1 **Distribution, chemical and molecular composition of high and**

2 **low-molecular-weight humic-like substances in ambient aerosols**

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Abstract

Humic-like Substances (HULIS) encompass a continuum of molecular weight (MW) ranges, yet our understanding of how HULIS characteristics vary with MW is still limited and not well-established. In this study, a combination of ultrafiltration and solid-phase extraction protocols was employed to fractionate the high MW (HMW, >1 kDa) and low MW (LMW, < 1kDa) HULIS fractions from ambient aerosols collected during summer and winter at a rural site. Subsequently, comprehensive characterization by using total organic carbon, high-performance size exclusion chromatography (HPSEC), UV-vis and fluorescence spectroscopy, Fourier-transform infrared spectroscopy (FTIR), negative electrospray ionization high resolution mass spectrometry (ESI- HRMS) were conducted. The results revealed that HMW HULIS were dominated by larger-sized chromophores, substantially constituting a higher fraction of total organic carbon and UV absorption at 254 nm than LMW HULIS. While both HMW and LMW HULIS shared similar fluorophore types and functional groups, the former exhibited higher levels of humification and a greater presence of polar functional groups (e.g., -COOH, >C=O). HRMS analysis further unveiled that molecular formulas within HMW HULIS generally featured smaller sizes but higher degrees of unsaturation and aromaticity compared to those within LMW HULIS fractions. This observation suggests the possibility of small molecules assembling to form the HMW HULIS through intermolecular weak forces. Moreover, HMW HULIS contained a higher proportion of CHON but fewer CHO compounds than LMW HULIS. In both HMW and LMW HULIS, the unique molecular formulas were

39 primarily characterized by lignin-like species, yet the former displayed a prevalence

40 of N-enriched and highly aromatic species. Additionally, HMW HULIS contained

41 more unique lipids-like compounds, while LMW HULIS exhibited a distinct presence

42 of tannin-like compounds. These findings provide valuable insights into the

43 distribution, optical properties, and molecular-level characteristics of HULIS in

44 atmospheric aerosols, thereby advancing our understanding of their sources,

45 composition, and environmental implications.

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1. Introduction

HUmic-Like Substances (HULIS) are complex and heterogeneous mixtures of water-soluble organic matters (WSOM) that are of great importance in the atmospheric environment. They usually share similar physicochemical properties (e.g., acidity, absorption, fluorescence, functional groups) with naturally occurring humic substances (Graber and Rudich, 2006; Zheng et al., 2013) and are prevalent in fog, clouds, rainwater and ambient aerosols (Birdwell and Valsaraj, 2010; Fan et al., 2016a; Santos et al., 2012). With substantial hygroscopic and surface-active properties, HULIS enhance the hygroscopic growth of particles, thereby contributing to the formation of the cloud condensation nuclei and ice nuclei (Chen et al., 2021a; Dinar et al., 2007). Moreover, acting as an important component of brown carbon (BrC), HULIS effectively absorb near-ultraviolet and visible light, thus influencing the global radiative balance and atmospheric chemistry processes (Bao et al., 2022; Zhang et al., 2020). Furthermore, HULIS have the potential to catalyze the formation of reactive oxygen species, leading to potential adverse health effects (Ma et al., 2019; Zhang et al., 2022b).

The chemical composition of atmospheric HULIS exhibit significant heterogeneity and typically comprises macromolecular compounds containing aromatic rings with highly conjugated structures, as well as long-chain hydrocarbon with polar groups (e.g., -OH, -COOH, -NO$_2$) (Fan et al., 2013; Huo et al., 2021). To unravel the structural characteristics and properties of HULIS, a range of analytical techniques, including absorption and fluorescence spectroscopy, Fourier transform

infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (^{1}H NMR),

have been utilized (Huo et al., 2021; Qin et al., 2022; Zou et al., 2020). These studies

have provided insights into the overall structural characteristics of complex HULIS,

including their abundances, chemical and optical characteristics (Huo et al., 2021;

Mukherjee et al., 2020; Win et al., 2018; Zhang et al., 2022b; Zheng et al., 2013). In

recent years, high-resolution mass spectrometry (HRMS) techniques, such as Fourier

transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and orbitrap

HRMS, in combination with electrospray ionization (ESI), have emerged as powerful

tools for elucidating the molecular-level characteristics of HULIS (Lin et al., 2012;

Sun et al., 2021; Wang et al., 2019; Zou et al., 2023). By utilizing HRMS, researchers

have gained deeper insights into the complexity and chemical heterogeneity of

HULIS at the molecular level.

Operationally, HULIS are defined as the hydrophobic fraction of water-soluble

organic matter (WSOM) typically extracted through solid-phase extraction (SPE)

protocol (Fan et al., 2012; Zou et al., 2020). Thus, the abundance and characteristics

of HULIS are contingent upon the chemical composition of WSOM. Previous studies

have shown that aerosol WSOM, often seen as water-soluble brown carbon (BrC), are

comprised of a continuum of molecular weight (MW) species, as revealed by

high-performance exclusion chromatography (HPSEC) analysis (Di Lorenzo et al.,

2017; Fan et al., 2023; Wong et al., 2019). These studies have highlighted that BrC

typically consist of both high-MW (HMW) and low-MW (LMW) chromophores in

various aerosols. For example, BrC emitted from fresh biomass burning (BB) are

95   dominated by low MW chromophores (Di Lorenzo et al., 2017; Wong et al., 2019).

96   However, BrC derived from aged BB aerosols and ambient aerosols tend to possess

97   more HMW chromophores that are highly chemically resistant (Di Lorenzo et al.,

98   2017; Fan et al., 2023; Wong et al., 2019). Further characterizations of different MW

99   BrC can be conducted using an ultrafiltration (UF) protocol (Fan et al., 2021). This

100   approach enabled researchers to obtain the distributions of content, chromophores and

101   fluorophores within various MW BrC fractions. Despite these advancements, the

102   chemical structures and molecular composition of different MW HULIS fractions

103   remain poorly understood. Consequently, a combination of UF and SPE protocols for

104   the fractionation and characterization of MW-separated HULIS is crucial, as it not

105   only provides insights into MW distributions but also illuminates the chemical

106   heterogeneities of aerosols HULIS.

107   In this study, a combination of UF-SPE isolation protocol was developed to

108   fractionate and characterize the MW HULIS fractions. Two distinct sets of ambient

109   $PM_{2.5}$ samples collected during summer and winter periods were utilized to facilitate a

110   comparative analysis of MW HULIS. Initially, the WSOM were fractionated into

111   high-MW (HMW, >1 kDa) and low-MW (LMW, <1 kDa) species using the UF

112   protocol. Subsequently, the resulting MW WSOM fractions underwent SPE to obtain

113   different MW HULIS fractions. The obtained HMW and LMW HULIS fractions were

114   comprehensively characterized using advanced analytical techniques, including total

115   organic carbon analysis, UV-vis and fluorescence spectroscopy, HPSEC, and HRMS

116   to unveil their abundances, absorption and fluorescence properties, and molecular

characteristics. The findings of this study hold great significance in advancing our understanding of the definition and molecular profiles of HULIS, as well as facilitating further investigations into their potential impacts on the atmospheric environment.

2. Materials and methods

2.1. Atmospheric fine particles sampling

Atmospheric $PM_{2.5}$ were sampled on the rooftop of a building within the campus of Anhui Science and Technology University (32.21ºN, 118.72ºE), around 20 m above ground level. Detailed information regarding the sampling site can be found in our previous studies (Cao et al., 2022; Fan et al., 2021). The $PM_{2.5}$ samples were collected using a high-volume $PM_{2.5}$ sampler (JCH-1000, Juchuang Ltd., Qingdao) onto prebaked quartz fiber filters (8 × 10 inches, Whatman). Sampling took place from July 25 to August 12, 2021, during summer, and from December 19, 2021 to January 6, 2022, during winter. Blank filters were also collected as control samples. All aerosol $PM_{2.5}$ filter samples were stored at -20 ºC in a freezer prior to analysis. The atmospheric pollutant data (NO_2, SO_2 and O_3) near sampling site during sampling period were obtained from the website (https://www.aqistudy.cn) and are summarized in Table S1. Additionally, the fire spots were investigated using data from the website (https://firms.modaps.eosdis.nasa.gov/map/) and are visualized in Fig. S1.

2.2. Application of UF-SPE for isolating MW HULIS fractions

139   Punches of summer and winter aerosol $PM_{2.5}$ filter samples were taken and

140   combined for the extraction of water-soluble organic matter (WSOM), respectively.

141   The filters were immersed in 300 mL of ultrapure water and subjected to

142   ultrasonication for 30 min. The resulting suspensions were then filtered through a 0.22

143   μm membrane (Φ 47 mm, Jinteng, China) to obtain bulk WSOM samples. These bulk

144   filtrates were further subjected to UF and SPE in tandem to obtain different MW

145   HULIS fractions. Please refer to Fig. S2 for a schematic representation of the

146   fractionation steps.

147   Before UF, the bulk WSOM were diluted to DOC concentration below 30 mg/L

148   to minimize the concentration effects and prevent the accumulation of organic matters

149   at the membrane surface during UF. The detailed UF procedure followed the profile

150   described in our previous study (Fan et al., 2021). Briefly, each bulk WSOM solution

151   was passed through a pre-cleaned 1 kDa cut-off membrane in a stirred UF cell

152   (Amicon 8200, Millipore, USA), with a pressure at 0.2 MPa applied by ultrapure N_2.

153   The concentration factor was ~10. The resulting retentate was considered as HMW

154   (>1 kDa) WSOM, while the permeate solutions represented the LMW (<1 kDa)

155   WSOM. Finally, each MW fraction was diluted to the initial volume for further

156   treatment and analysis. Mass balances of WSOM during one-step UF process

157   generally ranged from 92% to 99%, as determined by total organic carbon (TOC) and

158   UV absorption at 254 nm (UV_{254}), indicating good performance of UF without

159   substantial loss or organic contamination.

160   Subsequently, SPE was applied to isolate the so-called HULIS fractions from the

bulk and each MW fraction of WSOM, following the protocol proposed in our previous studies (Fan et al., 2013; Zou et al., 2020). Briefly, the acidified aqueous samples were passed through pre-activated HLB columns (Waters Oasis, 500 mg/6 mL, USA). The fractions retained on the resins (referred to as HULIS) were eluted with pure methanol and dried using a gentle stream of pure N_2. Finally, the bulk, HMW and LMW HULIS fractions were obtained. A blank filter control was performed using the same procedure described above, and the analysis signals of samples were corrected by blank control.

2.3. HPSEC analysis

The apparent MW distributions of MW HULIS fractions were analyzed using a high-performance liquid chromatography (HPLC) system (LC-20AT, Shimadzu, Japan) equipped with a refractive index detector (RID-10A, Shimadzu) and a diode array detector (SPD-M20A, Shimadzu). The wavelength of the diode array detector was set at 254 nm. Separation was performed using an aqueous gel filtration column (Polysep-GFC-P 3000, Phenomenex) preceded by a guard column (Polysep-GFC-P, Phenomenex). The mobile phase consisted of a mixture of water and methanol (90:10 v/v) containing 25 mM ammonium acetate (Di Lorenzo et al., 2017; Wong et al., 2019). The sample injection volume was 100 μL, and the flow rate was maintained at 1 mL min^{-1}. The HPSEC calibration was performed using a series of polyethylene glycol (PEG) standards (Kawasaki et al., 2011; Zhang et al., 2022c). The chromatographic peak areas were integrated to represent the abundances of

183   corresponding MW species. It should be noted that the MW values estimated here are

184   nominal rather than absolute due to the lack of appropriate standards for column

185   calibration (Fan et al., 2023; Wong et al., 2017).

186   The weight-average MW (Mw), number-average MW (Mn) and polydispersivity

187   (ρ), were determined using the following equations (Song et al., 2010):

$$Mw = \frac{\sum_{i=1}^{n}(h_i MW_i)}{\sum_{i=1}^{n} h_i} \tag{1}$$

$$Mn = \frac{\sum_{i=1}^{n} h_i}{\sum_{i=1}^{n}(h_i/MW_i)} \tag{2}$$

$$\rho = \frac{Mw}{Mn} \tag{3}$$

188   where h_i and MW_i are the absorption intensity of the chromatogram and the MW of

189   molecules corresponding to the ith retention time, respectively.

190

191   2.4. Measurements of WSOC content and optical properties

192   The concentration of water-soluble organic carbon (WSOC) in HMW and LMW

193   HULIS was measured using a Shimadzu TOC analyzer (TOC-VCPN, Japan)

194   following the non-purgeable organic carbon protocol.

195   The UV-vis spectra were recorded using a UV-vis spectrophotometer (UV 2600,

196   Shimadzu, Japan) over a wavelength range of 200-700 nm with 1 nm increments.

197   Excitation-emission matrix (EEM) spectra were determined using a fluorescence

198   spectrophotometer (F4600, Hitachi, Japan). The scanning ranges for excitation (Ex)

199   and emission (Em) wavelengths were 200-400 and 290-520 nm, respectively, with a

200   scanning speed was 12,000 nm/min.

201   To characterize the chemical and optical properties of MW HULIS fractions,

202 several commonly used spectra parameters were calculated, including the specific UV

203 absorbance at 254 nm (SUVA$_{254}$), the UV absorbance ratio between 250 and 365 nm

204 (E$_2$/E$_3$), spectra slope ratios (S$_R$), the absorption Angstrom exponent (AAE), and mass

205 absorption efficiency (MAE$_{365}$), fluorescence indices (FI), biological index (BIX), and

206 humification degree (HIX) (Fan et al., 2021; Li et al., 2022; Wu et al., 2021). Further

207 details can be found in Text S1 of the Supporting Information (SI).

208

209 2.5. HRMS analysis and data processing

210 The MW HULIS fractions were analyzed using a Q-Exactive mass spectrometer

211 (Thermo Scientific, Germany) equipped with a heated electrospray ionization (ESI)

212 source. The system operated in negative ESI mode with a resolution of 140,000 at m/z

213 = 200. The detection mass range was set from 60 to 900 m/z. To ensure accurate mass

214 measurements, mass calibration was carried out using a commercial standard mixture

215 of ESI-L Low Concentration Tuning Mix (G1969-85000, Agilent, USA).

216 The acquired mass spectra were processed using Xcalibur software (V2.2,

217 Thermo Scientific). The mathematically possible formulas for all ions were calculated

218 with a signal-to-noise ratio (s/n) $\geq$ 5 using a mass tolerance of 5 ppm. The assigned

219 molecular formulas followed specific constraints, with limitations on the following

220 elements: C $\leq$ 50, H $\leq$ 100, O $\leq$ 20, N $\leq$ 3, S $\leq$ 2. Additionally, the elemental ratios of

221 H/C, O/C, N/ C, and S/C were constrained to the ranges of 0.3–3.0, 0–3.0, 0–0.5, and

222 0–2.0, respectively. The double-bond equivalents (DBE) and modified aromaticity

223 index (AI$_{mod}$) values of the assigned neutral assigned formula (C$_c$H$_h$O$_o$N$_n$S$_s$) were

224   calculated using the equations (4-5) (He et al., 2023; Song et al., 2022):

$$\text{DBE} = 1 + \frac{1}{2}(2c - h + n) \qquad (4)$$

$$\text{AI}_{mod} = \frac{1 + c - 0.5o - 0.5n - 0.5h}{c - 0.5o - n} \qquad (5)$$

225   The intensity-weighted molecular parameters (X_w) of MW, H/C, O/C, DBE, and

226   AI values were calculated according to the equation (6) (He et al., 2023; Zhang et al.,

227   2021; Zou et al., 2023):

$$X_W = \frac{\sum(I_i \cdot X_i)}{\sum I_i} \qquad (6)$$

228   where X represents the aforementioned parameters, and I_i denote the intensity for each

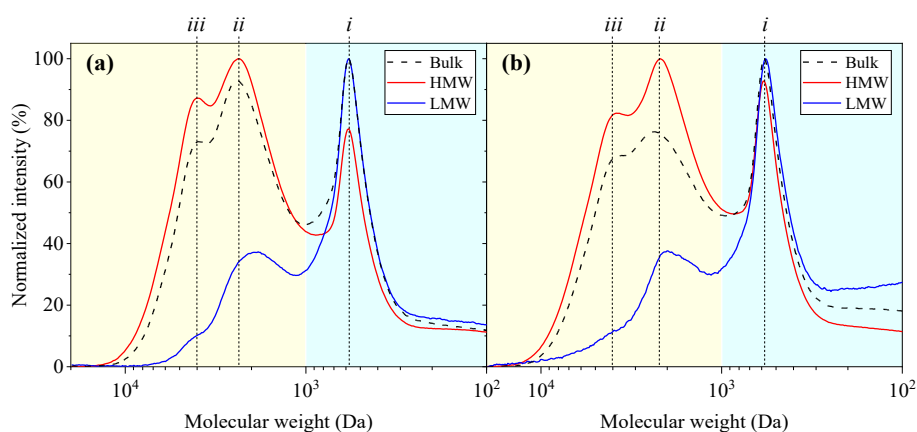229   assigned formula i.

230

231   **3. Results and discussion**

232   3.1. Size and distribution of MW HULIS fractions

233   3.1.1. Molecular size of HMW and LMW HULIS

234   Fig. 1 shows the HPSEC chromatograms of MW HULIS. Both HMW and LMW

235   HULIS exhibit MW continuum distributions ranging from 100 to 20,000 Da, which is

236   consistent with the reported distributions of BrC in BB-derived and various ambient

237   aerosol in previous studies (Di Lorenzo et al., 2017; Fan et al., 2023; Wong et al.,

238   2017). However, the chromatographic patterns for HMW HULIS clearly differ from

239   those observed for LMW HULIS in both aerosol samples. As seen in Fig. 1, HMW

240   HULIS display an additional and stronger absorption peak at around 4000 Da (peak

241   *iii*), along with a more pronounced peak at 2200 Da (peak *ii*) and a similar magnitude

242   peak at 570 Da (peak *i*) compared to LMW HULIS. This suggests that HMW HULIS

243   contain the majority of larger molecular size chromophores within the bulk WSOM.

244   In addition, the magnitude peak at 570 Da (peak i) in HMW HULIS may indicate the

245   incorporation of small molecule through weak interactions based on π–π and/or van

246   der Waals forces between the HULIS components (Fan et al., 2021; Piccolo, 2002).

247

248 **Fig. 1.** Average HPSEC chromatograms of bulk, HMW and LMW HULIS fractions in

249 (a) summer and (b) winter aerosol, respectively. The yellow and cyan shadows

250 represent MW size regions of >1 kDa and <1 kDa, respectively.

251

252   Moreover, the molecular size of MW HULIS can be further reflected by the

253 differences in Mw and Mn. As listed in Table 1, the average Mw and Mn of HMW

254 HULIS are 2233-2315 and 654-707 Da, respectively, greatly larger than that of LMW

255 HULIS (989-1071 and 293-394 Da, respectively). These differences indicate that the

256 sources and formation processes of HMW HULIS may differ from LMW HULIS.

257 Many previous studies have demonstrated that fresh BB HULIS generally consist of

258 small molecular-sized chromophores (Di Lorenzo et al., 2018; Di Lorenzo et al., 2017;

259 Wong et al., 2017; Wong et al., 2019). However, a notable enhancement in the

260 formation of large molecular-sized chromophores has been found when they undergo

261 intricate atmospheric processes (Di Lorenzo et al., 2018; Di Lorenzo et al., 2017;

262 Wong et al., 2017; Wong et al., 2019). Based on these limited studies, it is suggested

263 that HMW HULIS, characterized by higher proportions of large-size chromophores

264 and the resulting larger apparent molecular size, might be associated with the products

265 from atmospheric aging process rather than being emitted directly by primary sources.

266

267 **Table 1.** The summary of typical quantity and quality parameters of each MW HULIS

268 fraction from BB and ambient aerosols.

		Summer			Winter		
		Bulk	HMW	LMW	Bulk	HMW	LMW
HPSEC-derived parameters	M_w	1975±13	2315±38	1071±24	1918±56	2233±42	989±67
	M_n	591±53	707±48	394±13	525±57	654±17	293±32
	ρ	3.4±0.3	3.3±0.2	2.7±0.2	3.7±0.3	3.4±0.2	3.4±0.2
HULIS/WSOM (%)[a]	TOC	65±1	68±1	51±2	63±2	67±2	41±1
	UV_{254}	66±5	65±2	55±4	67±1	65±1	61±2
Optical parameters	E_2/E_3	12.02±0.54	11.72±0.31	14.98±0.98	6.30±0.24	6.54±0.16	7.24±0.43
	MAE_{365}	0.21±0.02	0.23±0.01	0.20±0.01	1.04±0.02	1.06±0.01	0.88±0.00
	AAE	7.11±0.32	7.59±0.00	8.25±0.23	6.66±0.06	6.25±0.06	7.28±0.03
	FI	2.00±0.04	1.99±0.03	2.04±0.05	2.06±0.01	1.97±0.03	2.25±0.02
	BIX	0.95±0.01	0.86±0.07	1.02±0.01	0.96±0.01	0.81±0.01	1.07±0.02
	HIX	2.42±0.06	2.43±0.04	2.40±0.05	3.13±0.25	5.64±0.34	1.94±0.16

269 [a] The ratios of contents of SPE-isolated HULIS fractions to that of corresponding

270 WSOM fractions determined by TOC and/or absorbance at 254 nm (UV_{254}).
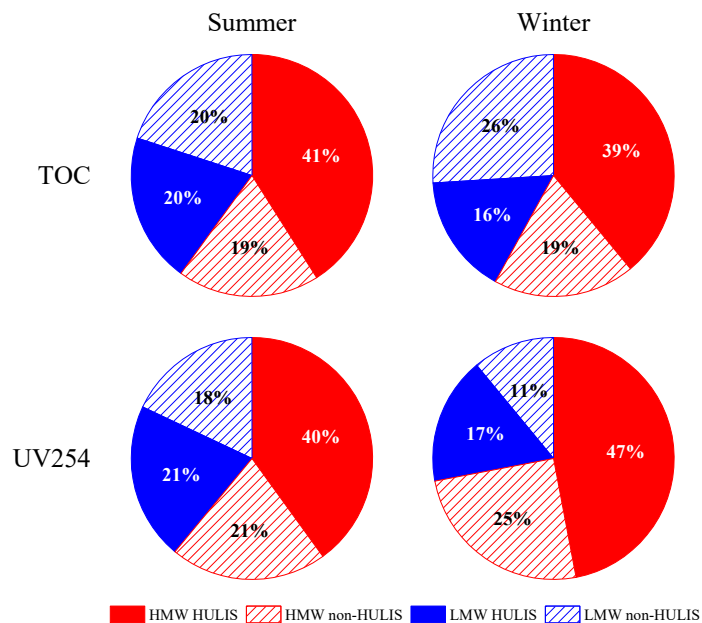
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272 3.1.2. Relative abundances of HMW and LMW HULIS

273 The contribution of MW-HULIS fractions to their corresponding MW-WSOM

274 fractions, quantified in terms of TOC and UV absorption at 254 nm for both summer

275 and winter aerosols are summarized in Table 1. In general, the ratios of

276 HULIS/WSOM of HMW fractions (in terms of TOC and UV254) (65-68%) were

277 higher than the ratios (41-61%) observed for LMW fractions. This finding suggests

278 that the higher presence of hydrophobic and conjugated aromatic structures in HMW

279 WSOM, but more hydrophilic OC and non-aromatic species (e.g., aliphatic

280 dicarboxylic acid) in the LMW WSOM (Fan et al., 2012; Zou et al., 2020).

281 Fig. 2 illustrates the distribution of distinct MW fractions within reconstructed

282 WSOM, wherein "non-HULIS" refers to the content differences between the MW

283 WSOM and its HULIS fractions. The HMW HULIS fraction contributed 39-41% of

284 TOC and 40-47% of UV254 to the bulk WSOM. In contrast, the LWM HULIS

285 fraction only make up a smaller proportion, accounting for 16-20% of TOC and 17-21%

286 of UV254 within the bulk WSOM. Specifically, the ratios between HMW HULIS and

287 LMW HULIS (H/L) ranged from 1.88 to 2.75 for both summer and winter aerosols in

288 terms of either TOC or UV254. These findings emphasize that HMW HULIS

289 significantly dominate the bulk aerosol HULIS fractions. Notably, the H/L ratio for

290 winter aerosols was higher than that for summer aerosols, suggesting that larger-sized

291 HULIS contributed more to the bulk HULIS fractions in winter aerosols.

Summer   Winter

TOC

UV254

■ HMW HULIS   ▨ HMW non-HULIS   ■ LMW HULIS   ▨ LMW non-HULIS

Fig. 2. Relative proportions of different MW fractions in summer and winter aerosols determined by TOC and UV254.

The non-HULIS fractions are also important constituents within aerosol WSOM, but exhibit some differences between HMW and LMW fractions. The contributions of HMW non-HULIS to bulk WSOM were ~19% as determined by TOC and 21-25% measured by UV254. In case of LMW non-HULIS, the contributions were higher in terms of TOC (20-26%) but lower in terms of UV254 (11-18%). These results indicate that the LMW WSOM contain a larger proportion of hydrophilic organic species with weaker light absorption.

3.2. Optical characteristics of MW HULIS fractions

3.2.1. Light absorption characteristics

The absorption spectra of MW HULIS fractions in ambient aerosols are shown in Fig. S3. These spectra exhibit a featureless shape with a general decrease in

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absorbance as the wavelength increases, which is a typical characteristic of HULIS found in rainwater, biomass burning (BB), and ambient aerosols (Huo et al., 2021; Santos et al., 2009; Zhang et al., 2022b). The E_2/E_3 ratio, commonly used as an indicator of the chemical characteristics of organic species, is inversely correlated with higher aromaticity and larger molecular weight (Fan et al., 2021; Li et al., 2022; Sun et al., 2021). As listed in Table 1, the E_2/E_3 of HMW HULIS fractions generally were lower than that of LMW HULIS in both ambient aerosols. This is consistent with the expectation that larger-sized HULIS generally possess more polyconjugated and polymeric structures (Fan et al., 2021; Zhang et al., 2022c), leading to greater aromaticity and larger molecular size.

MAE$_{365}$ and AAE are commonly used to characterize the light absorption capacity and the spectral dependence of light absorption by aerosol chromophores, respectively (Bao et al., 2022; Fan et al., 2016b; Kumar et al., 2017; Yuan et al., 2021; Zou et al., 2020). As listed in Table 1, the average MAE$_{365}$ values of HMW HULIS are 0.23 and 1.06 m^2 g^{-1} in summer and winter aerosol, respectively. These values are higher than the corresponding values of 0.20 and 0.88 m^2 g^{-1}, respectively, for LMW HULIS. In addition, HMW HULIS presented lower AAE values, being 7.59 and 6.25 in summer and winter aerosol, respectively, than the corresponding values of 8.25 and 7.28, respectively, for LMW HULIS (Table 1). These findings are consistent with the observation in our previous study that larger WSOM generally own higher MAE$_{365}$ but smaller AAE values than smaller WSOM (Fan et al., 2021). Generally, the results suggest that HMW HULIS exhibit stronger light-absorbing ability but with light

absorption showing a weaker wavelength dependence. It is worth noting that combustion sources, such as BB and coal combustion, usually emit primary HULIS with high MAE_{365} values due to the enrichment of poly-aromatic and unsaturated species (Cao et al., 2021; Fan et al., 2018; Huo et al., 2021; Zhang et al., 2021), but with small molecular weight distributions (Di Lorenzo et al., 2018; Di Lorenzo et al., 2017; Wong et al., 2017). Furthermore, subsequent pronounced photooxidation and photobleaching processes can lead to a enrichment and/or formation of large sized chromophores (Di Lorenzo et al., 2018; Di Lorenzo et al., 2017; Wong et al., 2017), concurrently resulting in a reduction in their absorption capacity and an enhancement of their spectra dependence on wavelength (Chen et al., 2021b; Fan et al., 2019; Wu et al., 2018; Wu et al., 2020; Zhang et al., 2022a). From this perspective, the SOA formation might induce the generation of HMW HULIS with lower light absorption capacity and weaker light absorption wavelength dependence. In contrast, LMW HULIS is more likely to represent small-sized primary HULIS and/or by-products resulting from the degradation and oxidation of primary large-sized HULIS. Considering the complex sources of ambient HULIS, future studies should explore the MW-dependent light absorption characteristics of HULIS from different sources.

3.2.2. Fluorescence characteristics

The EEM contours of MW HULIS fractions from both summer and winter aerosols are presented in Fig. S4. These HULIS fractions from both seasons exhibit similar EEM spectra features, with a predominance of humic-like fluorophores

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(Ex/Em = 210-235/395-410 nm). This observation suggest that humic-like fluorophores are fundamental constituents of both HMW and LMW HULIS, which are consistent with previous findings for aerosols MW WSOM (Fan et al., 2021) and bulk HULIS in BB-derived and ambient aerosols (Fan et al., 2020; Qin et al., 2018). The findings imply an assembly of small and heterogeneous molecules to form bulk HULIS through weak intramolecular forces (i.e., π–π, van der Waals, hydrophobic, or hydrogen bonds) (Fan et al., 2021; Piccolo, 2002) and/or charge-transfer interactions (Phillips et al., 2017; Qin et al., 2022). In this study, the fluorescence regional integration (FRI) method was applied to characterize the fluorescent composition of MW HULIS. Using FRI, EEM spectra were divided into five fluorescence regions (labeled as I to V) (Fig. S4), which were successively assigned to simple aromatic proteins (I and II), fulvic acid-like (III), soluble microbial byproduct-like (IV), and humic acid-like (V) substances, respectively, as established in previous studies (Chen et al., 2003; Qin et al., 2018; Wang et al., 2021b). As shown in Fig. S5, the large-size aromatic proteins (II) and fulvic acid-like substances (III) dominated the fluorophores within MW HULIS in both summer and winter aerosols, comprising approximately 62-64% of the total fluorescence intensity. This finding is consistent with previous reports on bulk HULIS in summer and winter aerosols from industrial and urban cities (Qin et al., 2018; Wang et al., 2021b). In comparison, the HMW HULIS in both summer and winter aerosols generally exhibited a higher proportion of humic acid-like substances (V), while having a lower abundance of small-size aromatic proteins I compared to LMW HULIS. These differences are particularly pronounced

in winter aerosols, with the humic acid-like substances accounting for 23% in HMW

HULIS compared to 13% in LMW HULIS, and small-size aromatic proteins I

comprising 9% in HMW HULIS compared to 17% in LMW HULIS (Fig. S5).

Furthermore, the higher HIX values of HMW HULIS (5.64) in comparison to LMW

HULIS (1.94) further support these differences (Table 1). The pronounced BB

emissions and potential NO_2-related oxidation of OA, as evidenced by the presence of

more hotspots (Fig. S1) and higher concentration of NO_2 (Table S1), are likely driving

these marked distinctions between HMW and LMW HULIS in winter aerosols. In

general, these findings imply that the HMW HULIS have a stronger level of

humification and oxidation, while the LMW HULIS appear to be of a simpler nature

and are more likely associated with fresh emissions (e.g., BB).

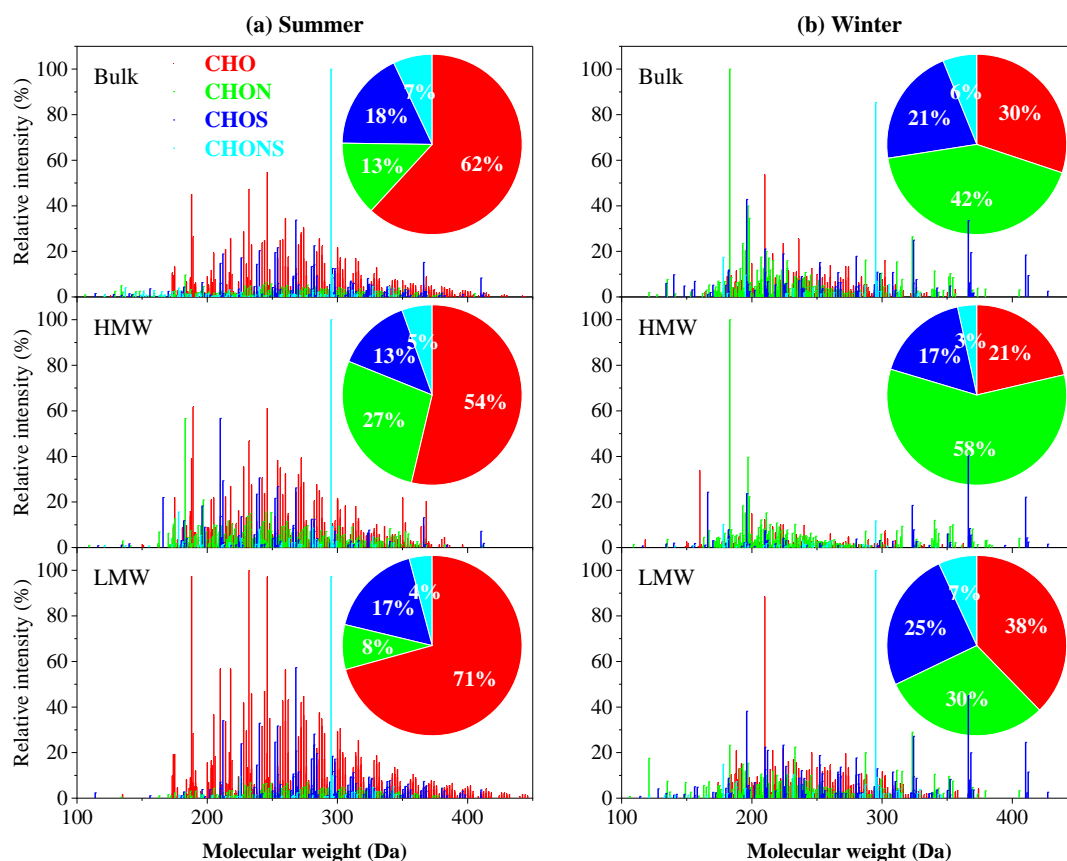3.3 Molecular-level insights into MW HULIS

3.3.1. Seasonal variations in the molecular composition of MW HULIS

The molecular-level characteristics of MW HULIS were examined using

negative ESI- HRMS analysis. It is worth noting that the ESI- HRMS could reveal

molecular composition of a subset of organic molecules that are biased ionized in the

negative ESI source rather than representing the entire HULIS composition (He et al.,

2023; Lin et al., 2012). Fig. 3 displays the reconstructed mass spectra of all HULIS

fraction in both summer and winter aerosols. Hundreds of peaks can be observed in

the spectra ranging from m/z 100 to 450 for all samples, with most ions being

abundant within the m/z 150-350 range. These spectrum characteristics are similar to

396   those previously reported for HULIS in ambient aerosols and BB emissions (He et al.,

397   2023; Song et al., 2022; Sun et al., 2021; Wang et al., 2019; Zhang et al., 2021; Zou et

398   al., 2023).

399

Fig. 3. Mass spectra of bulk and MW HULIS in (a) summer and (b) winter aerosols.
The pie charts represent the intensity distributions of four compound categories (CHO,
CHON, CHOS, and CHONS).

403

404   As listed in Table 2, the number of assigned formulas within MW HULIS in

405   summer aerosols were 655-672, which was higher than the range of 470-506 observed

406   in winter aerosols. This suggests that the MW HULIS in summer aerosols exhibited

407   greater diversity than those in winter aerosols, mainly due to the stronger SOA

408   formation that enhanced the heterogeneity of HULIS fractions in the summer. The

409 identified formulas were then classified into four groups (i.e., CHO, CHON, CHOS

410 and CHONS) according to their elemental composition. As depicted in pie charts in

411 Fig. 3, summer HULISs are predominantly composed of CHO (54-71%), while winter

412 HULISs feature a high concentration of both CHON (30-58%) and CHO (21-38%).

413 The notably higher content of CHO in summer HULISs are likely due to a wide

414 distribution of biogenic VOC-derived SOAs during the summer season (Li et al., 2022;

415 Sun et al., 2023). CHON content in winter HULISs is generally higher than in

416 summer ones, potentially due to more significant contributions from direct BB, as

417 well as secondary nitrogen-related chemical processes during the winter season (He et

418 al., 2023; Song et al., 2022; Zhang et al., 2021; Zou et al., 2023). This finding is

419 supported by the greater number of fire spots (Fig. S1) and higher concentrations of

420 NO_2 (Table S1) during winter. The higher proportions of CHON compounds in

421 aerosol HULIS typically lead to enhanced light absorption capabilities (He et al., 2023;

422 Song et al., 2022; Zeng et al., 2021). This provides a potential explanation for the

423 higher MAE_{365} values observed in winter HULIS compared to summer HULIS.

424 Additionally, CHOS is more abundant in winter HULISs (17-25%) than in summer

425 aerosols (13-18%). Previous studies have demonstrated that both coal combustion and

426 the oxidation initiated by SO_2 can lead to the generation of larger amounts of

427 S-containing compounds (Song et al., 2018; Song et al., 2022; Zou et al., 2023). This

428 finding suggested that the increased levels of coal combustion and SO_2-related SOAs,

429 as evidenced by higher concentration of SO_2 (Table S1), are significant contributors to

430 the presence of BrC in winter compared to in summer.

431 **Table 2.** The average values of intensity-weighted molecular weights (MW), elemental ratios, double bond equivalents (DBE), modified

432 aromaticity index (AI_{mod}) and carbon oxidation state (OS_C) for tentatively identified compounds of the bulk and MW HULIS samples.

		Elemental compositions	Number of formulas	MW_w	H/C_w	O/C_w	N/C_w	S/C_w	O/N_w	O/S_w	OM/OC_w	DBE_w	DBE/C_w	$DBE\text{-}O_w$	$AI_{mod,w}$	$OS_{C,w}$
Summer	BULK	CHO	376	276	1.39	0.59					1.91	4.69	0.39	-2.20	0.15	-0.21
		CHON	270	260	1.35	0.60	0.15		5.52		2.10	4.99	0.50	-1.17	0.27	-0.14
		CHOS	133	278	1.74	0.71		0.11		6.61	2.38	2.21	0.24	-4.41	0.02	-0.33
		CHONS	81	265	1.69	0.59	0.18	0.14	4.98	5.20	2.49	3.38	0.36	-1.95	0.09	-0.52
		Total	860	273	1.47	0.61	0.03	0.03	1.09	1.54	2.06	4.20	0.38	-2.44	0.14	-0.24
	HMW	CHO	270	264	1.41	0.55					1.85	4.51	0.38	-1.70	0.16	-0.32
		CHON	264	248	1.42	0.61	0.14		5.23		2.09	4.44	0.47	-1.33	0.24	-0.20
		CHOS	72	247	1.82	0.75		0.14		5.83	2.53	1.81	0.22	-4.07	0.01	-0.32
		CHONS	39	269	1.55	0.64	0.19	0.15	4.87	5.16	2.60	3.82	0.44	-1.65	0.14	-0.28
		Total	645	258	1.48	0.60	0.05	0.03	1.70	1.07	2.05	4.09	0.39	-1.91	0.16	-0.29
	LMW	CHO	365	275	1.34	0.64					1.97	4.82	0.42	-2.47	0.16	-0.05
		CHON	155	272	1.39	0.66	0.11		6.60		2.12	4.80	0.45	-2.09	0.18	-0.08
		CHOS	120	284	1.71	0.74		0.11		7.05	2.43	2.51	0.25	-4.58	0.02	-0.22
		CHONS	32	322	1.71	0.64	0.11	0.11	6.59	6.63	2.42	3.32	0.30	-3.41	0.06	-0.42
		Total	672	278	1.42	0.66	0.01	0.02	0.80	1.48	2.08	4.36	0.39	-2.84	0.13	-0.10
Winter	BULK	CHO	142	247	1.23	0.48					1.75	5.35	0.47	-0.05	0.32	-0.26
		CHON	194	231	1.24	0.53	0.16		3.96		1.99	5.17	0.57	0.52	0.48	-0.18
		CHOS	79	271	1.93	0.51		0.11		4.97	2.14	1.21	0.14	-3.80	0.04	-0.91
		CHONS	20	271	1.61	0.69	0.16	0.13	5.60	5.90	2.60	3.32	0.40	-2.65	0.09	-0.24
		Total	435	247	1.41	0.52	0.08	0.03	2.02	1.42	1.99	4.27	0.44	-0.77	0.31	-0.37
	HMW	CHO	138	232	1.36	0.47					1.74	4.79	0.41	0.02	0.28	-0.42

23

	CHON	244	232	1.29	0.53	0.16		3.89		2.01	4.87	0.55	0.23	0.44	-0.23
	CHOS	59	292	2.08	0.40		0.12		4.40	2.04	0.46	0.06	-4.06	0.02	-1.27
	CHONS	29	236	1.74	0.48	0.22	0.20	2.82	3.02	2.57	2.88	0.38	-0.57	0.27	-0.77
	Total	470	242	1.46	0.50	0.10	0.03	2.37	0.85	1.98	4.04	0.43	-0.57	0.33	-0.46
LMW	CHO	176	249	1.23	0.54					1.82	5.25	0.48	-0.65	0.30	-0.15
	CHON	195	239	1.34	0.49	0.16		3.99		1.95	4.88	0.52	0.29	0.45	-0.36
	CHOS	107	280	1.94	0.54		0.10		5.42	2.17	1.20	0.13	-4.25	0.02	-0.85
	CHONS	28	272	1.67	0.69	0.16	0.14	5.89	6.03	2.63	2.99	0.37	-3.15	0.13	-0.29
	Total	506	256	1.47	0.54	0.06	0.04	1.61	1.78	2.01	3.96	0.39	-1.45	0.26	-0.40

433   Table 2 summarizes the intensity-weighted molecular parameters for MW

434   HULIS in both summer and winter aerosols. Evidently, the MWw of summer HULISs

435   are 258-278, which are higher than the corresponding values of 242-256 for winter

436   HULISs. It is noted that these values are considerably smaller than those revealed by

437   HPSEC analysis. This difference could be explained by several factors: (1) ESI-

438   HRMS is based towards relatively small molecules that easily protonated in negative

439   ESI mode (He et al., 2023; Lin et al., 2012); (2) SEC can provide an apparent rather

440   than accurate molecular size of molecules due to the lack of appropriate standards for

441   column calibration (Fan et al., 2023; Wong et al., 2017), and (3) the potential

442   disassembly of larger molecules stabilized by weak forces during electrospray

443   ionization of HRMS (Fan et al., 2021; Phillips et al., 2017). Nevertheless, both HRMS

444   and HPSEC indicate that summer HULISs exhibit larger sizes than winter HULISs.

445   Moreover, summer HULISs exhibit higher O/Cw ranging from 0.60 to 0.66, as well as

446    $OS_{C,w}$ ranging from -0.29 to -0.10, which exceed the respective values of 0.50 to 0.54

447   and -0.46 to -0.37 observed in winter HULISs. Conversely, winter HULISs display

448   higher $AI_{mod,w}$ values (0.26-0.33) than those (0.13-0.16) for summer ones. These

449   findings suggest that summer HULISs are characterized by a high degree of oxidation,

450   while winter HULISs exhibit stronger aromaticity.

451

452   3.3.2. Comparison on molecular composition of HMW and LMW HULIS

453   **CHO compounds.** The CHO compounds are prominent constituents within

454   HULIS fractions, accounting for 54% and 21% in summer and winter HMW HULIS,

respectively, whereas these proportions increase to 71% and 38% in LMW HULIS

(Fig. 3). It is worth noting that CHO compounds that undergo deprotonation in ESI-

mode are likely associated with the presence of carboxyl, carbonyl, alcohol and ester

(Lin et al., 2012; Wang et al., 2018). Moreover, CHO compounds in LMW HULIS

exhibit a higher oxygenation level compared to HMW HULIS, as evidenced by the

higher O/C_w and $OS_{C,w}$ values. As shown in Table 2, the O/C_w for CHO in LMW

HULIS are 0.55-0.64, which are higher than 0.47-0.54 observed in HMW HULIS. In

contrast, the H/C_w for CHO in HMW HULIS were consistently higher than those in

LMW HULIS, with values of 1.41 vs. 1.34 in summer and 1.36 vs. 1.23 in winter

(Table 2). This disparity strongly suggests a higher saturation level of CHO

compounds within HMW HULIS. This conclusion is further corroborated by the

lower DBE_w and $AI_{mod,w}$ observed for CHO in HMW HULIS compared to LMW

HULIS (Table 2). It is known that these values serve as estimations of C=C density

and aromatic and condensed aromatic structures (Song et al., 2022; Zhang et al.,

2021). Taken together, the CHO compounds within HMW HULIS exhibit a more

aliphatic nature but lower aromaticity and oxidation levels when compared to those

within LMW HULIS.

CHON compounds. HMW HULIS fractions consist of a higher proportion of

CHON compounds compared to LMW HULIS, with proportions of 27% vs. 8% in

summer and 58% vs. 30% in winter (Fig. 3). This observation suggests that HMW

HULIS contain a higher content of N-containing components. It is noted that the

LMW HULIS are generally characterized by higher O/N_w values of 6.60 in summer

477 and 3.99 in winter compared to 5.23 in summer and 3.89 in winter for HMW HULIS.

478 This indicates that the CHON compounds within LMW HULIS are more highly

479 oxidized than those within HMW HULIS. In general, compounds with O/N $\geq$ 3 are

480 indicative of oxidized N groups such as nitro ($-NO_2$) or nitrooxy ($-ONO_2$), while

481 compounds with O/N < 3 may denote the reduced N-containing functional groups (i.e.,

482 amines) (He et al., 2023; Song et al., 2022; Zeng et al., 2021). In this study, a majority

483 of the CHON compounds, comprising 73-85% in summer and 59-64% in winter,

484 exhibited O/N $\geq$ 3 in both MW HULIS fractions. This suggests that high

485 concentrations of nitro compounds or organonitrates dominate the CHON compounds

486 (Sun et al., 2023; Wang et al., 2018; Zeng et al., 2021), especially in summer samples,

487 primarily due to the hydroxyl radical oxidation of biogenic or anthropogenic VOC

488 precursors, as well as BB emissions (Song et al., 2022; Sun et al., 2021; Zhang et al.,

489 2021; Zou et al., 2023). Furthermore, the CHON compounds exhibiting O/N $\geq$ 3 were

490 more abundant in LMW HULIS compared to HMW HULIS, accounting for 85% vs.

491 73% in summer and 64% vs. 59% in winter. In contrast, HMW HULIS contained

492 more CHON compounds with O/N < 3 compared to LMW HULIS. These findings

493 collectively indicate that the CHON within HMW HULIS possess lower content of

494 nitro compounds or organonitrates than LMW HULIS. Based on FTIR analysis (Text

495 S2 and Fig. S6 in SI), it is known that HMW HULIS contain more carboxylic groups

496 than LMW HULIS, which indicate a higher likelihood of HMW HULIS containing

497 more amino acids.

498   **CHOS and CHONS compounds.** In this study, we observed that CHOS

accounted for proportions of 13% to 25% in all MW HULIS fractions, while CHONS

had a lower proportion of 3% to 7% (Fig. 3). Notably, the distribution of CHOS

differed between HMW and LMW HULIS in both season samples. As depicted in Fig.

3, HMW HULIS contained fewer CHOS compounds compared to LMW HULIS, with

proportions of 13% vs. 17% in summer and 17% vs. 25% in winter. This finding

suggests that a greater number of CHOS compounds are incorporated into the LMW

HULIS fractions, which potentially leading to a reduction in the light absorption of

LMW HULIS (Zeng et al., 2021; Zhang et al., 2021). Furthermore, as indicated in

Table 2, both the CHOS and CHONS within LMW HULIS exhibited higher O/Sw

values than HMW HULIS in both seasonal samples. Consequently, the S-containing

compounds within LMW HULIS were characterized by a higher degree of oxidation,

primarily attributed to SO_2-related chemical oxidation process, in comparison to those

in HMW HULIS. Moreover, it was observed that 61% to 92% of CHOS compounds

exhibited O/S > 4, and 3% to 43% of CHONS compounds with O/S > 7 for all MW

HULIS fractions. Among them, HMW HULIS own lower proportions of CHOS with

O/S > 4 and CHONS with O/S > 7 than LMW HULIS, suggesting a reduced presence

of potential organosulfates and nitrooxyorganosulfates within HMW HULIS (Sun et

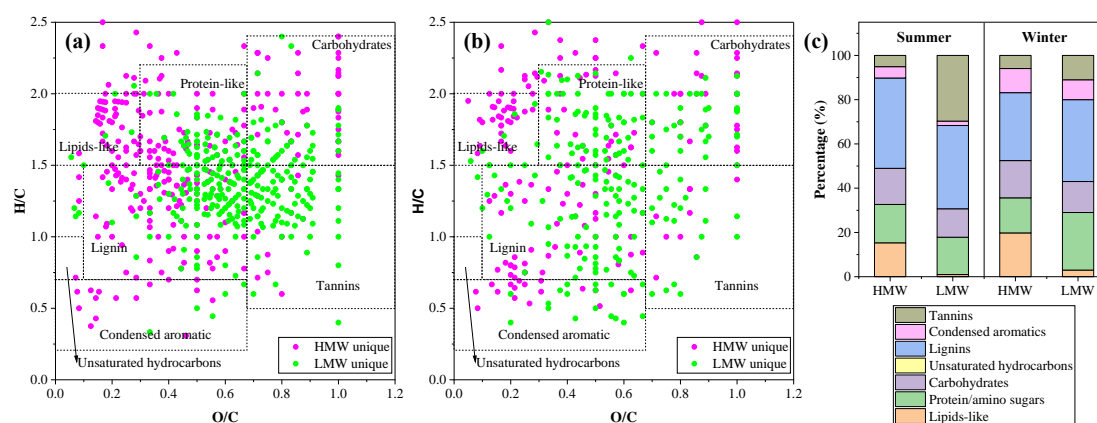al., 2023; Wang et al., 2018; Zeng et al., 2021; Zou et al., 2023).

3.3.3. Comparative analysis of unique molecular formulas in HMW and LMW

HULIS

In this study, particular emphasis was placed on the unique molecular formulas

within the HMW or LMW HULIS fractions. Fig. 4a, b illustrates the Van Krevelen

(VK) diagram depicting the distribution of unique molecular formulas within HMW

and LMW HULIS in summer and winter samples. It is evident that a majority of

unique formulas within LMW HULIS are concentrated around the origin with O/C >

0.5, accounting for 83% in summer and 64% in winter. In contrast, most formulas

within HMW HULIS exhibited O/C < 0.5, representing about 58% for both seasonal

samples. These findings indicate that the unique molecules within LMW HULIS

consist of more polar O-containing organic compounds than those within HMW

HULIS.

Fig. 4. Van Krevelen diagrams for the unique molecular formulas within HMW and LMW HULIS from (a) summer and (b) winter aerosols. (c) The contributions of major substances classes in unique formulas.

The molecular formulas are further categorized into seven groups based on

previous studies, including lignin-like species, protein/amino sugars, condensed

aromatics, tannin-like species, carbohydrate-like species, unsaturated hydrocarbons,

and lipid-like species (He et al., 2023; Sun et al., 2021; Sun et al., 2023). The

classification rules for these formulas can be found in Table S2. Fig. 4c provides an

overview of the relative contributions of the number of unique formulas from each of

the seven groups for HMW and LMW HULIS. The results indicate that the dominant

substance class in the unique formulas within both MW HULIS are lignin-like species,

accounting for proportions of 31-40%. This finding indicates that lignin derivatives

are fundamental components in both HMW and LMW HULIS either in summer or

winter aerosols. Additionally, there are notable differences in the molecular

characteristics of lignin-like species within HMW and LMW HULIS. As listed in

Table S3, lignin-like species within HMW HULIS exhibit lower MWw and O/Cw, but

higher N/Cw and AImod,w values than those within LMW HULIS in both seasonal

samples. These observations suggest that the unique lignin-like substances in HMW

HULIS likely contain more N-enriched and highly aromatic species, while those in

LMW HULIS tend to concentrate more aliphatic O-containing compounds. These

distinctions in composition and characteristics between HMW and LMW HULIS

fractions provide valuable insights into their origins and transformations in the

atmosphere.

Moreover, there are notable variations in the contributions of lipids-like,

protein/amino sugars, carbohydrates, condensed aromatics, and tannins species

between HMW and LMW HULIS. In general, HMW HULIS have a higher proportion

of lipids-like species, carbohydrates and condensed aromatics than LMW HULIS in

both summer and winter aerosols. Among these, the most remarkable difference in

composition between HMW HULIS and LMW HULIS is seen in lipids-like species,

562 accounting for 15% versus 1% in summer and 20% versus 3% in winter (Fig. 4). As

563 reported in previous studies, lipids-like species primarily originate from biogenic

564 emissions (He et al., 2023; Li et al., 2022; Sun et al., 2021). This suggests that there is

565 a stronger contribution from biogenic emissions to HMW HULIS. Additionally, these

566 species in HMW HULIS were usually characterized by lower DBE_w and slightly

567 lower $OS_{C,w}$ when compared to LMW HULIS (Table S3), indicating they present

568 stronger saturation and fewer oxidized substituents. On the other hand, tannins species

569 contribute a higher proportion to LMW HULIS, constituting 30% in summer and 11%

570 in winter, while comprising only 5%-6% in HMW HULIS in both season aerosols.

571 Tannin-like species are known to consist of various polyphenolic groups containing

572 hydroxyl and carboxylic functional groups (He et al., 2023; Li et al., 2022; Ning et al.,

573 2019; Sun et al., 2021). The slightly lower DBE_w but much higher $DBE\text{-}O_w$ for

574 unique tannin-like species within HMW HULIS were observed compared to LMW

575 HULIS (Table S3), suggesting that the former ones are enriched in more unsaturated

576 O-containing functional groups, particularly carboxylic functional groups.

577

578 3.4. Atmospheric implications

579   This study provides comprehensive comparison between HMW and LMW

580 HULIS regarding their distributions, chemical structures, molecular sizes and

581 compositions. HMW HULIS appear to be larger than LMW HULIS, as evidenced by

582 both ultrafiltration natures and the MW distributions of chromophores analyzed by

583 HPSEC. However, HRMS analysis revealed that the average MWw of identified

584 formulas within HMW HULIS were lower than those of LMW HULIS (Table 2). This

585 discrepancy can likely be attributed to the "assembled structures" that construct the

586 aerosol HULIS, as suggested in many previous studies focusing on HULIS and BrC

587 characterization (Fan et al., 2021; Fan et al., 2023; Phillips et al., 2017; Qin et al.,

588 2022). In fact, the results from EEM-FRI and FTIR analysis support the notion that

589 HMW and LMW HULIS likely consist of potential structures assembled by similar

590 basic fluorophores and functional groups. Based on this theory, HMW HULIS may

591 consist of macromolecular species primarily assembled from small molecules through

592 weak forces (i.e., π–π, van der Waals, hydrophobic, or hydrogen bonds) and/or

593 charge-transfer interactions (Fan et al., 2021; Phillips et al., 2017), which can

594 potentially disassemble during ESI ionization and form low MW molecules.

595 Based on the molecular-level characterization, significant distinctions in

596 properties between HMW HULIS and LMW HULIS become evident. HMW HULIS

597 generally exhibit stronger aromaticity but lower oxidation degree when compared to

598 LMW HULIS. In terms of molecular composition, HMW HULIS contain higher

599 quantities of CHON species but lower quantities of CHO compounds than LMW

600 HULIS. Furthermore, more lipids-like species were identified as unique molecules in

601 HMW HULIS, while more tannin-like species with abundant carboxylic groups were

602 observed as unique molecules in LMW HULIS. Given these pronounced differences

603 between HMW and LMW HULIS, it can be speculated that the higher levels of

604 aromatic structures, greater presence of CHON molecules and the presence of

605 lipids-like species may serve as driving factors in the formation of potential

assembled structures in HMW HULIS. Additionally, it is well-established that CHON can enhance the light absorption of organic aerosols (OA), while CHO species may have the opposite effect, weakening light absorption (He et al., 2023; Song et al., 2022; Wang et al., 2019; Zeng et al., 2021). Therefore, it is reasonable to conclude that HMW HULIS possess stronger light absorbing capability, which is consistent with their larger MAE_{365} values.

Importantly, HMW HULIS contain higher amounts of carboxylic functional groups, reduced nitrogen species (e.g., amines) and aromatic species than LMW HULIS. These functional groups have strong complexation abilities with transition metals (Wang et al., 2021a; Wang et al., 2021b), thus influencing the transformation and chemical behavior of metals. Moreover, the OA-metals complex can potentially enhance the catalytic generation of reactive oxygen species (ROS) in organic aerosols (Win et al., 2018; Zhang et al., 2022a), thereby playing significant roles in adverse health effects of OA. These results reinforce the significance of HMW HULIS in light absorption, metal complexation, and the potential ROS generation ability of aerosol BrC.

4. Conclusions

This study successfully isolated and characterized HMW and LMW HULIS in atmospheric aerosols using the UF-SPE technique, yielding insights into their distribution, optical properties and molecular-level characteristics. Both HMW and LMW HULIS exhibited a continuum of MW distributions ranging from 100 to 20,000

Da. However, HMW HULIS displayed more extensive and intricate MW distributions,

suggesting differences in their sources and formation processes compared to LMW

HULIS. In general, HMW HULIS constituted a higher percentage of TOC and UV254

in aerosols compared to LMW HULIS, indicating the prevalence of hydrophobic and

conjugated aromatic structures in the former. Moreover, HMW HULIS exhibited

higher aromaticity, stronger light absorption abilities, weaker spectra dependence, and

stronger humification and conjugation, compared to LMW HULIS. Interestingly,

HRMS analysis revealed slightly lower MWw values for HMW HULIS than LMW

HULIS, which contradicted the HPSEC results and the nature of UF fractionation.

This finding strongly suggests the possibility of small molecules assembling to form

macromolecules in HMW HULIS. Regarding molecular composition, HMW HULIS

contained a higher proportion of CHON compounds, constituting 27% vs. 8% in

summer and 58% vs. 30% in winter, while LMW HULIS were primarily composed of

CHO compounds, accounting for 71% vs. 54%% in summer and 38% vs. 21% in

winter. Both HMW and LMW HULIS featured lignin-like substances as major unique

molecular formulas, but HMW HULIS exhibited more N-enriched and highly

aromatic species, whereas LMW HULIS contained a higher proportion of polar

O-containing functional groups. Additionally, HMW HULIS included a greater

number of unique lipids-like compounds, while LMW HULIS tend to concentrate

more tannin-like compounds. These observations shed light on the complex nature of

MW HULIS, and their diverse sources and transformations. Future research should

expand the geographical and seasonal coverage to gain a more comprehensive

understanding of the molecular-level characteristics of MW HULIS in various

atmospheric environments. Furthermore, exploring additional physicochemical

properties of MW HULIS will provide valuable insights into their potential health and

environmental implications. Overall, this study offers valuable insights into the

molecular-level characteristics of aerosol HULIS, enhancing our understanding of

their evolution, sources and potential environmental effects.

Author contribution

Xingjun Fan: Methodology, Supervision, Funding acquisition, Writing-review & editing. **Ao Cheng:** Sampling, Data curation. **Xufang Yu:** Writing-review & editing. **Tao Cao:** Sampling, Investigation. **Dan Chen:** Investigation, Data curation. **Wenchao Ji:** Formal analysis. **Yongbing Cai:** Writing-review & editing. **Fande Meng:** Writing-review & editing. **Jianzhong Song:** Methodology, Writing-review & editing. **Pingan Peng:** Writing-review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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35

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673

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