An organoleptic survey of meads made with lactic acid-producing yeasts

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Abstract

We previously reported the isolation a suite of wild lactic acid-producing yeasts (LAYs) that enable "primary souring" during beer fermentation without the use of lactic acid bacteria. With sour meads gaining popularity in modern mead making, we were interested in exploring the same primary souring approach to traditional semi-sweet meads. In this study, we utilized 13 LAY strains to produce semi-sweet meads using a standardized batch of honey must to ensure consistent starting conditions. Thirteen 11-L batches of mead were prepared, and each was inoculated with one of the LAY strains, along with two control batches inoculated with champagne yeast. The initial pH and specific gravity were measured for each batch before inoculation. Traditional organic staggered nutrient addition was utilized for the first 72 h of fermentation with specific gravities being taken throughout the mead making process. Meads were racked, tasted, stabilized, cold crashed, bottled, and transported to the American Mead Maker's Association 2018 Conference in Broomfield, Colorado. There, organoleptic surveys were conducted on these meads utilizing an array of tasters with varying levels of mead sensory analysis experience. The results of the sensory analysis, focusing on aroma and flavor, are discussed.

Keywords: mead, yeast, fermentation, lactic acid yeast, sour mead, modern mead making

Abbreviations: LAY, lactic acid-producing yeast; ABV, alcohol by volume; OG, original gravity; FG, final gravity; SG, specific gravity; AMMA, American Mead Makers Association; TOSNA, traditional organic staggered nutrient addition

1. Introduction

Mead, and its origins, are the focus of many legends found deep within the lore of a variety of civilizations throughout the world. Mead is arguably the oldest alcoholic beverage known to humankind, preceding beer but perhaps not fruit-based wines, with mead evidence found in pottery dating to 6500-7000 BC in China (1) and in the Hindu texts of the Rigveda predating 1700 BC (2). The fact that both mead and fruit wines contain fewer ingredients and are easier to produce than beer, which requires the malting of grains to convert complex carbohydrates into readily fermentable sugars (3), also argues for the mead/wine before beer supposition.

The origin of mead is likely serendipitous as described by the Neolithic hypothesis (4): Honeycomb fell into a container of rain water (or rain water fell into a container of honey), wild yeasts initiated fermentation at mild ambient temperatures, and then a curious soul wandered by at the right time, tasted the elixir, and being ignorant of fermentation and alcohol, soon succumbed to the wiles of intoxication. No doubt a quest to replicate that magical elixir ensued, but it was not until the French chemist Louis Pasteur provided experimental evidence that yeast fermented sugars into ethanol in 1856 that alcoholic fermentation became more studied and understood (4-6). Until then, mead in its various forms became known for possessing sacred, mystical, and even healing properties. Receiving legendary notoriety as “ambrosia, or nectar, of the Gods” in ancient Greece, mead has notoriety as being the mythological source of the Norse god Odin’s strength when, as an infant, he drank mead from a goat’s udder (7).

Today, the fascination with mead and its mystical charm continues. Mead production is fast evolving with modern mead-making techniques riding on the coattails of craft brewing and distilling, rendering it easier to make and reducing fermentation times (8,9). More akin to wine making than beer brewing, mead has tapped into the creative minds of home and commercial mead makers alike (10). By replacing the grapes and grape juice typically found in wines with various honey varietals and adding

75 yeast, water, and nutrients, traditional meads are born. Meads can vary from being light-bodied and low
76 in alcohol to being sweeter, full bodied, and containing a higher alcohol by volume (ABV). They also vary
77 in sweetness from being dry, semi-sweet, or sweet, which is not dissimilar from typical wine
78 characteristics. Yeast choice has certain organoleptic influences on mead, impacting mouthfeel, aromas,
79 and flavors (11). A plethora of adjunct additions (*e.g.*, fruits and spices) and wood aging techniques may
80 also be used to make meads more complex. However, once again like wine, achieving the delicate
81 balance between sweetness, alcohol, tannins, and acidity is the key to making an exceptional mead.

82 The Beer Judge Certification Program (BJCP) guide on mead styles (12) provides insight into how
83 much creative license craft mead making provides. The Experimental Mead category provides the
84 creative mead maker with almost endless options for barrel aging, fortification, and experimenting with
85 microbes. It was with this creative inspiration that we sought to use heterofermentative lactic acid-
86 producing yeast (LAY) to make meads and perhaps develop a novel Sour Mead category in the BJCP
87 Mead Styles. As documented in the 2017 American Mead Makers Association (AMMA) Mead Industry
88 Report, sour meads currently account for 13.2% of the mead styles being commercially produced (9).
89 Current souring techniques for both beer and mead require either rapid kettle souring or slow
90 techniques using mixed culture (lactic acid bacteria and yeast) fermentation (13), both presenting risk of
91 infection across batches. Recently, the use of LAY has been suggested as an alternative to these
92 techniques (14), being as rapid as kettle souring but reducing the risk for contamination of other batches
93 because LAY are susceptible to traditional sanitation procedures. Here, we used a collection of 13 LAY
94 strains to produce sour meads and assessed their perceived organoleptic properties by surveying a
95 panel of mead sensory analysts at the 2018 AMMA Conference. The detailed fermentation and sensory
96 results are presented below, and we conclude that LAY strains expand the options available for mead
97 makers in producing sour meads.

98 **2. Methods and materials**

99 *2.1. Strains, media, and culture other reagents*

100 *S. cerevisiae* strain WLP715 (champagne yeast (15)) was purchased from White Labs (San Diego,
101 CA). The LAY strains were provided by Wild Pitch Yeast (Bloomington, IN) and are described in (14). All
102 yeast strains were routinely grown on yeast extract, peptone, and dextrose (YPD; 1% (w/v) yeast extract,
103 2% (w/v) peptone, and 2% (w/v) glucose) plates containing 2% (w/v) agar at 30°C and in YPD liquid
104 culture at 30°C with aeration unless otherwise noted. All strains were stored as 15% (v/v) glycerol stocks
105 at -80°C. Media components were from Fisher Scientific (Pittsburgh, PA, USA) and DOT Scientific
106 (Burnton, MI, USA). All other reagents were of the highest grade commercially available.

107 *2.2. Mead fermentation*

108 Fifteen 11-L batches of traditional mead must, with an original gravity (OG) of 1.112 and initial
109 pH of 4.13, were prepared using true source clover honey (Dutch Gold, Lancaster, PA) and spring water.
110 Each was inoculated with approximately 1.85 x 10^{10} cell of one of the 13 LAY strain or the WLP715
111 control and degassed using a degassing wand attached to a standard electric drill. To assist with
112 clarification, 22 g (1-3g/L) of dry Albumex Perl Bentonite (Max F. Keller GmbH, Mannaheim, Germany)
113 was added to each batch 12 h before inoculation. Traditional organic staggered nutrient addition
114 (TOSNA) (16) using a FermaidO (Lallemand Oenology, Canada) regimen and twice daily degassing were
115 utilized to provide the yeast strains with sufficient and consistent nutrients, as well as oxygenation
116 during the first 72 h of fermentation. The ambient and mead fermentation temperatures ranged from
117 17-20°C.

118 The meads were racked off the yeast 3 weeks after inoculation, and the specific gravity (SG) for
119 each batch was measured using a hydrometer. At 6 weeks after inoculation, each batch was racked for a
120 second time, and the SG was again recorded. Tastings were performed intermittently throughout the
121 process. In the ninth week, a 6-g (2 g/3.7-L) potassium sorbate (LD Carlson Co., Kent, OH) 0.5-g (1g/7.4-

L) potassium metabisulfite (LD Carlson Co., Kent, OH) combination was added to stabilize the meads. Fermfast DualFine Super Kleer (LD Carlson Co., Kent, OH) was added as a fining agent, and the meads were cold-crashed by being incubated at an ambient temperature of -2.2 to 3°C for 36 h before bottling. All mead making equipment was thoroughly cleaned and sanitized with Star San (Five Star Chemicals and Supply, Inc., Commerce City, CO.) prior to each step of the mead making process. The bottles were transported under refrigeration to the 2018 AMMA Conference in Broomfield, CO, to conduct tasting and sensory evaluations (below).

2.3. Sensory analysis

The goal of this experiment was to analyze the mead sensory characteristics produced by the 13 LAY strains utilizing a wide variety of taster sensory analysis experience levels, as well as variation in palate sophistication. The benefit of this, rather than only using expert sensory analysts, was to gather feedback from a population that would mimic the full range of craft mead consumers, from individuals tasting their first mead to long-term enthusiasts. After a brief verbal introduction to the LAY sour mead project, sensory analysis feedback sheets (see Supplemental Materials for examples) were provided to tasters for all 13 batches of experimental mead. Tasters were asked to provide their feedback the flavor, aroma, and mouthfeel, of each mead. A total of 490 sensory analysis feedback sheets were completed and gathered during the mass tasting session held at the 2018 AMMA Conference.

2.4. Statistical analysis

All sensory analysis data from the feedback sheets were entered into Microsoft Excel spreadsheets. Statistics were determined using Microsoft Excel and/or GraphPad Prism.

3. Results

144 *3.1. Fermentation performance*

145 To compare the fermentative abilities of all 13 LAY strains to the WLP715 control, 170 L of honey
146 must was prepared using clover honey and spring water, and 11 L was added to each of 15 individual
147 fermenters. These vessels were then each inoculated with a different LAY or the control strain (x2) and
148 allowed to ferment at ambient temperature (17-20°C). Samples of each batch were collected several
149 times during fermentation to measure SG and collect tasting notes (Supplemental Table S1). Table 1
150 shows the specific gravity, attenuation, ABV, and pH information for the 15 meads, including the two
151 control batches (C1 and C2) at the end of fermentation.

152 **Table 1.** Fermentation parameters.

Strain	Species	OG[a]	FG	Attenuation (%)	ABV (%)	Initial pH	Terminal pH
WYP39	*L. fermentati*	1.112	1.068	37	5.8	4.12	3.24
YH25	*L. fermentati*	1.112	1.062	42	6.56	4.12	3.33
YH26	*L. thermotolerans*	1.112	1.070	35	5.6	4.12	3.13
YH27	*L. thermotolerans*	1.112	1.034	68	10.2	4.12	3.15
YH72	*H. vineae*	1.112	1.080	27	4.2	4.12	3.07
YH73	*L. thermotolerans*	1.112	1.072	34	5.3	4.12	3.12
YH77	*L. fermentati*	1.112	1.066	39	6.0	4.12	3.35
YH79	*L. thermotolerans*	1.112	1.044	58	8.9	4.12	3.00
YH81	*L. thermotolerans*	1.112	1.038	64	9.7	4.12	3.02
YH82	*W. anomalus*	1.112	1.070	35	5.6	4.12	3.1
YH109	*L. thermotolerans*	1.112	1.056	48	7.4	4.12	3.01
YH144	*L. thermotolerans*	1.112	1.048	55	8.4	4.12	3.09
YH156	*S. japonicus*	1.112	1.038	64	9.7	4.12	3.35
C1	*S. cerevisiae*	1.112	1.006	94	13.9	4.12	3.51
C2	*S. cerevisiae*	1.112	1.002	98	14.4	4.12	3.70

153
154 [a] Abbreviations: OG, original gravity; FG, final gravity; ABV, alcohol by volume.

155

156 Comparing the LAY-fermented meads to the controls, it is obvious that they were significantly
157 less attenuated and, consequently, had lower ABVs than meads produced with WLP715 (p = 0.0003; Fig.
158 1A,B). This was not unexpected because WLP715 is an industrial strain known for its high levels of
159 attenuation (15), while the LAY have displayed variable levels of attenuation of beer wort (14) and mead

160 (17). Although all 15 meads had a final pH of 3.70 or below, those made with LAY were significantly
161 more acidic on average (p = 0.0004; Fig. 1C). This is consistent with their heterofermentative nature and
162 ability to sour beer by lactic acidification (14).

163 *3.2. Sensory analysis*

164 Various methods have been used for the sensory analysis of mead (*e.g.*, (18,19)). At its root,
165 sensory analysis is subjective, being influenced by multiple factors including the experience level of the
166 participants and various methods of analysis (20). To simulate consumer feedback, participants in our
167 sensory analysis were all mead enthusiasts but varied from self-described novices to experts. We
168 received and documented the results recorded on 490 sensory analysis feedback sheets. Overall, the
169 meads were ranked on a scale from 1-5, with 5 being most favorable, and the sums of these overall
170 impression scores are shown in Figure 2. Eight of the LAY strains yielded meads that scored better than
171 the average of 86 ± 7, with the YH77 mead scoring highest at 107 points. The mead fermented by YH26
172 scored lowest at 56. Further trends in aroma and flavor perception are detailed below.

173 *3.2.1. Aroma*

174 Next, we calculated the average general aroma rating for a series of descriptors: delicate, light,
175 mild, pleasant, pungent, rich, sharp, and strong. No two meads had identical profiles, though several
176 were quite similar (*e.g.*, YH73 and YH77 meads) to the average of the group, which tended toward light
177 and mild (Fig. 3). Overall, the aromas were favorable, though several LAY produced meads whose
178 aromatics stood out as being pungent (YH26 and YH156) and/or sharp (YH79 and YH156).

179 The sensory analysts also scored aromatic esters with the following descriptors: apple, banana,
180 berry, citrus, clean, floral, fruity, grape, mango, melon, nail polish, pear, ripe fruit, stone fruit, tropical,
181 and unripe fruit. On average, the meads displayed banana, citrus, fruity, and tropical notes (Fig. 4).
182 Among the 13 experimental meads, the results were more mixed here than above. Some were ranked

183 highly for apple aromatics (YH27, YH73, and YH156), while others had stronger grape notes (YH26 and
184 YH109) or were more variably scored (WYP39).

185 Finally, "other" aromas were scored for odors reminiscent of acetone, acidic, alcohol, bitter,
186 bread, bubble gum, buttery, candy, cinnamon, earthy, grassy, medicinal, oaky, phenolic, smoky, sour,
187 vegetal, vinous, and yeast descriptors. The average other aroma profile was dominated by the acidic
188 descriptor, consistent with this being the chief descriptor of all but one of the 13 experimental meads
189 (Fig. 5). The lone mead bucking this trend was that produced by YH156, which had a predominant aroma
190 of alcohol, with lesser contributions of acidic and candy aromas.

191 *3.2.2. Flavor*

192 As above for aroma, we also calculated the average general flavor ratings for the experimental
193 meads using a series of descriptors: bright, delicate, light, mild, pleasant, pungent, rich, sharp, strong,
194 and yeasty. The mean flavors were overall mild and pleasant, but there was some diversity among
195 individual meads (Fig. 6). For instance, the meads produced by YH27, YH81, YH109, and YH156 tasted
196 quite sharp, while the YH72 mead had a rich flavor.

197 Flavors were also scored for apple, banana, berry, Brett, citrus, fruity, pear, stone fruit, and
198 tropical descriptors. Here, Brett refers to "Brett character" or the rustic barnyard flavors characteristic of
199 beverages fermented with common strains of *Brettanomyces* spp. (13,21-23). On average, apple, citrus,
200 fruity, and pear flavors predominated, and several individual meads shared this general profile (YH27,
201 YH73, YH77, and YH82) (Fig. 7). Notably, some meads scored highly for citrus and/or pear flavors, such
202 as those made by YH79, YH81, and YH140.

203 Additional flavor descriptors (acetone, astringent, bitter, bread, bubble gum, buttery, earthy,
204 funk, grassy, leather, oaky, silky, smoky, sour, sweet, tart, vegetal, vinous, and yeast) were also
205 considered. Perhaps unsurprisingly, the average profile ranked as highly sour (Fig. 8). Indeed, this was

206 the case for all but two of the individual meads (YH25 and YH77), which were instead perceived as
207 buttery. Several meads were also ranked as bitter: YH27, YH79, and YH156.

208

209 **Discussion**

210 This project provided insight into the sensory characteristics LAY can provide in mead making. In
211 the overall organoleptic survey of sour meads, most sensory analysts preferred the mead fermented
212 with *L. fermentati* strain YH77, which produced citrusy, tropical aromas with mild, fruity, and pear
213 flavors. In contrast, the least preferred mead was produced by *S. japonicus* strain YH156, which
214 displayed pungent and phenolic aromas, as well as strong, bitter, and acetone flavors. It is interesting to
215 note that even though YH156 was the least favored strain, it flocculated the most rapidly and exhibited
216 some initially pungent ammonia aromas during fermentation that were not discernible in the finished
217 mead.

218 The LAY strains tested here tended to attenuate mead less than they do beer wort (17,24), with
219 *L. thermotolerans* strain YH27 yielding the highest attenuation, finishing with an ABV of 10.2%. *H.*
220 *vineae* strain YH72 had the lowest attenuation, with an ABV of 4.2%, which is in stark contrast to our
221 recent work with YH72 in mead production that yielded a finished product with 8.2% ABV (17). The
222 reason for this discrepancy is currently unknown but could be due to variations in the honey musts used
223 between experiments or the yeast nutrient supplementation regime used here. There was also some
224 stochastic variation in the attenuation of the meads (*e.g.*, the FGs of C1 and C2 were 1.006 and 1.002
225 despite identical must, nutrient, inoculation, and fermentation conditions). Additionally, the terminal pH
226 also varied among the experimental meads, with *L. thermotolerans* strain YH79 producing the lowest at
227 pH = 3.0 and the highest terminal pH being found in meads made by YH77 and YH156 (both finishing at
228 pH = 3.35). It is interesting to note that of the four strains with the lowest terminal pH (YH79, pH = 3.0;

229 YH109, pH = 3.01; YH81 pH = 3.02; and YH72 pH = 3.07), YH81 mead scored as having the highest
230 perceived sour flavors (Fig. 8), and YH72 mead had the highest perceived sour aromas (Fig. 5).

231 The sensory data gathered from this initial study of LAY-fermented meads makes the case for
232 future work with these organisms. For instance, it would be interesting to examine the effects of
233 fermentation temperature on the organoleptic properties of LAY meads, the effects of initial honey
234 must gravity on attenuation, and the alcohol tolerances of these yeast strains. We also suggest the need
235 for the addition of a sour mead category into the existing BJCP Mead Styles to encourage such
236 experimentation by professional and home mead makers using LAY and other more common souring
237 microbes.

238 Given that the yeast strains used here produced such an array of aromas and flavors in a
239 fundamental semi-sweet traditional mead, paired with the variety of attenuation levels and levels of
240 clarity yielded by traditional souring techniques, the addition of a new sour mead category will help to
241 encompass the LAY attributes. Indeed, a sour mead category would allow such meads produced by a
242 variety of souring methods to be further evaluated with regard to their unique organoleptic
243 characteristics, resulting in potential growth in what is already a popular style among all levels of mead
244 drinkers. Sour mead could potentially win favor with the already established sour beer enthusiasts and
245 encourage kombucha and other fermented product consumers and brewers to try sour meads, thus
246 expanding the already growing mead market.

247

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296 **Figure legends**

297 **Figure 1. Average attenuation, ABV, and terminal pH of the experimental meads.** Comparisons
298 between the mean attenuation, ABV, and pH of the meads produced in the control fermentations
299 (black) and LAY fermentations (gray). The error bars correspond to the standard deviations (SD). Values
300 were compared by *t* tests using the Holm-Sidak method, with a = 5% and without assuming a consistent
301 SD. Significant *p*-values are listed on the graphs.

302 **Figure 2. Overall impression scores for the LAY meads.** Each sensory analyst gave the LAY meads an
303 overall impression ranking from 1 (least favorable) to 5 (most favorable), and those scores are tallied
304 here. The dashed line indicates the average overall impression score of the 13 experimental meads.

305 **Figure 3. General aroma ratings.** The average scores for the indicated descriptors were calculated and
306 plotted as radar charts. Averages were calculated from between 26 and 43 evaluations for each mead.
307 The LAY strain used to ferment the mead is indicated above the plotted data. The "Average" label
308 indicates that the data are the means across all 13 experimental meads. Mild, light, and pleasant aromas
309 dominated the meads.

310 **Figure 4. Aromatic ester ratings.** The average scores for the indicated aroma descriptors were
311 calculated and plotted as described in Figure 3. The aromas were predominantly described as citrus,
312 fruity, and tropical.

313 **Figure 5. "Other" aroma ratings.** The average scores for the indicated aromatic descriptors were
314 calculated and plotted as described in Figure 3. All meads smelled acidic.

315 **Figure 6. General flavor ratings.** The average scores for the indicated flavor descriptors were calculated
316 and plotted as described in Figure 3. The meads tasted mild and pleasant overall.

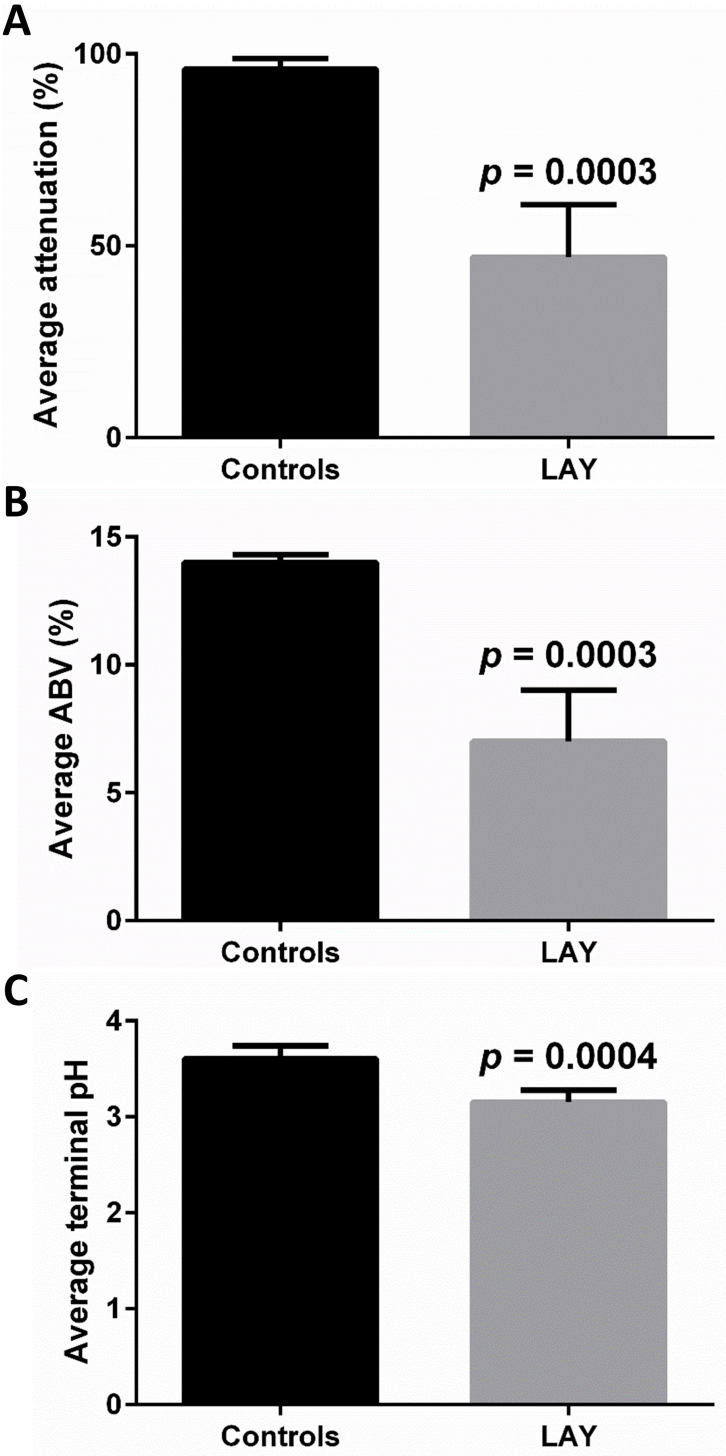
317 **Figure 7. Fruit and Brett flavor ratings.** The average scores for fruit-like and Brett character flavor

318 descriptors were calculated and plotted as described in Figure 3. Flavors of apple, citrus, fruit, and pear

319 were common.

320 **Figure 8. "Other" flavor ratings.** The average scores for the indicated flavor descriptors were calculated

321 and plotted as described in Figure 3. All meads scored highly for the sour flavor descriptor.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50
0
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Controls
LAY
B
Average ABV (%)
15
10
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3
2
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0
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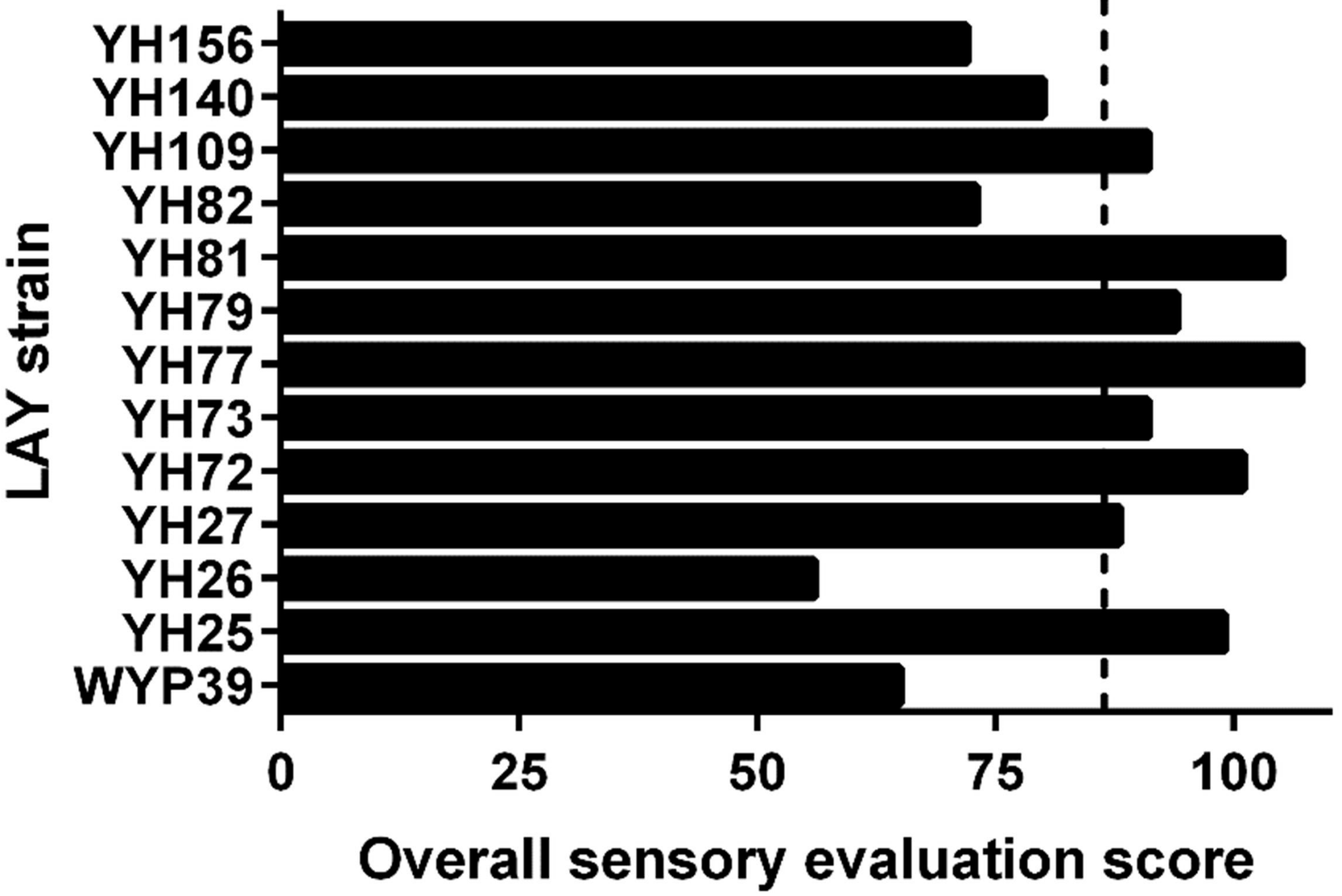
LAY


LAY strain
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YH140
YH109
YH82
YH81
YH79
YH77
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YH72
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YH25
WYP39
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50
75
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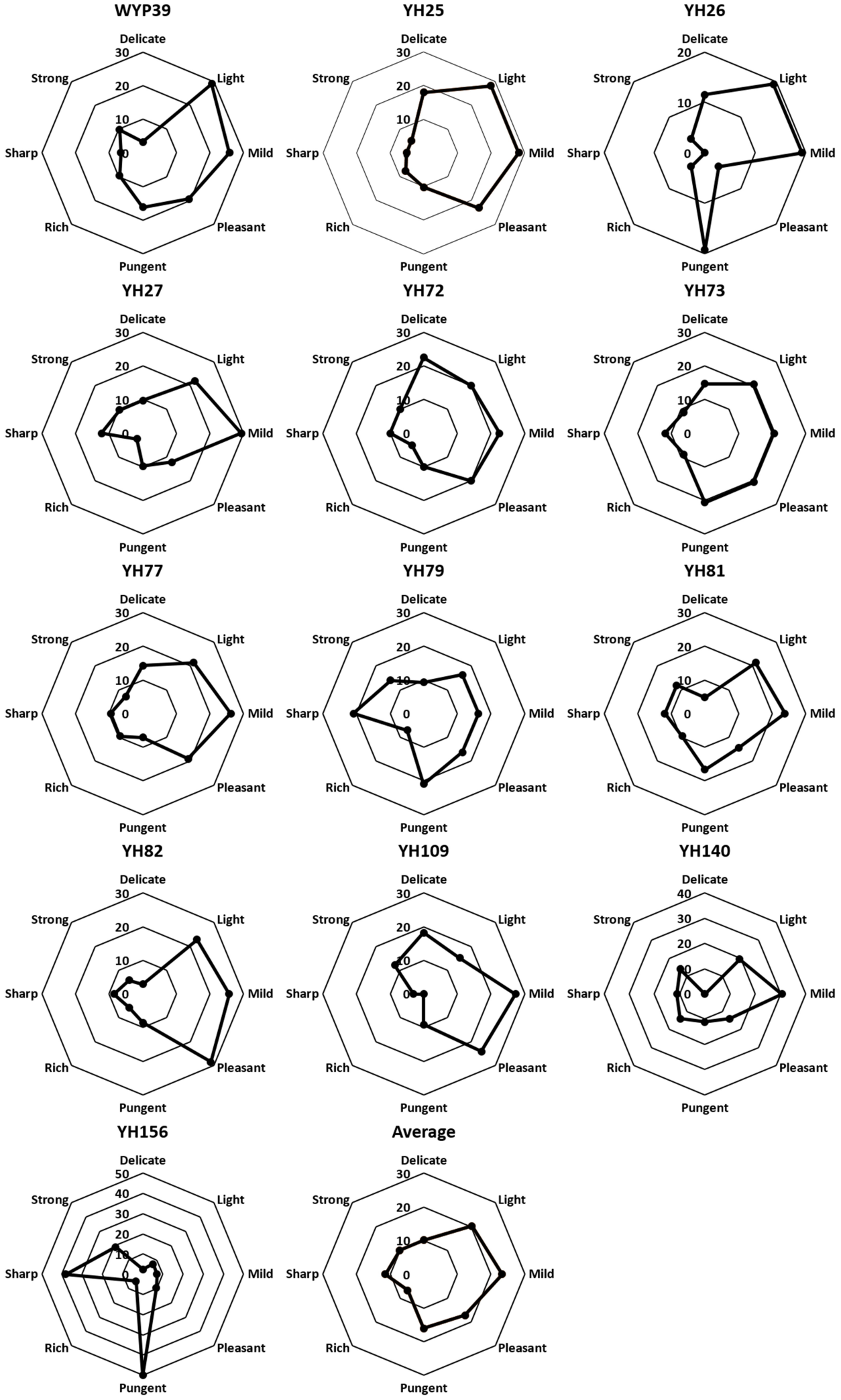
Overall sensory evaluation score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YH73
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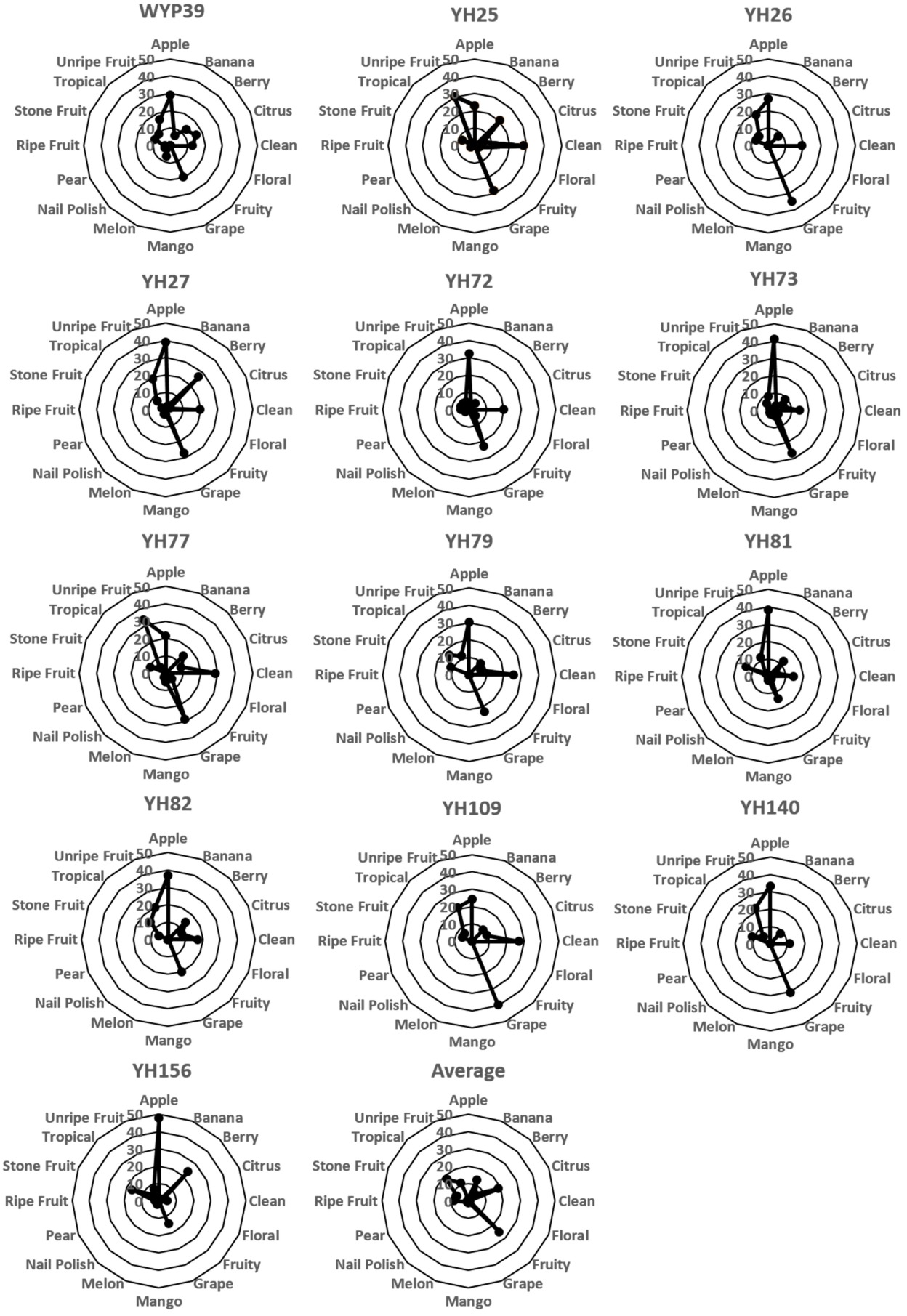
WYP39
YH25
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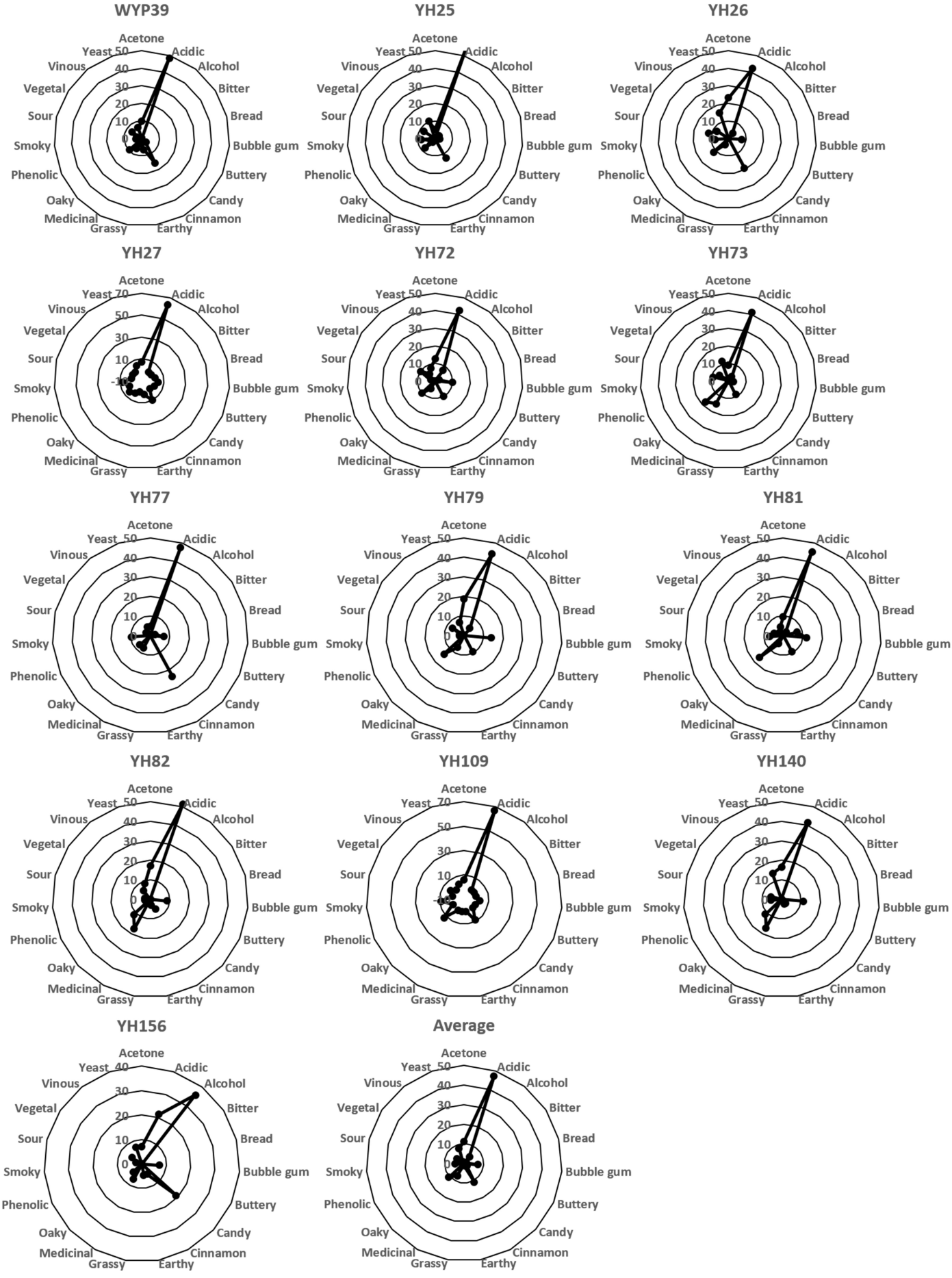
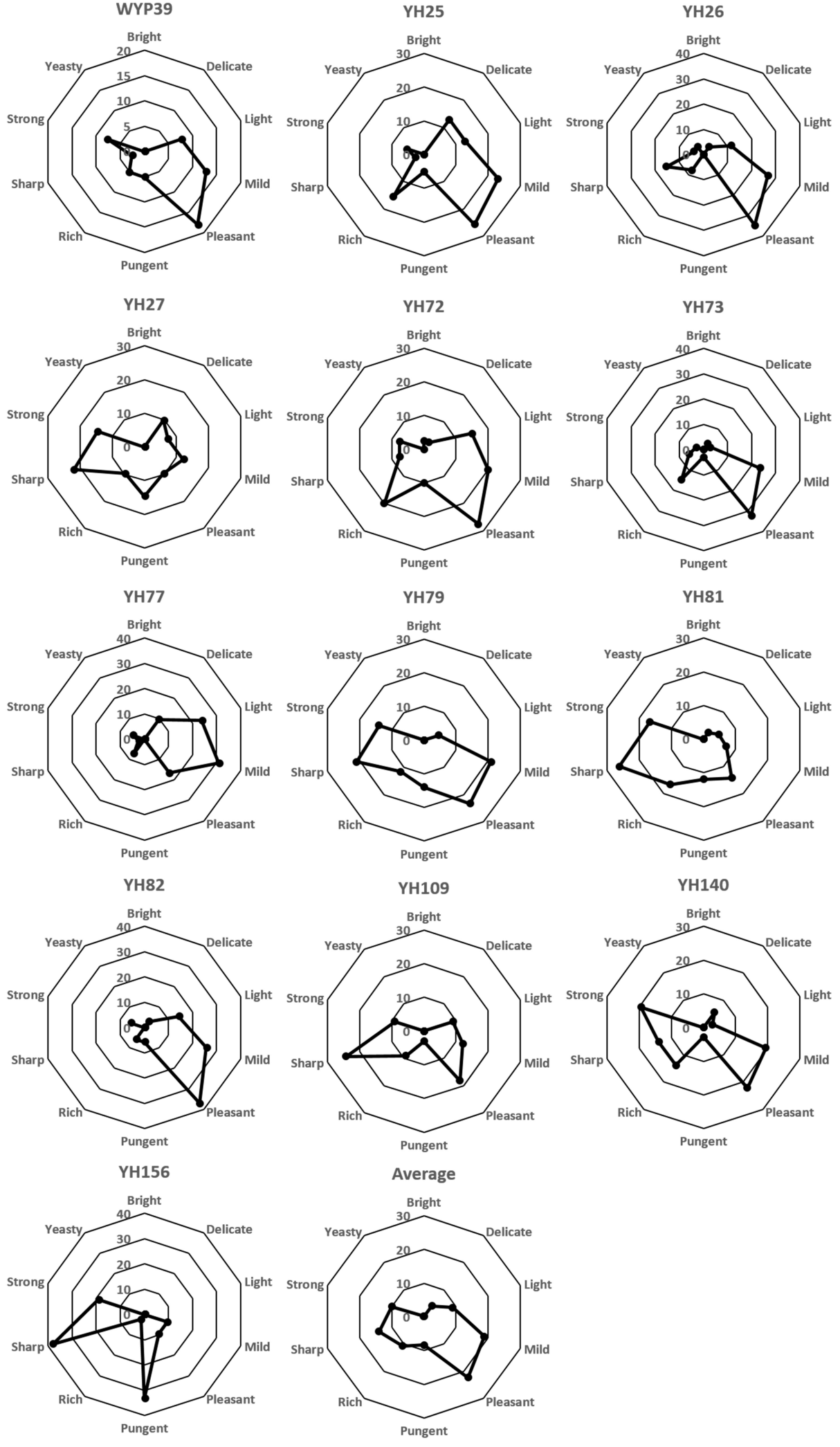
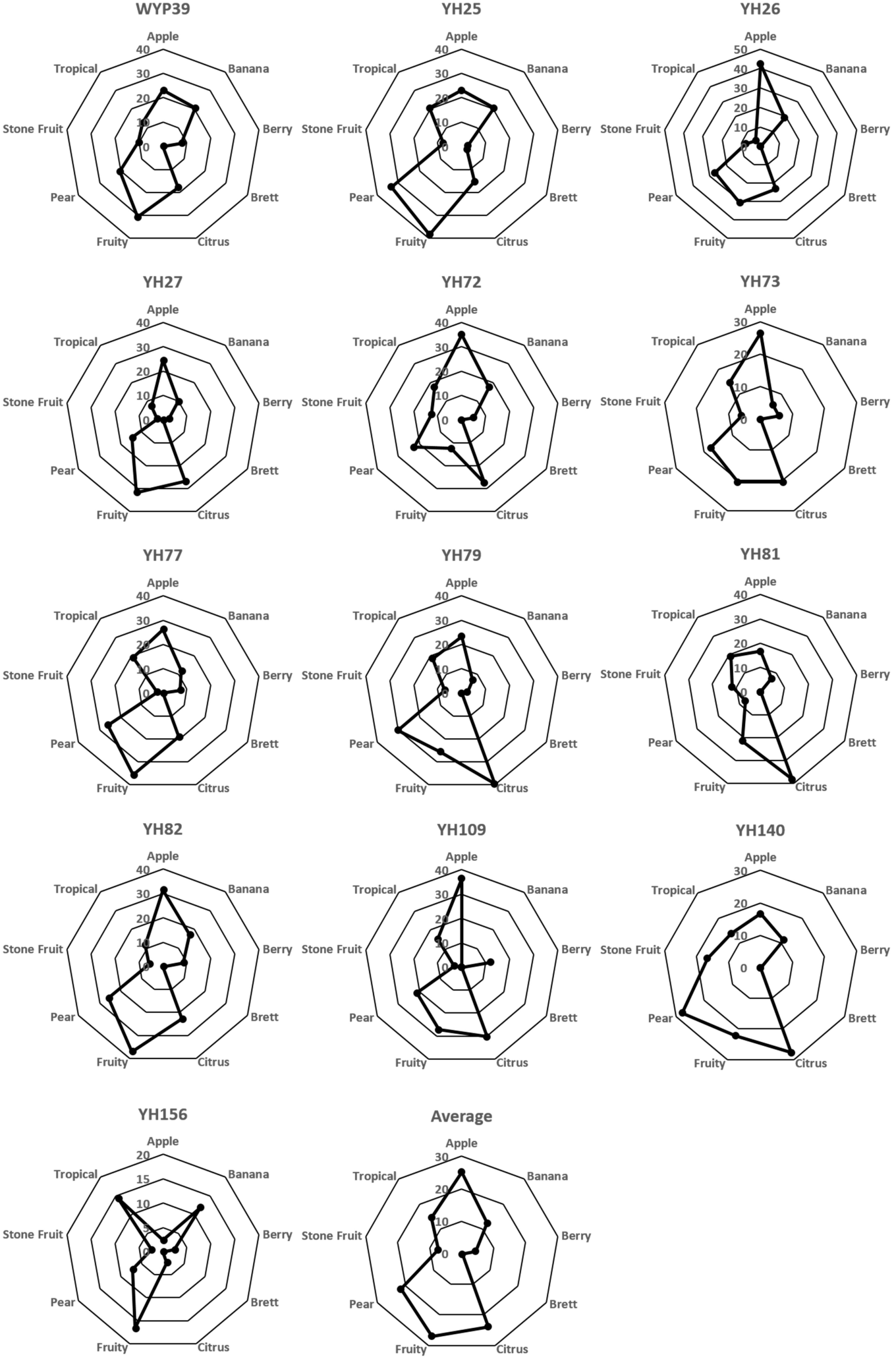
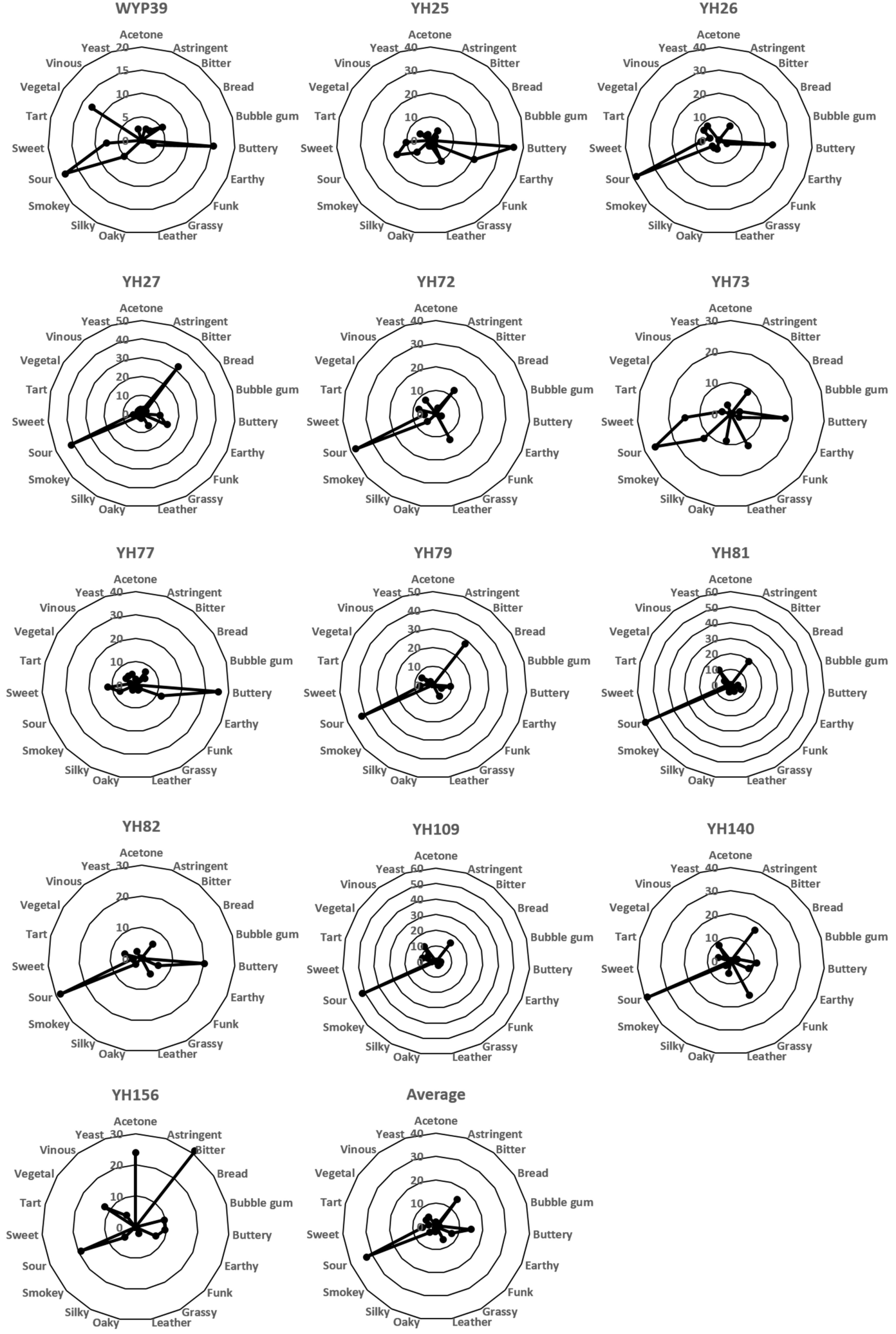
YH72
YH73
YH77
YH79
YH81
YH82
YH109
YH140
YH156
Average
Apple
Banana
Berry
Citrus
Clean
Floral
Fruity
Grape
Mango
Melon
Nail Polish
Pear
Ripe Fruit
Stone Fruit
Tropical
Unripe Fruit
0
10
20
30
40
50


WYP39
YH25
YH26
YH27
YH72
YH73
YH77
YH79
YH81
YH82
YH109
YH140
YH156
Average
Acetone
Acidic
Alcohol
Bitter
Bread
Bubble gum
Buttery
Candy
Cinnamon
Earthy
Grassy
Medicinal
Oaky
Phenolic
Smoky
Sour
Vegetal
Vinous
Yeast


WYP39
YH25
YH26
YH27
YH72
YH73
YH77
YH79
YH81
YH82
YH109
YH140
YH156
Average
Bright
Delicate
Light
Mild
Pleasant
Pungent
Rich
Sharp
Strong
Yeasty


WYP39
YH25
YH26
YH27
YH72
YH73
YH77
YH79
YH81
YH82
YH109
YH140
YH156
Average
Apple
Banana
Berry
Brett
Citrus
Fruity
Pear
Stone Fruit
Tropical


WYP39
YH25
YH26
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YH72
YH73
YH77
YH79
YH81
YH82
YH109
YH140
YH156
Average
Acetone
Astringent
Bitter
Bread
Bubble gum
Buttery
Earthy
Funk
Grassy
Leather
Oaky
Silky
Smokey
Sour
Sweet
Tart
Vegetal
Vinous
Yeast