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MICROWAVE-ASSISTED SYNTHESIS OF APIS MELLIFERA BEE CHITOSAN AND INVESTIGATION OF ITS RHEOLOGICAL PROPERTIES

Annotation

In this study, chitosan was synthesized from dead Apis Mellifera honeybees using a microwave-assisted deacetylation method. Compared to conventional thermal processing, this approach significantly reduces reaction time and energy consumption. The obtained chitosan was analyzed for its physicochemical properties, rheological behavior, molecular weight, and degree of deacetylation (DDA). The results indicate that chitosan derived from Apis Mellifera demonstrates high quality and promising potential for applications in biomedicine, pharmaceuticals, and biomaterials engineering.

Keywords: chitin, chitosan, demineralization, deproteinization, deacetylation, aminopolysaccharide, DDA, microwave-assisted extraction.

СИНТЕЗ ХИТОЗАНА ПЧЁЛ APIS MELLIFERA С ИСПОЛЬЗОВАНИЕМ МИКРОВОЛНОВОГО МЕТОДА И ИССЛЕДОВАНИЕ ЕГО РЕОЛОГИЧЕСКИХ СВОЙСТВ

Аннотация

В данной работе изучен процесс синтеза хитозана из мёртвых пчёл Apis Mellifera с применением метода деацетилирования под действием микроволн. По сравнению с традиционной термической обработкой, данный подход значительно сокращает время реакции и снижает энергозатраты. Полученный хитозан был исследован на предмет физико-химических свойств, реологических характеристик, молекулярной массы и степени деацетилирования (DDA). Результаты показывают, что хитозан, полученный из Apis Mellifera, отличается высоким качеством и обладает перспективным потенциалом применения в биотехнологии, медицине и фармацевтике.

Ключевые слова: хитин, хитозан, деминерализация, депротеинизация, деацетилирование, аминополисахарид, DDA, микроволновой метод.

APIS MELLIFERA ASALARI XITOZANINI MIKROTO‘LQIN YORDAMIDA SINTEZ QILISH VA REOLOGIK XOSSALARINI O‘RGANISH

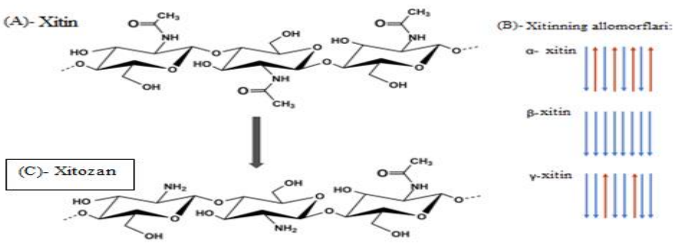
Annotatsiya

Ushbu maqolada *Apis Mellifera* asalaridan mikroto‘lqin yordamida deatsetilatsiya” - xitin molekulasidagi asetil (–$COCH_3$) guruhlarini ishqoriy muhitda (odatda NaOH eritmasida) olib tashlab, xitozan hosil qilish jarayoni ko’rsatilgan. Shuningdek hasharot manbalarining boshqa manbalarga nisbatan ustunliklari ta’kidlab o‘tildi. Maqolada olingan xitozanning reologik xossalari va fizik-kimyoviy analiz tahlillari xam o’rganilgan.

Mikroto‘lqin yordamida - jarayonda issiqlikni tez va bir xil taqsimlab, reaktsiya vaqtini sezilarli qisqartiradigan mikroto‘lqin energiyasidan foydalanish haqida ma’lumotlar keltirilgan.

Kalitli so‘zlar: Xitin, xitozan, demnerallash, deproteinlash, deatsetillash, aminopolisaxarid, N-atsetilglyukozamin, α-xitin, β-xitin, γ-xitin, deatsetillanish darajasi (DDA), ekstraksiya.

Kirish. Xitin – tabiatda sellulozadan keyin eng ko‘p uchraydigan polisaxarid bo‘lib, u β-(1,4) bog‘langan N-atsetilglyukozamin (GlcNAc) birliklaridan tashkil topgan to‘g‘ri zanjirli polimerdir[1]. Xitinda 3 xil shakl mavjud bo’lib, ular: α-xitin, β-xitin va γ-xitindir [2].



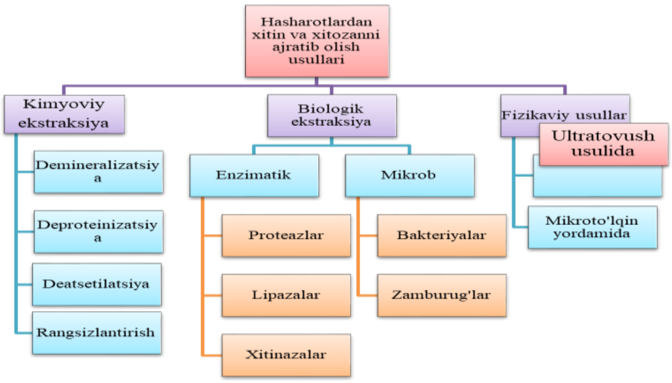
1-rasm. (A) Xitinning molekulyar tuzilishi. (B) Xitinning uchta kristallik allomorf shakli. (C) Xitozanning molekulyar tuzilishi.

Xitozan xitinning deatsetillangan shakli bo'lib, uning eruvchan hosilasi hisoblanadi [3]. Biologik parchalanadigan polimer xitozan ekologik toza mahsulotdir [4]. Xitozan tabiiy aminopolisaxarid bo’lib, tibbiyotda keng qo’llaniladi [5-7]. Xitozan xitindan olingan bo’lib, zamburug’larning hujayra devorlarining, qisqichbaqasimonlar va hasharotlarning ekzoskeletlarining asosiy komponenti sifatida uchraydi (2-rasm).

2-rasm. Xitindan xitozan olish tabiiy manbalari

Mavzuga oid adabiyotlar tahlili. Hasharotlar – xitin va xitozan uchun serob va barqaror manba bo‘lib, ular xitozan ishlab chiqarishda istiqbolli variant hisoblanadi. Birinchidan, hasharotlar tez ko‘payadi, qisqa hayot aylanishiga ega, va ularni yetishtirish uchun kam resurslar talab qilinadi, bu esa xitinning barqaror va uzluksiz manbasini ta’minlaydi [8]. Xitin hasharot organizmida, ayniqsa tashqi skeletda, balki qanotlar, mo‘ylovlar va traxeyalarda ham uchraydi [9]. Bunday keng tarqalganligi turli tana qismlaridan xitin olish imkonini beradi va hasharot biomassasidan to‘liq foydalanish imkonini yaratadi. Hasharotlarning tashqi skeleti asosan xitindan tashkil topgan bo‘lib, u tana uchun strukturaviy tayanch va himoya vazifasini bajaradi [10]. O’zbekistonda xitin tut ipak qurti g’umbagi [11] va asalarilardan oinadi [12-13]. Xitozan, kislotalarda yaxshi eruvchanligi va polikation tabiatga ega bo‘lishi sababli, biologik faollik va biomolekulalar bilan o‘zaro ta’sirlashuv nuqtai nazaridan ustunlik qiladi [14].

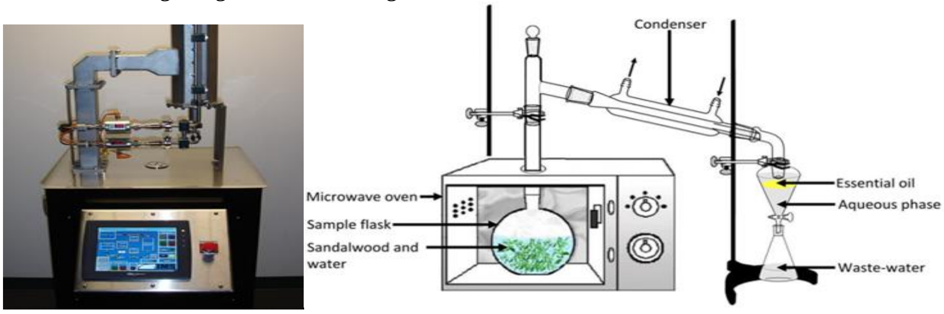
Hozirda xitozan olishning samarali turli xil sintez usullari bor (3-rasm). Bizning tadqiqotda *Apis Mellifera jonsiz* asalaridan xitozan ultratovush [15] va mikroto’lqin yordamida sintez qilindi. Bu usul asosan deatsetillash bosqichida qo’llaniladi. Ish jarayoni xuddi 3-rasmdagidek boradi.



3-rasm. Hasharotlardan xitin hamda xitozanni ajratib olishning turli usullari

Tadqiqot metodologiyasi. ***Apis Mellifera* asalaridan xitozan sintezi.** *Apis Mellifera* jonsiz asalaridan sintez jarayoni uchun 40 g olindi, 70-80^0 C da 7 % li HCl bilan 1 soat davomida deminerallash jaraoni olib borildi. Bu bosqichda barcha minerallar va yog’lar kislotada eriydi. Olingan namuna distillangan suvda pH=7 bo’lguncha yuvildi va bir kun davomida xona haroratida quritildi. Keyingi kun esa 7 % li NaOH bilan 80^0 C da 2 soat davomida deproteinlash jarayoni olib borildi. Bu bosqichda namuna oqsillardan tozalanadi va toza xitin olinadi. Xitin namunasini pH=7 bo’lguncha distillangan suvda yuvildi. So’ngra xona haroratida qirutildi.

Xitinni oqartirish uchun 3 % li H_2O_2 (vodorod peroksid) bilan 15 daqiqa magnitli mishalkada aralashtirdik (jarayon ko’pirib samarali oqarishi uchun biroz qizdirdik). Olingan xitinni distillangan suvda yuvib, xona haroratida qurutdik. So’ngra maydalagichda yanchib oldik. Olingan xitindan xitozan olish uchun deatsetillash jarayonini mikroto’lqinli pechda (4-rasm) 40 % li NaOH bilan 90^0 C da 3 soat davomida qizdirdik. Olingan namunani pH=7 bo’lguncha distillangan suv bilan yuvib, xona haroratida qurutdik. Xitozanning olingan massasi m=5 g ni tashkil etdi.



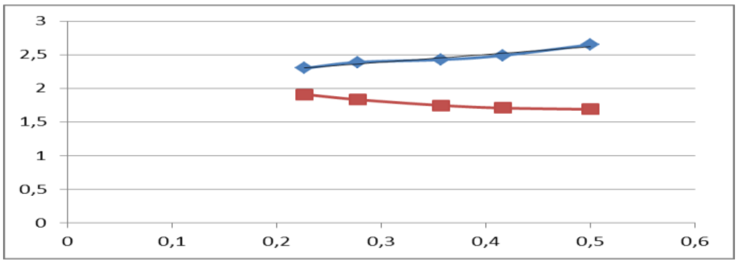
4-rasm. Mikroto’lqinli pech (microwave-assisted).

Xitozanning statistik chiqish unumi. Xitin olishda an’anaviy hamda enzim, mikroto‘lqin va ultratovush yordamida ekstraksiya usullarini solishtirildi. Natijalar shuni ko‘rsatdiki, an’anaviy usulda 9,7% xitin hosil bo‘lsa, enzim yordamida 42,3%, mikroto‘lqin yordamida 11,4% va ultratovush yordamida 13,7% xitin (quruq massa bo‘yicha) olingan [16].

Biz sintezlagan xitozanning chiqish unumi quruq massaga nisbatdan 12,5% ni tashkil qildi.

Natijalar va tahlillar. Qovushqoqlik hamda molekulyar massasini aniqlash. Xitosanning Mr ni o'lchash uchun turli usullar ishlab chiqilgan, jumladan, gel o'tkazuvchanlik xromatografiyasi (GPC), o'lcham bo’yicha ajratish xromatografiyasi va viskozimetriya [17,18].

Biz *Apis Mellifera* dan olgan xitozanimizni Ubbelode vizkozimetri yordamida aniqladik. Buning uchun 2% li 10 ml sirkaga 0,1 g xitozanni eritib, 2 soat aralashtirdik. So’ng 0,33 M li natriyli sirka bufferini tayyorlab oldik. Shundan so’ng ketma-ket suyultirish natijasida olingan turli polimer konsentrasiyali eritmalarning oqish vaqtini o’lchadik, so’ngra *[η]* aniqladik. Eritmalarning qovushqoqligi Ubellode viskozimetrida 25^{0}C da (kapillyar diametri 0,56 mm) o’lchadi. Olingan natija grafigi 5-rasmda keltirilgan.



5-rasm. Vizkozimetrda olingan qovushqoqlik grafigi

Olingan natijalarni Engler-Mark-Houwink($[\eta] = K\times M^{\alpha}$)tenglamasiga kiritish orqali xitozanning Mr aniqlandi. Bunda xitozanning Mr si 103 kDa ni tashkil etdi.

***Apis Mellifera* xitozanining DDA sini aniqlash**

Xitozanning deatsetillanish darajasini konduktometrik titrlash usuli orqali aniqladik. Konduktometriyada xitozan kuchli kislota (odatda HCl) bilan titrlanadi, so‘ngra kuchli ishqor (NaOH) bilan titrlanadi. O‘lchov jarayonida olingan konduktometriya grafigida ikki burilish nuqtasi hosil bo‘ladi. Buning uchun 0,15 g xitozan namunasi 10 ml 0,1 M HCl va 40 ml distillangan suvda suspenziya qildik. Olingan eritma 0,1 M NaOH bilan titrlanadi, har 30 soniyada 0,1 ml dan doimiy aralashtirib qo’shdik. Erkin aminokislotalar titrlash uchun zarur bo’lgan ishqorning ml ga miqdori eritmaning elektr o’tkazuvchanligining gidroksidi hajmiga bog’liqligi grafigini chizdik. Grafik 6-rasmda keltirilgan. Shundan so’ng konduktometriya titrlash bo’yicha DDA ni aniqlash formulasiga qo’ydik:

$$\mathrm{DDA}(\%) = \frac{(V_2 - V_1)\times C_{\mathrm{NaOH}}\times 161.16}{m} \times 100$$

bu yerda:

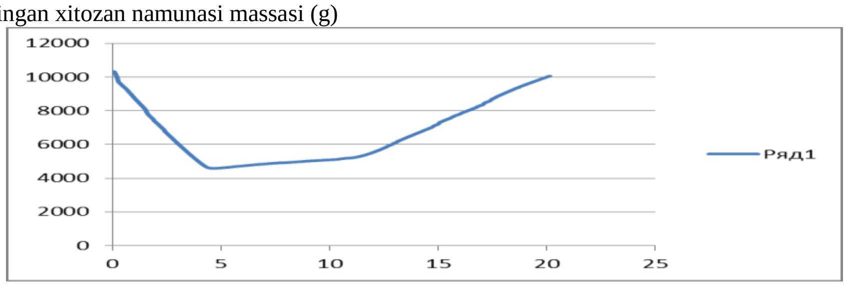
V_1 - birinchi ekvivalent nuqtaga qadar ishlatilgan NaOH hajmi (L)

V_2 - ikkinchi ekvivalent nuqtaga qadar ishlatilgan NaOH hajmi (L)

C_{NaOH} - NaOH eritmasining molyar konsentratsiyasi (mol/L)

161.16 - D-glukozamin birligining molyar massasi (g/mol)

m - tahlil qilingan xitozan namunasi massasi (g)



6-rasm. Konduktometrdan olingan qiymatlar grafigi

Sintezdan olgan qiymatlarni formulaga qo’yib DDA ni hisoblaymiz.

$$x = \frac{(11,6 - 4,3) \times 0,1 \times 161,16}{150} \times 100$$

x= 78 % ga, demak *Apis Mellifera* dan olingan xitozanning DDA darajasi 78 %

Izoh: — bu titrlash jarayonida amin guruhlarini neytrallash uchun ketgan NaOH miqdori. Bu qiymatdan DDA hisoblanadi, chunki DDA — amin guruhlari miqdoriga to‘g‘ridan-to‘g‘ri proporsional.

Xulosa va takliflar. Xitin va xitozanning strukturaviy xususiyatlari ularning fizik-kimyoviy xossalari hamda qo‘llanilish sohalarini aniqlab beradi. Xitinning kristallik tuzilishi va uchta allomorf shaklining noyob joylashuvi uning mexanik mustahkamligi, eruvchanligi va parchalanish holatiga ta’sir qiladi. Xitozanning biomimetik tadqiqotlarda keng imkoniyatlarini ochib beradi. Xususan, biologik to‘qimalar, hujayra tashqi matritsasi va hujayra muhitini taqlid qiluvchi materiallarni yaratishda xitozan asos bo‘lishi mumkin. Biosensorlar va diagnostik ilovalarda xitozanning biomolekulalar bilan o‘zaro ta’siri va biokompatibilligi uni bioaktiv sirtlar, biosensorlar va diagnostik testlar uchun istiqbolli materialga aylantiradi.

Demineralashda namunaga kuchli kislotalar bilan uzoq vaqt ta’sir qildirish yoki yuqori harorat sharoitida ishlov berish xitin va xitozanning polimer zanjirini qisqartirib, ularning molekulyar massasi va qovushqoqligini kamaytirishi mumkin. Deproteinizatsiya jarayonini samarali olib borish uchun reagent konsentratsiyasi va haroratni hasharot turining xususiyatlari va mo‘ljallangan mahsulot xossalariga muvofiq optimallashtirish zarur.

Mahalliy xom-ashyo *Apis Mellifera* asalaridan xitin va xitozan olish optimal texnologiyasi sintez qilingan namunalar fizik-kimyoviy xossalaridan – deatsetillanish darajasi, qovushqoqligi, molekular massasi o’rganilgan va natijalar tahlil qilingan. Demak mikroto’lqinli usulda olish afzalliklari quyidagicha:

Reaksiya vaqti an’anaviy issiqlik bilan solishtirganda bir necha barobar tezroq bordi. Ishqor konsentratsiyasi kamaytirildi. Xitozanning molekulyar massasi va deatsetilatsiya darajasini aniqroq boshqarish oson bo’ldi.

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