

Figure S1. Aliphatic and C α -methine region of ^{13}C CP/MAS NMR spectrum of fingernail plates (red) and second derivative spectrum of the same region of this sample (blue). Black lines show signal positions. Second derivative spectrum is presented inverted and multiplied 2000 times for better comparison.

Table S1. Mean (\pm SE) chemical shifts, relative areas and widths of the ^{13}C CP/MAS NMR signals of the female (F) and male (M) groups.

The areas and line-widths have been tested for the gender differences using the Student's *t*-test for unpaired samples (normal data distribution) or the non-parametric Kolmogorov–Smirnov test for unpaired samples (non-normal data distribution; only FWHM for Signals 1 and 13). The statistically significant differences are shown in bold. The degree of significance *p* is denoted by one and two asterisks for weak ($0.05 < p < 0.01$) and strong significance ($p < 0.001$), respectively.

Signal number	Chemical shift ^I [ppm]		Area ^{II}		FWHM ^{III} [Hz]	
	F	M	F	M	F	M
1	12.9	12.5	0.0225±0.0009	0.0136±0.0015**	962±54	567±51**
2	16.5	16.3	0.0239±0.0013	0.0247±0.0012	448±17	427±13
3	20.5	20.3	0.0481±0.0012	0.0474±0.0015	391±5	389±6
4	25.1	25.1	0.1100±0.0017	0.1162±0.0019*	439±6	458±7*
5	29.8	29.7	0.0871±0.0019	0.0844±0.0021	430±7	426±6
6	33.1	33.0	0.0306±0.0016	0.0325±0.0018	300±11	305±12
7	35.9	35.7	0.0206±0.0010	0.0153±0.0009**	331±6	287±6**
8	40.7	40.4	0.1192±0.0012	0.1315±0.0014**	793±8	848±9**
9	48.1	48.0	0.0126±0.0007	0.0132±0.0007	297±8	303±7
10	53.4	53.5	0.1550±0.0023	0.1567±0.0025	755±9	748±8
11	56.5	56.4	0.0067±0.0005	0.0074±0.0006	250±11	241±8
12	59.8	59.9	0.0766±0.0020	0.0776±0.0015	664±11	640±9
13	65.0	64.9	0.0587±0.0016	0.0534±0.0013*	1394±47	1123±38**
14	129.0	129.0	0.0308±0.0005	0.0307±0.0006	579±7	591±6
15	157.7	157.6	0.0153±0.0003	0.0144±0.0003*	374±5	361±4
16	171.6	171.9	0.0914±0.0016	0.1116±0.0022**	531±4	541±3*
17	175.2	175.7	0.0782±0.0012	0.0537±0.0013**	563±8	451±6**
18	180.7	180.1	0.0119±0.0008	0.0137±0.0006	510±24	525±16

^ISE \leq 0.1 ^{II}Fractions of the total area of the 18 studied signals. ^{III} Abbreviation FWHM: full width at half maximum.

Appendix S1. Detailed analysis of the ^{13}C CP/MAS NMR signal areas

The detailed comparative analysis of the signal areas between the female (F) and male (M) groups, presented below, refers to Tables 1 and S1. The discussed content of amino acids in the fingernail α -keratin is expressed in molecular fractions. The abbreviation F \rightarrow M denotes a change from the group of females to the group of males. The protein α -helix, β -sheet and random coil secondary structures are designated by H, S and C, respectively. The order in which the signals were examined arises from the logical reasoning.

Signals 16 and 17

Signals 16 and 17 are assigned to the S/C and H secondary structures, respectively. For males, the former signal has a higher area and the latter a lower signal (strong significance). Therefore, males have more S/C and fewer H structural regions in the fingernail proteins than females.

Signal 9

Signal 9 is exclusively assigned to Pro. Its area does not change for F \rightarrow M, therefore nor is the Pro content.

Signal 6

Signal 6 has contributions from Lys and Glu. Since its FWHM is about 3 ppm, we assume that the C_β -Lys resonances for H, S and C all contribute to this signal. The more important circumstance is that the Lys content is about four times lower than that of Glu.⁵¹ Therefore, it is Glu that governs the intensity of Signal 6. As the signal area does not change for F \rightarrow M, we conclude that the Glu molecular fraction does not vary either.

Signal 1

Signal 1 is produced by only one amino acid. Its area is significantly lower for males (strong significance), indicating that the Ile content is lower for this group.

Signal 2

The area of Signal 2 does not change for F \rightarrow M. The Ile content for males is lower, so the Ala content for them must be higher.

Signal 18

The area of Signal 18 and the Glu content do not change for F \rightarrow M, so the Asp content does not change either.

Signal 5

The area of Signal 5 does not change for F \rightarrow M and the mean content of His is negligibly low.⁵¹ As the molecular ratio of Glu and Pro does not change, the content of Val is the same for both groups.

Signal 3

The area of Signal 3 and the content of Val do not change for F → M. Therefore, the Thr content must be the same for the compared groups.

Signal 14

The area of Signal 14 does not change for F → M. The content of His is negligibly low, while Tyr and Phe have similar mole percentages of 2.9 and 2.2, respectively.⁵¹ Thus, there are four resonances from Tyr and Phe to interpret, two for each amino acid. Three of them have similar CP kinetics, because all are from CH-aromatic groups. The remaining fourth resonance is from aromatic non-protonated carbon and this one must have considerably slower CP kinetics than the others.⁵⁰ In this situation it is improbable that for F → M the content of one amino acid increases and the content of the other decreases, and that those effects compensate each other to keep the signal area unchanged. Therefore, we believe that the contributions from Tyr and Phe stay unchanged.

Signal 15

The area of Signal 15 decreases for F → M (weak significance). As the Tyr content does not change, the content of Arg must be lower for males.

Signal 4

The area of Signal 4 increases for F → M (weak significance). The content of redCys is expected to decrease, because the total Cys content is lower for males as deduced from Dittmar et al.'s work.¹⁷ Furthermore, we observe the significant increase of Signal 8, well explained by the increase of the contribution from oxCys (see the forthcoming discussion of Signal 8). If the oxCys content grows, the redCys content is reduced. Signal 4 has many attributed amino acids, whose contributions are listed here (order as in Table 1) together with the formerly determined gender effects: Arg (decrease), redCys (decrease), Ile (decrease), Pro (unchanged), Arg (decrease), Lys (?), Leu (?), Leu (?) and Lys (?). The influence of Lys on the global signal area is probably smaller than that of Leu, considering their respective molar percentages of 3.1 and 8.0.⁵¹ Thus, the Leu content must be sufficiently higher for the male group in order to boost the common signal of those six amino acids.

Signal 7

The area of Signal 7 decreases for F → M (strong significance). As the Asp, Phe and Tyr contributions are unaffected, this change can only be accounted for by the decrease in the Ile content. This is in agreement with the previous conclusion for Signal 1.

Signal 8

The area of Signal 8 increases for F → M (strong significance). This complex signal has a troublesome contribution from the α carbon of Gly. However, it is known from the solution NMR spectroscopy that with the change of the protein secondary structure this resonance moves in the carbon-13 spectrum by not more than 2 ppm (see Table S1 in Wong et al.'s paper³³). Thus, the C_{α} -Gly peak is less sensitive to the H ↔ S/C structural changes than the

corresponding peaks of other amino acids. The FWHM value of Signal 8 increases from 7.9 to 8.5 ppm on average when going from the female to male group. Considering that Signal 8 is very broad, it is very likely that even under the H ↔ S/C structural changes the C_α-Gly peak will remain inside the common envelope of Signal 8. Therefore, only a change in the Gly content may affect the total area of Signal 8. Other involved resonances are from side chains of oxCys, Leu, Arg and Lys, so they are less sensitive to the protein structural changes. For two amino acids, we anticipate a cancelling effect on the global signal area: an increase in Leu and a decrease in Arg. We still know nothing about any changes in the contributions from Gly and Lys, the amino acids with the mean molar percentages of 6.9 and 3.1,⁵¹ respectively. Presumably, Lys is less influential because of its lower content. If so, Gly and/or oxCys seem to be primarily responsible for the increase in the Signal 8 integral intensity. However, the total Cys content decreases for males, as deduced from Dittmar et al.'s work.¹⁷ Therefore, either there is an unprecedented jump in the Gly content for F → M, or there is a prominent increase in the content of Cys disulphide linkages, that is in the oxCys content. The latter conjecture appears more reasonable, if the increase already deduced in the S/C secondary structure in α-keratin from male nails is considered in reference to β-keratin, which is rich in β-sheet secondary structure and simultaneously in the disulphide bonds.³ Besides, the Gly content is reasonably stable in nail keratins, because it is similar in fibrils and in matrix.⁵¹

Signal 13

The area of Signal 13 decreases for F → M (weak significance). This signal has contributions from α carbons (Ile, Thr, Val) and β carbons (Ser, Thr). Their resonances move with a change in the protein secondary structure (more for α carbons, see Results) and we know from the earlier interpretation that males have a greater content of the S/C secondary structure than females. Therefore, the interpretation of Signal 13 in terms of the gender differences is difficult. To proceed further, one should consider the following conditions: (1) Signal 13 is very broad (FWHM over 10 ppm); (2) the neighbouring Signal 12 with the lower chemical shift does not change for F → M; (3) there is no neighbouring peak with the higher chemical shift (Fig. 1B), from which an intensity transfer to Signal 13 caused by the C_α(H) → C_α(S/C) would have been possible. Therefore, we believe that the C_α-resonances of Ile, Thr and Val together with the smaller shifted C_β-resonances of Ser and Thr all remain within the broad line of Signal 13 without regard to the existing secondary structure. Then, from the former discussion, we know that the Thr content and the Val content are both the same for the analysed groups. We speculate that because of the lower content of Ile for males, Signal 13 may have a lower area for this group, although this change is not as significant as in the case of Signal 1. The question is whether there is a damping effect of Ser, that is its slight increase in opposition to the decrease in Ile (mean contents of Ser and Ile: 10.2 and 3.3 mole%, respectively⁵¹).

Signal 12

The area of Signal 12 does not change for F → M. The postulated higher content of Ser for males is consistent with the behaviour of Signal 12, provided that for males there is no shift of the included C_α-resonances outside the signal envelope (FWHM about 6 ppm) and/or

no accumulation of the extraneous C_{α} -resonances from the neighbouring peaks. Under this assumption and with the unchanged Pro content (see Signal 9), the area of Signal 12 would not differ between the two studied groups, if there was a compensation effect between Ser (increase) and Cys (decrease).

Signals 10 and 11

These signals include C_{α} -resonances from various amino acids and any interpretation of their integral intensity would be too speculative.

In summary, the presented comparative analysis of the signal areas demonstrates some modification of the amino acid composition of fingernail α -keratin, when going from females to males (molecular fractions are considered): an increase in Leu, Ala and possibly in Ser; a decrease in Cys, Arg and Ile; no change found in Glu, Asp, Pro, Thr, Val, Tyr and Phe; undetermined variations in Gly, Lys and His.

M21 M23
M3 M27
M21 M23 M31
F28 F29
M16 M17
M1 M14
F41 F46
M26 M32
F10 F21
M18 M19
M5 M6
F35 F36
F14 F32
M20 M25
M8 M10
M9 M30
F40 F43
F26 F44
F3 F7
F6 F37
F28 F29 F39
M3 M27 M4
F13 F18
M28 M29
F4 F16
M7 M24
M9 M30 M12
M1 M14 M15
F19 F34
F8 F12
M21 M23 M31 M28 M29
F23 F27
M9 M30 M12 M11
F35 F36 F42
M21 M23 M31 M28 M29 M26 M32
F10 F21 F13 F18
F41 F46 F45
M3 M27 M4 M5 M6
F14 F32 F17
F11 F33
F4 F16 F28 F29 F39
F6 F37 F40 F43
F2 F15
M8 M10 M9 M30 M12 M11
F19 F34 F22
M2 M7 M24
F26 F44 F41 F46 F45
F8 F12 F25
F10 F21 F13 F18 M13
F1 F30
M18 M19 M20 M25
F6 F37 F40 F43 F24
F20 F23 F27
F9 M2 M7 M24
F5 F20 F23 F27
F4 F16 F28 F29 F39 F31
F11 F33 F35 F36 F42
F2 F15 F14 F32 F17
F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13
F3 F7 M22
M3 M27 M4 M5 M6 M18 M19 M20 M25
F6 F37 F40 F43 F24 F26 F44 F41 F46 F45
M1 M14 M15 M16 M17
F3 F7 M22 F5 F20 F23 F27
F1 F30 F8 F12 F25
F6 F37 F40 F43 F24 F26 F44 F41 F46 F45 F11 F33 F35 F36 F42
F9 M2 M7 M24 M1 M14 M15 M16 M17
F3 F7 M22 F5 F20 F23 F27 F38
F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13 F19 F34 F22
F1 F30 F8 F12 F25 F2 F15 F14 F32 F17
F9 M2 M7 M24 M1 M14 M15 M16 M17 M8 M10 M9 M30 M12 M11
M3 M27 M4 M5 M6 M18 M19 M20 M25 M21 M23 M31 M28 M29 M26 M32
F3 F7 M22 F5 F20 F23 F27 F38 F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13 F19 F34 F22
F9 M2 M7 M24 M1 M14 M15 M16 M17 M8 M10 M9 M30 M12 M11 M3 M27 M4 M5 M6 M18 M19 M20 M25 M21 M23 M31 M28 M29 M26 M32
F1 F30 F8 F12 F25 F2 F15 F14 F32 F17 F3 F7 M22 F5 F20 F23 F27 F38 F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13 F19 F34 F22
F1 F30 F8 F12 F25 F2 F15 F14 F32 F17 F3 F7 M22 F5 F20 F23 F27 F38 F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13 F19 F34 F22 F6 F37 F40 F43 F24 F26 F44 F41 F46 F45 F11 F33 F35 F36 F42

Sex-related chemical differences in keratin from fingernail plates: A solid-state carbon-13 NMR study

Paulina Brzózka * and Waclaw Kolodziejski

Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy with the
Laboratory Medicine Division, Medical University of Warsaw, Warsaw, Poland.

*Corresponding author: e-mail: pnykiel@wum.edu.pl

Statistical Report S1.
Cluster Analysis. Amalgamation Schedule

F1 F30 F8 F12 F25 F2 F15 F14 F32 F17 F3 F7 M22 F5 F20 F23 F27 F38 F4 F16 F28 F29 F39 F31 F10 F21 F13 F18 M13 F19 F34 F22 F6 F37 F40 F43 F24 F26 F44 F41 F46 F45 F11 F33 F35 F36 F42 F9 M2 M7 M24 M1 M14 M15 M16 M17 M8 M10 M9 M30 M12 M11 M3 M27 M4 M5 M6 M18 M19 M20 M25 M21 M23 M31 M28 M29 M26 M32

Plot of Linkage Distances across Steps

Euclidean distances

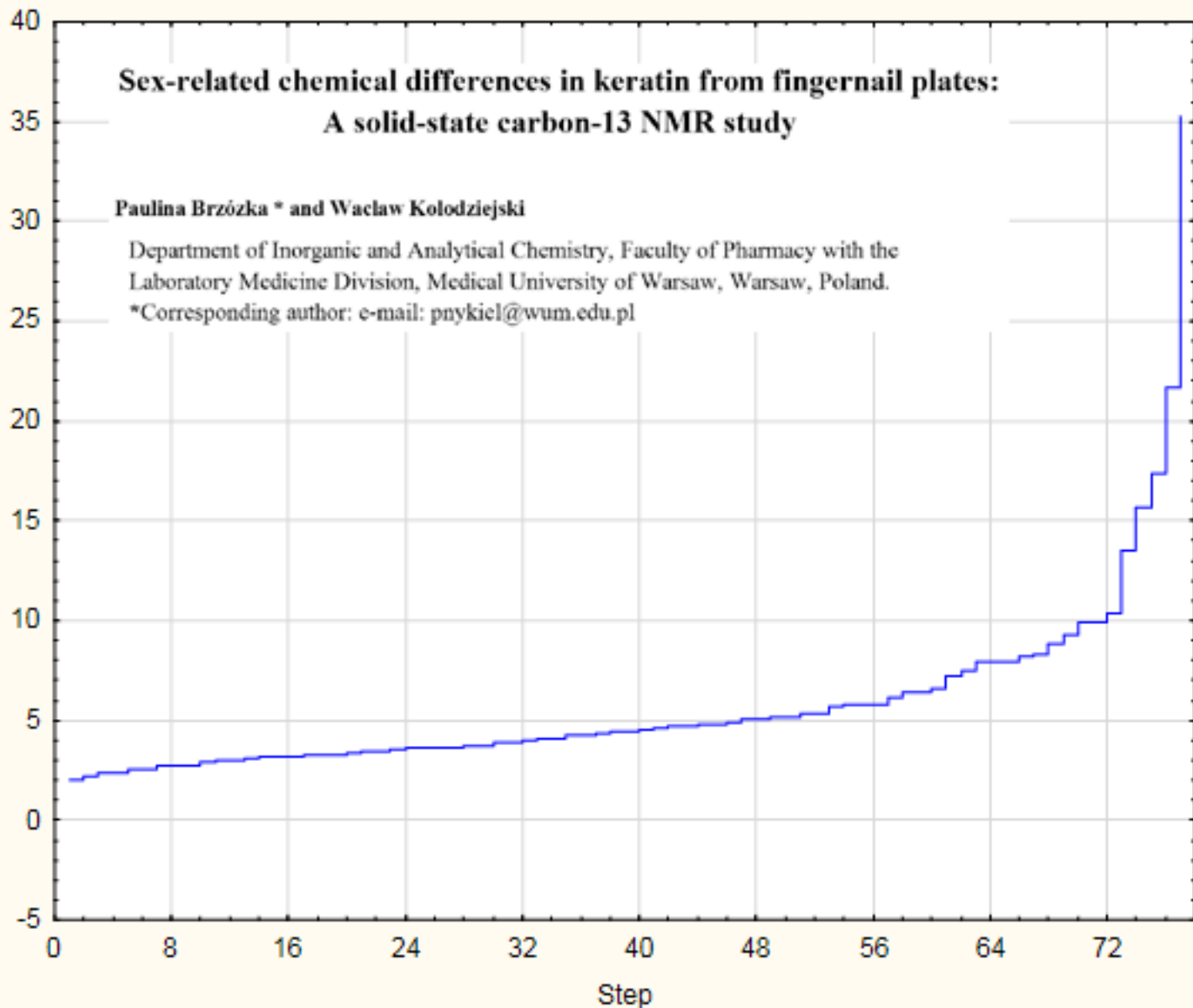
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Paulina Brzózka * and Wacław Kołodziejcki

Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy with the
Laboratory Medicine Division, Medical University of Warsaw, Warsaw, Poland.

*Corresponding author: e-mail: pnykiel@wum.edu.pl

Linkage Distance



— Linkage Distance

Statistical Report S2

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Paulina Brzózka * and Waclaw Kolodziejcki

Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy with the Laboratory Medicine Division, Medical University of Warsaw, Warsaw, Poland.

*Corresponding author: e-mail: pnykiel@wum.edu.pl

Principal Component Analysis

Descriptive Statistics

	Mean	Std. Deviation	Analysis N
Area_171.8	847721557.30	212606352.65	78
Area_175.4	595420214.52	232007007.22	78
Area_180.5	111377191.52	59831977.88	78
Area_157.6	132015689.04	45625587.54	78
Area_129.0	264945244.50	79694176.85	78
Area_64.9	491391922.64	183687791.06	78
Area_59.9	656957864.68	183966161.19	78
Area_53.4	133233528.74	393423416.03	78
Area_40.5	1057481394.11	258988502.48	78
Area_29.7	740523054.93	215184346.59	78
Area_25.1	962833880.03	274301397.90	78
Area_20.4	411866798.49	124135784.07	78
Area_12.7	168192956.86	103453022.70	78

Correlation Matrix

	Area_171.8	Area_175.4	Area_180.5	Area_157.6	Area_129.0	Area_64.9	Area_59.9	Area_53.4	Area_40.5	Area_29.7	Area_25.1	Area_20.4	Area_12.7
Correlation Area_171.8	1.000	.656	.583	.719	.711	.573	.692	.767	.837	.660	.812	.737	.530
Area_175.4	.656	1.000	.494	.799	.768	.738	.695	.797	.761	.753	.768	.706	.784
Area_180.5	.583	.494	1.000	.477	.661	.765	.622	.569	.647	.603	.595	.555	.447
Area_157.6	.719	.799	.477	1.000	.797	.672	.715	.811	.829	.752	.806	.780	.576
Area_129.0	.711	.768	.661	.797	1.000	.791	.729	.844	.845	.820	.817	.694	.647
Area_64.9	.573	.738	.765	.672	.791	1.000	.742	.742	.752	.771	.735	.658	.629
Area_59.9	.692	.695	.622	.715	.729	.742	1.000	.686	.813	.720	.761	.741	.591
Area_53.4	.767	.797	.569	.811	.844	.742	.686	1.000	.872	.755	.887	.713	.647
Area_40.5	.837	.761	.647	.829	.845	.752	.813	.872	1.000	.786	.888	.767	.597
Area_29.7	.660	.753	.603	.752	.820	.771	.720	.755	.786	1.000	.632	.796	.603
Area_25.1	.812	.768	.595	.806	.817	.735	.761	.887	.888	.632	1.000	.617	.627
Area_20.4	.737	.706	.555	.780	.694	.658	.741	.713	.767	.796	.617	1.000	.554
Area_12.7	.530	.784	.447	.576	.647	.629	.591	.647	.597	.603	.627	.554	1.000

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.905
Bartlett's Test of Sphericity	Approx. Chi-Square	1220.104
	df	78
	Sig.	.000

Anti-image Matrices

		Area_171.8	Area_175.4	Area_180.5	Area_157.6	Area_129.0	Area_64.9	Area_59.9	Area_53.4	Area_40.5	Area_29.7	Area_25.1	Area_20.4	Area_12.7
Anti-image Covariance	Area_171.8	.177	.005	-.059	.032	.012	.071	.029	.016	-.023	-.033	-.051	-.077	-.005
	Area_175.4	.005	.173	.036	-.043	.009	-.027	.007	-.009	.006	-.025	-.012	-.011	-.130
	Area_180.5	-.059	.036	.315	.040	-.045	-.123	-.005	.018	-.015	.016	.006	-.014	.003
	Area_157.6	.032	-.043	.040	.163	-.028	.019	.019	.014	-.009	-.022	-.035	-.073	.047
	Area_129.0	.012	.009	-.045	-.028	.157	-.004	.007	-.018	-.009	-.057	-.015	.019	-.029
	Area_64.9	.071	-.027	-.123	.019	-.004	.162	-.018	-.001	.008	-.049	-.029	-.020	-.005
	Area_59.9	.029	.007	-.005	.019	.007	-.018	.221	.063	-.033	-.031	-.043	-.070	-.028
	Area_53.4	.016	-.009	.018	.014	-.018	-.001	.063	.116	-.016	-.031	-.043	-.038	-.009
	Area_40.5	-.023	.006	-.015	-.009	-.009	.008	-.033	-.016	.099	-.027	-.022	-.012	.022
	Area_29.7	-.033	-.025	.016	-.022	-.057	-.049	-.031	-.031	-.027	.147	.050	-.008	.003
	Area_25.1	-.051	-.012	.006	-.035	-.015	-.029	-.043	-.043	-.022	.050	.060	.051	-.006
	Area_20.4	-.077	-.011	-.014	-.073	.019	-.020	-.070	-.038	-.012	-.008	.051	.170	-.010
	Area_12.7	-.005	-.130	.003	.047	-.029	-.005	-.028	-.009	.022	.003	-.006	-.010	.351
	Anti-image Correlation	Area_171.8	.875	.028	-.249	.187	.071	.420	.149	.113	-.175	-.206	-.494	-.444
Area_175.4		.028	.934	.152	-.259	.055	-.160	.034	-.061	.050	-.159	-.119	-.063	-.529
Area_180.5		-.249	.152	.897	.179	-.200	-.547	-.020	.096	-.084	.075	.042	-.059	.008
Area_157.6		.187	-.259	.179	.917	-.178	.117	.100	.101	-.068	-.142	-.358	-.439	.198
Area_129.0		.071	.055	-.200	-.178	.959	-.027	.039	-.132	-.072	-.373	-.155	.117	-.124
Area_64.9		.420	-.160	-.547	.117	-.027	.894	-.095	-.007	.059	-.317	-.296	-.122	-.023
Area_59.9		.149	.034	-.020	.100	.039	-.095	.916	.392	-.224	-.171	-.371	-.360	-.100
Area_53.4		.113	-.061	.096	.101	-.132	-.007	.392	.918	-.147	-.239	-.512	-.272	-.045
Area_40.5		-.175	.050	-.084	-.068	-.072	.059	-.224	-.147	.964	-.223	-.288	-.095	.118
Area_29.7		-.206	-.159	.075	-.142	-.373	-.317	-.171	-.239	-.223	.893	.534	-.049	.014
Area_25.1		-.494	-.119	.042	-.358	-.155	-.296	-.371	-.512	-.288	.534	.816	.510	-.039
Area_20.4		-.444	-.063	-.059	-.439	.117	-.122	-.360	-.272	-.095	-.049	.510	.866	-.042
Area_12.7		-.022	-.529	.008	.198	-.124	-.023	-.100	-.045	.118	.014	-.039	-.042	.924

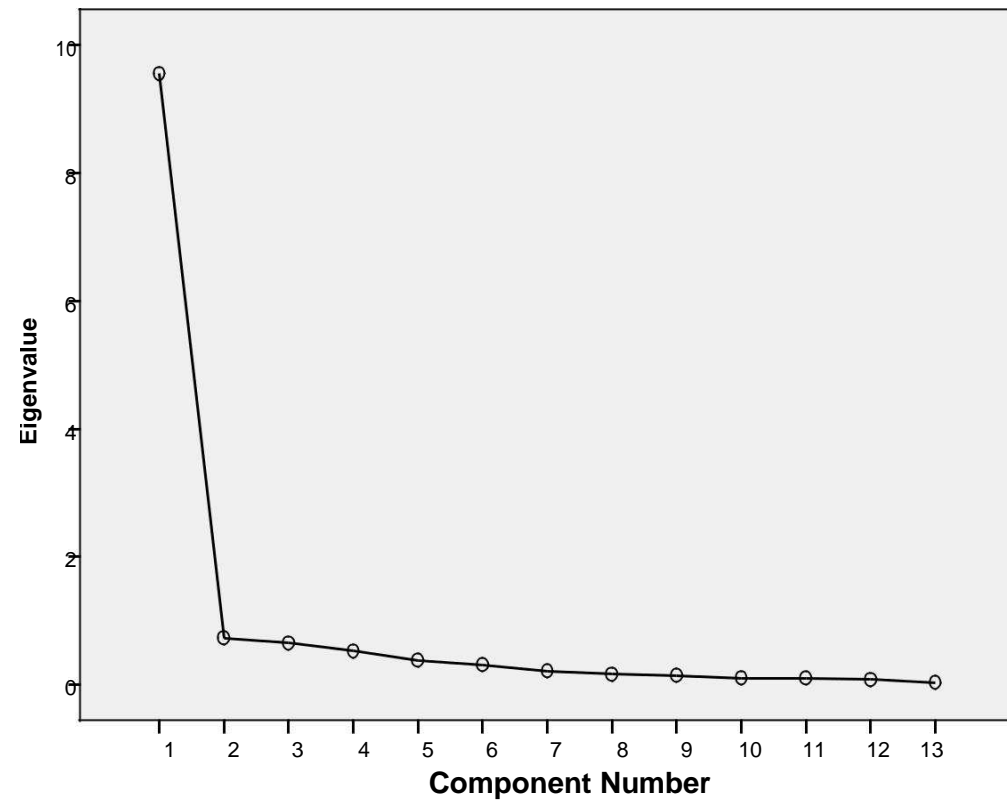
Communalities

	Initial	Extraction
Area_171.8	1.000	.698
Area_175.4	1.000	.765
Area_180.5	1.000	.509
Area_157.6	1.000	.773
Area_129.0	1.000	.832
Area_64.9	1.000	.737
Area_59.9	1.000	.730
Area_53.4	1.000	.829
Area_40.5	1.000	.879
Area_29.7	1.000	.754
Area_25.1	1.000	.805
Area_20.4	1.000	.702
Area_12.7	1.000	.540

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	9.554	73.495	73.495	9.554	73.495	73.495
2	.732	5.628	79.124			
3	.655	5.035	84.159			
4	.529	4.070	88.228			
5	.382	2.936	91.164			
6	.311	2.390	93.554			
7	.212	1.629	95.183			
8	.163	1.257	96.440			
9	.143	1.096	97.536			
10	.106	.814	98.350			
11	.100	.768	99.118			
12	.079	.609	99.727			
13	.035	.273	100.000			

Scree Plot



Component Matrix

	Component
	1
Area_171.8	.835
Area_175.4	.874
Area_180.5	.714
Area_157.6	.879
Area_129.0	.912
Area_64.9	.858
Area_59.9	.855
Area_53.4	.911
Area_40.5	.938
Area_29.7	.868
Area_25.1	.897
Area_20.4	.838
Area_12.7	.735