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Gel Formation of Pectin from Okra Leaves, Pulp and Seeds

Dhakane Priti Subhash, Mr.V.G.Rokade, Dr.L.D.Hingane
Aditya Pharmacy College Beed-431122

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ABSTRACT
Okraplantparticularlyitsfruitishighlymucilagewhich composedofpectinandhighcontentofcarbohydrate.B yproductsofokraplantsuchasleavesandmaturedfruits willbediscardedwhenevertheyoungfruitsareharveste dwhicheventuallyleadstoenvironmentalpollution.Th osebyproductshavepotentialtobecomeplant-basedalternativeforbovineandporkrelatedgelatin.Thi sstudyaimedtodeterminethegelformationofpectinext ractedfromokraplantbyproductsparticularlytheleave s,pulp(skinwithoutseeds)andseeds.Pectinwasextract edusingasequentialextractionwiththeapplicationsof hotbuffer(HB)andhotbufferwithchelatingagents(CH).CHextractiongavethehighestpectinyield(>40%)co mparedtoHBandDA.TheHBfractionharboredhighly purifiedpectinduetohighanhydrouronicacidcontenta nddegreeofesterification.Thehighestpectinyieldwas extractedfromseedswithanoverallfractionyieldof86 %,followedbytheleaves(75%)andpulp(71%).Thepe ctinwasblendedwithkonjacglucomannan(KG)in5.0: 1.6ratiotoformgelandstoredfor16-18hrat4°C±1.0.ThegelformedusingHBextractionwa sfoundtohavesignificantlylower($p<0.05$)gelstrength thanHBwithCHextraction.

I. INTRODUCTION

Okra(Abelmoschusesculentus(L.)Moench) isknownasthelady'sfingers,'bhindi'(India),'gumbo' (Africa),and'bendiorkacangbendi'(Malaysia).Theo kraleavesareslightlybiggerthantealeaveswithlongers tem;theyalternateatwidespaceintervalsuptothetopoft hestemandtheyaredeeplydividedwithtoothedmargin s.Okraiswidelyknownasaviscousmucilaginousplant .

Pectinisamethylatedesterofpolygalacturoni cacidthatcontains1,4-linkedα-Dgalacturonicacidresiduesandcanbefoundinmanyty pesofplants.Forexample,heteropolysaccharidesderi vedfromthecellwallofhigherterrestrialplantsandthep eelsofcitrusfruit,guavasandapples(Sengkhamparnet al.,2009).Itcanformgelinthepresenceofsugarandanac idsolutionundersuitableconditions.Itistypicallyadde dtojamsandjelliesasagellingagent.

Severalstudieshaveconductedsequentialext ractionofpectinfromokraleaves,pulp,andseedsandid entifieditsfunctionalgroups.Besides,therewasastudy involvedoptimizationextractionandrheologicalprop ertiesofpectinfromokra.

However,limitedstudieshavebeendoneupo ncomparingthegellingformationofanimalbased(gela tin)andplant-based(pectin).

Thisstudyappliedtheconceptof"wasteintog old"whichinvolvedutilizationofokraby-productthateventuallyreducethewastefrompost-harvestcultivatedintovaluableplant-basedhydrocolloid.

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BOTANICALDESCRIPTION

ScientificName	ABELMOSCHUSESCULENTUS(HIBISCUSESCULENTUS)
PlantFamily	MALVACEAE(MALLOWS)
HindiName	BHINDI
SanskritName	TINDISHA,PITALI,GANDHAMULA
EnglishName	OKRA,LADIES'FINGER,EDIBLEHIBISCUS,OCKRO
OtherNames	BENDI,RAMTURAI,DUK

DISCRIPTIONOFOKRAPLANTS

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BotanicalClassification(Taxonomy)

Kingdom	PLANTAE
Sub-Kingdom	VIRIDIPLANTAE
InfraKingdom	STREPTOPHYTA (landplants)
SuperDivision	EMBRYOPHYTA
Division	TRACHEOPHYTA(TRACHEOPHYTESorVascularPlants)
SubDivision	SPERMATOPHYTINA(SPERMATOPHYTESorSeedPlants)
Class	MAGNOLIOPSIDA
SuperOrder	ROSANAE
Order	MALVALES
Family	MALVACEAE(MALLOWS)
Genus	ABELMOSCHUS(OKRA)
Species	ABELMOSCHUSESCULENTUS(OKRA)

PlantDescription

TypeofPlant	Perennialfloweringplants
NativeRange (GeographicDistribution)	India,NorthAfrica,SouthAfrica
Height (growsupto)	1to2meter
Habitat (typeofenvironment)	Warmtemperateandtropicalregions
Leaves	10–20cmlonghaving5to7palmatelobesandbroadinshape
Flowers	4–8cmindiameterwith5lightyellowpetals
Fruits/Pods	5to10cmlongFibrousfruits(seedpods)containingmucilagesubstanceandwhiteseeds
Seeds	Roundandwhiteseeds

THERAPEUTICINDICATIONS

Okra(Bhindi)ishelpfulinfollowinghealthconditions.

DigestiveHealth

Constipation

Chronicdysentery

Skin&Hairs

Usedassoothingandsofteningpoultice

Kidney&Bladder

Dysuria(painfulurination)

Gonorrhealcystitis

Cystitis

MEDICINALPROPERTIES

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Okra(Bhindi)hasfollowinghealingproperties.
Emollient(softeningandsoothingeffectonskin)–blandviscidmucilage
Demulcent(soothestheinflamedskin)–blandviscidmucilage
Diuretic
Aphrodisiac(mucilagesubstanceinunripepods)
Nutritious

Nutritionalcompositionofokra

Okraismoreadietfoodthanstaple.
Okraseedshavebeenusedonasmallscaleforoilproduction.Lipidcomponentsgreatlycontributetothenutritionalandsensoryvalueofalmostalltypesoffoods.
Natureprovidesalargenumberoffatsthatdifferintheirchemicalandfunctionalproperties.
Fourclassesoflipidsarehabituallyfoundinvegetableoils:triacylglycerols,diacylglycerols,polarlipids,andfreefattyacids.
Thefattyacidcompositiondeterminesthephysicalproperties,stability,andnutritionalvalueoflipids.

Monoenoicfattyacidsandpolyunsaturatedfattyacidsarestructurallydistinguishedbythepresenceofrepeatingmethyleneunits.
Theseunitsproduceanextremelyflexiblechainthatrapidlyreorientsthroughconformationalstatesandconstitutesaninfluentialgroupofmoleculesthatpromotehealth.

Theoilmainlyconsistsoflinoleicacid(upto47.4%).Okraseedoilisarichsourceoflinoleicacid,apolyunsaturatedfattyacidessentialforhumannutrition.
Proteinsplayaparticularlyimportantroleinhumannutrition.
Theaminoacidcontents,proportions,andtheirdigestibilitybyhumanscharacterizeaprotein'sbiologicalvalue.
Okrahasbeencalled"aperfectvillager'svegetable"becauseofitsrobustnature,dietaryfiber,anddistinctseedproteinbalanceofbothlysineandtryptophanaminoacids(unliketheproteinsofcerealsandpulses).
Okraseedisknowntoberichinhighqualityproteinespeciallywithregardstoitscontentofessentialaminoacidsrelativetootherplantproteinsources.
Hence,itplaysavitalroleinthehumandiet.Okraalsocontainscarbohydratesandvitamins,andplaysavitalroleinhumandiet.

Consumptionofyoungimmatureokrapodsisimportantasfreshfruits,anditcanbeconsumedindifferentform.

Themaincomponentsaregalactose(25%),rhamnose(22%),galacturonicacid(27%)andaminoacids(11%).

Themucilageishighlysolubleinwater.Itssolutioninwaterhasanintrinsicviscosityvalueofabout30%.

Driedokrasauce(podsmixedwithotheringredientsandregularlyconsumedinWestAfrica)doesnotprovideanybetacarotene(vitaminA)orretinol.

Okraseedaspotentialedibleoilandfloursource

Okraseedscontainabout20to40%oil

Okraseedoilyieldiscomparabletomostoilseedcropsexceptoilpalmandsoybean.Moreover,okraseedoilhaspotentialhypocholesterolemiceffect.
Thepotentialforwidecultivationofokraforedibleoilaswellasforcakeisveryhigh.
Forexample,supplementingmaizeogiwithokramealincreasesprotein,ash,oilandfibercontent.

Okraseedflourhasbeenusedtosupplementcornflourforaverylongtimeincountrieslike Egypttomakebetterqualitydough.

Theenormousnutritionalandotherbiologicalactivitiesinthepodsandseeds.
Okraseedsweredeterminedtohaveappreciableprotein content.Thevariationsinpolysaccharidesfoundinthemucilagearehigherinokrapods.

Healthbenefitsofokra

Inrecentyears,increasingattentionhasbeenpaidtotheroleofdietinhumanhealth.Thehighintakeofplantproductsisassociatedwithareducedriskofanumberofchronicdiseases,suchasatherosclerosisandcancer.

Themajorantioxidantsofvegetablesarevitamins C and E,carotenoids,andphenoliccompounds,especiallyflavonoids.

Theseantioxidantsscavengeradicalsandinhibitthechaininitiationorbreakthechainpropagation(thesecond defenseline).VitaminEandcarotenoidsalsocontributetothefirstdefenselineagainstoxidativestress,becausetheyquenchsingletoxygen.

FlavonoidsaswellasvitaminCshowedaprotectiveactivitytoα-atocopherolinhumanLDL,andtheycanalsoregeneratevitaminE,fromtheα-chromanoxyradical.

Nutrientantioxidantsmayacttogethertoreducereactiveoxygenspieceslevelmoreeffectivelythansingledietaryantioxidants,becausetheycanfunctionassynergists.

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Inaddition,amixturecontainingbothwater-solubleandlipid-solubleantioxidantsiscapableofquenchingfreeradicalsinbothaqueousandlipidphases.

.Combinationsofα-tocopherolorvitaminCplusphenoliccompoundsalsoprovidedsynergisticeffectsinhumanerythrocytemembraneghostsandphosphatidylcholineliposomesystems.

.Okraseedisrichinproteinandunsaturatedfattyacidssuchaslinoleicacid.Insomecountries,okraalsoisusedinfolkmedicineasantiulcerogenic,gastroprotective,diureticagents.

.However,littleinformationonantioxidantcapabilitiesofmajorphenoliccompoundsfromokraseedisavailable.Okraisalsoapopularhealthfoodduetoitshighfiber,vitaminC,andfolatecontent. Okraisalsoagoodsourceofcalciumandpotassium.

Okrapodcontainsthickslimypolysaccharides,whichareusedtothickensoupsandstews,asaneggwhitesubstitute,andasafatsubstituteinchocolatebarcookiesandinchocolatefrozendairydessert.

Okraisalsoknownforbeinghighinantioxidantsactivitywithdifferentpartsoftheplant.Atawodihasreportedinvitroantioxidantassayofmethanolextractofokrafruits.

PHYTOCONSTITUENTS
VitaminsCandE,carotenoids,andphenoliccompounds,especiallyflavonoids.

VitaminEandcarotenoidsalsocontributetothefirstdefenselineagainstoxidativestress,becausetheyquenchsingletoxygen.

Phenoliccompoundsonantioxidantcapabilitiesofmajorfromokraseedisavailable.

FlavonoidsaswellasvitaminCshowedaprotectiveactivitytoα-atocopherolinhumanLDL,andtheycanalsoregeneratevitaminE,fromtheα-chromanoxyradical.

Chemicalconstituents

Petalsyieldflavanoidglycosides;gossypetinandhibiscetinglucosides.

Freshfruitsarerichinpectinandmucilage;itcontainsoxalicacid,protein,fat,minerals(potassium,sodium,magnesium,sulphur,copper,manganeseandiodine),carbohydrate,calciumandphosphorus.

FreshfruitsalsocontainvitaminA,thiamine,riboflavin,ascorbicacidandniacin.d-Galactose,lrhamnoseandd-dalacturonicacidalsoisolatedfromthemucilageofthefruit.

Flavonoidcompoundhasbeenreportedfromfruits.

Essentialoilisolatedfrompodsandseedscontainaliphaticalchohols,cyclohexanol,ptolualdehyde(infruits),a-terpenylacetate(inseeds)andcitral;nonvolatileneutralpartcontainsβ-sitosterol&its3β-galactoside(inseeds).

Leaveshavegotmoreorlesssameconstituents.Ripeseedscontain10-22%edibleoil.

EdibleUses:
Immaturefruit-cookedontheirownoraddedtosoupsetc.

Theycanbeusedfreshordried.Mucilaginous,theyarecommonlyusedasathickeningforsoups,stewsandsauces.

Seed-cookedorgroundintoamealandusedinmakingbreadormadeinto'tofu'or'tempeh'.Theroastedseedisacoffeesubstitute.

Theseedcontainsupto22%ofanedibleoil.(1)

MedicinalUsesOfOkara

Antipasmodic:-
Thisqualityintheseedsisbeneficialtothegastrointestinaltract.

Demulcent:
Thisqualityoftherootsisveryactiveduetothemucilagewhichcanbeusedtoreplaceplasmid.
Itisqqualitythatispresentintheleaves,theskinandyoungpods.

Diuretic:

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Theyoungpodsactasadiureticandemollient.Releases accumulationofwaterthatleadstoswelling/waterreten tion.

Emollient:

Soothesandsoftenstheskininfusionoftherootsisusedi nthetreatmentofsyphilis.

ThejuiceoftherootsisusedexternallyinNepaltotreatcu ts,woundsandboils.

Theleavesfurnishanemollientpoultice.Adecoctionof theimmaturecapsulesisdemulcent,diureticandemolli ent.

Itisusedinthetreatmentofcatarrhalinfections,dysuria andgonorrhoea.

Theseedsareantispasmodic,cordialandstimulant.

OkrainEthnomedicine:

FRUITS	Infusionoffruitmucilage	Indianetnomedicine	Fortreatingdysenteryanddiarrhoeainacuteinflammationandirritationofthestomach,bowel,andkidneycatarrhalinfection,ardourinae, andgonorrhoea
	Infusionoffruitmucilage	Indianethnomedicine	Antipyreticandpalcereplacement
	Adecoctionoftheimmaturefruits	Indianethanomedicine	Demulcentandemollientpoultice
	Extractofleavesandfruits	Indianethanomedicine	Demulcent,toughlesssothanthatofokrafruit
LEAVES	Extractofleaves	Indianethanomedicine	Extractofleavesmixedwitheggalbuminandappliedonhairwhichmakesblackandsilkyhair
	Leaves	LatinAmerica	Remediesfortumour
ROOT	Extractofroots	Indianethanomedicine	Demulcentandemollientpoultice
	Thejuiceoftheroots	Nepal	Totreatcuts,woundsandboil
	Infusionofthe roots	TraditionalmedicineofNicoraguaAtlanticcoastandTurkey	Usedasstomachic,totreatdiabetes,ulcer,usedaslaxativeandtreatmentofjaundice
SEED	Seeds	Indianethnomedicine	Antispasmodic,cordialandstimulant
	Infusionofthe roastedseeds	Indianethnomedicine	Hassudorificproperties
	Okraseed	Indianethanomedicine	Treatmentofspermatorrhoea
	Seedsinfusionofroastedokraseed	LatinAmerica	Remediesfortumour

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METHODOFPREPRATIONOFGELPECTIN
Plantmaterial
Plantmaterialsusedwereokrafruitsandokraleaves.
.Theplantmaterialswerecleanedandtheokrapulp(fruit withoutseed)wasseparatedmanuallyfromtheseeds.Then,theywereimmediatelyfreeze-driedfor96hrusingafreezedrier.
Commercialpectin(CP)ofapplepomaceandgelatin(bovine)waspurchasedfromSigmaAldrichCo.
Thesolventsandchemicalsemployedinthisstudywere obtainedcommerciallyandwereofanalyticalgrade

Preparationof6.67%concentrationofgelatinandcommercialpectin
Thegelatinandcommercialpectinof6.67%concentrationwerepreparedbydissolving2.66gofdriedgelatinandcommercialpectinseparatelyin40mLdistilledwaterat60°C.
Thedissolvedsampleswereheldinarefrigerator(7°C)for16–18hrandwereusedinviscosityanalysis.

Fractionationofalcohol-insolublesolids
Leaves,pulpandseedswerehomogenizedtwicewith70%(v/v)aqueousethanolatroomtemperaturefor1hr.
Afterfiltration,theinsolubleresidueswerepooledtogetherandsoakedinchloroform/methanol(1:1v/v)withagentlestirringfor30mintoremovelowmolecularweight(colored)compound

SequentialextractionofokraAIS
Thesequentialextractionmethodwasconductedaccordingto Vierhuisetal.,(2000)withseveralmodifications.
TheokraAIS(20g)wereextractedusing600mLofthefollowingextractants:0.05Msodiumacetatebuffer,pH5.2(hotbuffer,HB)at70°C,0.05Methylenediaminetetraaceticacid(EDTA)and0.05Msodiumacetatein0.05Msodiumoxalate,pH5.2at70°C(chelatingagent,CH)ofsolublesolids.
After30minofextraction,theextractwasseparatedfromtheinsolubleresiduebycentrifugationat18,500xgfor25min.
Then,thesupernatantwascoagulatedwithisopropanolandlyophilized
Sugarcomposition
Thenaturalsugarcompositionofallfractions(HBandCHfractionsofokraleaves,pulp,andseeds)weredeterminedbyhigh-performanceliquidchromatography.
Tenmilligramsofeachfractionweredissolvedin10mLof2MHClinmethanolandhydrolysedinitiallyat80°Cfor16hrandsubsequentlyincreasedthetemperatureto121°Cfor1hr.
Thesolventwasevaporatedusingrotaryevaporator.
Monosaccharides(rhamnose,xylose,arabinose,mannose,fructose,glucose,andgalactose)weredetermined usingaHPLCandrefractiveindex(RI)wasusedasadetector.
Themobilephaseusedwasacetonitrile/water(75:25,v/v),withaflowrateof1mL/min.
Amonosaccharidestandardsconsistingofrhamnose,xylose,arabinose,mannose,fructose,glucoseandgalactosewereusedatconcentrationsbetween1and5mg/mLinordertogettheequationofcalibrationcurve.
Thequantificationofuronicacidinthefractionswasperformedusingagalacturonicacid

Gelformation
Gelformationwascarriedoutbymixingthefractionswithkonjacglucomannan(KG)intheratioof5.00:1.67(w/w).
ThefractionsandKGwereweighedseparatelyandmixedtogetherinabeaker.
Then,waterwasaddedtosolubilizethemixturesandstirredcontinuouslyfor15mininawaterbathat60°Cuntilitdissolvedcompletely.
Next,themixturewasstoredintherefrigeratorfor16-18hr.
Thegelmixturewasanalysedtodetermineitsmeltingpoint,gelstrengthandviscosityat25°C

II. OBSERVATION

Determinationofmeltingpoint
Solutioncontainedatotalof6.67%(w/v)offractions(5.00%)andKG(1.67%)waspreparedinathinwall(12mm×75mm)screwcaptesttubes.

Thetesttubeswerefilledtoleavesomeheadspaceforgasreleasedduringgelformationandclosed.

Determinationofgelstrength
Gelstrengthwasmeasuredaccordingtothemethodrecommendedbythemanufacturerofthetexturemeasuringinstrument(Gómez-GuillénandMontero,2001
Thepreparedsamplewillalsobeusedforviscositydetermination.
Thedimensionofthesampleinthecontainerwas6cmindiameterand2cminheight.
Thesolutionwascooldowninarefrigeratorat7°Cfor16-18hr.

Measurementsweredoneat25°CusinganInstronmodel4501UniversalTestingMachine(InstronCo.,Canton,Mass.,USA)witha5kNofloadcell,cross-headspeed1mm/s,equippedwitha1.27cmdiametercylindricalTeflonplunger.

Maximumforce(expresseding)wasdeterminedwhentheplungerhadpenetrated4mmintothepectingels.
Threereplicateswereperformedforeachfraction.

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Determinationofviscosity
Thesimilarpreparedsample(6.67%at60oC)fromprev iousgelstrengthtestwasused.
TheviscosityofthesamplewasdeterminedusinganAR - G2rheometer(TAInstruments,WestSussex,England) equippedwithaheatingcirculator(Julabo,modelF12, Germany).
Aconeandplategeometry(60mmdiameter,1°angle)w asusedforthemeasurements.
Approximately1mLofeachsamplewastransferredtot hePeltierplateandexcessmaterialwastrimmedoff.
Aplatinumresistancethermometer(PRT)sensorwasp ositionedinthemiddleofthelowersampleplatetoensur eaccuratesamplemeasurement(25±0.1°C).
Flowcurveswithincreasingshearrate(0.01–100s-1)weremeasuredat25°C.
ThedownwardcurvewasanalyzedusingTARheology AdvantageDataAnalysisSoftware(versionV5.7.0)(S engkhamparnetal.,2010)

Statisticalanalysis
Alldatawereexpressedasamean±standarddeviation.
Datawereanalyzedusingone-wayANOVAusingSPSS15.0.Duncan'smultiple-rangetestwasusedtoassessthedifferencebetweenthe means.
Pearson'scorrelationtestwasusedtoassessthecorrelat ionsbetweenthemeans.

III. CONCLUSION

Lady'sfingeralsoknownasokraisaplantwithhighnatu ralviscousmucilageparticularlyinthefruits.
Okrawasselectedinthisstudy,duetothehighavailabilit y,easytogetallyearroundatlowcost.
Vegetarians,forinstance,couldnotacceptanimalbased ingredient.Inthiscase,pectinwhichisplantbasedisthe mostsuitablealternativeforgellingagent.
Inthisstudy,agelcouldnotbeformedbyokrapectinitsel f;therefore,anotherpolysaccharideknownaskonjacgl ucomannan(KG)wasused.
Toconclude,basedonthegelproperties,HBandCHfrac tionderivedfromokraleaves,pulp,andseedshavegood potentialtobecomeplant-basedgellingagent

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