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Fractionation of Agarose and *Gracilaria verrucosa* Agar and Comparison of Their IR Spectra with Different Agar

Agaroz ve *Gracilaria verrucosa* Agarının Fraksiyonlandırılması ve IR Spektrumlarının Değişik Agar ile Karşılaştırılması

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Abstract

Agarose and *Gracilaria verrucosa* agar were fractionated by Sephadex G-25 and SPE columns (butyl, octadecyl, alumina and quaternary amine). IR spectrophotometric and metachromatic methods were used for the identification of the fractions. In contrary to findings of Tsuchiya and Hong, (1965) IR spectrum of agar and agarose were not the same. Agar showed different IR bands than agarose as at 505, 516, 534, 580, 617, 667 cm^{-1}. Agar was not unique compound, after fraction various agars gave different IR spectra. However agar and agarose gave the same metachromatic β band. Hence differenciation of agar and agarose is possible by IR spectra but not metachromatic method.

Keywords: Agarose, *G. verrucosa* agar and agars, fractionation, SPE column, Sephadex, IR Spectra

Introduction

Gracilaria verrucosa (Huds.) Papenfuss, a red alga, is abundant in Turkish coastline. It is one of the main sources of raw material for the manufacture of agar.

Agar is a sulphated polysaccharide and contains maximum 9 % sulphate group. It is a mixture of polymers as agarose and agaropectin containing 30.000-120.000 Dalton molecular weight. The structure of agar consists of alternating β-1,3 and α-1,4 linked D - and L - galactose units (Araki *et al.*, 1967). Charged residues are sulphated esters and pyruvated ketal groups also present on the polysaccharide chain. The gel properties are highly dependent on the amount and position of sulphated groups as well as the amount of 3,6-anhydrogalactose fraction of the polysaccharide. The repeating sugar unit may be substituted by methoxyl, pyruvate and sulfate groups (Araki, 1966). The red algal polysaccharides are important in practical use due to their well-known gel-forming ability. The gel strength is often being changed considerably by its impurities. Therefore, a simple and effective purification method is required to improve its gel-forming properties.

Agarose consists of alternating units of 3.6 anhydro α – L- galactose and β – D- galactose. Agarose was separated from agar by acetylation method (Araki, 1937a), Sephadex chromatography (Duckworth and Yaphe, 1971), treating with chitosan (Allan *et al.*, 1971), rivanol (Sviridov *et al.*, 1971), acrinol (Fuse and Goto, 1971), cetyl-pyridinium chloride (Hjerten, 1962) and precipitation with polyethylene glycol (Russel *et al.*, 1964). Santos and

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Doty (1983) obtained agarose from *Gracilaria cylindrica* by precipitation with benzothonium chloride or with *Eucheumea striatam.*

Agarose contain low sulfate value and high 3,6- anhydrogalactose. Low organic sulfate content is an important criterion in determining the quality of agarose since sulfate group is the major contributory factor to the ionic character of agarose (Santos and Doty, 1983). The acceptable value is 0.7 % for sulfate content (Guiseley and Renn, 1970). The most current applications of agarose require value is 0.3 % or less. *Gracilaria cylindrica* agarose have sulfate content of 0.17- 0.42 % (Santos and Doty, 1983). Methoxyl group content of agarose differs due to the origin. Analysis of algal polysaccharides was made by infrared spectrophotometry (Stanley, 1963; Bellion *et al.*, 1981; Rochas *et al.*, 1986; Sur and Güven, 2002).

Agar was extracted from the algae by hot water, dilute acid or alkali media. Agar was widely used in medicene, pharmaceutics, cosmetics and food industry etc.

Agaropectin was obtained by precipitation with ammonium hydroxide (Barteling, 1962), and ammonium sulfate (Egerov *et al.*, 1970).

Agarose in the from of aqueus gels has been widely used in electropheresis, chromatography, culture media, immunological analysis and gel cloning as well.

IR spectrum has a role for differenciation of algal polysaccharides. The IR absorption bands of sulfate groups of algal polysaccharide are shown at 1240-1250 cm^{-1} generally for ester sulfate and 805 cm^{-1} attributed to sulfate on C_2 of 3.6 anhydro galactose (Anderson *et al.*, 1968). The band at 705 cm^{-1} is probably due to sulfate on C_4 galactose (Rohas *et al.*, 1986).

The weak absorption peak at 850 cm^{-1} indicate the presence of a low content of 4 sulfate in the 1.3- linked galactose units (Zablackis and Santos, 1986).

The peaks at 1960 and 1180 cm^{-1} (Cross, 1964) were attributed to sulfate ester linked.

Absorbances at 2960, 2845, 1640 and 895- 900 cm^{-1} were also observed in IR spectra of agar. Absorbance at 2960 cm^{-1} is associated with CH_2, absorbance at 2845 cm^{-1} due to O- CH_3 occurs as a shoulder and the band at 2920 cm^{-1} in spectra of highly methylated agar (Ji *et al.*, 1985). The peaks at 2830 and 2815 cm^{-1} were attributed to O-CH_3 group (Araki *et al.*, 1967) and the peak at 1780 was 6 mono methyl group of agar (Christiaen and Bodard, 1983). The band at 1640 cm^{-1} was attributed to water (Zundel, 1969). The band at 930 cm^{-1} (Stanley, 1963) and also at 1070 cm^{-1} were usually attributed to 3.6 anhydro- galactose (Christiaen and Bodard, 1983).

A sharper band at 930-940 cm^{-1} indicated O ether bond of 3.6 anhydro – D- galactose. The band at 897 cm^{-1} was attributed to 1.3 linked β- D galactose pyranosyl units (Barker *et al.*, 1956).

The metachromatic method was used for identification of algal polysaccharides. Metachromasy is a case of the λ_{max} (α-band) of the dye changes and another λ_{max} appears (β-band) which can be observed visually and by using spectrophotometer. Metachromasy was used in histological staining of tissues first by Ehrlich (1887). Lison (1935) showed that agar gave metachromatic reaction with cresyl blue. Metachromatic phenomenon of algal polysaccharides was studied by various workers (Michaelis, 1947; Shubert and Levin, 1953; Stone *et al.*, 1963; Suzuki *et al.*, 1969; Graham, 1971; Stone, 1972; Gangolli *et al.*, 1973). Identification of the algal polysaccharides such as agar, carragenan and alginate has been studied in detail by Güven and Güvener, (1985a,b). Agar gave one metachromatic band with acridine orange, toluidine blue, two bands with Azur A and finally three bands with methylene blue (Güven and Güvener, 1985a). The method can be also used for qualitative and quantitative identification of agar

fractions.

The method used for fractionation of agar in this work are as follows: Sephadex is a modified dextran which forms cross-linked three-dimensional network of polysaccharide chains. It is suitable for gel filtration chromatography which is usually used for separating biological macromolecules according to their molecular weights. The subtances are eluted from a Sephadex bed in the order of decreasing molecular size (Annon. 1966).

Solid phase extraction (SPE) technique based on fractionation of the sample has been used since 1970, for analysis.

This paper reports the fractionation of agar and agarose and comparison of IR spectra and also different agar and their metachromatic properties.

Material and Method

Agarose (Sigma),
Pure agar (Merck),
Commercial agar,
Difco agar,
Pasteur agar,
Aqua agar,
Gracilaria verrucosa agar obtained in our laboratory.

Gracilaria verrucosa (Huds.) Papenfuss was collected, in Izmir Bay, Turkey in September 2001. The sample was cleaned from foreign materials, washed with distilled water, dried and powdered. Agar was extracted with water from algae at 110°C in autoclave for 30 min. It was filtered from cheese cloth and the filtrate was put in the freezer (Freezing drying technique or the extract was precipitated by adding of ethyl alcohol (95%) or isopropyl alcohol).

Fractionation of agar

1. Sephadex G25 column

The volume of the column was determined by using 0.5% dextrane-blue solution.

100 mg of crude agar was fractionated by Sephadex G-25 (AB Pharmacia, Uppsala) in a 1x50cm glass column; distilled water used as mobile phase. The sample was dissolved in 4mL water and applied to the column. The flow rate was adjusted to 6 drops/min. Each 5mL of fractions was collected and controlled with 0.5% Azur A solution for metachromatic reaction. Each fraction was lyophilized and its IR and UV spectra were taken.

2. SPE column (J.T.Baker)

The columns used and solvent system are;

2.1. Butyl column, elution solvent:
2.1.1. 1mL distilled water, 1N NaOH and 2mL distilled water
2.1.2. 1mL distilled water, 1N NaOH and 2mL distilled water and 1mL 96% alcohol
2.1.3. Acetonitrile: distilled water (1:1), acetonitrile:distilled water (1:2) and 1mL 96% alcohol
2.1.4. Acetonitrile:distilled water (1:1), acetonitrile:distilled water (1:2) and isopropyl alcohol

2.1.5. Isopropyl alcohol / %96 ethanol / acetonitrile – distilled water (1:1) / distilled water, 0.1 N NaOH, distilled

2.2. Alumina column, elution solvent:
1mL distilled water/ 1N NaOH, 2mL distilled water.

2.3. Amino column, elution solvent :
2 ml acetonitrile distilled water (1:1), 2 ml 0.1 N HCl.

2.4. Quarterner column, elution solvent:
Distilled water / 0.1 NaOH/distilled water / acetonitrile- distilled water (1:1), 2 ml 0.1 N HCl

2.5. Octadecyl column, elution solvent:
Distilled water.

UV spectrum was taken after addition of 1 drop 0.5% Azur A (Gurr) solution. The analysis was made by UV-Visible spectrophotometer (Shimadzu-UV 1601).

IR spectra were taken on agar fractions in KBr tablet by FTIR spectrophotometer (Shimadzu-PC8601).

Results and Discussion

IR spectrum of agarose, *Gracilaria verrucosa* agar and commercial agar are shown in Fig 1-3.

The differences on the IR bands between agarose with various agar and *G. verrucosa* agar are: at 505, 516,534, 580, 617, 650, 667 cm^{-1} observed in IR spectra of agar but was not found on the spectra of agarose.

The absorbance of sulfate groups at 705, 805 and 1070 cm^{-1} were not observed on IR spectra of agarose and also of sulfate groups at 1240-1250, 705 and 850 cm^{-1} are not found in agarose due to it low contains of sulfate groups.

The band at 2920 cm^{-1} indicated high methylated group not found in agarose.

This study on IR spectra of various agars gave not the same absorption band.

The absorption bands of *G. verrucosa* agar were not observed in various agars as:

Pure agar: 443, 457, 584, 607, 634, 642, 665, 705, 750, 790, 848, 875, 935, 1066, 1126, 1236, 1325, 1488, 1496, 1506, 1569, 1635, 1647, 1716 and 1739 cm^{-1};

Commercial agar: 534, 578, 771, 869, 931, 968, 1072, 1157, 1373, 1643, 2115, 2343, 2513 and 2898 cm^{-1}.

Pasteur agar:424, 580, 617, 650, 869, 891, 1045, 1157, 1218, 1251, 1525, 1544, 1643, 2043, 2898 cm^{-1}

Difco agar: 424, 650, 690, 713, 771, 869, 891, 931, 989, 1045, 1072, 1218, 1251, 1525, 1544, 1643, 2933 cm^{-1}

Aqua agar: 424, 435, 501, 617, 650, 869, 891, 1045, 1072, 1157, 1218, 1251, 1525, 1544, 1647, 2358, 2933, 3419 cm^{2}

According to these findings agars are not completely similar products. The composition of agars varies according to the algae used and also extraction techniques.

The absorbance of the IR bands of agars provide information on the presence of 3. 6 anhydro galactose (930 cm^{-1}), sulfate 1370 cm^{-1}, galactose –4- sulfate (845 cm^{-1}), galactose –2- sulfate 830 cm^{-1}, galactose –6- sulfate 820 cm^{-1} and 3.6 anhydro galactose – 2- sulfate 805 cm^{-1}.

Absorbance at 805 cm^{-1} is attributed to sulfate on C_2 of 3.6 anhydro galactose (Anderson *et al.*, 1986). It was not observed in IR spectra of agarose and agar.

The absorbance at 930 cm^{-1} and 1070 cm^{-1} is attributed to 3.6 anhydro- galactose (Stanley, 1963). These were found in IR spectra of agarose and agar.

The absorbance at 1060, 1180, 1070 cm^{-1} and 1370 cm^{-1} (Cross, 1964), 1250 cm^{-1} (Akahane and Izumi, 1976) are attributed to the O-CH_3 group (Araki *et al.*, 1967). The bands 1060 and 1180 cm^{-1} were not observed in IR spectra of agarose.

The absorption bands of sulfate groups as 1240- 1250, 705 and 850 cm^{-1} are not found in agarose while it contains lower sulfate groups.

The band at 2920 cm^{-1} indicated highly methylated group was not found in agarose.

Santos and Doty (1983) are investigated of gel strenghts of agarose and found some differences.

Tsuchiya and Hong, (1965) have studied IR spectra of agar, agarose and agaropectin from *Gelidium amansii* and *Gracilaria sp.* and found that the IR spectra of all tested compounds are similar. In contrary to this findings we found that IR spectra of agar and agarose are not similar. The different band are observed on the IR spectra of agar as 505, 516, 534, 580, 617 and 667 cm^{-1}.

The metachromatic properties of agarose and agar fraction were also studied. When agar precipitated by addition of 96% ethyl alcohol on crude agar solution their UV spectrum of gave no difference at α and β bands.

λ max of the metachromatic β band of agar and fractions and α band of dye are listed below.

Agar obtained

	β band	α band
Agarose	565	634
Precipitated by ethanol	565	639
by isopropyl alcohol	567	634
Agar fractioned by		
SPE (butyl)	546	634
SPE octadecyl	551	615
SPE Alumine	547	634

As can be seen in the table, the metachromatic bands of agar and fractions and agarose are the same. Hence differenciation of agar and agarose is possible by IR spectra but not metachromatic method.

Table 1. The IR bands of various agars, its fractions and agarose

AGAR	cm^{-1}																						
pure agar	418 443 457 472	516 584	607 634 642 665	705 750 790	848 875	935	1066	1126	1236	1325 1338 1365 1388 1396	1417 1436 1456 1473 1488 1496	1506 1521 1558 1569 1575	1624 1635 1647 1670 1683 1697	1716 1739									
commercial agar	470	534 578	667 690	713 740 771	869 891	931 968 989	1045 1072	1157		1373	1413	1544	1643					2115		2343 2358	2513	2898	
G. verrucosa agar(pure)	410 464		669	744 773	869 893	931 968	1047 1080	1159		1363 1373	1427	1552	1643					2150		2343 2360			2902
Agarose (sigma)	420 459 470	516	690	740 771	864 891	929 968	1041	1161 1191		1315 1338 1361 1373 1396	1419 1431 1473 1488	1508 1519 1542 1558	1651 1681 1697	1716 1747 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2017 2040 2063			2349 2383		289	
Frozen agar filt. Fr. I	420 470	516 594	617 671 690	717 744		991	1045	1107 1191	1269	1315 1338 1361 1396	1419 1434 1458 1473	1519 1542 1558 1573	1624 1651 1670 1685 1697	1731 1747 1770 1793	1828 1843 1867	1921 1944 1967 1990				2322 2360		2835	2939
Frozen agar filt. Fr. II	420 470	524	617 667	744	864 887	937 991	1041	1107 1188		1315 1342 1373 1396	1419	1519 1542	1651 1681	1712 1731	1867		2075			2341 2360			2943
Frozen agar filt. Fr. III	470	528	617 667 686	744	883	933 991	1045	1107 1191		1338	1415 1454	1519	1635				2086			2341 2360		2839	2943
Frozen agar filt. Fr. IV	420 470	520	617 667	744	864 883	937 991	1041	1107 1188		1338 1373 1396	1419 1458	1519 1542	1651 1670 1693	1712 1731	1828 1843 1867	1944	2075			2329 2360			2954
Frozen agar filt. Fr. V	420 470	516 543	617 667 690	744	887	937 991	1045	1103 1195		1315 1338 1373 1396	1419 1458 1473	1504 1519 1542 1558 1573	1623 1651 1681	1732 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990				2326 2360			2954
Frozen agar filt. Fr. VI	420 470	528	617	744	883	933 991	1045	1110 1191		1315 1338 1396	1419 1458 1488	1519 1542 1558	1651	1732 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2017 2067			2326 2360			2931 2958
Frozen agar filt. Fr. VII	420 470	520	617 690	744	887	937 991	1041	1107 1195		1315 1338 1396	1419 1458 1488	1519 1542 1558	1651	1701 1732 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2086			2326 2360			2943
Frozen agar filt. Fr. VIII	420 470	516	617 667	744	860 883	933 991	1041	1103 1195		1615 1338 1373 1396	1419 1458 1473 1488	1519 1542 1558	1624 1651 1681 1697	1732 1770 1793	1843 1867 1890	1921 1967 1990	2017 2607			2326 2360			2943
Frozen agar filt. Fr. IX	420 470	524	617 667 690	744 771	813 860 887	933 995	1041	1107 1188		1338 1373 1396	1419 1458	1519 1542 1558	1651	1716 1731	1828 1867	1944	2075			2341 2360			2935
Frozen agar filt.. Fr. X	420 459 470	516	617 667 686	744	860 887	933 991	1045	1107 1191		1315 1338 1373 1396	1419 1458 1473 1488	1504 1519 1542 1558	1623 1651 1670 1681 1697	1732 1747 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990				2326 2360			2939
Frozen agar filt. Fr.XI	420 470	516	617 667	744 783	887	937 991	1045 1076	1107 1191		1315 1338 1373	1419 1458 1473	1519 1542 1558	1651 1681	1732 1770 1793	1828 1843 1867	1921 1944 1967	2067			2322 2360			2939

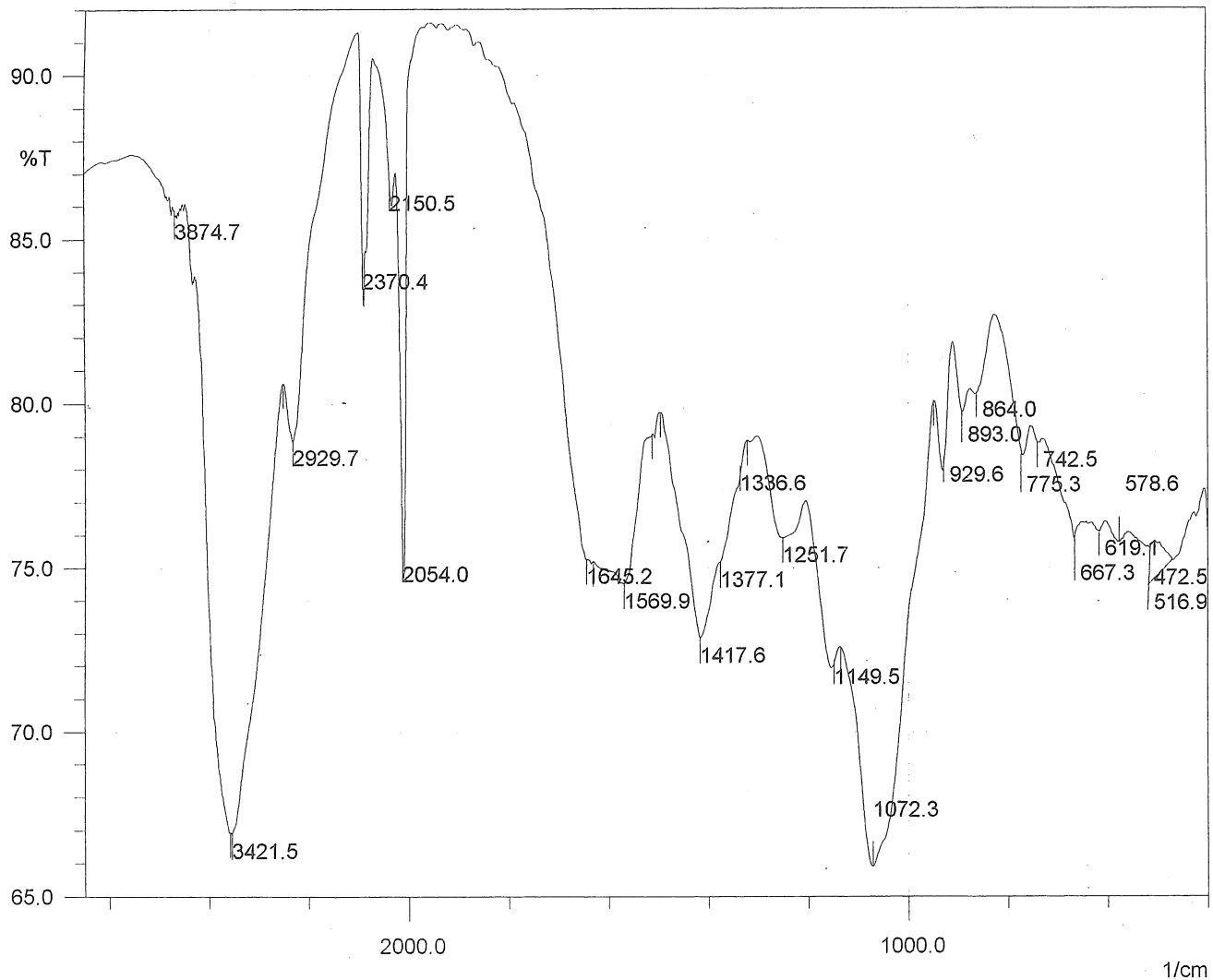
Table 1. Continued

										1396	1488				1890	1990									
Frozen agar filt. Fr. XIII	420 459 470	516 547	617 671 690	744	860 887	933 991	1045	1114 1191	1269 1288	1315 1338 1373 1396	1419 1458 1473	1519 1542 1558 1573	1624 1651 1681 1697	1716 1731 1747	1828 1843 1867 1890	1921 1944 1967 1990	2017			2322 2360		2831 2896	2939
Frozen agar filt. Fr. XIV	420 443 455 470	505 516 547	621 667 690	721 744 775	840 860	933 991	1045	1114 1195	1269	1315 1338 1396	1419 1458 1473	1519 1542 1558	1624 1651 1681 1697	1716 1731 1747 1770	1828 1843 1890	1921 1944 1967 1990	2017			2322 2360		2839 2896	2935
Frozen agar filt. +Alcohol. Fr.XII	420 459 470	516 547 578	617 667 690	740 771	813 891	933 987	1045 1080	1118	1218	1315 1338 1373 1396	1419 1458 1473	1519 1542 1558 1573	1620 1651 1670 1681 1697	1716 1747 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990				2322 2360		2893	2935
Frozen agar filt. +IPA- aqueous part	420 459 470	516 594	617 671	705 744	813	918 937	1045	1118 1195	1207 1288	1319 1338 1396	1419 1458 1473 1488	1519 1542 1558	1651 1681 1697	1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2017 2040 2063			2322 2349 2372		2835	2935
Frozen agar filt. +EA-aqueous part	420 459 470	516 578	617 667 686	705 721 744	864	933	1045 1076	1118 1157 1191	1269 1288	1315 1338 1373 1396	1419 1458 1473 1496	1519 1542 1558	1624 1651 1681 1697	1716 1747 1770 1793	1828 1843 1867 1890	1917 1944 1967 1990	2017			2318 2360		2858	2931
Frozen agar filt. +EA-precipitate	420 470	516 547	617 659 694	756 763		902 918 983		1114		1315 1338 1396	1419 1458 1473 1488	1508 1542 1558	1624 1651 1681 1697	1732 1770 1793	1828 1843		2079			2322 2376			2900 2943
Frozen agar filt. +IPA- precipitate	420 470	516	617 659	705 756	825 856 891	929 983	1014	1118 1149	1269	1315 1338 1361 1396	1419 1458 1473 1488	1508 1542 1558	1624 1651 1685 1697	1732 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2086		2268	2322 2349 2372		2835	2900 2943
Autoclaved agar +IPA- aqueous part	420 470	578	613	767 786	856 891	929	1029	1161		1396	1458 1473	1508 1542 1558	1651 1681 1697	1732 1744	1870 1894					2314 2360		2893	2927
Autoclaved agar +EA- aqueous part	420 459 470	516 543 578	617 671 690	705 721 744 771	864 894	933 968	1037		1257 1269	1315 1338 1373 1396	1419 1458 1473	1519 1542 1558 1569	1624 1651 1685 1697	1747 1770 1793	1828 1840 1870	1921 1940 1990				2326 2364		2835 2896	2939
Autoclaved agar +IPA- precipitate	420 443 459 470	505 516 543 578	617 671 690	705 744	840 867	933 991	1045	1114 1191	1269 1288	1315 1338 1373 1396	1419 1458 1473 1488	1508 1542 1573	1651 1681	1701 1716 1747 1793	1828 1843 1867 1890	1921 1944 1967 1990	2017 2063			2322 2360		2854	2931
Autoclaved agar +EA- precipitate	420 459 470	516 578	617 671 690	717 740 771	864 894	933 968 991	1045 1076	1188	1269 1284	1315 1338 1373	1419 1458 1473	1519 1542 1558	1624 1651 1681	1747 1770 1793	1828 1843 1867	1921 1944 1967	2017 2040 2063			2322 2360		2854	2900 2931
Sephadex G-25 (5-10) Fr.1	416 470	516 578	671	740 771	856 894	933 987	1041 1072	1157	1218 1265	1311 1342 1373 1396	1458	1519 1542	1651 1681	1735 1797	1843 1867	1921 1944		2183		2360		2854	2939
Sephadex G-25 (11-15) Fr.2	416 478	524	671	740 771	856 894	933 979	1041 1072	1157	1218 1257	1342 1373	1419 1458	1519 1542	1651	1735 1797	1859 1867					2360			2923
Sephadex G-25 (16-20) Fr.3	470	578	640 678		840 879		1056		1265 1288	1319 1342 1373 1396	1458	1512 1542	1651 1681	1735 1797	1828 1867	1928 1944	2036	2144		2360		2854	2923
Sephadex G-25 (21-25) Fr.4	470	547	601 671	748	894	925	1041 1095	1134	1203 1265	1311 1334 1342 1373 1396	1419 1458	1519 1550	1651 1681	1743 1766 1797	1843 1867	1921 1982				2360		2831	2900
Sephadex G-25 (26-30) Fr.5	416 470	547 594	640 671		833 879		1056	1134	1265	1319 1334 1373	1434 1458	1512 1535	1620 1681	1743 1774 1797	1843 1859	1921	2036	2137		2360		2862	2931 2962

Table 1. Continued

										1396													
Sephadex G-25 (31-35) Fr.6	470	516 547 594	640 671	756 771 794	848		1064	1188	1265	1319 1342 1373 1396	1419 1458 1473	1519 1542	1620 1651 1681 1697	1743	1843		2036	2137		2360			
SPE-Al_2O_3 (d.water +NaOH+d. water)	420 470 489	516 547 578	636 671 686	717 740	867	902	1072		1242 1269	1315 1338 1357 1373 1396	1458	1508 1542	1620 1651 1685 1697	1747 1770 1793	1843 1867 1890	1921 1944 1967 1990			2295	2368	2457		
SPE-C4 (d. water +NaOH+d.water)	420 470		698	779	879		1041	1141	1203 1272	1319 1338 1377	1438 1458 1488	1539	1624 1651 1685 1697	1747 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990				2322 2349	2495	2873	2931 2958
SPE-C4 (d. water +NaOH+d.water+alcohol)	470		636 698	744 786	840 879		1072		1272	1319	1458	1542 1558	1623 1651	1732 1770	1823 1843 1867					2349 2383	2495	2873	2931 2962
SPE-C4 (acetonitril/ dist.water+EA)	420 474	524	617 659 698	744	813 864	945 995	1041	1107 1191		1342 1377	1419 1458	1542 1558	1651 1681	1716 1731			2086			2322 2360		2854	2923
SPE-C4 (acetonitril/ dist.water+IPA)			617 671	717	837 875	937 995	1045 1099	1199		1315 1338 1361 1396	1419 1458 1473 1488	1508 1542 1558	1651 1685 1697	1747 1770 1793	1828 1843 1867	1921 1944				2322 2360		2858	2931
SPE-C4 (acetonitril/ dist.water+IPA+precip.)	420 470	516 520	617 659 690	756	856 894	925 983	1014 1095	1122 1195		1315 1338 1361 1396	1419 1458 1473	1504 1519 1542	1651 1685 1697	1747 1770 1793	1828 1843 1867 1890	1921 1944 1967 1990	2063			2322 2368		2831	2900 2939

Fig 1. IR spectra of *Gracillaria verrucosa* agar



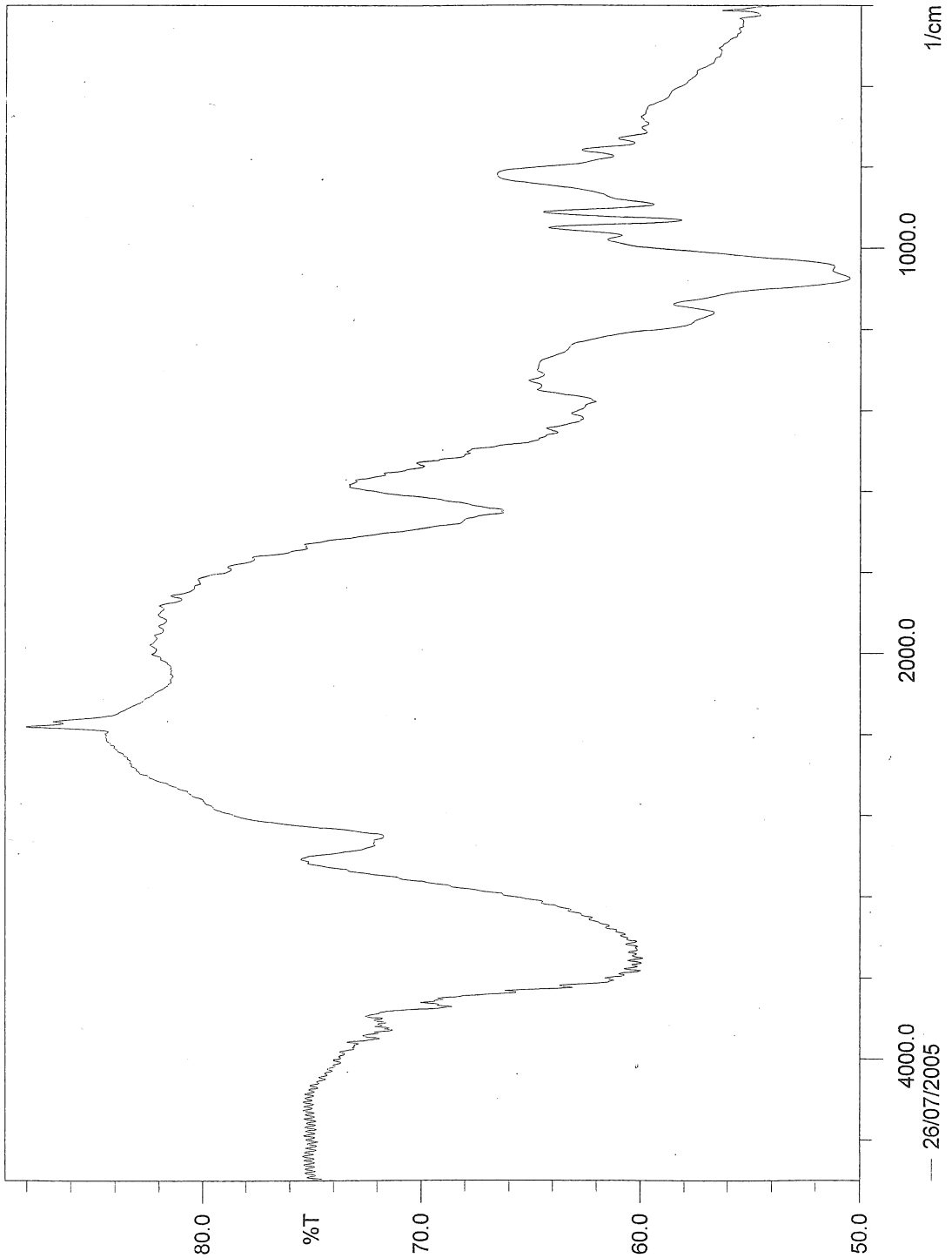
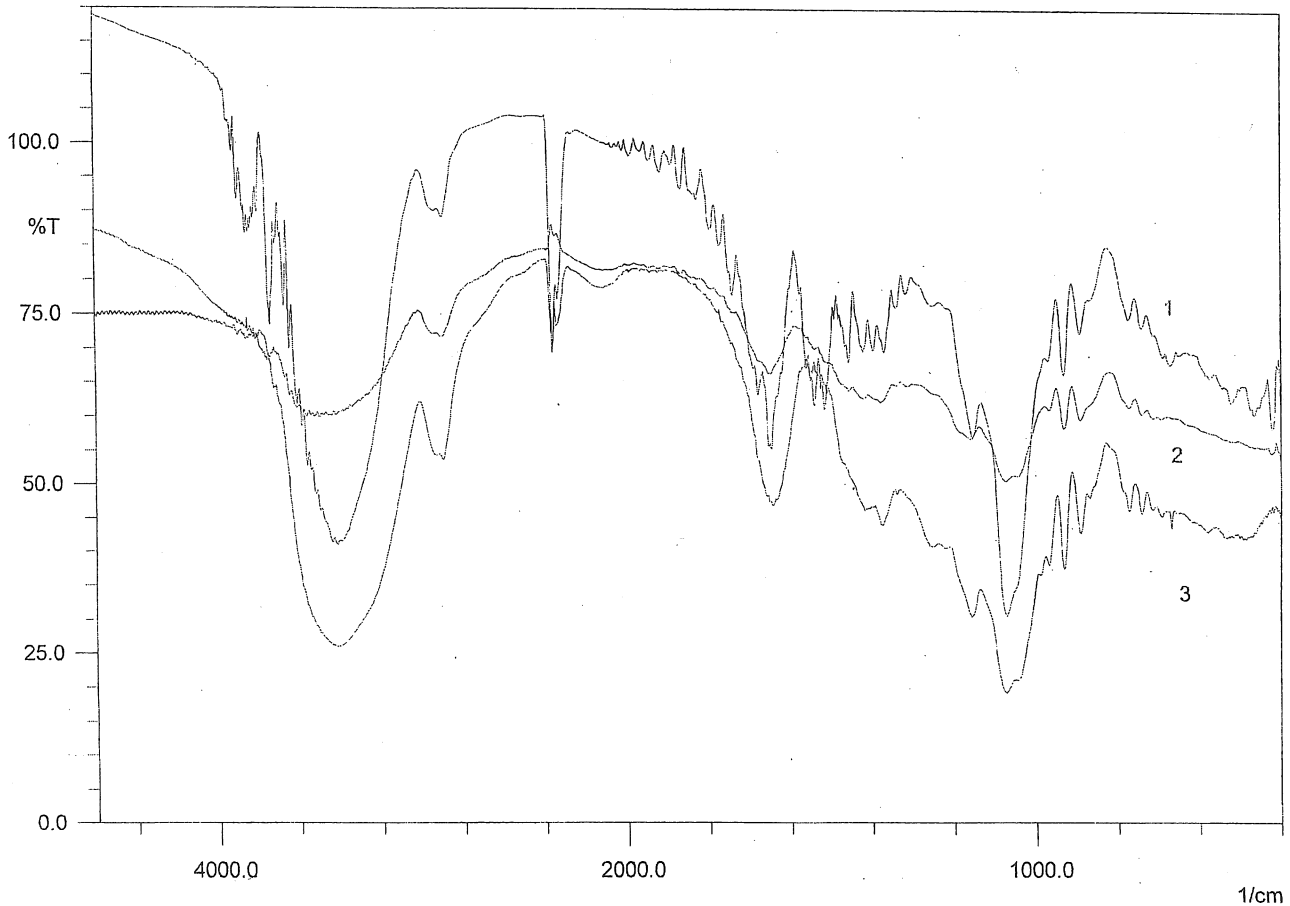


Fig 2. IR spectra of agarose

Fig 3. Comparison of IR spectra of various agars and agarose

1. *Gracillaria verrucosa*, 2. Agarose, 3. Commercial agar



Özet

Bu çalışmada Türkiye sahillerinde bulunan *Gracilaria verrucosa* (Huds.) Papenfuss agarı ile değişik agarın, agarozun IR spectrumları incelendi. Agar değişik kromatografi yöntemler ile fraksiyone elde edildi. Bunun için Sephadex G-25 ve SPE kolonları (bütil, oktadesil, alumina ve kuarterner) kullanıldı. Fraksiyonları belirleme tayininde metakromazi tekniği uygulandı. Agar ve agarozun IR spektrumları karşılaştırıldı. Tsuchiya and Hong, (1965) agar ve agarozun IR spectrumlarının farklı olmadığı görüşüne aykırı olarak bu çalışmada agarozun IR spektrumunun 505, 516, 534, 580, 617 and 667 cm^{-1} arasında farklı bantlar taşıdığı saptanmıştır.

Bu çalışmada ayrıca agarın tek bir madde olmadığı ve fraksiyonlanması sonucunda farklı IR spektrumlarına sahip maddelerden oluştuğu tespit edilmiştir.

Diğer taraftan agarın ve agarozun ve agar fraksiyonlarının UV'de metakromatik ile spektrumları alındı burada ise bir farklılık saptanmadı.

Sonuçta agar ve agarozun IR spectrunları arasındaki fark ile ayrılabileceği ve fakat metakromatik yol ile ayrılamayacağı saptandı.

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