, respectively dropped significantly to 7.52, 1.78, 5.65 and 8.84 after 4 h of hydrolysis (Fig. 7a). Compared with the alcalase, papain, and pepsin fractions, the decrease rate of pH in trypsin was higher, followed by the pepsin fractions.

The hydrolysis process of BCM protein is shown in Fig. 7b, which reveals that initially, DH increased in the first 2 hours; thereafter, the DH increased more slowly and stabilized. The hydrolysis rate decreases with the reaction time, which corresponds to lowering quantity of available peptide bonds. Four enzymes had higher hydrolyzing rate for the early 2 hours resulting in gradual fall.

It was evaluated the antioxidant activity of four BCM peptides using scavenging abilities on DPPH and ABTS (Table 9). At the 10 mg/ml concentration, the peptides scavenging effect on DPPH was in the following order: TF > PF > AF > PaF, the scavenging effect on ABTS was TF > AF > PaF > PF.

Amino acid composition of peptides along with their structure determine their antioxidative property; low molecular weight peptides tend to have strong antioxidant activity. It was selected the trypsin fraction for the separation, purification and identification of antioxidant peptides. After hydrolysis, TF was separated into two different MW fractions, labeled as TFI (< 3 kDa) and TFII (> 3 kDa). TFI exhibited the highest antioxidative activity both on DPPH and ABTS compared to TFII (Table 10).

Table 10. Purification steps of antioxidative peptides, yields and antioxidant activities (IC_{50})

Fractions	Steps	Yield / (mg/g)	Antioxidant activities (IC_{50}) (mg/mL)	
			DPPH	ABTS
TF	After hydrolysis	770	6.6±0.03	7.8±0.09
TFII (> 3kDa)	After ultrafiltration	450	5.9±0.01	7.2±0.01
TFI (< 3kDa)	After ultrafiltration	320	5.3±0.01	6.9±0.01
TFI-a	After HW-55F	175	5.1±0.05	6.6±0.03
TFI-b	After HW-55F	145	3.8±0.01	4.2±0.03
TFI-b1	After RP-HPLC	7.8	1.9±0.04	2.4±0.07
TFI-b2	After RP-HPLC	4.5	1.2±0.07	1.8±0.04
TFI-b3	After RP-HPLC	3.2	0.6±0.02	0.9±0.02

TFI fraction exhibited the high antioxidant activity, and was fractionated to two fractions (TFI-a and TFI-b) using a TSK HW-55F column (2.5 x 100 cm, Whatman, England) (Fig. 6d). The scavenging activities were measured on DPPH and ABTS+ of two fractions, and fraction TFI-b showed the highest activity (IC_{50} =3.8 mg/ml on DPPH, IC_{50} = 4.2 mg/ml on ABTS+). The low molecular weight peptides significantly increase their antioxidant activity. Therefore, the molecular weight of peptide not only

plays a key role in antioxidant activity, but also plays an important role in other factors such as peptide sequence.

TFI-b with high antioxidant activity was purified by RP–HPLC using a semi-preparative C_{18} Agilent column. The DPPH and ABTS+ scavenging activities (Fig. 8a, Table 11) of the three individual peptides (TFI-b1, TFI-b2, and TFI-b3) were determined. IC_{50} values of DPPH are TFI-b1 1.9, TFI-b2 1.2, TFI-b3 0.6 mg/ml and IC_{50} values of ABTS were 2.4, 1.8 and 0.9 mg/ml, respectively. TFI-b3 exhibited higher DPPH and ABTS scavenging activity compared to that of other fractions. Table 12 also lists the steps of purification. In general, the IC_{50} value of antioxidative peptides was increased 5 to 10 times by three-step purification.

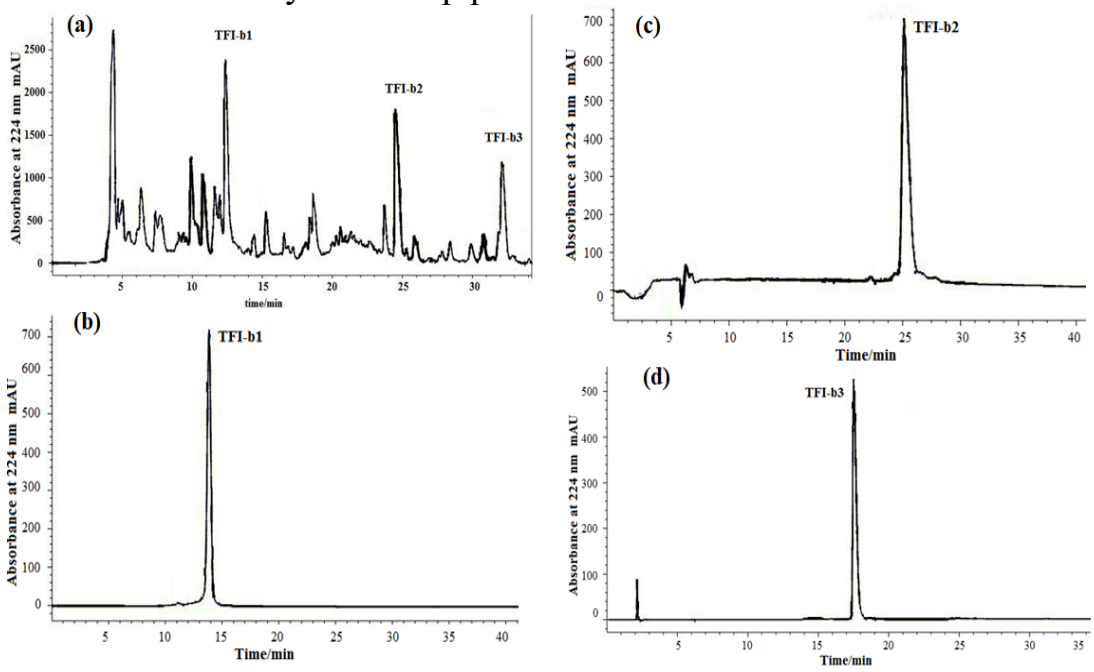

Fig. 8 HPLC analysis of: (a) TFI-b; (b) TFI-b1; (c) TFI-b2; (d) TFI-b3 antioxidant peptides.

The amino acid sequences of the individual three peptides (TFI-b1, TFI-b2, and TFI-b3) (Table 11) were identified by MALDI-TOF-MS/MS. Molecular masses of TFI-b1, TFI-b2 and TFI-b3 were 1665.94, 2122.13 and 2489.09 Da, respectively.

Studies have shown that milk peptides generally has hydrophobic (Tyr, Pro, Trp and His), acidic amino acids and its amides (Asn and Asp, Gln and Glu). Milk protein hydrolysates could be used to prevent oxidation in food processing.

Table 11. Antioxidant activity of TFI-b1, TFI-b2 and TFI-b3 purified from camel milk

Peptides	Sequence	IC_{50}(mg/ml)		Hydrophobec amino acids, (%)
		DPPH	ABTS	
TFI-b1	RLDGQGRPRVWLGR	1.9	2.4	42.9
TFI-b2	TPDNIDIWLGGIAEPQVKR	1.2	1.8	52.6
TFI-b3	VAYSDDGENWTEYRDQGAVEGK	0.6	0.9	59.1

Conclusion:

BCM proteins were hydrolyzed under optimal conditions using 4 nypes of enzymes: pepsin, alcalase, papain, trypsin and formed yields of peptides were 67, 59, 75 and 82%, respectively. After separation of TF (which was 82%) by 3 step chromatography (ultrafiltration, gel filtration and HPLC), and 3 antioxidant active peptides TFI-b1, TFI-b2 and TFI-b3 were identified. IC50 values of DPPH are TFI-b1 1.9, TFI-b2 1.2, TFI-b3 0.6 mg/ml and IC50 values of ABTS were 2.4, 1.8 and 0.9 mg/ml, respectively. TFI-b3 exhibited higher DPPH and ABTS scavenging activity compared to that of other fractions. Primary structures and molecular mass of peptides TFI-b1, TFI-b2 and TFI-b3 by MALDI TOF-MS/MS determined as: RLDGQGRPRVWLGR, TPDNIDIWLGGIAEPQVKR, and VAYSDDGENWTEYRDQGAVEGK respectively. Studied peptides may be considered as antioxidant/free radical scavenging remedy in developing of functional foods.

Part 5. Characterization of scorpion proteins

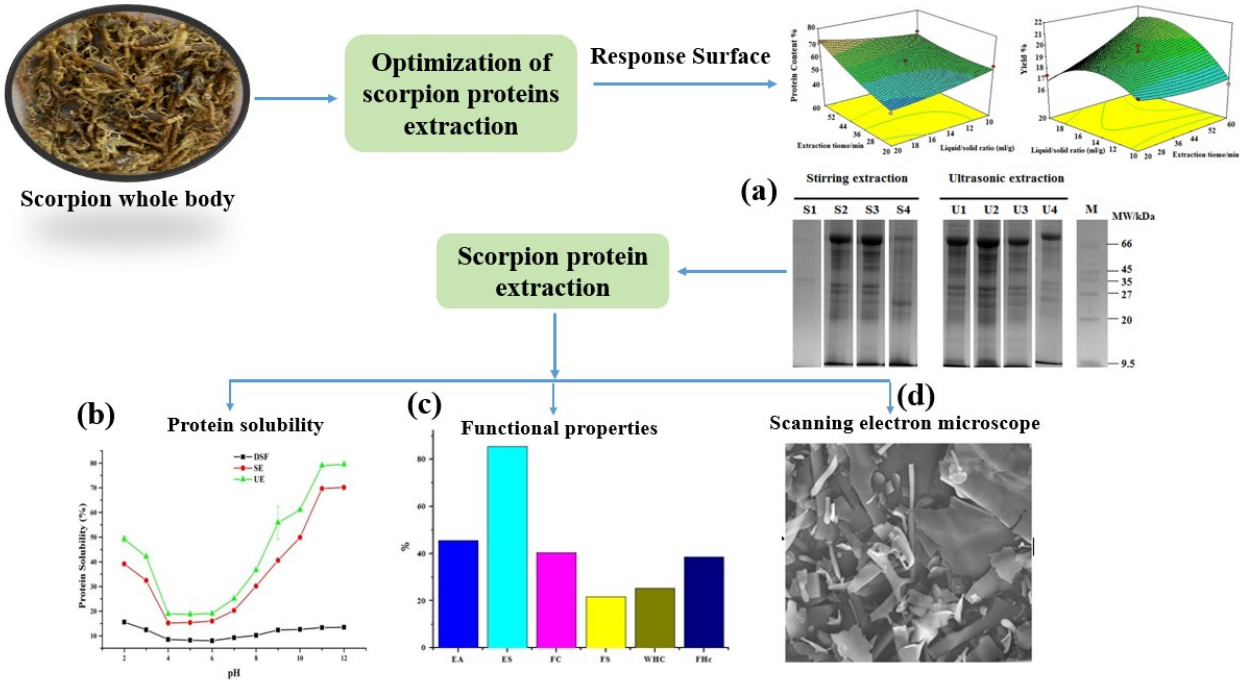

Fig. 9 Processing and study steps of scorpion proteins

The main aim of the study was to develop an optimized protein extraction process from scorpion whole body using response surface methodology (RSM), evaluate the amino acid composition and functional characteristics of the extracted proteins, and assess the potential of scorpion proteins as a new source of bioactive ingredients with possible industrial and nutritional value.

In this study, the effects of diverse buffer solutions for the extraction of scorpion protein were studied. Meanwhile, the effects of ultrasonic and stirring extraction on the yield and protein content were compared. Fig. 9a and Table 14 shows the order of the effects of four buffer solutions on yield and protein content is 0.5 M NaCl > 20 mM PBS > 0.02 M NaOH > water. 0.5 M NaCl buffer solution (yield 14.64 ± 0.08%, protein content 79.06 ± 0.05%) with ultrasonic been better than other buffers for extracting

scorpion protein, followed by 20 mM PBS (yield 18.29 ± 0.05%, protein content 60.98 ± 0.07%).

Table 12 Effects of ultrasonic and stirring methods on extraction of total proteins from scorpion body

Extraction method	Extraction on Yield (%)		Extraction on Protein (%)	
	Ultrasonic	Stirring	Ultrasonic	Stirring
Water	34.85 ± 0.06	34.15 ± 0.09	31.14 ± 0.04	18.08 ± 0.06
0.5 M NaCl	14.64 ± 0.08	11.80 ± 0.03	**79.06 ± 0.05**	35.26 ± 0.08
20 mM PBS	18.29 ± 0.05	13.45 ± 0.10	60.98 ± 0.07	37.25 ± 0.09
0.02 M NaOH	7.70 ± 0.11	7.57 ± 0.13	50.87 ± 0.07	51.91± 0.17

For extraction, ultrasound power - 200 W, the liquid / solid ratio - 15, and time – 20-60 minutes (Fig. 10a). Increase of time, the yield and protein content rapidly increased and reached to maximum at 50 minute to 17.95% and 66.35%, respectively. Further increase of time did not affect the yield and amount of protein.

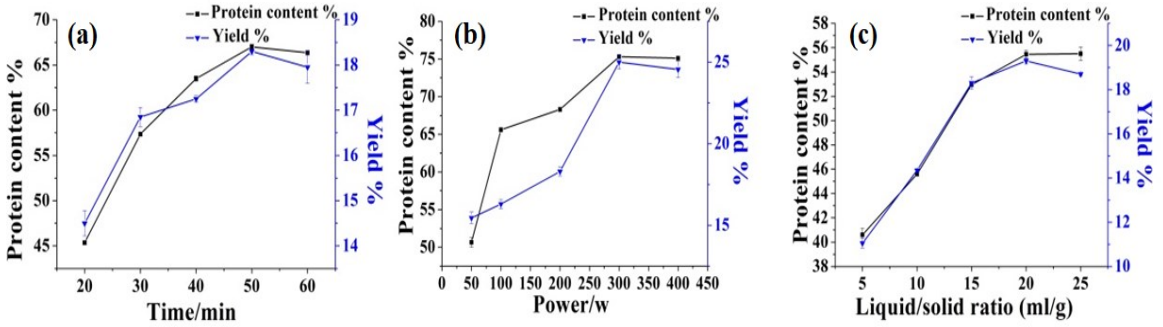

Fig. 10 Effects of extraction time (a), ultrasonic power (b), and liquid/solid ratio (c) on yield and protein content.

To estimate the effect of ultrasonic power on yield and protein content extraction time was 50 min, liquid/solid ratio (mL/g) is 15, the ultrasonic powers were 50 − 400 w (Fig. 10b). Obtained data show that increase in power, the yield and protein content increased rapidly and reached to maximum at 300 w to 23.60% and 75.30%, respectively. Further increase of time did not affect the yield and amount of protein.

Effects of liquid/solid ratio on yield and protein content were studied under ultrasound power - 200 W, extraction time - 50 minute, and the liquid / solid ratio was evaluated in the range between 5 − 25 mL/g (Fig. 10c). Increase of liquid/solid ratio until the 15 mL/g the yield and protein content rapidly increased and when reached to 20 mL/g it can be seen lower increase and reaching to maximum 19.21% and 55.32%, respectively. Further increase of time did not affect the yield and amount of protein.

Optimization of extraction parameters by RSM

When the factor sign is positive, it shows that the amount of the response variable increases with an increase in its value and vice versa. Fig. 11 (a, c) can also be used to compare the model predicted and the experimental results. Fig. 11 (b, d) is the normal probability chart indicating that the points follow a narrow linear pattern. The analysis of variance of the regression equation is shown in Table 3. F - value of the regression model on protein content and yield were 17.49, 34.12 and the P-value was 0.0005, < 0.0001, respectively. These values showed that the model obtained was significant. F-

value and P-value of Lack of Fit on protein content and yield were 1.54, 2.04 and 0.3354, 0.2506, respectively, which shows that the equation has a good fitting degree and high reliability. In addition, the decision coefficient (R2) of the model were 0.9574, 0.9777 and the adjusted coefficient of determination (Adj-R2) were 0.9027, 0.9491, respectively.

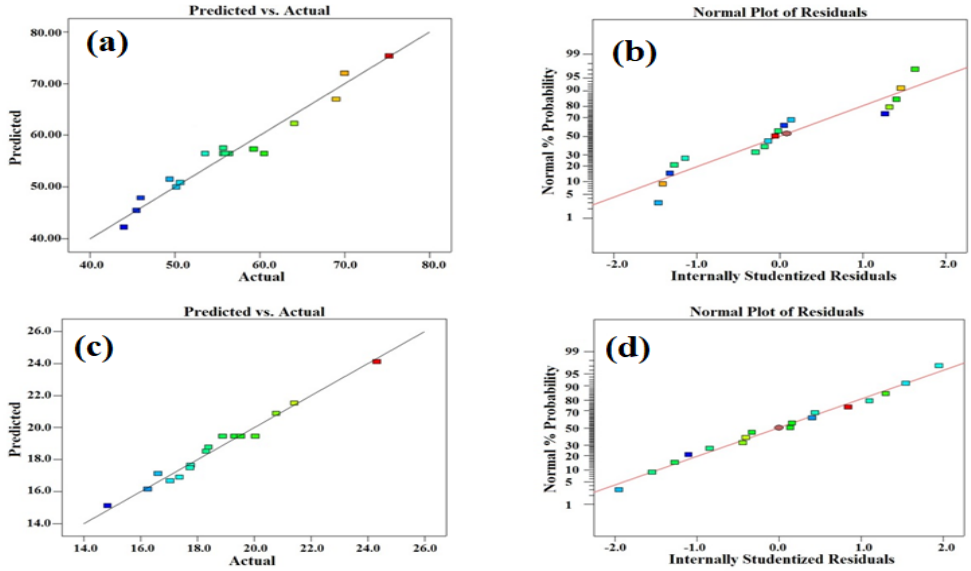

Fig. 11 Standard statistical diagrams for model verification. (a,c) model-predicted values versus actual data. (b,d) normal probability plot of the residuals.

Contour plots for each fitted models that display the effects of the three variables (to visualize the combined effects of the three factors on protein content and yield) were generated. Fig. 12 illustrates the 2D plots of the binary interactions of the variables on protein content and yield via the contour plots.

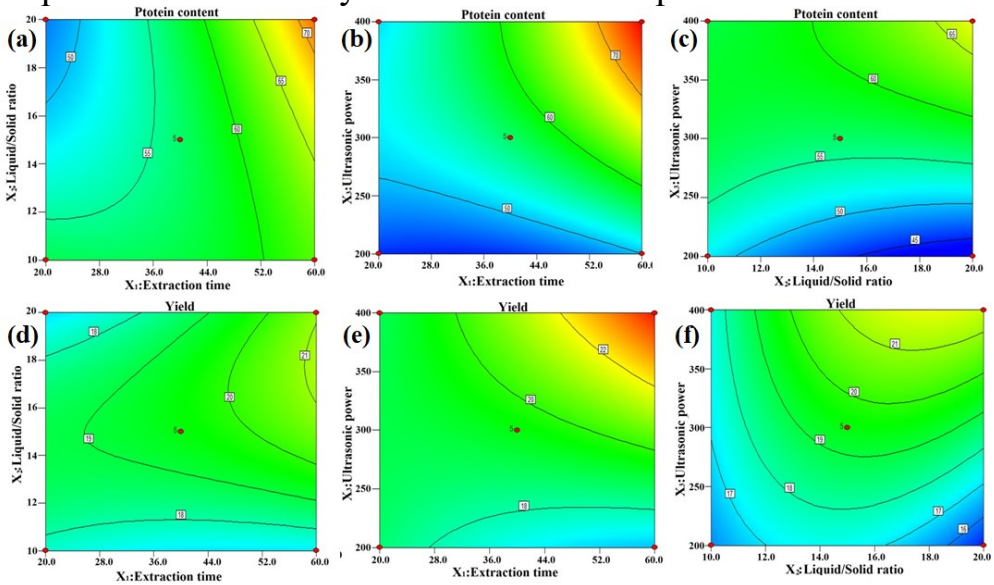

Fig. 12 Effects of operational variables on protein content and yield Interaction between: (a) liquid/solid ratio and extraction time; (b) ultrasonic power and extraction time; (c) ultrasonic power and liquid/solid ratio to protein content; (d) liquid/solid ratio and extraction time; (e) ultrasonic power and extraction time; (f) ultrasonic power and liquid/solid ratio to yield.

The interaction between the extraction time and the liquid/solid ratio is shown in Fig. 12 (a, d). This plot indicates that protein content and yield depends more on X1 than on X2. Fig. 12 (a, d) reveals that at low values of X1, maximum protein content and yield occurs at higher values of X2. However, at higher values of X1, maximum protein content and yield occurs at lower values of X2. As seen in Fig. 12 (a, d), the interaction between the two factors is weak. The interaction between the extraction time and ultrasonic power is shown in Fig. 12 (b, e). This plot indicates that protein content and yield depends more on X3 than on X1. In Fig. 12 (c, f), the effect of the interaction between the liquid/solid ratio and ultrasonic power is depicted. Fig. 12 (c, f) shows that increasing the liquid/solid ratio at different ultrasonic power has no important effect on the protein content and yield, so the plot shows that protein content and yield depends more on X3 than on X2. Therefore, the optimum values of X1, X2, and X3 as determined by the software are 50 min, 400 w, and 18 mL/g, respectively.

Determination and validation of optimal extraction conditions

Optimum conditions, which were obtained by Design-Expert V8.0.6 software, as follows: extraction time - 47.68 min; ultrasonic power - 395.84 w; and solid/liquid ratios - 18.01 mL/g. In view of the feasibility of the experiment, the optimum conditions of extraction proteins were adjusted as follows: extraction time 50.00 min, ultrasonic power 400 w and solid/liquid ratio 18.00 mL/g. After several tests ($n > 3$), the protein content and yield were 78.94%, and 24.80%, respectively. It is shown that the regression equation and the optimal conditions obtained by response surface method are reliable.

Functional properties of SP

The protein solubility profiles of de-oiled scorpion flour (DSF), ultrasonic extraction (UE) and stirring extraction (SE) in the water at different pH range (2.0-12.0) were shown in Fig. 10b. PS of DSF, UE and SE were significantly different and Fig. 10a showed the same U-shaped curves. When the pH value is in the range of 2-4, the solubility of DSF, UE and SE decreased, but when in the range of 6-10, the solubility of DSF, UE and SE were significantly increased. The minimum protein solubility of DSF, UE and SE were presented at pH 4.0 with values of 8.05%, 15.25% and 18.75%, respectively. While the maximum protein solubility was presented at pH 12 with values of 13.5%, 70.15% and 79.5%, respectively. So, scorpion protein extracted by ultrasonic method showed exceptional solubility at alkaline pH.

Obtained results by water absorption capacity (WAC) and oil absorption capacity (OAC) of proteins extracted by ultrasonic and stirring are presented in the Table 13. WAC and OAC of both methods were significantly different, with UE having the highest WAC (40.3) and OAC (27.70) than SE with WAC (33.45) and OAC (18.80) at pH 7.0.

Results of emulsifying properties of UE and SE are shown in Table 13. At pH 7.0, the emulsifying properties of UE and SE were significantly different to each other with values of 45.55%, 40.25%, respectively. However, the emulsifying property of UE was significantly different and was higher (85.50%) than that of SE (69.45%). The UE had pronounced effects on the emulsifying property since it might be exposing more

hydrophobic groups to water and oil interface, giving rise to increased emulsifying capacity and stable emulsion.

Foam formation is similar to the emulsion formation. In foam formation, water molecules surround the air droplets, which is a nonpolar phase. At pH 7.0, the foam capacity (FC) of UE was considerably higher than SE, with the values of 40.30% and 33.45%, respectively. The foam stability (FS) of UE was significantly higher than SE with the values of 21.70% and 18.80%, respectively (Table 13). Results obtained show, that the UE to compare of SE has more flexible protein structure in aqueous solution and to form more stable foams for interaction in air-water interface. High protein concentration will improve foam capacity, stability, increase the viscosity and promote the formation of the interfacial multilayer membrane.

Table 13. Functional properties of scorpion proteins

Properties	Ultrasonic extraction	Stirring extraction
Water holding capacity (g/g)	25.25 ± 0.21	20.45 ± 0.07
Oil holding capacity (g/g)	38.50 ± 0.14	30.65 ± 0.64
Emulsifying activity (%)	45.55 ± 0.64	40.25 ± 0.07
Emulsion stability (%)	85.50 ± 0.28	69.45 ± 0.35
Foam capacity (%)	40.30 ± 0.28	33.45 ± 0.07
Foam stability (%)	21.70 ± 0.14	18.80 ± 0.15

Protein samples also were studied by the SEM method. Fig. 13 below, shows the surface states of protein samples extracted from whole body de-oiled protein (DSF) powder, stirred extraction (SE), and ultrasonic extraction (UE).

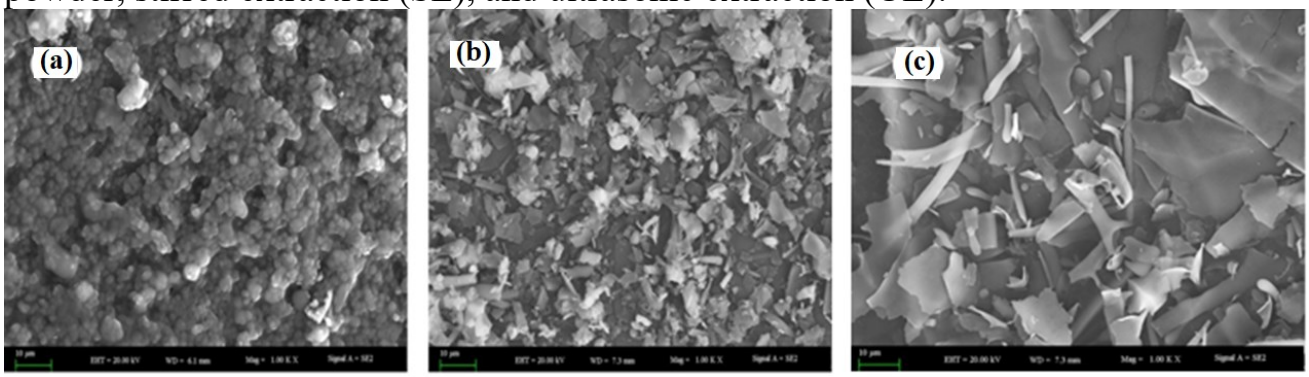

Fig. 13 Scanning electron microscope of: (a) DSF; (b) SE; (c) UE

In Fig. 13a can be seen adhesive surface layers of protein molecules. It is a collapse form, which shows high crystalline degree. In Fig. 13b, on the dried SE protein layers, plurality of elements, similar to “wood shavings” can be seen. In contrast to Fig. 13a, and these elements were formed in a result of separation from the collapsed surface. Protein molecules during drying has transition from crystalline to amorphous state, as shown in Figure 19b. Also in Fig. 13c, it can see the results of the drying of protein molecules obtained after its UE.

Conclusion：

The effects of solvents 0.5M NaCl, 20mM PBS, 0.02M NaOH and H2O on the extraction of scorpion protein were studied. Ultrasound with 0.5M NaCl showed better results than others (yield 14.64%, protein content 79.06%). The optimal extraction values for the RSM method were 50 min, 400 W and 18 ml/g for X1, X2 and X3, with

protein content and yield of 78.94% and 24.80%, respectively. The minimum protein solubility of defatted scorpion protein (DSF), ultrasonic extraction (UE) and agitation extraction (SE) at pH 4.0 was 8.05%, 15.25%, 18.75%, respectively, and the maximum solubility at pH 12 was 13.5%, 70.15% and 79.5%, respectively. The emulsification properties of UE and SE at pH 7.0 were 45.55% and 40.25%, respectively, and the foaming properties of UE at pH 7.0 were 40.30% and 33.45% higher than SE, respectively. SEM showed a highly crystalline structure of the samples resembling "wood chips". It was shown that protein molecules change from a crystalline state to an amorphous state during drying.

Part 6. Antioxidant peptides of Buthus martensii protein hydrolysates

The task of this research was to separate, purify, and characterize antioxidant peptides obtained from enzymatically hydrolyzed scorpion protein (*Buthus martensii* Karsch). The study aimed to identify specific peptides with strong antioxidant activity and to optimize the enzymatic hydrolysis conditions for maximum peptide yield and activity.

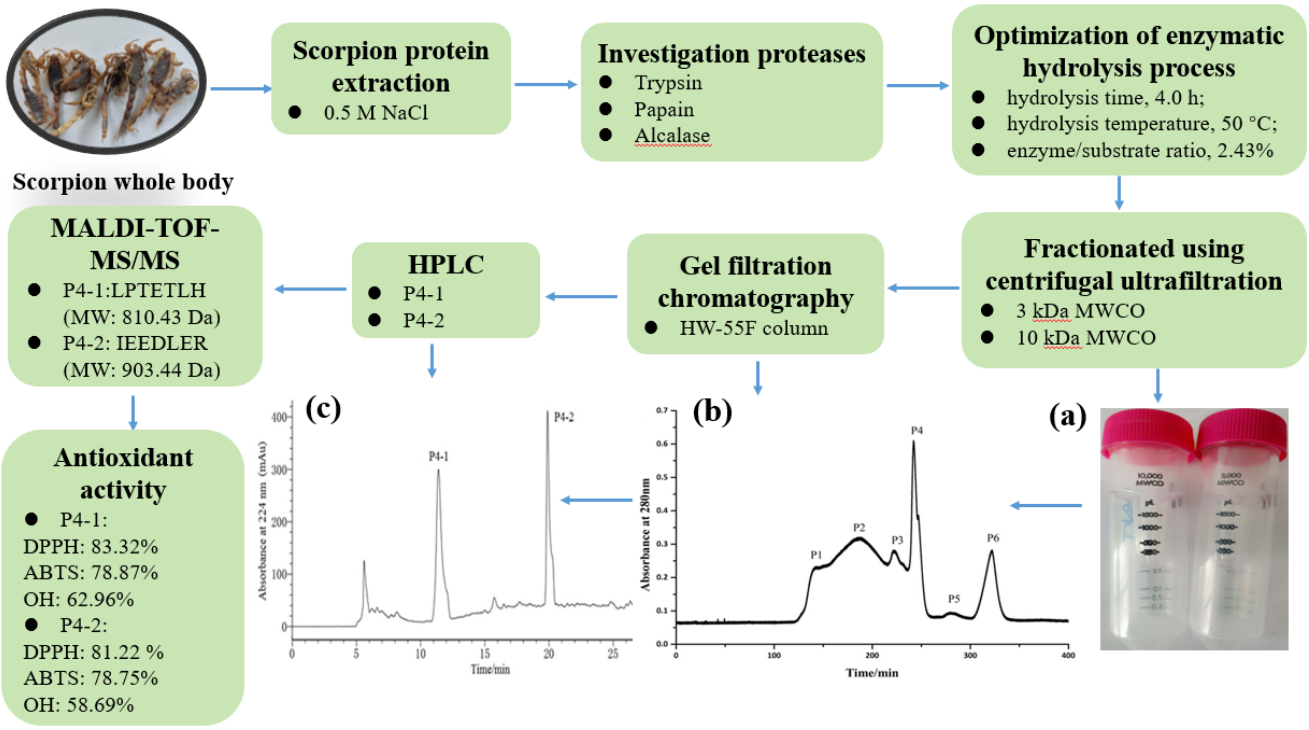

Fig. 14 Processing and study steps of scorpion protein hydrolysates

Scorpion protein (SP) was hydrolyzed by trypsin, papain, and alcalase at optimal conditions. The SDS-PAGE profiles of SPH showed that the SP was gradually hydrolyzed to peptides over the hydrolysis time. SP above 20 kDa was mostly hydrolyzed by papain in 2 h and the complete digestion of the main proteins was achieved at 3 h.

The DH was monitored by the estimation of the DH, and the DH evolution as time function as shown in Fig. 15a. The order of DH hydrolyzed by single enzyme is as follows: Papain > Alcalase > Trypsin. As shown in Fig. 15a, the peptides hydrolyzed by three enzymes showed different hydrolytic processes, and the DH of SPHs increased

with the hydrolysis time. Papain had a significantly higher hydrolysis effect compared with other enzymes, reaching a final value of 26.46% after 4 h hydrolysis times. It was supposed that different cleavage sites of the three enzymes result in the difference of DH value.

Antioxidant activitie of three scorpion protein peptides was measured using .OH, ABTS·+ and DPPH· radical scavenging assays as shown in Fig. 15b. As shown in Fig. 15b, at 5.0 mg/mL of concentration, the scavenging effect of peptides on ABTS•+ was in this order;papain (77.45%) >> alcalase (37.15%) > trypsin (30.75%), on DPPH• the order is papain (75.54%) > trypsin (58.65%) > alcalase (48.60%), and on the .OH the order is papain (49.44%) ≈ alcalase (48.35%) > trypsin (30.95%). Papain fraction has the most antioxidant activity among other peptide fractions. DH and antioxidant activity indicates that the hydrolysis of SP with papain was noticeably different from the other enzymes and papain can be effectively used to prepare SPHs. Meanwhile, for the trypsin and alcalase enzyme treatments, the hydrolysis of the SP to peptides needed a longer time.

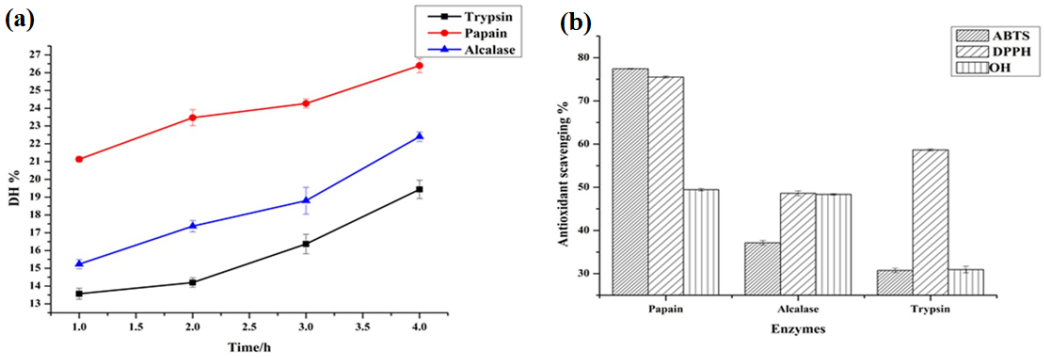

Figure 15 (a) Hydrolysis of SP; (b) DPPH, OH, and ABTS radical scavenging activities.

Isolation and purification of antioxidant peptides

In considering scavenging effects on ABTS·+ scavenging activity, .OH scavenging activity, and DPPH scavenging activity, papain fraction was selected for isolation and purification of the antioxidant peptide, As shown in Fig. 16a, PF-III (< 3 kDa) exhibited stronger ABTS·+ scavenging activity (77.45 ± 0.08%), .OH scavenging activity (49.44 ± 0.23%), and DPPH· scavenging activity (75.54 ± 0.30%) compared to PF-II (3-10 kDa) and PF-I (> 10 kDa) fractions at 5.0 mg/mL of concentration. Oun Ki et al., Bing et al. and Liu et al. reported that lower molecular weight peptides had higher antioxidant activity, which agrees with previously reported data showing that usually antioxidant peptides contain 2-20 amino acids. This might be why the PF-III fraction showed higher antioxidant activity than other two fractions. The peptide fraction (PF-III) with the best antioxidant activity was further fractionated by a gel filtration chromatography HW-55F column (1.5 cm × 100 cm), and six remarkable fractions, P1, P2, P3, P4, P5, and P6 were collected (Fig. 16b). When compared with the other five fractions, fraction P4 showed the best antioxidant activity, with scavenging activities of 80.75 ± 0.35, 85.90 ± 0.14%, and 64.50 ± 0.71% on ABTS·+, DPPH·, and .OH scavenging activity, respectively, at a concentration of 2.0 mg/mL,

and followed by fraction P6 (Fig. 16c). Therefore, the fraction P4 with stronger antioxidant scavenging activity was freeze-dried and further purified by the preparation RP–HPLC method.

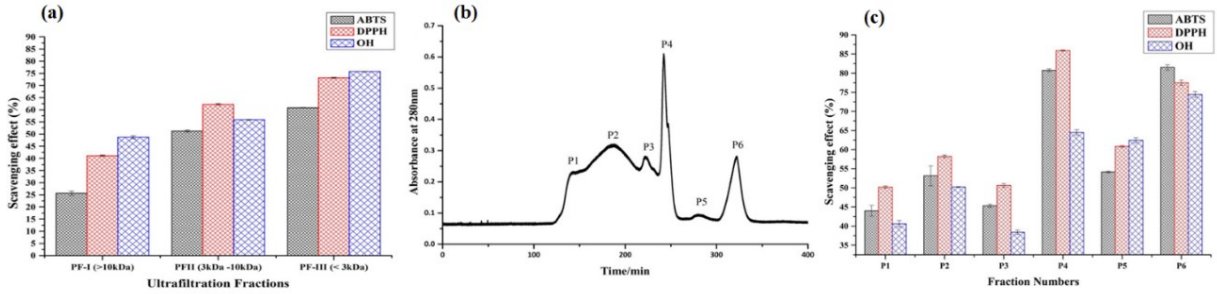

Fig. 16 (a) A Scavenging effects (%) of fractions of papain hydrolysate at different MWCO obtained from SP. (b) Gel filtration of papain fraction by TSK HW-55F (1.5x100 cm) column. (c) DPPH, OH, and ABTS radical scavenging activities.

In Fig. 17a, two single P4-1 and P4-2 peptides were collected and used to determine antioxidant activity. Fig. 17b showed that the antioxidant scavenging activity of P4-1 on ABTS·+, DPPH·, and .OH are 83.32, 78.87, and 62.96%, respectively, while activity of P4-2 on ABTS·+, DPPH, and .OH is 78.75, 81.22, and 58.69%, respectively. The yield of each purification procedure of P4-1 and P4-2 are shown in Table 14. The final yield of P4-1 is 1.04 %, and P4-2 is 1.39%.

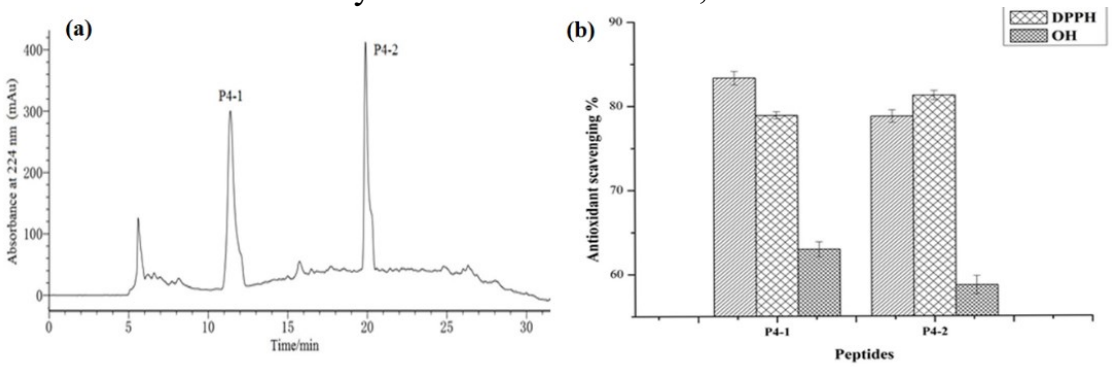

Fig. 17 (a) HPLC profile of fraction P4; (b) Antioxidant activities of P4-1 and P4-2.

Amino acid composition of SP, SPHs, and each purification procedure fractions were determined by HPLC. As shown in Table 15, 16 amino acids were detected and the total amino acids content was 544.24, 346.24, 301.99, and 273.56 mg/g, respectively. With the increase of purification process, the content of hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, and Phe) was increased, especially for PF-III (72.96%) and P4 (75.49%). The hydrophobic aromatic amino acids, Tyr, Trp, His, and Phe, also increased. The percentage of Ala and Met was increased from PF-III to P4. Therefore, these hydrophobic amino acids in the SPHs peptides were mainly responsible for the antioxidant activity. It has been reported that the rich content of Tyr and Pro in peptide sequence could obtain a higher antioxidant activity.

Table 14. Yield of each purification procedure of P4-1 and P4-2

Fractions	Procedures	Yield (mg/g protein)	Yield (%)
SP	After extraction of protein	796.30	100
SPHs	After enzymatic hydrolysis	414.24	52.02
PF-III	After ultrafiltration (UF)	293.75	36.89
P4	After purification by HW-55F	91.48	11.49
P4-1	After purification by RP-HPLC	8.27	1.04
P4-2	After purification by RP-HPLC	11.04	1.39

The amino acid sequences of the P4-1 and P4-2 peptides were identified by MALDI-TOF-MS/MS. Sequence of P4-1 was LPTETLH, the sequence of P4-2 was IEEDLER, the molecular mass of these peptides were 810.43 Da and 903.44 Da, respectively. The sequences of the two peptides were searched in BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The LPTETLH peptide showed the highest ABTS·+ scavenging activity, with a value of 83.32%, the IEEDLER peptide showed the highest DPPH·scavenging activity, with a value of 81.22%. Moreover, .OH scavenging activities of these peptides were not significant, with a value of 62.96% and 58.69%, respectively, lower than that of the ABTS·+ and DPPH·.

Table 15. Amino acid composition (mg/g) of each purification procedure fractions

Amino acids	SP	SPHs	PF-III	P4
Ala	36.47	29.68	2.40	39.3
Val	20.40	7.56	34.66	27.29
Phe	50.89	17.86	61.35	48.15
Pro	27.38	22	2.98	46
Met	12.59	3.32	13.15	35.85
Ile	22.83	7.5	33.11	17.37
Leu	58.06	9.92	72.69	60.26
Tyr	15.75	8.76	18.10	16.39
Gly	20.32	17.06	2.28	3.38
Ser	27.28	20.1	2.49	3.42
Thr	28.47	20.94	1.76	2.60
Asp	82.39	53.72	4.44	9.35
Glu	102.48	83.18	9.12	17.30
Lys	38.73	1.36	39.51	30.73
His	19.02	10.08	0.53	1.05
Arg	44.66	33.2	3.42	4.80
Total	544.24	346.24	301.99	363.24

Conclusion:

Buthus martensii Karsch scorpion protein was hydrolyzed with papain, alcalase and trypsin enzymes. Papain hydrolysate had a significantly higher hydrolysis effect, reaching a yield of 26.46%. Papain fraction at a concentration of 5.0 mg/ml showed high antioxidant activity (ABTS•+ - 77.45%; DPPH• - 75.54% and •OH - 49.44%). Separation of papain fraction by ultrafiltration, gel filtration and HPLC methods resulted to obtain peptides P4-1 and P4-2. Amino acid sequences of the P4-1 and P4-2 peptides were determined by MALDI-TOF-MS/MS to be LPTETLH and IEEDLER, respectively. It was found that the ABTS activity of the P4-1 peptide was 83.22% and

the DPPH activity of the P4-2 peptide was 81.22%. The activities of the P4-1 and P4-2 peptides against the OH radicals were 62.96% and 58.69%, respectively, which showed lower values than the ABTS•+ and DPPH• activities.

Part 7. Study of antifungal peptide against *Candida albicans*

Due to the increasing drug-resistant of Candida albicans, there is an urgent need to develop a novel therapeutic agents induced inflammatory disease treatment. Antimicrobial peptides (AMPs) are regarded as one of the most promising antifungal drugs. However, most of the designed AMPs showed side-effects. In the present study, 10 novel peptides were designed based on the sequence of frog skin secretions peptide (Ranacyclin AJ).

Functional screening of the designed peptides

Ten new peptides were designed based on the amino acid sequence of a 20-residue peptide Ranacyclin AJ from frog skin secretions. The peptides were referred to as AKK1, AKK2, and so on up to AKK10. The amino acid sequences, physicochemical properties, and MICs of the 10 designed peptides are listed in Table 16, and Table 17.

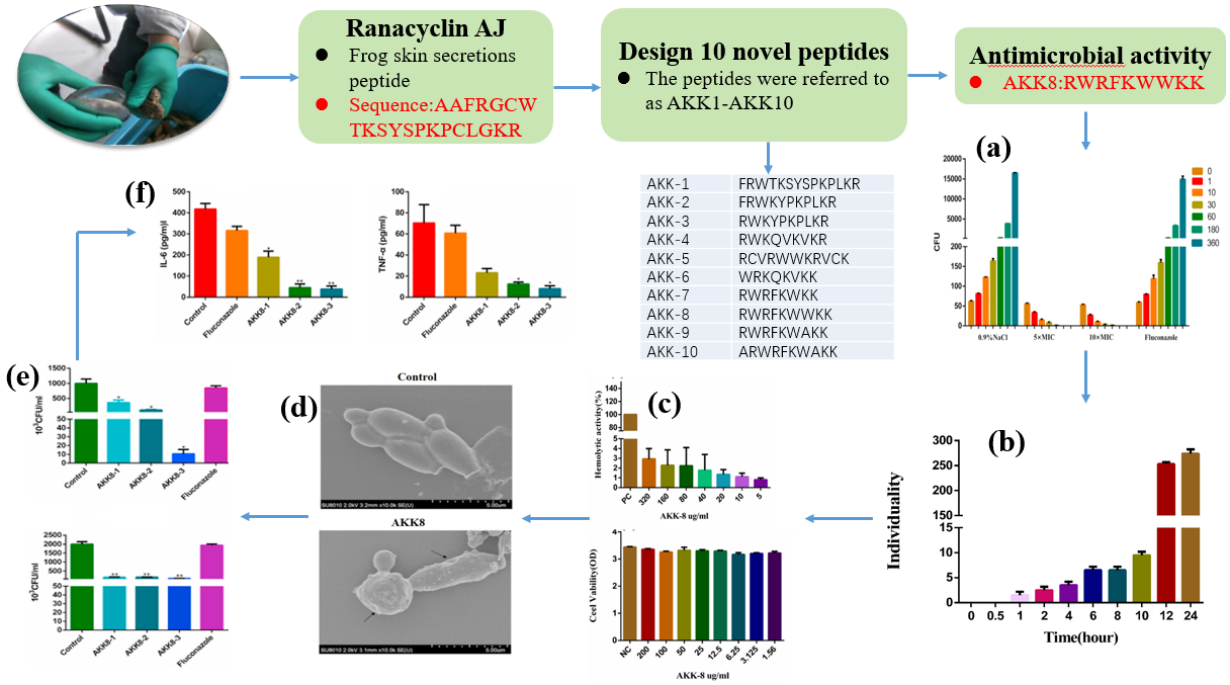

Fig. 18 Processing and study steps of frog skin secretions

Among the 10 peptides, AKK8 exhibited the highest antimicrobial activity against the four tested strainsd *E.coli*, *S.aureus*, *B.subtilis*, and *C.albicans*. The MIC of AKK8 against *C.albicans* was 18.5 μg/mL, which is lower than that of AKK8 against *E.coli*, *S.aureus*, and *B.subtilis* (37.5 μg/mL), indicating that AKK8 exhibits higher inhibition of *C.albicans*, compared with the other 3 strains. Owing to the superior killing effect of AKK8, we tested its activity against the clinically drug resistant *C.albicans* strains ATCC2002, 08032815, and 08030401. As shown in Table 17, ampicillin showed no antimicrobial activity against the 3 aforementioned strains; meanwhile, fluconazole exhibited antimicrobial activity against *C.albicans* ATCC2002 with a MIC of 0.8 μg/mL but not against *C.albicans* 08032815 and 08030401. The results indicated that *C.albicans* 08032815 and 08030401 were resistant to fluconazole. Notably, AKK8

inhibited the growth of not only *C.albicans* ATCC2002 but of the other 2 strains as well and exhibited the same MIC as those of standard strains (Tables 16 and 17). These observations suggest that AKK8 exerts a significant antibacterial effect against pathogens with clinical drug resistance.

Table 16. Physicochemical Properties of the Designed Peptides

Peptide	Sequence	MW	NC	PR/n%	NPR/n%
Ranacyclin AJ	AAFRGCWTKSYSPK PCLGKR	2256.67	+5	11/55	9/45
AKK-1	FRWTKSYSPKPLKR	1794.13	+5	9/64.28	5/35.71
AKK-2	FRWKYPKPLKR	1518.87	+5	6/54.54	5/45.45
AKK-3	RWKYPKPLKR	1371.69	+5	6/60	4/40
AKK-4	RWKQVKVKR	1227.52	+5	6/66.66	3/33.33
AKK-5	RCVRWWKRVCK	1519.90	+5	7/63.63	4/36.36
AKK-6	WRKQKVKK	1100.38	+5	6/75	2/25
AKK-7	RWRFKWKK	1234.52	+5	5/62.5	3/37.5
AKK-8	RWRFKWWKK	1420.73	+5	5/55.55	4/44.44
AKK-9	RWRFKWAKK	1305.59	+5	5/55.55	4/44.44
AKK-10	ARWRFKWAKK	1376.67	+5	5/50	5/40

Table 17. The MIC (μg/mL) of peptides against several strains of microorganisms

Peptide	*C.albicans* ATCC10231	*B.Subtilis* ATCC6633	*E.coli* ATCC25922	*S.aureus* ATCC25923
Ranacyclin-AJ	-	-	-	-
AKK-1	-	-	-	-
AKK-2	>100	>100	>100	>100
AKK-3	75	37.5	75	>100
AKK-4	37.5	75	75	>100
AKK-5	100	75	100	100
AKK-6	-	-	-	-
AKK-7	>100	>100	>100	>100
AKK-8	18.5	37.5	37.5	37.5
AKK-9	>100	>100	>100	>100
AKK-10	75	75	75	75

Killing kinetics of AKK8

Instead of delaying the bactericidal growth as traditional antifungal drugs, AKK8 could kill C. albicans 08032815 rapidly. After treatment with fluconazole, the *C.albicans* 08032815 markedly increased from 52 to 160 in 0.5 h and further to 14,887 after incubation for 6 h (Fig. 18a). In contrast to fluconazole, AKK8 killed *C.albicans* 08032815 at concentrations of 5 x and 10 x MIC within 30 min. The antifungal effect of AKK8 was long-lasted; no colony of *C.albicans* 08032815 was observed, continuing for 24 h (Fig. 18a).

Effects of human plasma and salt ion on the antifungal activity of AKK8

Plasma and salt ion were regarded as important factors that influence MIC. Thus, we determined the stability of AKK8 in human plasma and salt ion by an antifungal assay. Notably, the antifungal activity of AKK8 was not significantly affected by human plasma. Even after incubation for 10 h with blood plasma, AKK8 still exhibited

antimicrobial activity against the tested *C. albicans*, suggesting the stability of AKK8 in plasma (Fig. 18b). To assess the effect of salt ion on the antibacterial activity of AKK8, the peptide was dissolved in ddH2O, PBS, and 150 mM NaCl, sequentially. The result showed that AKK8 with MIC of 18.5 μg/mL inhibited *C.albicans* activity. Salt ions did not alter the antimicrobial activity of AKK8 against *C.albicans*, suggesting its suitability for clinical applications.

Hemolytic and cytotoxic assays

The hemolytic activity of AKK8 against human red blood cells was assessed based on the release of hemoglobin from fresh human erythrocytes. The results showed that AKK8 at a concentration of 320 μg/mL, which was 17 times higher than its MIC to *C.albicans* 08032815, exhibited only about 3% hemolytic activity in human red blood cells (Fig.18c). To verify the safety of AKK8, its potential toxicity was evaluated in vitro. The results showed no apparent toxicity to RAW267.4 and L6 cells after treatment with AKK8 at concentrations in the 1.56-200 μg/mL range (Fig. 18c). Together, AKK8 may be an ideal candidate for drug development.

Membrane morphology of *C. albicans*

To further investigate the mechanism underlying the killing activity of AKK8s against *C.albicans*, the morphological difference between the AKK8-treated and untreated *C.albicans* (08032815) was examined by SEM. The untreated *C.albicans* showed an intact cell wall, cytoplasmic membrane, and smooth outer membranes. By contrast, the AKK8-treated *C.albicans* exhibited perforations in both pseudohyphae and hyphae (Fig. 19). These observations indicate that AKK8 may have been bound to the cell wall and then caused damage to *C.albicans*.

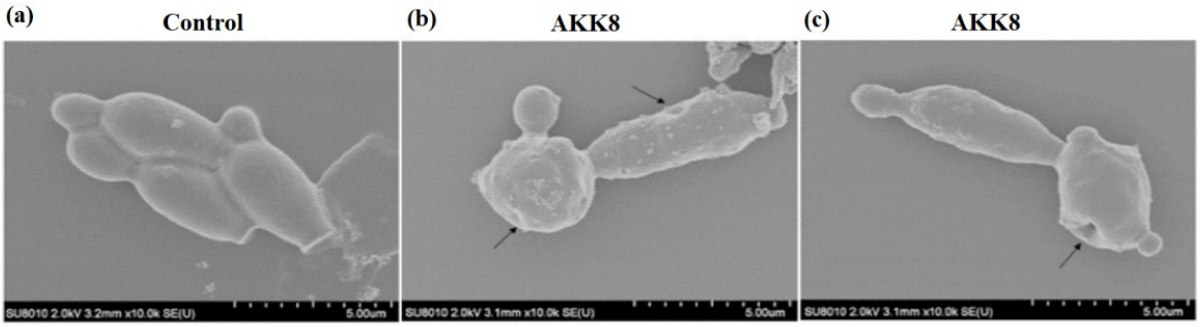

Fig. 19 Effect of peptide AKK8 on the morphologies of *C.albicans*. (a) control; (b) 2.5 x MIC peptide AKK8 caused cell wall breakages on pseudohyphae; (c) hyphae. The arrows indicate the typical damage to the plasma of *C.albicans*.

The effect of AKK8 on mice C.albicans model

To confirm the clinical safety and therapeutic effect of AKK8, it's in vivo antifungal activity was evaluated using the mouse model for systemic fungal infection. The mice were administered with *C.albicans* (08032815) at 5.0 x 107 CFU/mouse via intravenous injection and then treated with 2, 4, and 8 mg/kg AKK8, fluconazole, and 0.9% saline. After 2 d, the inflammatory cytokine levels in plasma and the colony count of *C. albicans* in main organ tissues were tested. The results indicated that the mice infected with 0.9% saline showed invasive growth of *C.albicans* (08032815) in the kidney, liver, heart, lung, and spleen.

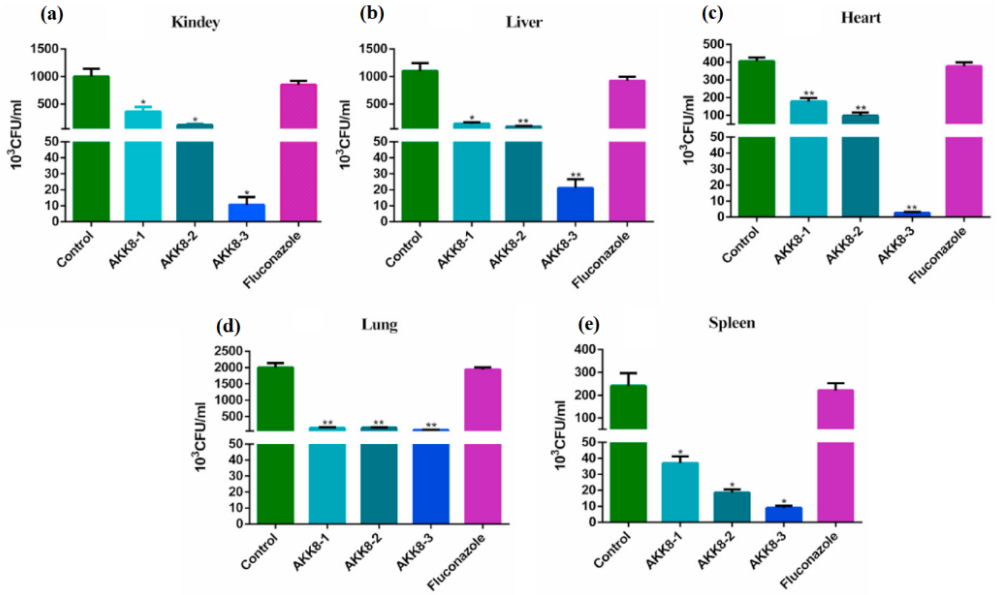

Fig. 20 (a) Effect of AKK8 on the number of *C.albicans*. The number of *C.albicans* colonized in the main organs kidney; (b) liver; (c) heart; (d) lung; (e) spleen of the mice after AKK8 treatment (*p < 0.05, **p < 0.001) (AKK8-1:2 mg/kg, AKK8-2: 4 mg/kg, and AKK8-3: 8 mg/kg).

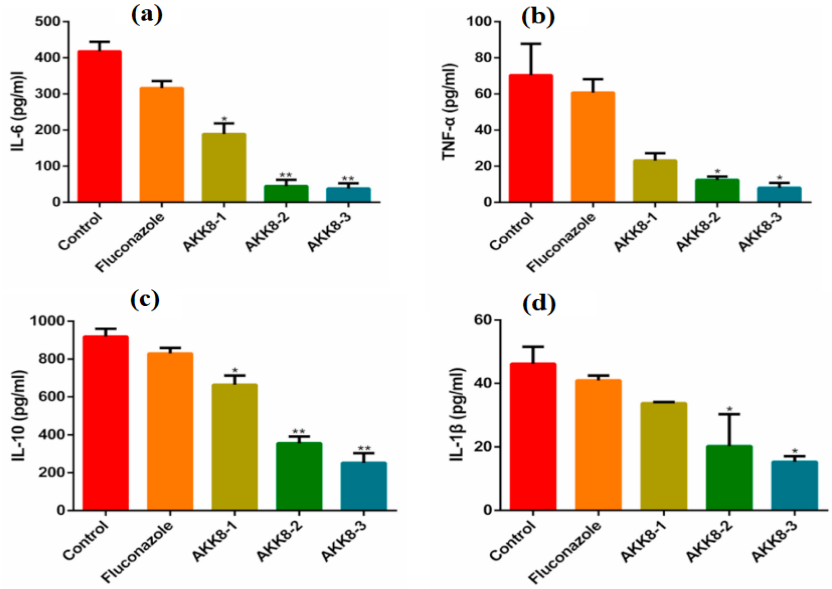

Fig. 21 (a) Effect of AKK8 on serum IL-6; (b) TNF-a; (c) IL-10; (d) IL-1b production induced by *C.albicans*. Significant difference from the values treated by 0.9% saltwater (*p < 0.05, **p < 0.001) (Control: Fluconazole: 2 mg/kg, AKK8-1: 2 mg/kg, AKK8-2: 4 mg/kg, and AKK8-3: 8 mg/kg).

Treatment with fluconazole also exerted no significant therapeutic effects on infected mice. Notably, the 3 groups treated with 2, 4, and 8 mg/kg of AKK8 exhibited significant inhibition of *C.albicans* growth in the tissues of the tested organes relative to the groups treated with 0.9% saline and fluconazole (Fig. 18e). The inflammatory cytokines, including IL-6, TNF-a, IL-10, and IL-1b, are among the most important indicators of systemic inflammation. Our results indicated that the concentrations of IL-6, TNF-a, IL-10, and IL-1b in the plasma of the control mice were significantly higher than the normal levels (85.4,

15.3, 218.5, and 20.2 pg/mL, respectively) (Fig. 20), indicating that systemic inflammation was successfully induced via injection with *C. albicans* (08032815). Treatment with 2, 4, and 8 mg/kg of AKK8 significantly reduced the amounts of IL-6, TNF-a, IL-10, and IL-1b in the plasma of the infected mice. Specifically, treatment with 4 mg/kg of AKK8 reduced the concentrations of inflammatory cytokines to normal levels in infected mice (Fig. 21).

Conclusion:

Ten new peptides were designed based on the amino acid sequence of a 20-residue peptide Ranacyclin AJ from frog skin secretions. The peptides were referred to as AKK1 - AKK10. Among the 10 peptides, AKK8 exhibited the highest antimicrobial activity against *E.coli*, *S.aureus*, *B.subtilis*, and *C.albicans*. Activity of AKK8 against to *C.albicans* was 18.5 μg/mL higher than others. These observations suggest that AKK8 exerts a significant antibacterial effect against pathogens with clinical drug resistance. Salt ions did not alter the antimicrobial activity of AKK8 against *C.albicans*, suggesting its suitability for clinical applications. Injection of AKK8 peptide (4 mg/kg) reduced inflammatory cytokines to normal levels in infected mice. Considering its simple structure、little hemolytic activity、low cytotoxicity, and high stability in the physiological environment, the AKK8 might be an excellent template for the development of therapeutic agents to treat drug-resistant C.albicans..

Part 8. Study of protein from Cervus elaphus abomasumr

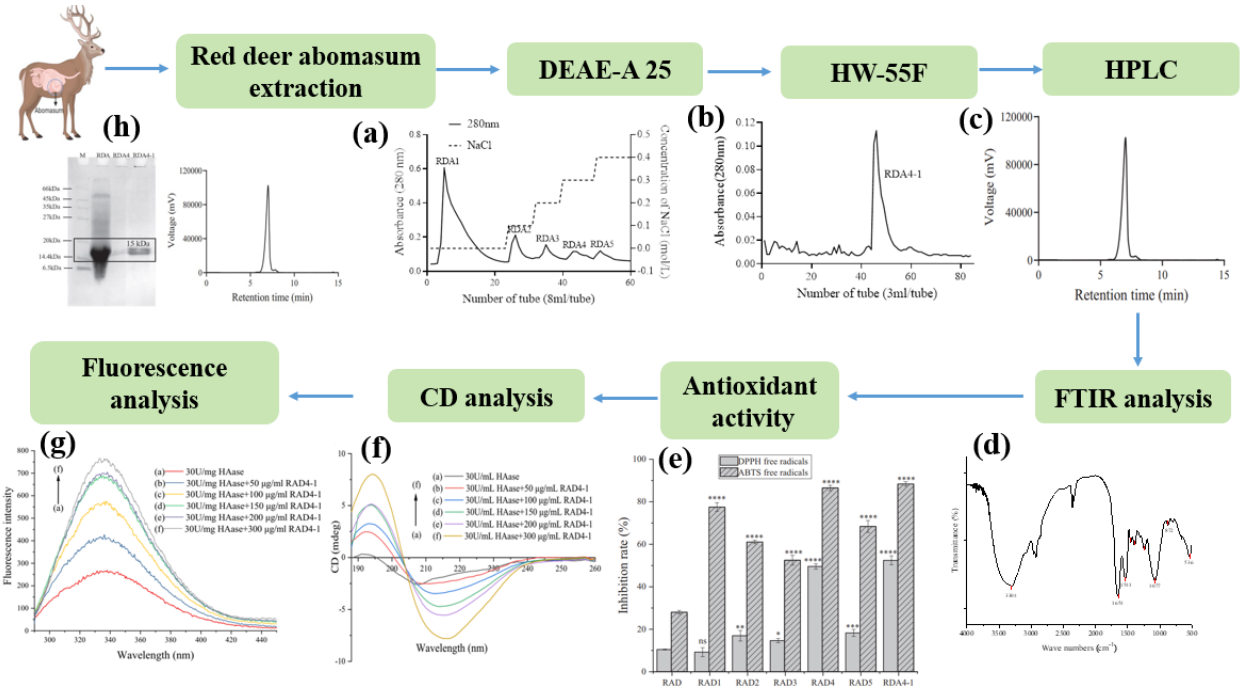

Fig. 22 Processing and study steps of Cervus elaphus abomasum protein

Preparation of homogenised protein

The aim of the study was to enhance the utilisation of red deer abomasum (RDA), a by-product of deer farming, identify and characterise a homogenised protein with potential antioxidant and hyaluronidase inhibitory properties, investigate the interaction mechanism between the purified protein and hyaluronidase to evaluate its potential application in nutraceuticals and anti-aging formulations.

The DEAE-Sephadex A-25 anion-exchange chromatography elution profile revealed five major peaks: RDA1, RDA2, RDA3, RDA4, and RDA5 (Fig. 22a). RDA4 was further purified using HW-55F gel chromatography to obtain RDA4-1 (Fig. 22b).

SDS-PAGE analysis (Fig. 22h) showed an increase in the purity of RDA4-1 during the purification process. After two steps of purification, the homogenised protein RDA4-1 was obtained, and its molecular weight was 15 kDa. The absorption peak of RDA4-1 was a single peak with an area greater than 95% according to HPLC analysis (Fig. 22h), demonstrating the efficacy of the purification process.

Chemical composition analysis

The protein content of RDA4-1 was 91.63%, with a polysaccharide content of 3.09%. It was hypothesised that RDA4-1 may be a glycosylated protein, which was also supported by the results of amino acid and monosaccharide analyses. Glycosylated proteins are more stable and biologically active compared to non-glycosylated proteins. They provide protective, stabilizing, organizing, and barrier effects on cells.

Amino acids composition analysis

The composition of amino acids is shown in Table 18.The peptide chain of RDA4-1 consists mainly of alanine, glycine, lysine, tyrosine, and valine. These amino acids play a crucial role in determining the structure and function of the protein. Alanine, for example, is responsible for maintaining the structure of the peptide chain. Tyrosine and valine, on the other hand, are hydrophobic amino acids that make up the hydrophobic core of the peptide chain. Meanwhile, as essential amino acids, the content of lysine and valine exceeds 40%. This suggests that RD4-1 meets the definition of a high-quality protein that is more easily absorbed and utilised by the body.

Table 18. Amino acids composition of RDA4-1 (in %)

Amino acids	**Asp**	**Glu**	**Ser**	**Gly**	**His**	**Thr***	**Ala**	**Pro**
Relative molarity	0.33	0.64	0.42	11.1	2.75	0.61	10.5	1.76
Amino acids	**Tyr**	**Val***	**Cys**	**Ile***	**Leu***	**Phe***	**Lys***	
Relative molarity	15.1	20.0	13.7	0.26	0.16	0.21	22.2	

Monosaccharide composition analysis

The monosaccharide analysis results are displayed in Table 19. RDA4-1 mainly comprises glucuronic acid, glucose, and amino galactose. Amino saccharides are typically located at the position where the polysaccharide chain connects to the peptide chain in glycosylated proteins, indicating that RDA4-1 is a glycosylated protein. Additionally, RDA4-1 contains L-fucose and mannose. The presence of L-fucose suggests that RDA4-1 may have antibacterial and antiviral activities, whereas mannose indicates that RDA4-1 has immunomodulatory properties.

Table 19. Monosaccharide composition of RDA4-1 (in %)

Monosaccharide	**Man**	**Glu**	**GalUA**	**GluN**	**L-Fuc**
Relative molarity	8.76	29.86	38.55	20.13	2.71

FT-IR analysis

FT-IR absorption peaks can be utilized to analyse the functional groups of a compound and characterise its structure. As shown in Fig. 22d, a distinct broad peak is observed at 3304 cm^{-1}, representing a superimposed stretching vibration of oxygen-hydrogen (O-H) bonds. The absorption at 1654 cm^{-1} is a deformation vibration of nitrogen–hydrogen (N-H), indicating the presence of protein structure in the sample. The absorption band at 1541 cm^{-1} corresponds to a carbon–nitrogen (C-N) stretching vibration. The carbon–oxygen (C-O) stretching vibration has absorption peaks at 1077 cm^{-1}. The presence of α-type glycosidic linkages was indicated by the absorption band at 872 cm^{-1}. The secondary structure of RDA4-1 was determined by analysing the amide I band (1700 cm^{-1} to 1600 cm^{-1}). RDA4-1 consists of 65.22% β-sheet and 34.78% β-turn. The β-turn structure is essential for maintaining the stability of protein sites. The presence of β-sheetenhances the stability of protein structures and helps in resisting denaturation.

Antioxidant activity

Oxidative stress can cause issues such as aging. Particularly, skin aging problems are linked to the degradation of collagen and elastin by hyaluronidase. Therefore, the search for compounds with both antioxidant and hyaluronidase inhibitory activities is essential in addressing aging. The antioxidant activity assay indicated that the abomasum protein displayed DPPH• and ABTS• + scavenging activities. Enhanced purification was correlated with higher antioxidant activity (Fig. 23a). At a concentration of 1.5 mg/mL, the homogenised protein RDA4-1 exhibited a maximum scavenging capacity of 50.97% and 88.37%. Previous studies found that the half-maximal inhibitory concentration (IC_{50}) of antioxidant peptides from sheep abomasum was 6.92 mg/mL, suggesting lower antioxidant activity compared to RDA4-1. This difference could be attributed to the presence of glycan chains, which enhance the stability of protein chemical structures over peptides, enabling them to scavenge free radicals more effectively. The antioxidant experiments demonstrated that RDA4-1 is a promising antioxidant raw material for active addition to nutraceuticals.

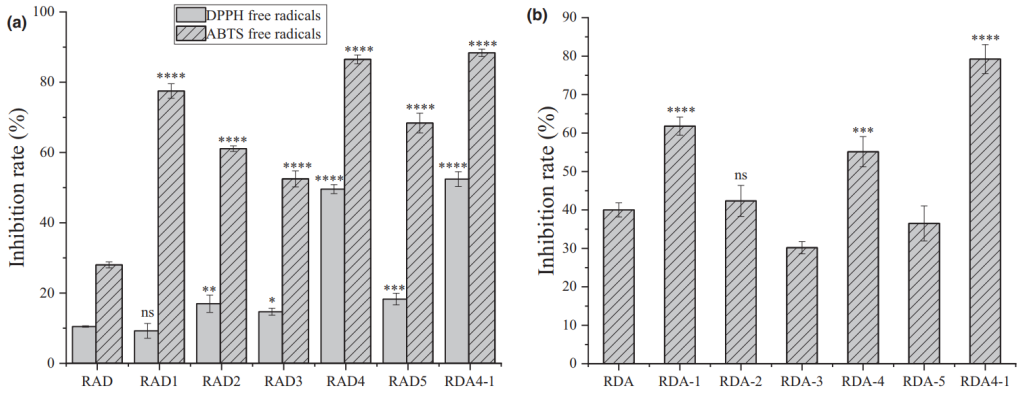

Fig. 23 (a) Anti-oxidation activity and (b) HAase inhibition of samples; (RDA was crude extract, RDA1-RDA5 were fractions purified by DEAE-Sephadex A-25 from RDA, RDA4-1 was homogenised protein purified by HW-55F from RDA4; '*' represents significance compared to crude extract RDA, 'ns' represents no significance, '*' represents P ⩽ 0.05, '**' represents P ⩽ 0.01, '***' represents P ⩽ 0.001, and '****' represents P ⩽ 0.0001, and unlabelled represents lower activity than crude extract RDA).

HAase inhibition activity

1) Inhibitory effect on HAase

The inhibitory activity of the sample against HAase is illustrated in Fig. 23b. HAase inhibitory activity increased with purity. The inhibition rate of HAase by RDA4-1 at a concentration of 1 mg/mL was 79.23%. Peptides derived from marlin skin collagen inhibited HAase by 39.83%. RDA4-1 inhibited HAase more effectively than Marlin skin collagen hydrolysed peptide.

2) Inhibition reaction process CD analysis

The CD analysis of various concentrations of RDA4-1 mixed with HAase are shown in Fig. 22f. In the absence of RDA4-1, two negative peaks with similar intensities were observed at 208 nm and 222 nm. A strong negative absorption broad peak appeared at 207 nm, indicating that HAase is primarily composed of α-helix. Upon the addition of RDA4-1, the wavelength at which the negative peak appeared gradually increased, and the absorption of the positive peak also increased. This indicates that the secondary structure of the system into β-sheet structure, showing concentration dependence. Higher concentrations of RDA4-1 resulted in the formation of more β-sheet structures and fewer α-helix structures. This suggests that when RDA4-1 acts as a HAase inhibitor, it modifies the secondary structure of the protein docking body, thereby preventing the enzyme from binding to hyaluronic acid.

3) Fluorescence spectroscopy analysis

Fluorescence spectroscopy offers low dosage and high sensitivity. Fluorescence spectra reflect changes in the fluorescence structure of enzyme protein molecules. A positive emission peak near 348 nm is typically observed in the fluorescence spectrum of enzyme protein molecules containing tryptophan. The fluorescence spectrum of HAase mixed with various concentrations of RDA4-1 is depicted in Fig. 22g. The HAase fluorescence spectrum exhibited a broad positive emission peak around 340 nm, consistent with the fluorescence spectral properties of enzymes and proteins. No new emission peak emerged in the fluorescence spectrum upon the addition of RDA4-1, and the peak position remained unchanged. The fluorescence intensity of the emission peak at 340 nm increased with the rising concentration of added RDA4-1, indicating a correlation between intensity and RDA4-1 concentration. The emission peak position remained unchanged, suggesting that RDA4-1 did not impact the tryptophan residue of HAase, indicating a non-covalent binding of RDA4-1 to HAase.

Fluorescence intensity with consistent trends in concentration implies the reversibility of the binding. These experiments confirmed the potential of abomasum protein to inhibit HAase activity, suggesting that abomasum protein could be a new candidate as a HAase inhibitor.

Conclusion:

From proteins of red deer abomasum (RDA) by the DEAE-Sephadex A-25 anion-exchange chromatography were obtained 5 fractions marked as: RDA1, RDA2, RDA3, RDA4, and RDA5. RDA4 was further purified using HW-55F gel chromatography and obtained RDA4-1 peptide with Mm 15 kDa. The protein content of RDA4-1 was 91.63%, with a polysaccharide content of 3.09%. The peptide chain of RDA4-1

consists mainly of Ala, Gly, Lys, Tyr, Val and monosaccharides, like: Man, Glu, GalUA, GluN, L-Fuc in a concentration of 8.76, 29.86, 38.55, 20.13 and 2.71, respectively. FT-IR analysis showed the absorption at cm-1: 1654 (N-H), 1541 (C-N), 1077 (C-O). The presence of α-type glycosidic linkages was indicated by the absorption band at 872 cm-1. The secondary structure of RDA4-1 was determined by analysing the amide I band (1700 cm-1 to 1600 cm-1). RDA4-1 consists of 65.22% β-sheet and 34.78% β-turn. Antioxidant activity assay indicated that the abomasum protein displayed DPPH• and ABTS• + scavenging activities. RDA4-1 (1.5 mg/mL) had maximum scavenging capacity of 50.97% and 88.37%. The inhibition rate of HAase by RDA4-1 (1 mg/mL) was 79.23%. Peptides derived from marlin skin collagen inhibited HAase by 39.83%. The results of this study demonstrate that red deer abomasum may have physiological activity for the treatment of gastritis and other drug development.

Part 9. Biological activity and function of modified sericin

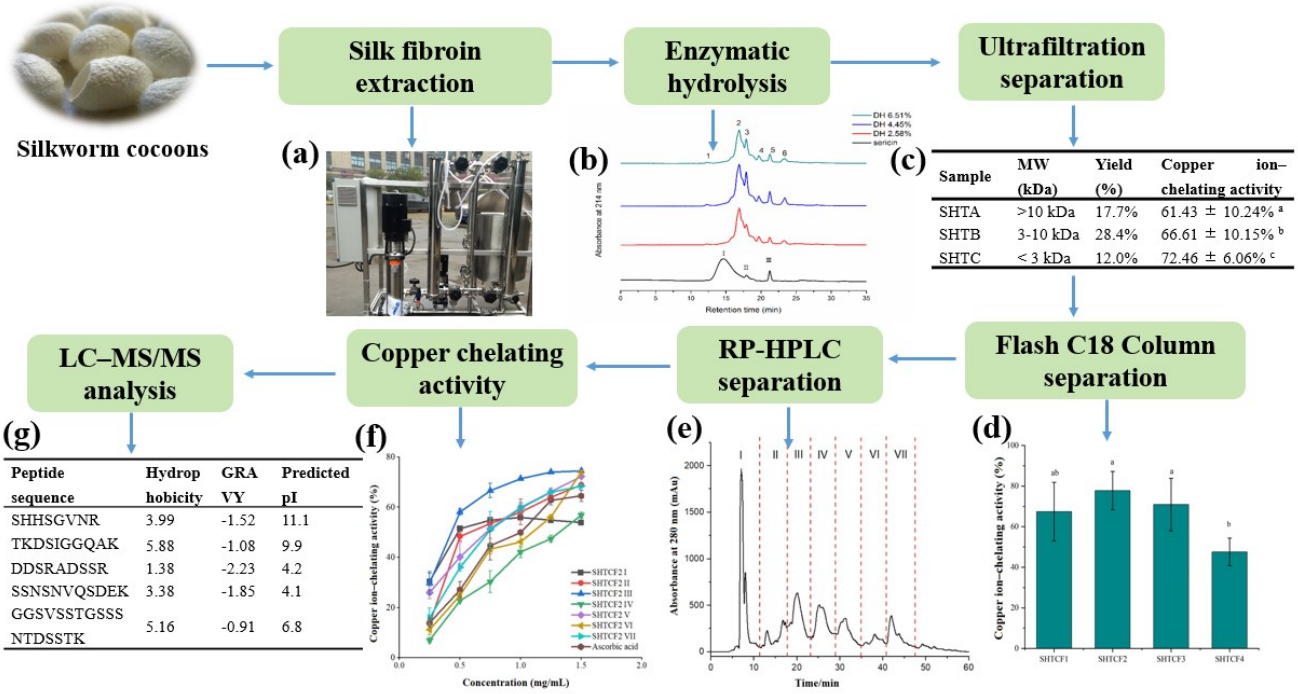

Fig. 24 Processing and study steps of sericin

Sericin is an inexpensive by-product of the silk industry, and about 50,000 tons of unused sericin is discharged with industrial wastewater every year. Sericin has a broad application prospect in the fields of food, cosmetics medicine. This work aims to improve the physical, chemical and functional properties of sericin by its structural modification.

Preparation of sericin and its hydrolysates

10g of silkworm cocoons were immersed in 500 mL of water (1:50, w/v) and heated in an autoclave at 121°C for 60 min. After cooling, extract was centrifuged at 10000g for 10 min. Supernatant was incubated at 37°C for 5 min, and the pH was adjusted to 8.0 using 0.5 M NaOH solution. Enzymatic hydrolysis reaction was

initiated by adding trypsin at an enzyme-to-substrate ratio (E/S) of 1:50. During hydrolysis, the pH was maintained at 8.0 by continuous addition of 0.1 M NaOH solution, and the mixture was stirred at 37°C at 1000 rpm. Reaction was terminated by heating the mixture in a water bath at 80°C for 15 min and hydrolysates were freeze-dried. Obtained powders were stored at −20°C.

Sericin (without enzymatic treatment) was prepared under identical conditions. Protein yield was 95.15%.

The degree of hydrolysis (DH) during the trypsin-induced hydrolysis of sericin was monitored for 90 min using the pH-stat method. As shown in Figure 25, the DH increased rapidly within the first 10 min, indicating an intense hydrolytic reaction, followed by a relatively slow increase during the subsequent 80 min. Hydrolysates with DH values of 2.58%, 4.45%, and 6.51% were prepared for further investigation on the effects of trypsin-induced hydrolysis on the structural and functional properties of sericin.

Ultrafiltration separation of copper ion–chelating peptides

Using the ultrafiltration method, sericin trypsin hydrolysate (SHT) was separated into three fractions: SHTA (>10 kDa), SHTB (3–10 kDa), and SHTC (<3 kDa), with yields of 17.7%, 28.4%, and 12.0%, respectively. At a concentration of 2 mg/mL, the copper ion–chelating abilities of SHTA, SHTB, and SHTC were 61.43 ± 10.24%, 66.61 ± 10.15%, and 72.46 ± 6.06%, respectively (Fig. 24c). The fraction with a molecular mass below 3 kDa (SHTC) exhibited significantly higher copper ion–chelating activity than the >10 kDa fraction (SHTA) and the 3-10 kDa fraction (SHTB).

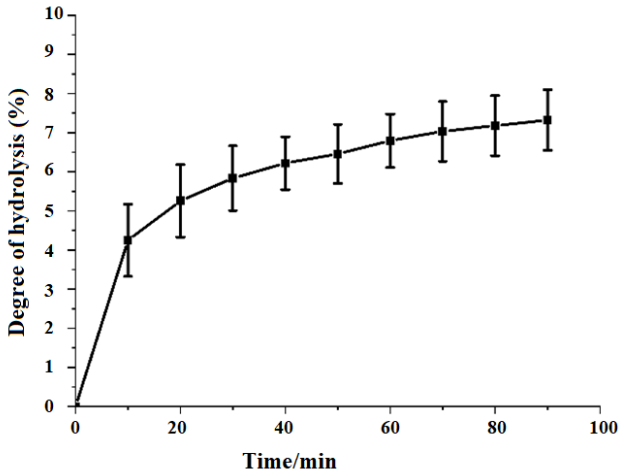

Fig. 25 Degree of hydrolysis of sericin during 90 min

Peptides with molecular weights below 3 kDa have been reported to exhibit antioxidant, ACE-inhibitory, anti-inflammatory, and antimicrobial activities. Therefore, the SHTC fraction was selected for further investigation.

Separation of copper ion–chelating peptides using a Flash C18 Column

SHTC was further separated using a SePaFlash C18 reversed-phase column. In a result four fractions were eluted. Each fraction was lyophilized and then dissolved in ultrapure water to a final concentration of 2 mg/mL for the determination of copper ion–chelating activity.

As shown in Fig. 24d, at a concentration of 2 mg/mL, the fraction eluted with 30% ethanol (SHTCF2) exhibited the highest copper ion–chelating activity (77.80 ± 9.42%), while the fractions eluted with 10%, 50%, and 70% ethanol showed chelating activities of 67.43 ± 14.39%, 70.91 ± 12.91%, and 47.61 ± 6.75%, respectively. These results indicate that more hydrophilic peptides exhibit most copper ion–chelating abilities. The fraction SHTCF2 is collected for further purification and analysis.

Purification by high-performance liquid chromatography (HPLC)

The SHTCF2 fraction was further purified using RP-HPLC (Fig. 24e). A total of seven fractions were obtained. Each fraction was collected, lyophilized, and then subjected to activity evaluation.

The RP-HPLC separated fractions SHTCF2I, SHTCF2II, SHTCF2III, SHTCF2IV, SHTCF2V, SHTCF2VI, and SHTCF2VII were lyophilized and tested for copper ion–chelating activity, with ascorbic acid used as a positive control (Fig. 24f). The IC_{50} values for copper ion ‑ chelating activity were 0.7839, 0.7028, 0.4013, 1.280, 0.6499, 0.9664, 0.7723, and 0.9406 mg/mL, respectively. Among these, SHTCF2III exhibited the most copper ion–chelating ability. Therefore, SHTCF2III was selected for LC–MS/MS analysis to identify amino acid sequence.

LC–MS/MS analysis of the SHTCF2III fraction

The SHTCF2III fraction was analyzed using LC–MS/MS, and the obtained spectra were searched against the Swiss-Prot database using Mascot software. Peptide identification was performed based on standard scoring algorithms. The five peptides with the highest matching scores exhibited mass-to-charge ratios (m/z) of 298.3860, 503.2090, 504.6280, 597.6640, and 823.2990, corresponding to molecular mass of 892.1362, 1004.4034, 1007.2414, 1193.3134, and 1644.5834 Da, respectively.

The identified peptide sequences were as follows: SHHSGVNR, TKDSIGGQAK, DDSRADSSR, SSNSNVQSDEK, GGSVSSTGSSSNTDSSTK.

Table 20. The mass-to-charge ratios and molecular weights of identified peptides

Peptide sequence	Mass/charge (m/z)	Molecular weight (Da)
SHHSGVNR	298.3860	892.1362
TKDSIGGQAK	503.2090	1004.4034
DDSRADSSR	504.6280	1007.2414
SSNSNVQSDEK	597.6640	1193.3134
GGSVSSTGSSSNTDSSTK	823.2990	1644.5834

These results provide valuable molecular information about the SHTCF2III fraction and contribute to further studies on its biological functions and potential pharmacological activities.

The hydrophobicity, grand average of hydropathicity (GRAVY), and theoretical isoelectric point (pI) of the peptide sequences were calculated using Thermo Fisher's Peptide Analyzing Tool (Fig. 24g).

Hydrophilicity/hydrophobicity is one of the most important factors influencing the function and activity of peptides and proteins. In general, the GRAVY values of most peptides or proteins range from -2 to +2. A negative GRAVY value indicates

hydrophilicity, with lower values representing stronger hydrophilic character. Conversely, a positive GRAVY value indicates hydrophobicity, and higher values represent stronger hydrophobic properties. The calculation results showed that all five peptides are hydrophilic. Based on their hydrophilicity from highest to lowest, the order is as follows: DDSRADSSR > SSNSNVQSDEK >SHHSGVNR > TKDSIGGQAK > GGSVSSTGSSSNTDSSTK.

The metal ion chelation capacity of peptides mainly depends on their amino acid sequences. The chelation mechanism between metal cations and peptides involves coordination and covalent bonding of the metal ions with various functional groups such as the N-terminal amino group, C-terminal carboxyl group, side chains of amino acids, as well as carbonyl and imino groups within the peptide chain, forming ring-like structures. Copper ions mainly bind to nitrogen atoms from different functional groups, such as the guanidino group in arginine (Arg), the imidazole group in histidine (His), and the ε-amino group in lysine (Lys). Copper ions have a strong affinity for Arg, Lys, and His. Moreover, studies have reported that aspartic acid (Asp) and glutamic acid (Glu) can also chelate metal ions.

As shown in Table 20, the relative molecular weights of the five identified peptides are all below 2000 Da. Among them, peptides TKDSIGGQAK, SSNSNVQSDEK, and GGSVSSTGSSSNTDSSTK have lysine residues at their C-termini, while SHHSGVNR and DDSRADSSR have arginine residues at their C-termini. Notably, SHHSGVNR contains two histidine residues, which are excellent chelating agents for transition metals and enhance the binding to copper ions. DDSRADSSR contains three aspartic acid residues, and SSNSNVQSDEK contains one aspartic acid and one glutamic acid residue.

Conclusion:

Using the ultrafiltration method, sericin trypsin hydrolysate (SHT) was separated into three fractions: SHTA (>10 kDa), SHTB (3–10 kDa), and SHTC (<3 kDa), with yields of 17.7%, 28.4%, and 12.0%, respectively. At a concentration of 2 mg/mL, the copper ion–chelating abilities of SHTA, SHTB, and SHTC were 61.43%, 66.61%, and 72.46%, respectively. SHTC fraction with Mm 3 kDa exhibited higher copper ion–chelating activity than others. In a result of separation SHTC fraction by HPLC were obtained 4 fractions. At a concentration of 2 mg/mL, fraction eluted with 30% ethanol (SHTCF2) exhibited the highest copper ion–chelating activity (77.80%) than others. SHTCF2 fraction was separated to 7 fractions by RP-HPLC. The IC_{50} values for SHTCF2III copper ion–chelating activity was more active and equal to 0.4013 mg/mL, respectively. Amino acid sequence of th SHTCF2III peptide, identified by LC–MS/MS was DDSRADSSR.

Part 10. Antioxidant peptides of sheep abomasum protein hydrolysates

The aim of this study to isolate, purify, and identify antioxidant peptides derived from sheep abomasum protein hydrolysates (SAPH), and to characterize their molecular structures and antioxidant activities in order to explore their potential as natural antioxidant agents for food and pharmaceutical applications.

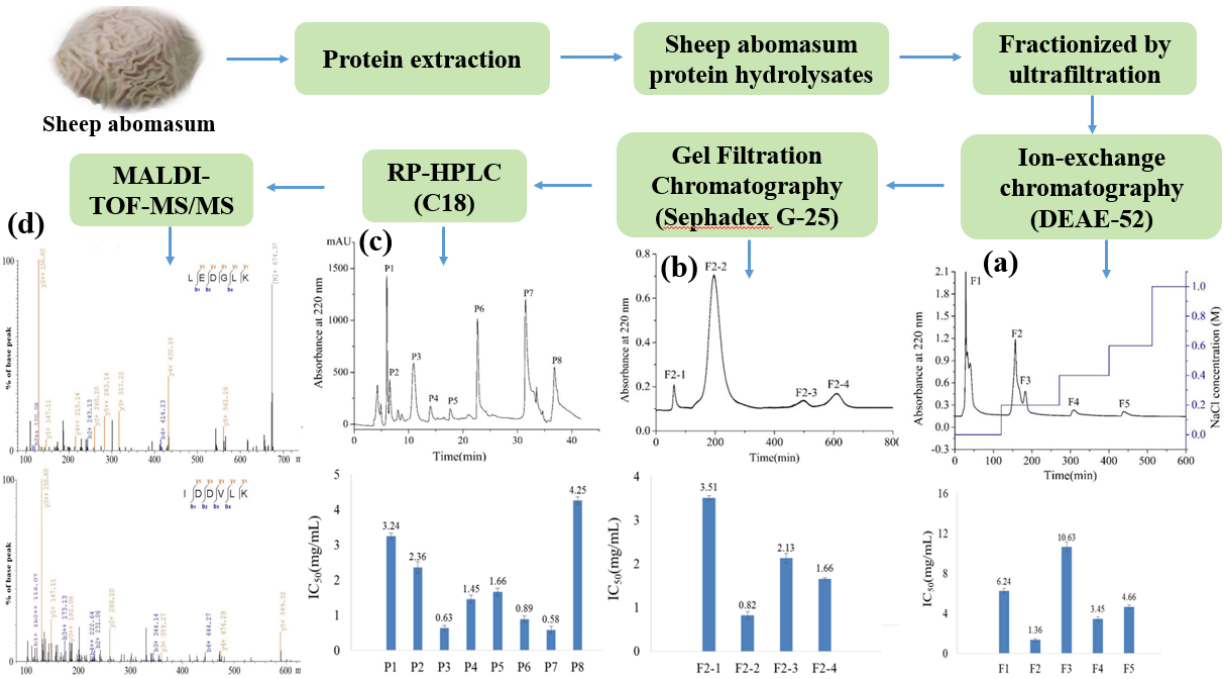

Fig. 26 Processing and study steps of sheep abomasum protein hydrolysates

Preparation of sheep abomasum protein hydrolysates (SAPH)

Sheep abomasum protein concentration (SAPC) was dissolved in distilled water, and the pH was adjusted to 6.0. The solution was preheated for 30 minutes, followed by the addition of papain to carry out enzymatic hydrolysis at 46°C with an enzyme-substrate ratio of 1.5% (w/w). During the reaction, the pH maintained using the pH-stat method by adjusting with 0.1 mol/L HCl or 0.1 mol/L NaOH. After 4 hours of hydrolysis, the reaction was terminated by heating the mixture in a 95°C water bath for 5 minutes. Obtained hydrolysate centrifuged at 10000 r/min for 10 minutes, and the supernatant was lyophilized to obtain SAPH.

Separation of antioxidant peptides by ultrafiltration

SAPH was separated using ultrafiltration membranes with molecular mass 3 kDa and 10 kDa, yielding three fractions, designated as UF-I (MW > 10 kDa), UF-II (3 kDa < MW < 10 kDa), and UF-III (MW < 3 kDa). As shown in Fig. 27, the DPPH radical scavenging activity of UF-III (2.13 mg/mL) was significantly higher than that of UF-I (5.68 mg/mL) and UF-II (3.46 mg/mL).

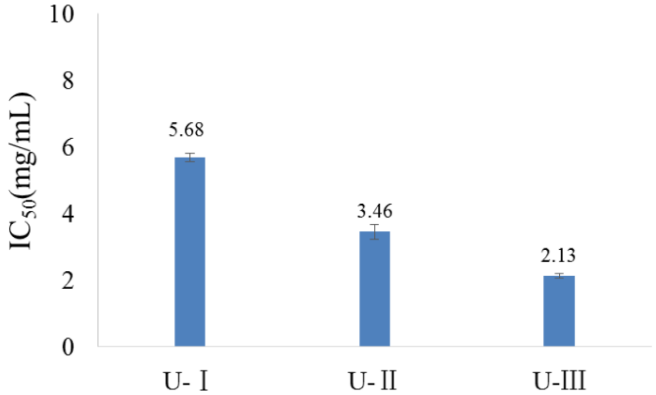

Fig. 27 The DPPH radical scavenging activity of each fraction by ultrafiltration

The DPPH radical scavenging activity of UF-III was 2.99 times higher than that of the original SAPH (Table 21). These results indicate that the low-molecular-weight fraction UF-III exhibited the highest antioxidant activity.

Table 21. Purification degree and anctivities of fractions

Sample	Yield (%)	IC_{50} (mg/mL) DPPH	Purification fold
SAPH	100	6.36	-
Ultrafiltration (U-III)	25.82	2.13	2.99
DEAE-52 (F2)	4.13	1.36	4.68
Sephadex G-25 (F2-2)	2.32	0.82	7.76
RP-HPLC (P3)	0.43	0.63	10.10
RP-HPLC (P7)	0.66	0.58	10.97

Summary of yield (%) and DPPH radical scavenging activity (IC_{50}, mg/mL) and purity after a series of purifications of antioxidant peptides of sheep abomasum protein hydrolysates (SAPH). Similar findings have been reported in previous studies, where the low-molecular-weight fractions (below 3 kDa) of hydrolysates from defatted peanut meal, egg white, blue mussel, and ovomucin showed the strongest antioxidant activities. Therefore, UF-III, which contains low-molecular-weight peptides, was selected for further purification and analysis.

Ion-exchange chromatography (DEAE-52)

As shown in Fig. 26a, UF-III was further separated and purified using a DEAE-52 anion-exchange chromatography column, resulting in five fractions (F1–F5). Fraction F1 was eluted with deionized water, F2 and F3 with 0.2 M NaCl, F4 with 0.4 M NaCl, and F5 with 0.6 M NaCl. As illustrated in Fig. 26a, the DPPH radical-scavenging activities of these fractions were 6.24, 1.36, 10.63, 3.45, and 4.66 mg/mL, respectively. Among them, the F2 fraction exhibited the strongest DPPH radical-scavenging activity, with an IC_{50} value 4.68 times lower than that of SAPH (Table 21).

This enhanced antioxidant activity may be attributed to the presence of acidic amino acid residues, as anion-exchange chromatography typically separates negatively charged molecules from neutral and basic ones. Therefore, peptides adsorbed on the anion-exchange resin are likely to contain one or more acidic groups responsible for their strong antioxidant activity. In summary, fraction F2 was selected for further purification.

Gel Filtration Chromatography (Sephadex G-25)

As shown in Fig. 26b, the F2 fraction, which exhibited significant antioxidant activity, was further purified using a Sephadex G-25 gel filtration chromatography column, yielding four fractions (F2-1 to F2-4). The DPPH radical-scavenging activities of these fractions were 3.51, 0.82, 2.13, and 1.66 mg/mL, respectively (Fig. 26b). Among them, F2-2 showed the highest DPPH radical-scavenging activity, with an IC_{50} value 7.76 times lower than that of SAPH (Table 21).

Generally, low-molecular-weight peptides exhibit stronger antioxidant activity than high-molecular-weight ones. However, in this study, F2-2, which had a relatively higher molecular weight than the other fractions, demonstrated stronger antioxidant

activity. Therefore, antioxidant activity is not solely dependent on molecular weight but is also influenced by other factors, such as peptide sequence and composition.

RP-HPLC

The F2-2 fraction, which exhibited the highest antioxidant activity, was further purified by semi-preparative RP-HPLC. Repeated injections were performed, and the eluted fractions were collected to evaluate their DPPH radical-scavenging activities. As shown in Fig. 26c, the chromatogram of F2-2 monitored at 220 nm revealed eight distinct peaks, designated as P1 to P8. The DPPH radical-scavenging activities of these fractions were 3.24, 2.36, 0.63, 1.45, 1.66, 0.89, 0.58, and 4.25 mg/mL, respectively (Fig. 26c). Among them, fractions P3 and P7 exhibited the highest DPPH radical-scavenging activities.

Based on the four-step purification procedure employed in this study, the IC_{50} values of the antioxidant peptides P3 and P7 were increased by 10-fold and 11-fold, respectively, compared with the initial SAPH (Table 21). These results indicate that RP-HPLC effectively isolated peptides with potent antioxidant potential.

Determination of amino acid sequences and molecular masses

In this study, MALDI-TOF-MS and TOF-MS/MS were employed to determine the molecular weights and amino acid sequences of the antioxidant peptide fractions P3 and P7. The MALDI-TOF-MS spectra of P3 and P7 representing the primary mass spectra of the peptides. As illustrated, the x-axis represents the mass-to-charge ratio (m/z), while the y-axis indicates ion intensity. The prominent peak at m/z 674.37 corresponds to the protonated molecular ion $[M+H]^+$ of peptide P3, indicating that the relative molecular weight of P3 is 674.37 Da. Similarly, in Figure 48, the peak at m/z 703.41 corresponds to the protonated ion $[M+H]^+$ of peptide P7, indicating a relative molecular weight of 703.41 Da.

MALDI-TOF-MS/MS tandem mass spectrometry allows the selection of precursor ions with the highest signal intensity from the MS spectrum for subsequent fragmentation in the MS/MS mode. The precursor ions collide with inert gas molecules, resulting in peptide bond cleavage and generation of fragment ions. The resulting spectra can be automatically analyzed using the instrument's software to deduce the amino acid sequence of the peptides. In this study, MALDI-TOF-MS/MS was employed to determine the amino acid sequences of the antioxidant peptides P3 and P7. As shown in Fig. 28d, the sequence of P3 was identified as Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK), while Figure 49b shows that P7 was identified as Ile-Asp-Asp-Val-Leu-Lys (IDDVLK). Database searches in SWISS-PROT and BIOPEP revealed no matches for either peptide, suggesting that the two peptides identified in this study may represent novel antioxidant peptides.

Preliminary analysis of the antioxidant Peptides P3 and P7

The relative molecular weights of P3 and P7 were 674.37 and 704.31 Da, respectively, classifying them as low-molecular-weight peptides. Numerous studies have reported that peptides with lower molecular weights generally exhibit higher antioxidant activity. Sequence analysis showed that both P3 and P7 were rich in acidic and hydrophobic amino acid residues such as Ile, Asp, Val, Leu, and Glu. Previous

studies have demonstrated that peptides enriched with acidic or hydrophobic residues possess strong radical-scavenging abilities. Moreover, the presence of hydrophobic residues (Val, Leu, Ile, Ala, Phe, and Lys) at the N- or C-termini has been associated with enhanced antioxidant activity. Therefore, the antioxidant activity of P3 and P7 may be attributed to their hydrophobic terminal residues, such as Ile, Lys, and Leu.

The -Gly-Leu- (GL) motif in P3 may play a key role in its potent antioxidant activity. Rajapakse et al. reported that the peptide Asn-Gly-Leu-Glu-Gly-Leu-Lys, isolated from giant squid muscle, exhibited high antioxidant capacity primarily due to the presence of the GL sequence. Hydrophobic dipeptide motifs are known to facilitate interactions with free radicals, thereby enhancing antioxidant activity.

Similarly, the presence of the repeated dipeptide sequence -Asp-Asp- (DD) in P7 may contribute to its strong radical-scavenging potential. Jin et al. also demonstrated that repeated di- or tri-residue motifs in peptides often confer higher antioxidant activity. Taken together, the strong antioxidant activities of P3 and P7 are likely attributed to their low molecular weights, hydrophobic terminal residues, and characteristic amino acid motifs acting synergistically.

Conclusion:

By ultrafiltration, ion exchange chromatography, gel filtration chromatography, and reverse-phase high-performance liquid chromatography two novel peptides (P3 and P7) with high antioxidant activity were purified from SAPH. Peptide sequences were determined as Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK, SAPHP-A) and Ile-Asp-Asp-Val-Leu-Lys (IDDVLK, SAPHP-B) with molecular weights of 674.37 and 703.41 Da, respectively. SAPH peptides could be used as food additives and pharmaceutical products.

CONCLUSIONS

1. Among the hydrolysis of chickpea (*Cicer arietinum* L.) proteins, the most effective method is the enzyme neutrazoprotease, peptides less than 20 kDa were obtained with an 80% yield. Through three-stage chromatographic purification, a peptide NF2-4-1 with a purity of 92.8%, Mm 685.41 Da, and an LTEIIP sequence was isolated. The IC50 values of the peptide in relation to the DPPH˙ and •OH radicals were 0.24 and 0.57 mg/ml, respectively, which confirmed its high antioxidant activity. This peptide is recommended as a promising bioactive substance for the neutralization of free radicals in functional foods.

2. An effective three-stage method for isolating peptides from the seeds of *Cuminum cyminum* L. has been developed, consisting of extraction, ion-exchange, and gel chromatography. It was established that the F4-c fraction obtained by gel chromatography exhibits strong antimicrobial activity against pathogens such as E. coli, C. albicans, and S. aureus. From this fraction, 10 homogeneous peptides in the range of Mm 1158.61 - 3312.26 Da were isolated using HPLC, and their primary structures were fully determined. It has been established that the obtained peptides are natural antibiotics and can be used in the future as safe preservatives for food products.

3. The technology for isolating proteins and peptides from the bone marrow of sheep, cattle, horses, and camels has been improved by aqueous extraction and fractionation in 30-70% saturated solutions of (NH4) 2SO4. The highest yield of protein from horse bone marrow was obtained, and its yield in aqueous extract reached 90.47%. At a 50% saturation of (NH4) 2SO4, the protein content in the bone marrow of cattle and camels was 52.3 and 56.5 mg/ml, respectively. It was found that salt extracts are richer in protein than aqueous extracts. Bone marrow protein exhibits strong antioxidant properties, with 83.9% radical neutralization and IC50 = 0.573 mg/ml.

4. BCM proteins were hydrolyzed with pepsin, alkalase, papain, and trypsin, and peptides were obtained with a yield of 67%, 59%, 75%, and 82%, respectively. The peptide TFI-b1, TFI-b2, and TFI-b3 were isolated as a result of three-stage purification (ultrafiltration, gel filtration, and HPLC) of the TF fraction with the highest yield (82%). Their antioxidant activity according to DPPH is IC50 = 1.9; 1.2; 0.6 mg/ml, and according to ABTS IC50 = 2.4; 1.8; 0.9 mg/ml. The peptide TFI-b3 showed the highest radical suppression activity. Through MALDI TOF-MS/MS, their primary structures were determined, and the sequences of RLDGQGRPRVWLDR, TPDNIDIWLGGIAEPQVKR, and VAYSDDGENWTEYRDQGAVEGK were recorded, respectively. These peptides are recommended for use as powerful antioxidants in functional foods.

5. When studying the conditions of extraction of scorpion protein, ultrasound 0.5 M NaCl solution showed the best results (output 14.64%, protein 79.06%). The optimal parameters for extraction according to RSM optimization were 50 min, 400 W, and 18 ml/g, with a protein yield of 78.94% and a yield of 24.80%. In the DSF, UE, and SE methods, the minimum solubility of the protein at pH 4.0 was 8.05%, 15.25%, 18.75%, and the maximum solubility at pH 12 was 13.5%, 70.15%, 79.5%, respectively. It was

established that at a pH of 7.0, the emulsifying (45.55%) and foaming (40.30%) properties of UE are higher than in SE. SEM images showed that during the drying process, the protein transitions from a crystalline state to an amorphous structure and forms a structure similar to high-crystalline "wood shavings."

6. The *Buthus martensii* scorpion protein was hydrolyzed with papain, alkalase, and trypsin, and the highest hydrolysis yield was achieved with papain (26.46%). Papain fraction at a concentration of 5.0 mg/ml exhibited strong antioxidant activity (ABTS - 77.45%, DPPH - 75.54%, •OH - 49.44%). As a result of three-stage purification of this fraction, P4-1 and P4-2 peptides were isolated. Through MALDI-TOF-MS/MS, their structure was determined to be equal to the LPTETLH and IEEDLER sequences, respectively. The peptide P4-1 showed 83.22% activity for ABTS, and P4-2 - 81.22% activity for DPPH. Activity in relation to the ON radical was 62.96% and 58.69%, respectively, which is lower than in relation to other types of radicals.

7. Among the 10 new peptides created from the secretion of Ranacyclin J frog skin, AKK8 showed the highest antimicrobial activity and had a strong effect against E. coli, S. aureus, B. subtilis, and especially C. albicans (MIC = 18.5 μg/ml). Salt ions did not reduce its activity, which confirms the stability of the ACC8 peptide in clinical conditions. When administered to infected mice at a dose of 4 mg/kg, it normalized inflammatory cytokines. Simple structure, low hemolytic and cytotoxic effect, stability in the physiological environment allow the use of AKK8 as a promising therapeutic template against drug-resistant C. albicans infections.

8. Using DEAE-cephadex A-25, 5 fractions were isolated from the red deer abomasum protein, and the peptide RDA4-1 with Mm 15 kDa was obtained by gel chromatography. RDA4-1 is a high-purity protein (91.63%), containing monosaccharides such as Ala, Gly, Leu, Tyr, Val and Man, Glu, GalUA, GluN, L-Fuc. Analysis of IR spectroscopy confirmed its protein-polysaccharide complex structure, while the secondary structure consisted of 65.22% β-delamination and 34.78% β-delamination. The antioxidant activity of RDA4-1 (1.5 mg/ml) was 50.97% according to DPPH, 88.37% according to ABTS, and the inhibition of NAase was 79.23%. This indicator is significantly higher than marlin collagen peptides (39.83%), which indicates the promising use of peptides derived from red deer abomasum in the treatment of gastritis and other inflammatory diseases.

9. Sericin trypsin hydrolyzate was separated by ultrafiltration into SHTA (>10 kDa), SHTB (3-10 kDa), and SHTC (<3 kDa) fractions, the chelating activity of which at a concentration of 2 mg/ml was 61.43%, 66.61%, and 72.46%, respectively. The SHTC fraction with the highest chelating activity was purified with HPLC, and the SHTCF2 fraction was eluted in 30% ethanol with a chelating ability of 77.80%. As a result of further fractionation, the peptide SHTCF2III was obtained, which has the highest activity in chelating the copper ion among all fractions with IC_{50} = 0.4013 mg/ml. LC-MS/MS analysis confirmed that the amino acid sequence of this peptide is DDSRADSSR.

10. Two new peptides (R3 and R7) with high antioxidant activity were purified from SAPH by ultrafiltration, ion exchange, gel chromatography, and high-performance reverse-phase liquid chromatography. The amino acid sequence of the peptides was determined as Leu-Glu-Asp-Gly-Leu-Lys (LEDGLK, SAPHP-A) and Ile-Asp-Asp-Val-Leu-Lys (IDDVLK, SAPHP-B) with molecular weights of 674.37 and 703.41 Da, respectively. SAPH peptides are recommended as food additives and pharmaceutical products.

НАУЧНЫЙ СОВЕТ DSc.02/30.12.2019.К/В.37.01 ПО ПРИСУЖДЕНИЮ УЧЁНЫХ СТЕПЕНЕЙ ПРИ ИНСТИТУТЕ БИООРГАНИЧЕСКОЙ ХИМИИ

ИНСТИТУТ БИООРГАНИЧЕСКОЙ ХИМИИ

ВАЙЛИ АЙХЕМИДИН

СТРУКТУРА И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ПЕПТИДОВ ЖИВОТНОГО И РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ, ДЛЯ РАЗРАБОТКИ ФУНКЦИОНАЛЬНЫХ ПРОДУКТОВ ПИТАНИЯ И ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ

02.00.10 – Биоорганическая химия

ПРЕЗЕНТАЦИЯ
на соискание ученой степени доктора химических наук (DSc)
на основании статей, опубликованных в научных журналах, имеющих соответственно высокий импакт-фактор, и включенных в международные научные базы данных
(без защиты диссертации)
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Автореферат диссертации на трех языках (узбекский, английский, русский) размещен на веб-странице (www.biochem.uz) и на Информационно-образовательном портале «ZiyoNet» (www.ziyonet.uz).

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ВВЕДЕНИЕ (аннотация презентации)

Актуальность и востребованность темы исследования. Научное изыскание и высокоэффективное использование биоактивных макромолекул и пептидов из региональных животных и растительных ресурсов стали глобальным приоритетом в области исследований в области науки о продуктах питания, биотехнологии и химии биоресурсов. Природные белки и пептиды, полученные из верблюжьего молока, нута, яда скорпиона, тканей желудка жвачных животных (например, сычуга ягнёнка и тянь-шаньского благородного оленя), семян тмина, фиброина шёлка и костного мозга быка, проявляют разнообразную и значимую биологическую активность, включая антиоксидантную, противовоспалительную, гипогликемическую, антимикробную и иммуномодулирующую. Эти результаты демонстрируют их широкий потенциал в разработке функциональных продуктов питания, нутрицевтиков и биомедицинских материалов.

В последние годы как местные, так и зарубежные исследования были сосредоточены на поиске новых биоактивных пептидов с помощью ферментативного гидролиза, хроматографии и масс-спектрометрической протеомики. Однако по-прежнему отсутствуют систематические, экологически безопасные и масштабируемые технологии разделения для получения высокочистых функциональных пептидов из этих сложных биологических матриц. Более того, молекулярные механизмы, лежащие в основе их биологической активности, и взаимосвязи между структурой и функцией до сих пор не полностью изучены.

На территории Синьцзяна (Синьцзяян-Уйгурский автономный район, КНР) обитает множество уникальных растений и животных, которые представляют собой важную ресурсную базу для традиционной уйгурской медицины. Наша группа ранее добывала активные ингредиенты различных растений и животных, что служит важным источником информации для расширения применения китайской медицины. В частности, мы очистили и изучили активные ингредиенты нижеперечисленных растений и животных. Что касается растений, мы исследовали семена тмина (*Cuminum cyminum* L.), семена нута (*Cicer arietinum* L.) и некоторые растительные ингредиенты (кверцетин и рутин, ковалентно связанный гидролизат серицина шелка). Что касается животных, мы исследовали белки, полученные из костного мозга овец, коров, лошадей и верблюдов, гидролизаты молока двугорбых верблюдов, белки скорпиона (общий белок и антиоксидантные пептиды), серицин шелка, гликопротеин из сычуга ягненка и белки сычуга благородного оленя. Наша работа дает важные справочные данные для глубокого понимания лекарственных ресурсов растений и животных в районах Синьцзяна, и мы предложили больше потенциальных вариантов традиционных китайских лекарственных средств для лечения соответствующих заболеваний.

Исследование имеет большое значение для устойчивого использования биоразнообразия Синьцзяна и Центральной Азии и вносит вклад в реализацию

национальных приоритетов в области развития биоресурсов, инноваций в области функциональных продуктов питания и экологичного биопроизводства. С научной точки зрения, оно способствует пониманию взаимосвязи между структурой пептидов и биологической активностью. С практической точки зрения, оно закладывает технологическую основу для промышленного производства высококачественных биологически активных ингредиентов, способствуя локальному преобразованию сельскохозяйственных и животных ресурсов в функциональные продукты.

Данная диссертация в определенной мере служит выполнению задач, которые были установлены Указами Президента Республики Узбекистан от 7 февраля 2017 года УП-4947 «О Стратегии действий по дальнейшему развитию Республики Узбекистан» и от 29 октября 2020 года УП-6097 «Об утверждении Концепции развития науки до 2030 года», Постановлением Президента от 12 июля 2018 года УП-3847 «О мерах по совершенствованию деятельности Института биоорганической химии Академии наук Республики Узбекистан имени А.С. Садыкова», а также других задач, предусмотренных в нормативно-правовых документах, связанных с вышеуказанной деятельностью.

Кроме того, данная работа способствует достижению долгосрочной цели – созданию международной исследовательской платформы для изучения природных биоактивных макромолекул в рамках Совместной лаборатории технологий макромолекулярных соединений, созданной Центральноазиатским центром по исследованию и разработке лекарственных препаратов Китайской академии наук и Институтом биоорганической химии Академии Наук Узбекистана. Она также обеспечивает прочную исследовательскую базу и технический резерв для будущих проектов высокого уровня, таких как Национальный фонд талантливых молодых учёных (NSFC-Excellent Young).

Целью исследования является выделение, очистка и структурная характеристика пептидов из семян тмина, проростков нута, костного мозга (бычьего, верблюжьего, конского и овечьего), верблюжьего молока, белков скорпиона, фиброина шелка, белков сычуга ягненка и благородного оленя, выявление их потенциальной биологической активности для разработки функциональных продуктов питания, нутрицевтиков и биомедицинских материалов.

Объектом исследования является разнообразная коллекция природных биологических материалов, в том числе растительные материалы семена тмина *(Cuminum cyminum* L.), проростки нута (*Cicer arietinum* L.), ткани и продукты животного происхождения (костный мозг овец, коров, лошадей и верблюдов, молоко верблюдицы-бактриана, тело скорпиона *Buthus martensii Karsch*, тело лягушки, сычуг ягненка и благородного оленя *Cervus elaphus*), выделения и побочные продукты животного происхождения (яд скорпиона *Buthus martensii Karsch*, выделения кожи лягушки (в качестве матрицы для синтеза), серицин шелка из коконов тутового шелкопряда).

Предметом исследования являются биоактивные молекулы, выделенные, охарактеризованные или синтезированные из вышеупомянутых объектов, включая природные антимикробные и антиоксидантные пептиды и белки, гликопротеины с противовоспалительной, противоопухолевой и фермент-ингибиторной активностью, нейротоксические пептиды с модуляторной активностью ионных каналов, новые синтетические противогрибные пептиды, ковалентно связанные конъюгаты серицина шелка с флавоноидами и фенольными соединениями.

Научная новизна исследования заключается в следующем:

Впервые обнаружены и охарактеризованы новые биоактивные пептиды из различных источников (семена *Cuminum cyminum*, молоко двугорбого верблюда, гидролизаты скорпиона, проростки Cicer arietinum L.), а также разработанный противогрибный пептид (AKK8) против лекарственно-устойчивого штамма *Candida albicans*;

Были идентифицированы биоактивные макромолекулы из тканей животных, включая белки костного мозга с антибактериальной/антиоксидантной активностью у четырёх видов домашних животных, гликопротеины, ингибирующие ЦОГ-2, из сычуга ягнёнка, белок, ингибирующий гиалуронидазу, из сычуга *Cervus elaphus*, и оптимизирована экстракция антиоксидантного белка из лягушки благодаря новаторскому исследованию;

Ковалентной конъюгацией серицина шелка с флавоноидами/фенолами достигнуто расширение его функциональных возможностей и значительное улучшение противовоспалительных и эмульгирующих свойств;

Разработаны инновационные методики выделения ключевых биоактивных белков, протоколы очистки новых пептидов/гликопротеинов, проведена стематическая оптимизация процесса экстракции белков из скорпиона (*Buthus martensii Karsch*);

Впервые систематически исследованы белковые ресурсы сычуга ягненка и благородного оленя *Cervus elaphus* и выявлен биоактивный потенциал выделенных белков;

Новые функциональные белки с противовоспалительной, противоопухолевой и гиалуронидаза - ингибирующей активностью, а также антиоксидантные пептиды (например, SAPHP-A, SAPHP-B, LPTETLH, IEEDLER), не описанные в существующих базах данных белков, были выявлены путем интеграции хроматографических, спектроскопических, комбинацией биотестов и вычислительных подходов.

Обнаружены четкие корреляции между структурными параметрами молекул (молекулярной массой, гидрофобностью, аминокислотным составом) и антиоксидантной или металлохелатирующей активностью.

Были получены новые знания о взаимосвязи структуры и функции белков животного и растительного происхождения, а также предложены новые теоретические и практические основы для эффективного использования биоактивных белков и пептидов из недоиспользуемых природных ресурсов.

Внедрение результатов исследований. На основе научных результатов, полученных при изучении биоактивных пептидов лекарственных растений и животных, обладающих антибактериальной, антиоксидантной и противовоспалительной активностью, а также разработки пептидов:

Компания Xinjiang Biochemical Pharmaceutical Co., Ltd. (KHP) разработала и вывела на рынок препарат «Капсулы с витамином B12 из сычуга ягненка» на основе белкового экстракта сычуга ягненка.

Получены 12 патентов Китайской Народной Республики, в которых представлены методы конструирования и их практическое применение для повышения биологической активности пептидов:

ZL201510191380.6. Preparation method of chickpea peptides and their application;

ZL201811122673.9. Preparation method of antioxidant peptides from camel milk and their application;

ZL202111360384.4. Preparation method of tianshan red deer abomasum glycoproteins with hyaluronidase inhibitory activity;

Результаты исследований цитировались в международных научных журналах с высоким импакт-фактором, включая «Journal of nanobiotechnology» (ИФ 12,6), «LWT-food science and technology» (ИФ 6,6), «Phytotherapy research» (ИФ 6,3), «Food bioscience» (ИФ 5,9), «Nutrients» (ИФ 5,0), «Molecules» (ИФ 4,6), «Scientific reports» (ИФ 3,9), «Journal of food science» (3,4), «Journal of food science and technology-mysore» (ИФ 3,3), «Journal of food measurement and characterization» (3,3), « Frontiers in sustainable food systems» (ИФ 3,1), «Journal of food quality» (ИФ 2,9), «Journal of separation science» (ИФ 2,8), «Current microbiology» (ИФ 2,6), « International journal of peptide research and therapeutics» (ИФ 2,4), « Biotechnology letters» (ИФ 2,1) и т. д. Результаты работы были процитированы более 280 раз (“h” индекс - 11, ResearchGate).

ЭЪЛОН ҚИЛИНГАН ИШЛАР РЎЙХАТИ
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