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INFLUENCE OF *SACCHAROMYCES CEREVISIAE* AND *WILLIOPSIS SATURNUS* VAR. *MRAKII* ON MANGO WINE CHARACTERISTICS

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Saccharomyces cerevisiae MERIT.ferm was used as mono- and mixed-cultures with *Williopsis saturnus* var. *mrakii* NCYC500 in mango wine fermentation. A ratio of 1:1000 (*Saccharomyces*:*Williopsis*) was chosen for mixed-culture fermentation to enable longer persistence of the latter. The monoculture of *S. cerevisiae* and mixed-culture was able to ferment to dryness with 7.0% and 7.7% ethanol, respectively. The monoculture of *W. mrakii* produced 1.45% ethanol. The mango wines fermented by *S. cerevisiae* alone and the mixed-culture were more yeasty and winey, which reflected their higher amounts of fusel alcohols, ethyl esters and medium-chain fatty acids. The mango wine fermented by *W. mrakii* alone was much less alcoholic, but fruitier, sweeter, which corresponded to its higher levels of acetate esters.

Keywords: mango wine, *Saccharomyces*, *Williopsis*, flavour, yeast

Saccharomyces cerevisiae is usually used in wine fermentation with regard to its high tolerance to ethanol, ease of control and homogeneity, but wine produced by monocultures of *S. cerevisiae* often lacks complexity (Rojas et al., 2003). More reports claimed that many non-*Saccharomyces* yeast strains could positively affect wine quality (Romano et al., 2003). However, monocultures of non-*Saccharomyces* lack fermentative power and may lead to off-flavour (Ciani et al., 2009). Researchers now tend to use mixed-starters of *S. cerevisiae* and non-*Saccharomyces* to exhibit the advantages of both (Ciani et al., 2009).

Application of *Saccharomyces* in mango wine fermentation has been reported elsewhere (Reddy & Reddy, 2005; Li et al., 2011). However, there is very little information on mango wine production using mixed-culture fermentation (Sadineni et al., 2011). *W. mrakii*, formerly known as *Hansenula mrakii*, is an efficient producer of acetate esters (Li et al., 2012). The yeast has been inoculated to improve the fruity character of Japanese sake (Inoue et al., 1997), grape wine (Erten & Tanguler, 2010) and papaya wine (Lee et al., 2010). This research is to assess the influence of *S. cerevisiae* and *W. mrakii* as both mono- and mixed-cultures on mango wine characteristics.

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1. Materials and methods

1.1. Yeast strain, juice preparation, pre-culture and fermentation

Williopsis saturnus var. *mrakii* NCYC500 (National Collection of Yeast Cultures, Norwich, UK) and *S. cerevisiae* MERIT.ferm (Chr.-Han., Denmark) were used in this study. The pre-culture and mango juice preparation were shown in the previous report (Li et al., 2012). A ratio screening (*Saccharomyces*:*Williopsis* = 1:1, 1:10, 1:100 and 1:1000) was carried out to determine the ratio that would be used in subsequent mixed-culture fermentation. The inoculums for 1:1, 1:10, 1:100 and 1:1000 of *S. cerevisiae* and *W. mrakii* were: 2.06×10^5 CFU·ml^{-1} *S. cerevisiae* and 1.90×10^5 CFU·ml^{-1} *W. mrakii*, 1.04×10^4 CFU·ml^{-1} *S. cerevisiae* and 1.47×10^5 CFU·ml^{-1} *W. mrakii*, 1.66×10^3 CFU·ml^{-1} *S. cerevisiae* and 2.14×10^5 CFU·ml^{-1} *W. mrakii*, 2.25×10^2 CFU·ml^{-1} *S. cerevisiae* and 2.06×10^5 CFU·ml^{-1} *W. mrakii*. The ratio of 1:1000 was selected, since it yielded the longest persistence of *W. mrakii* and the highest concentration of acetate esters.

Triplicate mango juice (250 ml) fermentations with *Saccharomyces* to *Williopsis* at a ratio of 1:1000 were carried out. The juices were inoculated with 2.5 ml of a pre-culture of *W. mrakii* NCYC500 and 2.5 ml of a 1000-time diluted pre-culture of *S. cerevisiae* MERIT.ferm. In addition, monocultures of *Saccharomyces* and *Williopsis* were started as controls. Fermentation was conducted at 20 °C statically for 21 days.

1.2. Analysis

Sugars and organic acids were measured by HPLC according to Li and co-workers (2012). Volatiles were identified using headspace (HS) solid phase microextraction (SPME) method coupled with gas chromatography (GC)–mass spectrometer (MS) and flame ionisation detector (FID) (HS-SPME-GC-MS/FID). The volatiles on day 0, 2, 4, 7, 10, 14 and 21 were analysed. The GC conditions and GC quantification method were described in the previous report of Li and co-workers (2012). Odour activity values (OAVs) were determined from the concentration of volatiles on day 21 and their reported odour threshold in literature.

1.3. Statistical and sensory test

Test of significance of difference among the three kinds of fermentation for the experimental data was accomplished by employing one-way analysis of variance (ANOVA, P=0.05). The mango wines were evaluated by a panel of eight experienced flavourists from Firmenich Asia. The test was single-blinded, and 20 ml of samples were presented in wine-testing glasses with a random number for sniffing only. Ten sensory descriptors were selected by consensus to describe the mango wine aroma. The panelists used a 5-point scale to rate the intensity of each attribute.

2. Results and discussion

2.1. Yeast growth

Williopsis mrakii persisted for the longest time, reached the highest cell count and produced the highest amounts of acetate esters after 21 days when its ratio was 1:1000, therefore ratio 1:1000 was selected for subsequent mixed-culture fermentation. *S. cerevisiae* reached ~10^8 CFU·ml^{-1} after 10 days in both mono- and mixed-cultures, but the growth of *S. cerevisiae* was delayed by the presence of *W. mrakii* in the first 4 days (Fig. 1). *W. mrakii* reached ~10^8

CFU·ml^{-1} after 10 days in the mono-culture, but in mixed-culture its cell count was 10–100 times lower. The growth delay of *S. cerevisiae* in the mixed-culture was probably due to killer toxin produced by *W. mrakii* (Yamamoto et al., 1986), while the growth arrest of *W. mrakii* might be due to its weak tolerance to ethanol (Inoue et al., 1994).

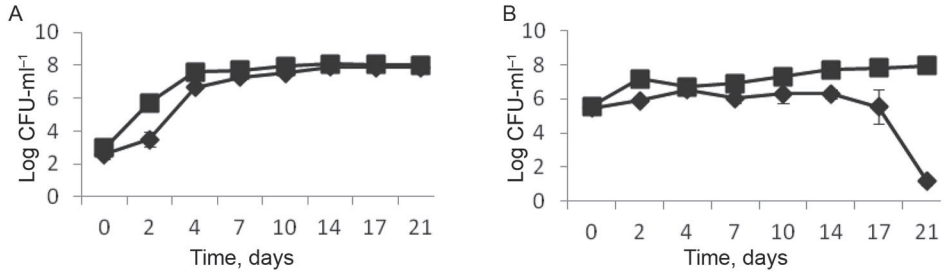

Fig. 1. Yeast cell populations in monoculture (■) or mixed-culture (◆) at a ratio of 1:1000 of *S. cerevisiae* MERIT.ferm to *W. saturnus* var. *mrakii* NCYC500. A: *S. cerevisiae*; B: *W. mrakii*

2.2. Physicochemical properties

Sugars were almost exhausted in monoculture of *S. cerevisiae* and mixed-culture, but not in monoculture of *W. mrakii* (Table 1). Correspondingly, ethanol and glycerol was higher in monoculture of *S. cerevisiae* and in the mixed-culture (Table 1). The pH varied 3.4–3.6 for the three wines. Citric, malic and tartaric acids showed no significant changes in the mango wine fermented with *W. mrakii*, but these acids significantly decreased in the wine fermented by *S. cerevisiae* and by the mixed-culture (Table 1). The amounts of succinic acid increased slightly in all three fermentations. The reduction of malic acid could be due to passive diffusion of D-malic acid into yeast cells (Coloretti et al., 2002). The decrease of tartaric acid was likely the result of tartrate salt precipitation.

Table 1. Changes of sugars, organic acids, ethanol and glycerol in mango wines before and after fermentation

	Day 0	Day 21		
	Uninoculated Juice	*S. cerevisiae* MERIT.ferm	*W. mrakii* NCYC500	Mixed-culture (ratio of *S.*:*W.*=1:1000)
Sugars (g l^{-1})				
Fructose	44.3±2.3^{A}	0.00±0.00^{B}	14.1±3.1^{C}	0.03±0.01^{B}
Glucose	15.1±0.7^{A}	0.00±0.00^{B}	2.4±1.6^{C}	0.00±0.00^{B}
Sucrose	69.8±3.7^{A}	0.03±0.01^{B}	50.7±1.8^{C}	0.02±0.01^{B}
Organic acids (g l^{-1})				
Citric acid	2.16±0.22^{A}	1.09±0.01^{B}	2.29±0.01^{A}	1.09±0.02^{B}
Tartaric acid	1.05±0.09^{A}	0.46±0.02^{B}	1.05±0.05^{A}	0.36±0.02^{B}
Malic acid	6.88±0.53^{A}	3.25±0.03^{B}	5.79±0.03^{A}	3.29±0.11^{B}
Succinic acid	1.01±0.02^{A}	1.41±0.01^{B}	1.23±0.01^{C}	1.44±0.02^{B}
Miscellaneous				
Ethanol (%, v/v)	0.10±0.00^{A}	7.01±0.68^{B}	1.45±0.08^{C}	7.69±0.34^{B}
Glycerol (g l^{-1})	0.00±0.00^{A}	4.76±0.20^{B}	0.21±0.02^{C}	4.40±0.09^{B}

ANOVA (n=6) was done at 95% confidence level with same letters (A, B or C) indicating no significant difference

2.3. Evolution of volatiles

The major terpenes in 'Chok Anan' mango juice were monoterpene hydrocarbons ($C_{10}H_{16}$). Most of the terpenes decreased rapidly in the mono-culture of *S. cerevisiae*, in the mono-culture of *W. mrakii* the decrease was much slower (Fig. 2). This phenomenon might be due to the difference of the fermentation power of the two yeasts. Terpenes were easily drawn off by CO_2, and lost during the metabolite exchange taking place between the medium and the atmosphere (Arévalo Villena et al., 2006).

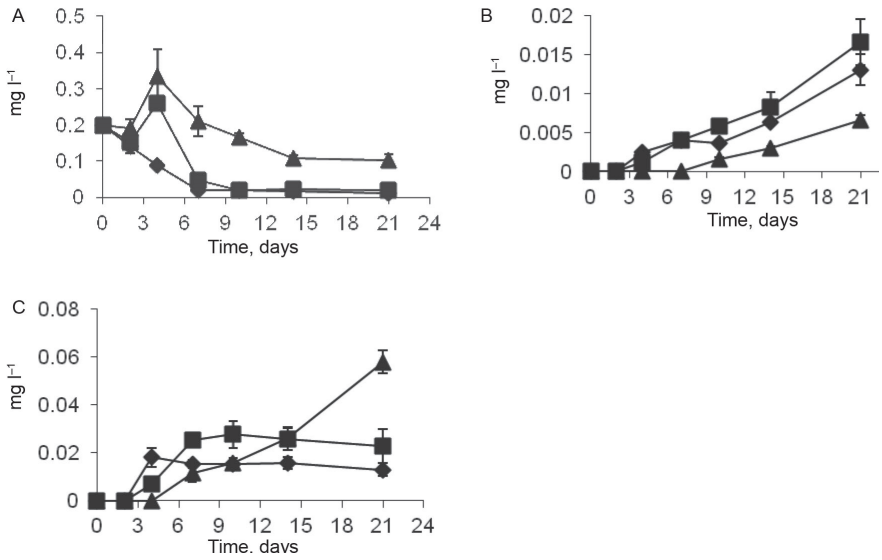

Fig. 2. Evolution trend of terpenoids throughout the fermentation of mango juices: monoculture of *S. cerevisiae* (♦), monoculture of *W. mrakii* (▲) and mixed-culture at a ratio of *S*:*W*=1:1000 (■). A: α-Terpinolene; B: β-citronellol; C: citronellyl acetate

The major terpenol in mango wine was β-citronellol, it was present in low amounts in the juice but its concentration increased throughout the fermentation (Fig. 2). The increase of terpenol was likely due to glycoside hydrolysis by yeast-derived glycosidases (Ugliano et al., 2006). The amount of β-citronellol was the highest in the mixed-culture followed by the monoculture of *S. cerevisiae* and then the monoculture of *W. mrakii* (Table 2). The lower level of β-citronellol in the monoculture of *W. mrakii* was probably due to β-citronellol being converted to citronellyl acetate by this yeast known to possess higher acetate ester synthesizing activities.

Table 2. Concentrations (day 21), odour thresholds, odour activity values (OAVs) of typical odourants in mango wines

Compounds	Retention index	*S. cerevisiae* MERIT (mg l^{-1})	OAV[a]	*W. mrakii* NCYC500 (mg l^{-1})	OAV	Mixed-culture (mg l^{-1})	OAV	Odour threshold (mg l^{-1})
Isobutyl alcohol	1089	89.1±11.9^{A}	2.2	20.3±0.98^{B}	0.5	78.8±13.6^{A}	2	40[b]
Isoamyl alcohol	1226	101.5±5.7^{A}	3.4	21.7±5.0^{B}	0.7	93.8±3.4^{A}	3.1	30[b]
2-Phenylethyl alcohol	1952	54.0±5.9^{A}	5.4	6.7±0.6^{B}	0.7	58.2±10.0^{A}	5.8	10[b]
Total alcohols		244.6		48.7		230.3		
Ethyl acetate	906	3.93±1.19^{A}	0.5	349.5±33.7^{B}	46.6	30.3±4.3^{C}	4	7.5[b]
Isobutyl acetate	1020	0.026±0.012^{A}	0.016	0.22±0.04^{B}	0.14	0.027±0.019^{A}	0.017	1.6[d]
Isoamyl acetate	1106	0.27±0.07^{A}	9	3.54±0.19^{B}	118	0.51±0.05^{C}	17	0.03[b]
Hexyl acetate	1275	0.003±0.000^{A}	1.5	0.034±0.001^{B}	17	0.004±0.000^{A}	2	0.002[c]
Citronellyl acetate	1682	0.013±0.003^{A}	0.05	0.058±0.004^{B}	0.2	0.023±0.007^{C}	0.09	0.25[e]
2-Phenylethyl acetate	1853	0.59±0.15^{A}	2.4	3.30±0.18^{B}	13.2	0.51±0.10^{A}	2	0.25[b]
Ethyl hexanoate	1229	0.27±0.05^{A}	19.3	0.006±0.001^{B}	0.4	0.25±0.08^{A}	17.9	0.014[c]
Ethyl octanoate	1445	1.13±0.30^{A}	565	0.053±0.002^{B}	26.5	0.95±0.39^{A}	475	0.002[b]
Ethyl decanoate	1657	1.26±0.25^{A}	6.3	0.059±0.002^{B}	0.3	0.84±0.23^{A}	4.2	0.2[d]
Ethyl dodecanoate	1867	1.21±0.45^{A}	0.2	0.083±0.005^{B}	0.01	0.89±0.05^{A}	0.15	5.9[c]
Total esters		8.7		356.9		34.3		
Acetic acid	1473	452±49^{A}	2.3	680±14^{B}	3.4	647±105^{B}	3.2	200[b]
Hexanoic acid	1871	1.39±0.15^{A}	0.46	0.32±0.02^{B}	0.11	2.17±0.56^{C}	0.72	3[c]
Octanoic acid	2087	4.64±0.76^{A}	0.53	0.24±0.03^{B}	0.03	3.87±0.59^{A}	0.44	8.8[d]
Decanoic acid	2303	1.57±0.27^{A}	0.16	0.35±0.01^{B}	0.04	1.45±0.03^{A}	0.15	10[c]
Dodecanoic acid	2607	0.65±0.05^{A}	0.065	0.48±0.02^{B}	0.048	0.66±0.04^{A}	0.066	10[c]
Total acids		460.2		681.4		655.2		
α-Terpinolene	1270	0.01±0.00^{A}	0.05	0.1±0.02^{B}	0.5	0.02±0.00^{A}	0.1	N.A.
β-Citronellol	1791	0.013±0.002^{A}	0.13	0.006±0.000^{B}	0.06	0.017±0.003^{A}	0.17	0.1[b]
Total terpenes		0.023		0.106		0.037		
Total volatiles		713.5		1087		919.8		

ANOVA (n=6) was done at 95% confidence level with same letters (A, B or C) indicating no significant difference. N.A.: not available; [a]: Odour activity values (OAV) were calculated by dividing concentration by the odour threshold value of the compound; [b]: The odour threshold was obtained from Guth (1997). The value was determined in 10% of ethanol solution.[c]:The odour threshold was obtained from Pino & Queris (2011). The value was determined in 11% of ethanol solution; [d]:The odour threshold was obtained from Bartowsky & Pretorius (2009). The value was determined in 10% of ethanol solution. [e]:The odour threshold was obtained from Yamamoto et al. (2004). The value was determined in 0.05% of ethanol solution.

Amounts of ethanol and fusel alcohols increased throughout the fermentation (Fig. 3). The monoculture of *S. cerevisiae* and the mixed-culture produced considerable amounts of the alcohols (Table 2); however, the production was much slower in the monoculture of *W. mrakii* (Fig. 3). The fusel alcohols were likely produced through the Ehrlich pathway from their corresponding amino acids (Derrick & Large, 1993) or could be produced from sugar metabolism (Pietruszka et al., 2010).

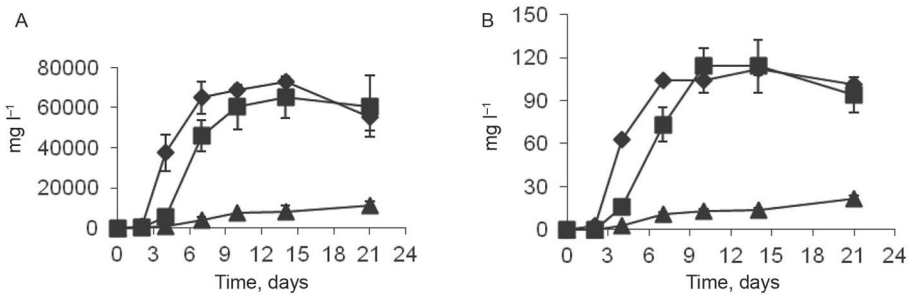

Fig. 3. Evolution trend of alcohols throughout the fermentation of mango juices: monoculture of *S. cerevisiae* (♦), monoculture of *W. mrakii* (▲) and mixed-culture at a ratio of *S*:*W*=1:1000 (■). A: Ethanol; B: isoamyl alcohol

The major volatile acids were acetic acid and medium-chain fatty acid (MCFA). The amounts of all the volatile acids increased (data not shown). The production of acetic acid was slightly higher in the mono-culture of *W. mrakii*, and this was consistent with a previous study in grape wine (Erten & Tanguler, 2010). Amounts of MCFAs were higher in the monoculture of *S. cerevisiae* and the mixed-culture (Table 2), but all MCFAs were below threshold levels (Table 2).

The monoculture of *W. mrakii* rapidly produced large amounts of acetate esters, such as isoamyl acetate (Fig. 4). Acetate esters in the mixed-culture had a surge on day 4 and started to decrease when *S. cerevisaie* dominated, while acetate esters were only produced at low levels in the monoculture of *S. cerevisiae*. The OAVs of acetate esters were much higher in the monoculture of *W. mrakii* (Table 2), but excess amount of acetate esters could impart off-flavour (Jackson, 2000). In *S. cerevisiae*, alcohol acetyl-transferase (AATase) was labile (Minetoki, 1992), but it was stable in *W. mrakii* (Inoue et al., 1997). Acetate ester hydrolyzing enzyme in *S. cerevisiae* is activated when it is in mixed-culture (Kurita, 2008). Therefore, mixed-culture fermentation was able to control acetate esters in a desirable range if suitable fermentation duration was selected.

Ethyl esters of MCFA (Fig. 4) were rapidly produced in the wine fermented by *S. cerevisiae* only, and their production was also enhanced in mixed-culture fermentation when *S. cerevisiae* dominated. The final concentrations of ethyl esters in *S. cerevisiae* alone and in the mixed-culture were generally higher than their threshold levels (Table 2). Excess amount of ethyl esters could impart waxy and soapy notes to wine, and mixed-culture by selecting optimal fermentation time could mitigate ethyl ester production. It was reported that the relative concentration of fatty acid precursors was the limiting factor for ethyl ester production (Saerens et al., 2008). Thus, the low production of ethyl esters in the monoculture of *W. mrakii* was probably due to its lower level of fatty acid precursors.

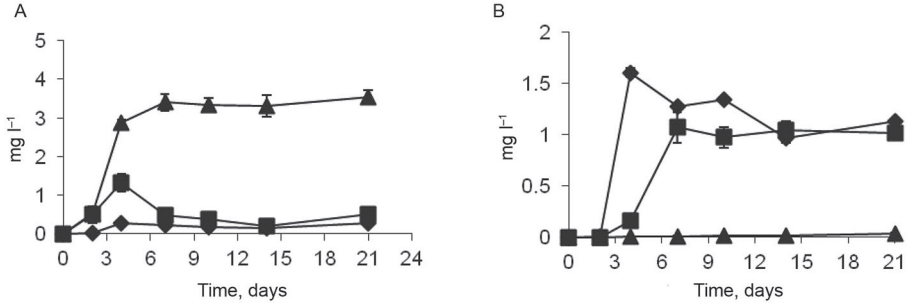

Fig. 4. Evolution trend of acetate and ethyl esters throughout the fermentation of mango juices: monoculture of *S. cerevisiae* (♦), monoculture of *W. mrakii* (▲) and mixed-culture at a ratio of *S*:*W*=1:1000 (■). A: Isoamyl acetate; B: ethyl octanoate

2.4. Sensory test

The sensory profile of the mango wines is presented in Fig. 5 and scores are compared by one-way ANOVA (P=0.05, data not shown). The mixed-culture wine had similar sensory attributes to the wine fermented with *S. cerevisiae* alone, but the monoculture wine of *W. mrakii* could significantly differentiate itself from the other two wines. In general, the mango wine fermented by *W. mrakii* alone was considered as more fruity and sweet and this corresponded to its higher levels of acetate esters. The mango wines fermented by *S. cerevisiae* alone and in mixed-culture were both considered as significantly more yeasty, winey and slightly waxy, which reflected their higher amounts of ethanol, fusel alcohols, ethyl esters and MCFA. To maximize the advantages of mixed culture, the duration of fermentation should be optimized in future.

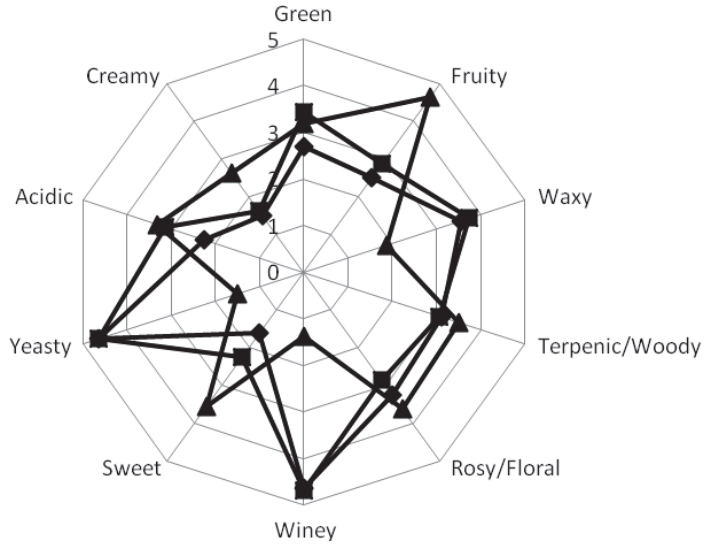

Fig. 5. Sensory profile of mango wines: monoculture of *S. cerevisiae* (♦), monoculture of *W. mrakii* NCYC500 (▲) and mixed-culture at a ratio of *S*:*W*=1:1000 (■)

3. Conclusion

The mixed-culture of *W. mrakii* and *S. cerevisiae* provided the opportunity to not only complete the fermentation but also improve aroma complexity and balance, but this needs the winemaker to apply the right ratio of *S. cerevisiae* and *W. mrakii* and to control the duration of fermentation. However, the results obtained from the laboratory-scale studies are not necessarily the same as what might be expected in larger-scale fermentations. Thus, larger-scale studies should be performed to confirm the results obtained in this work.

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