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Evaluation of physicochemical, sensory, and antimicrobial properties of small-scale produced fruit vinegars

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ABSTRACT

Hungarian fruit vinegars were characterised in terms of physicochemical attributes (total polyphenol content, antioxidant characteristics/FRAP, CUPRAC, ABTS/, ascorbic acid content, pH, total soluble solids), sensory profiles, and antimicrobial properties.

Both compositional and sensory profiles showed distinct patterns depending on the type of vinegar (Tokaj wine, balsamic or apple) and the additional fruit used. Balsamic vinegars matured on rosehip, sea buckthorn, and raspberry showed outstanding antioxidant performances. Rosehip, raspberry, and quince vinegars, as well as vinegars produced from Tokaji aszú and balsamic apple obtained high scores for fruity and sweet notes.

Antimicrobial activities were tested on Gram-negative and Gram-positive organisms, including probiotic bacteria. Generally, only weak activities were obtained, which was attributed to the natural sugar content of the samples, depending on the type of the vinegar and the fruit. Similar results, but more pronounced bacterial growth inhibitions were obtained for probiotic strains, however, some probiotic strains were resistant to at least two of the vinegars. Based on these, balsamic apple, raspberry, rosehip,

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quince, and sea buckthorn may qualify as potential functional components of probiotic preparations containing some of the strains tested.

1. INTRODUCTION

Being more than a versatile food ingredient, its bioactive components maintain vinegar in the focus of current research. Recently, the demand for fruit vinegars has increased due to their reputation as health-promoting food products, fitting into the global trends demanding enrichment of food with natural antioxidants (Dziki et al., 2014). Within fruit vinegars, small-scale produced vinegars represent a valuable quality category, enabling the selection of specific raw material and adequate technology appropriate to local conditions and requirements (Boonsupa et al., 2019). Fruits are used not only for flavour, aroma, and colour addition, but also as precious sources of phytochemicals, including phenolic compounds. In addition to products fermented directly from fruits as carbohydrate sources, vinegar-derived condiments obtained by maceration with fruits are gaining an increasing interest due to the various polyphenols, micronutrients, and other bioactive compounds present in these vinegars (Zhang et al., 2020). Phenolics transferred from fruits into vinegars considerably enhance the antioxidant potential of the product (Liu et al., 2019; Stagos, 2020). Fruit polyphenols may also exert their specific antimicrobial effects in vinegars, proven on various microorganisms, including food-borne pathogens like *Escherichia coli* and *Salmonella* Typhi (Priyadarshini et al., 2014; Soorya et al., 2019).

Next to their health-promoting properties, fruit-derived compounds also define the main sensory attributes of vinegars, thus playing a key role in formation of taste, aroma, and colour (Cejudo-Bastante et al., 2016; Boonsupa, 2019). Nevertheless, only limited information is available on the comparative sensory evaluation of vinegars matured on different fruit beds.

In the present research, a range of selected small-scale domestic vinegar products were studied in terms of their physicochemical, antioxidative, sensory, and antimicrobial properties.

2. MATERIALS AND METHODS

2.1. Reagents, solvents, and standards

All solvents and reagents were supplied by Sigma-Aldrich except ascorbic acid (Riedel-de-Haën), potassium persulphate (Acros Organics), copper(II) chloride (Alfa Aesar), ammonium acetate (Molar Chemicals Ltd.), hydrochloric acid (Carlo Erba), and distilled water. For ascorbic acid content determination an enzymatic kit from Megazyme was used.

2.2. Samples

A total of 8 wine- or cider-based small-scale vinegars from the Hungarian Tokaj region were analysed: a group of vinegars matured on fruit bed (quince – TQ, rosehip – TRH, raspberry –

TRB, sea buckthorn – TST) and a group of naturally fermented vinegars (cider – TC) including balsamic ones (balsamic apple – TAB, Tokaj aszú – TA) and a thyme-infused product (TT). These were compared to commercial samples (plain vinegar – V, white balsamic vinegar – WB, cider vinegar – OC, red wine vinegar – RW) (Supplementary Table 1S). Wine- or cider-based Tokaj vinegars were naturally fermented and matured for a minimum of 6 months in oak barrels. In the case of balsamic vinegars, concentrated grape or apple musts were added to the base vinegars upon maturation. For maceration on fruit beds, matured vinegars were subsequently transferred to stainless steel reservoirs, where fresh fruits were added in amounts according to the individual recipes of the manufacturer, then maturation on fruit beds (or thyme) was accomplished for 1–2 months. Results are reported for 1–1 bottle per sample, however, antioxidant measurements were performed on more bottles for most of Tokaj vinegars (Supplementary Table S2). Vinegars from Tokaj were kindly donated by Borecet Művek Ltd., Bodrogkeresztúr.

2.3. Analytical methods

Analytical measurements were performed in five replicates (except for vitamin C content – two replicates). For spectrophotometry, a Thermo Helios Alpha UV-VIS instrument (± 0.001 units of absorbance, 1 cm light path) was used. Proper dilutions (10- or 100-fold) were applied for antioxidant assays.

2.3.1. Total polyphenol content, ferric reducing antioxidant power (FRAP), cupric reduction antioxidant capacity (CUPRAC), and ABTS assays.
Total polyphenol content was evaluated following a method adapted from Singleton and Rossi (1965). In FRAP experiments, the procedure described by Benzie and Strain (1996) was adapted. ABTS antioxidant capacity was performed according to Re et al. (1999). CUPRAC assay was accomplished according to Apak et al. (2007).

2.3.2. Ascorbic acid content.
Ascorbic acid content was determined by Megazyme enzymatic kit for L-ascorbic acid. The assay is based on the selective determination of 3-(4,5-dimethylthiazolyl-2)-(2,5-diphenyltetrazolium bromide)-formazan formed exclusively from L-ascorbic acid (UV-VIS, 578 nm).

2.3.3. Acetic acid content.
Acetic acid content for small-scale vinegars was determined by titration of acidity with 0.1 N NaOH against phenolphthalein, reporting the results as m/V % acetic acid.

2.4. Sensory analysis

Tokaj vinegar samples (50 mL of each) were presented to panel members undiluted, in plastic cups, with 3-digit random codes. Two groups of samples were set up based on their production technology: vinegars matured on fruit beds (4 samples) and other vinegars (4 samples). Twelve trained panellists were given a complete list of the pre-defined taste and aroma descriptors (see Figs 1 and 2) and were asked to score them on scales from 0 to 100. Measurements were performed in two replicates. Neutral mineral water was used as taste neutraliser. Sensory tests were carried out meeting all criteria of ISO 13299 (ISO, 2016). For determination of sensory
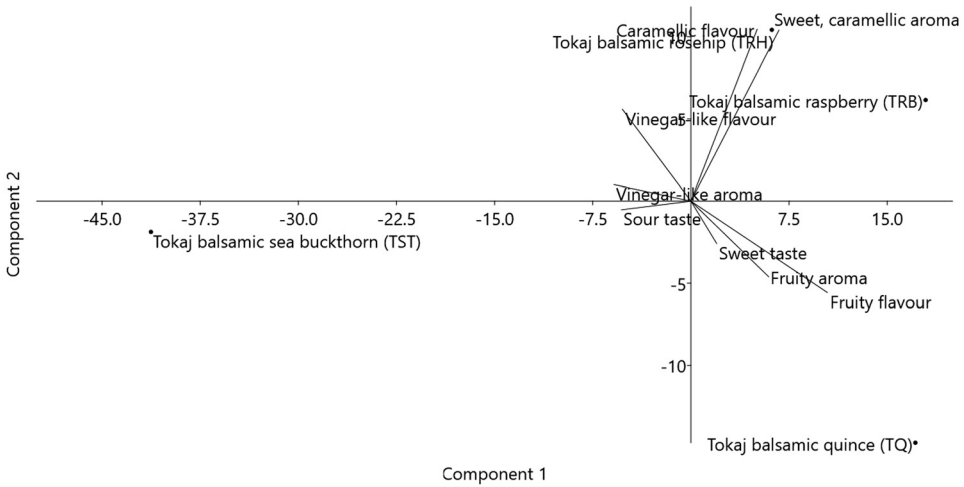
Fig. 1. Biplots of vinegar samples matured on fruit beds based on sensory properties (PC1/84%/-PC2/13%/)
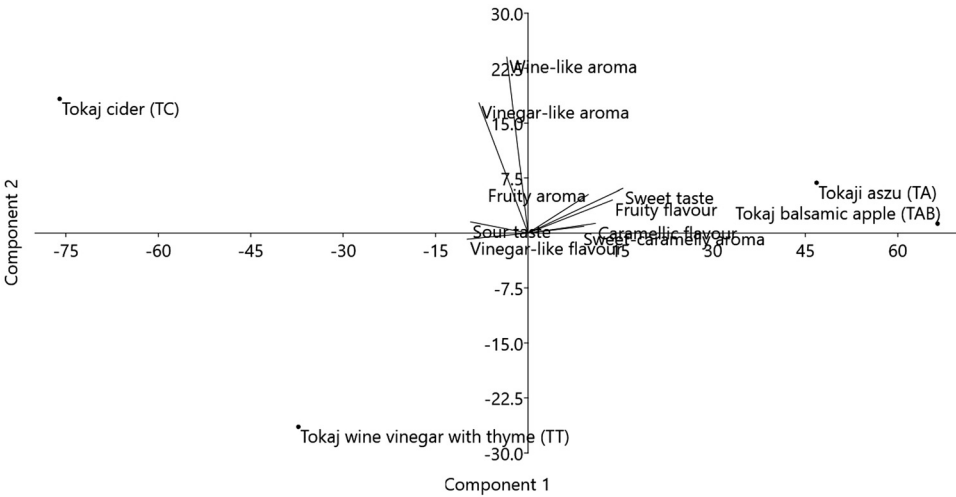

Fig. 2. Biplots of vinegar samples based on sensory properties (PC1/92%/-PC2/7%/)

profiles, the intensity values for the reference samples (sea buckthorn and cider vinegars) were pre-defined in both groups for each attribute on the evaluation scales.

2.5. Microbiological analysis

Antimicrobial activity of vinegars was tested on both Gram-positive (*Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* T1, and *Listeria monocytogenes* CCM 4699) and Gram-

negative (*E. coli* T1, *Proteus mirabilis* T1) spoilage and pathogenic bacteria. The following probiotic strains were also tested: *Lactobacillus acidophilus* LA-5, *Lactobacillus casei* 01, *Lactobacillus acidophilus* 150 Exqium, and *Lactobacillus plantarum* 299V. Details regarding the origin of the strains used are presented in Supplementary material Table S3. Agar well diffusion tests were performed on CASO agar plates for non-probiotic strains and MRS agar plates (20 mL/plate) for probiotic strains. Plates were inoculated with a 10^6 CFU mL^{-1} initial count inoculum of the test microorganism by spread plating. The volume of inoculum was 0.1 mL. The wells were prepared by punching the inoculated plates with a 5 mm diameter standard sterile stainless steel cork borer. The wells were filled up with 50 µL of each vinegar sample, and the plates were incubated at 37 °C. After 24 h of incubation, the diameter of the zone of inhibition was measured using a ruler, then results were quoted by subtracting the diameter of the well and dividing the results by two. Interpretation of inhibition zones of test culture was adopted from Priyadarshini et al. (2014): zone of inhibition diameters of 10 mm or less indicate resistance of test organisms, diameters of 11–15 mm indicate intermediate resistance, while diameters of 16 or more mm indicate test organisms sensitive to the product.

2.6. Statistical methods

Pearson correlation ($P = 0.05$) was used for verifying correlations between antioxidant properties, significant differences were checked by one-way analysis of variance (ANOVA) completed by Tukey's post-hoc test (https://www.statskingdom.com/180Anova1way.html). Sensory tests were evaluated by ProfiSens v. 2012 software (Kókai et al., 2002). Profile analysis was evaluated by one-way ANOVA ($P = 0.05$) followed by LSD (least significant differences) post-hoc tests. Principal Component Analysis (PCA) on sensory data was performed with the Past statistical package (Hammer et al., 2001).

3. RESULTS AND DISCUSSION

3.1. Polyphenol content and antioxidant capacity

More *in vitro* assays (Folin–Ciocalteu TPC, FRAP, CUPRAC, and ABTS) were used for a complex characterisation of the antioxidant properties of the vinegars (Table 1). ABTS, FRAP, and CUPRAC methods resulted in very similar patterns, all showing all strong correlations with TPC values (0.94, 0.89, and 0.96, respectively at $P = 0.05$), thus polyphenols are considered to dominate in the development of the antioxidant properties. Vinegars matured on fruit beds showed much better performances than the rest of the samples, rosehip vinegar producing the highest values for all parameters measured followed by raspberry and sea buckthorn vinegars. Also Tokaj aszú and balsamic apple vinegars performed generally better than commercial red wine vinegar. Our high results are attributed not only to the polyphenols extracted from the fruit beds, but also to the concentrated must added, however, more research is needed to investigate the effect of the base vinegar. The TPC values for vinegars on fruit beds were higher than those obtained by others for vinegar fruit macerates (57 mg GAE/100 mL for raspberry and 303 mg GAE/100 mL for rosehip) (Kalemba-Drożdż et al., 2020), probably due to the effect of added must concentrate in the Tokaj vinegars. Traditionally fermented fruit vinegars led to lower values (102–244, 65.72–117.8, or 327.98 mg GAE/L) (Kong et al., 2018; Boonsupa, 2019; Boonsupa et al., 2019). TPC and ABTS values measured for balsamic vinegar are in good

Table 1. Total polyphenol content (TPC), antioxidant capacities by FRAP, ABTS, and CUPRAC methods, and ascorbic acid (AA) content (average $\pm$ standard deviation, $n = 5$, $n = 2$ for AA)

Sample	TPC (mg GAE/L)	FRAP (mg AAE/L)	CUPRAC (mmol TE/L)	ABTS (mmol TE/L)	AA (mg L^{-1})
TAB	1,311 ± 18[d]	271 ± 6[f]	6.8 ± 0.3[c]	7.8 ± 0.2[d]	0.0
TA	1,319 ± 61[d]	292 ± 8[f]	5.2 ± 0.9[d]	6.8 ± 0.5[de]	0.0
TQ	1,451 ± 34[cd]	387 ± 8[c]	5.9 ± 0.2[cd]	10.9 ± 0.3[c]	11.3 ± 0.4
TRH	4,983 ± 156[a]	1,222 ± 24[a]	36.0 ± 1.0[a]	65.4 ± 3.6[a]	44.3 ± 4.7
TRB	2,669 ± 153[b]	865 ± 23[b]	10.7 ± 0.3[b]	14.7 ± 0.1[b]	18.7 ± 1.4
TST	1,570 ± 88[c]	393 ± 9[c]	6.1 ± 0.4[cd]	11.8 ± 0.2[c]	21.6 ± 0.7
TC	289 ± 13[g]	62 ± 2[h]	2.1 ± 0.4[h]	2.3 ± 0.1[gh]	0.0
TT	709 ± 21[f]	333 ± 6[e]	3.6 ± 0.3[e]	4.9 ± 0.2[ef]	0.0
V	0.0 ± 0.3[h]	0.0 ± 0.0[i]	0.0 ± 0.0[i]	0.8 ± 0.1[i]	ND
WB	436 ± 24[g]	46 ± 7[h]	1.4 ± 0.3[h]	3.1 ± 0.1[fg]	ND
OC	1,020 ± 57[e]	125 ± 8[g]	4.0 ± 0.4[e]	6.9 ± 0.3[de]	ND
RW	1,027 ± 12[e]	385 ± 11[d]	5.1 ± 0.2[d]	11.0 ± 0.5[c]	ND

ND = not determined; superscript letters indicate significant differences ($P < 0.05$).

agreement with those reported for Modena balsamic vinegar (1901–3216 mg GAE/L and 4.49–7.30 µmol TE mL^{-1}, respectively), for Tokaj cider the values are even higher than those reported for ciders (43.75–256 mg GAE/L and 0.03–1.01 µmol TE mL^{-1}, respectively) (Liu et al., 2019).

3.2. Ascorbic acid content

The highest ascorbic acid contents were measured in rosehip followed by sea buckthorn and raspberry, showing the valuable contribution of fruit extracts, partly still preserved in the final products. Vitamin C in vinegars originates exclusively from fresh fruits, it has not been detected in any of the other Tokaj vinegars (Table 1). Ascorbic acid content is also strongly correlated with antioxidant capacity (correlation coefficients: 0.82, 0.89, and 0.90 for FRAP, CUPRAC, and ABTS, respectively, $P = 0.05$). Our results also correlate with those obtained for a papaya-based fermented product (2.32 mg/100 mL) (Kong et al., 2018).

3.3. Sensory analysis

Sensory characteristics of vinegars result from the complex effect of raw materials, technology and various biochemical processes, as parts of a dynamic equilibrium (Cejudo-Bastante et al., 2016). Sensory profile of vinegars matured on fruit bed (see Supplementary material, Fig. 1S) showed that vinegar-like aroma and flavour, as well as sour flavour were the most pronounced for sea buckthorn vinegar (TST; even though its acidity was not higher than of the other samples), their values having been significantly higher ($P = 0.05$) than those obtained for quince (TQ), and for raspberry (TRB) (vinegar-like flavour) or rosehip (TRH) (vinegar-like aroma) samples or both of these (sour taste). These findings are also supported by the PCA biplot (Fig. 1), where these attribute vectors point towards the quadrant where the TST sample is. Fruity flavour, fruity aroma, and sweet taste were the most intensive for TQ. Both TRB and TRH showed significantly higher scores ($P = 0.01$) than TST, however, TRH was less fruity than TQ ($P = 0.05$). The same

applies for fruity aroma, where values were significantly higher for TQ and TRB compared to TST ($P = 0.01$ and $P = 0.05$, respectively). Mild sweet-caramellic aroma and taste were the strongest for TRB and TRH, both preceding TST ($P = 0.01$ and $P = 0.05$, respectively). TQ was found to be the sweetest (significantly sweeter than TST and TRH, $P = 0.05$) and the least sour. A significant dominance of caramellic flavour was noticed for TRB and TRH over TST ($P = 0.01$ and $P = 0.05$, respectively).

Sensory profile of vinegars not maturated on fruit bed (see Supplementary material, Fig. 2S) showed much higher differences, reflecting the different character of the samples; i.e. cider (TC), balsamic apple (TAB), Tokaj aszú (TA), and thyme-flavoured (TT) wine vinegars. Although TT had the highest acidity, vinegar-like notes and sour taste were the most pronounced for TC ($P = 0.01$, Fig. 2). The same applies for wine-like aroma ($P = 0.01$). On the other hand, sweet-caramellic aroma and taste and sweet taste were the most pronounced for TA, significantly more intense than for TT and TC ($P = 0.01$). Fruity notes were the strongest for TAB, both aroma and taste were significantly more intensive than in the case of TC and TT ($P = 0.01$).

3.4. Microbiological analysis

3.4.1. Antimicrobial activity of vinegars against selected spoilage organisms and pathogens.
Antimicrobial activity of vinegars was tested on Gram-positive and Gram-negative spoilage and pathogenic bacteria. The results (Table 2) showed that all vinegars possessed antimicrobial activity against all bacteria, however, only a few microorganisms were highly sensitive to some of the vinegars tested. Gram-positive bacteria, especially *L. monocytogenes* and *E. faecalis*, were the most susceptible, while both Gram-negative species were less sensitive. Although vinegar is reported to be generally efficient against many microorganisms (e.g. *L. monocytogenes, S. aureus*, and *E. coli* (Medina et al., 2007)), these effects were not proven for the fruit vinegars tested, even though many of the polyphenols transferred from fruits have their own antimicrobial effects as well, although this effect is strain-dependent (Bouarab-Chibane et al., 2019). The relatively weak antimicrobial activity of these products is attributed to the antagonistic effect induced by the fairly high sugar content (from both maceration on fruits and addition of concentrated musts). This sugar content is reflected in the Brix values, which correlate with the antimicrobial properties, i.e. the highest inhibition zones were obtained for cider and thyme-flavoured vinegars, these having the lowest Brix

Table 2. Antimicrobial inhibition zones of small-scale produced vinegars (mm, average ± standard deviation, $n = 3$)

Microorganism/ Sample	*S. aureus* ATCC 6538	*E. faecalis* T1	*E. coli* T1	*L. monocytogenes* CCM 4699	*P. mirabilis* T1
TAB	4.00 ± 0.70	6.00 ± 0.70	2.50 ± 0.00	5.00 ± 0.50	1.75 ± 0.36
TA	4.25 ± 0.36	6.50 ± 0.70	3.00 ± 0.70	5.50 ± 0.70	3.50 ± 0.00
TQ	3.25 ± 0.36	4.75 ± 0.36	2.25 ± 0.36	4.00 ± 0.70	1.75 ± 0.36
TRH	4.00 ± 0.00	6.00 ± 0.70	3.00 ± 0.00	4.25 ± 0.36	2.75 ± 0.36
TRB	3.75 ± 0.36	5.25 ± 0.36	2.75 ± 0.36	1.83 ± 0.36	2.75 ± 0.36
TST	3.75 ± 0.36	5.50 ± 0.70	3.00 ± 0.00	6.25 ± 0.36	3.25 ± 0.36
TC	5.00 ± 0.00	7.00 ± 0.70	4.00 ± 0.00	6.75 ± 1.06	5.50 ± 1.00
TT	6.00 ± 0.70	5.00 ± 0.00	5.25 ± 0.36	7.75 ± 1.61	3.50 ± 0.00

Table 3. Inhibition zones of small-scale produced vinegars on probiotic bacteria
(mm, average ± standard deviation, $n = 3$)

Probiotic bacteria/Sample	*L. casei* 01	*L. acidophilus* 150	*L. acidophilus* LA-5	*L. plantarum* 299V
TAB	7.50 ± 1.50	6.25 ± 0.36	5.75 ± 1.07	4.50 ± 0.50
TA	9.00 ± 0.00	5.25 ± 1.06	6.25 ± 1.06	6.75 ± 0.36
TQ	8.38 ± 0.76	5.50 ± 0.00	4.75 ± 0.36	4.50 ± 0.00
TRH	7.00 ± 0.00	5.75 ± 0.36	5.00 ± 0.86	5.34 ± 0.27
TRB	6.75 ± 0.36	3.75 ± 1.06	5.00 ± 0.00	5.50 ± 0.00
TST	6.00 ± 0.00	4.34 ± 0.63	0.00 ± 0.00	3.75 ± 0.36
TC	9.25 ± 0.36	8.83 ± 0.76	5.75 ± 0.36	6.50 ± 0.50
TT	12.00 ± 0.00	8.25 ± 1.06	8.50 ± 0.71	7.50 ± 0.71

values and containing neither fruit extracts nor fruit must. The stronger antimicrobial effect of thyme-flavoured vinegar can also be explained by its active compounds (carvacrol and thymol) (Kiskó and Roller, 2005).

3.4.2. Antimicrobial activity of vinegars against selected probiotics. Based on the weak antimicrobial effects observed above, fruit vinegars were tested on probiotic strains as well (Table 3). Our main goal was to select probiotic bacteria not susceptible to the action of these products and thus, to be able to utilise the potential of fruit vinegars as functional ingredients in probiotic food preparations. In the case of *Lactobacillus* strains the effect of sugar content was also observed, similarly to spoilage and pathogenic strains, while cider and thyme-flavoured vinegars had the most inhibitory effects against probiotic strains. Due to the known higher sensitivity of probiotics, the inhibition zones were generally larger, especially for *L. casei* 01, where thyme vinegar reached a moderate antimicrobial effect. However, most of the vinegars exerted only a weak antibacterial effect on most *Lactobacillus* strains. The most promising results were obtained for *L. acidophilus* 150 and *L. plantarum* 299V, which were practically resistant to at least two of the vinegars used (raspberry and sea buckthorn; balsamic apple, quince and sea buckthorn, respectively). Quince vinegar showed no growth inhibition on *L. acidophilus* LA-5 either. Thus, some of these probiotic strains can be used successfully in functional foods enriched with selected fruit vinegars as valuable sources of phytochemicals and minerals, while also delivering additional flavour and aroma.

4. CONCLUSIONS

High polyphenol contents and antioxidant capacities were detected in vinegars matured on fruits with exceptionally high values obtained for rosehip balsamic vinegar followed by raspberry and sea buckthorn products.

Sensory properties were determined by the type of the fruit macerated: sea buckthorn delivered vinegar-like notes, sourness; raspberry and rosehip provided sweet-caramellic taste and aroma, whereas fruity notes dominated in quince-based vinegar. For vinegars not matured on fruit bed, the highest vinegar-like scores were obtained for cider vinegar. Vinegar enriched

with Tokaj aszú showed a pronounced sweet-caramellic flavour and aroma, while balsamic apple vinegar was dominated by fruity notes.

Microbiological analysis revealed that fruit-containing vinegars exert only weak antimicrobial actions against the selected food spoilage and foodborne pathogens, this diminished effect being attributed to their sugar content (from either fruit or must concentrate). Their antimicrobial effects were generally more pronounced on probiotic strains, though some of these showed promising resistance in the presence of vinegars matured on fruits.

In addition to their versatile sensory features, the remarkable nutritional assets of small-scale vinegars highlighted here (especially those matured on fruit beds and, to some extent, balsamic vinegars), i.e. high polyphenol content and antioxidant capacity or even ascorbic acid content for the fruit-bed ones, qualify these products as valuable food ingredients, contributing to dietary antioxidant intake. Moreover, some of the vinegars matured on fruit beds may have a potential as functional additives in probiotic food preparations.

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APPENDIX A

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1556/066.2021.00077.

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