SUBERIC ACID – A POTENTIALLY FLAVOR-ACTIVE CONTAMINANT RELEASED BY SOME AGGLOMERATED CORKS

Cordes R.[1*], Schneider V.[2], Schwack W.[3], Haase-Aschoff P.[4]

[1] Wein-Cordes, 84453 Mühldorf, Germany
[2] Schneider-Oenologie, 55413 Weiler bei Bingen, Germany
[3] Justus-Liebig-Universität Gießen, 35392 Gießen, Germany
[4] Labor Dr. Haase-Aschoff, 55543 Bad Kreuznach, Germany

* Corresponding author: face-of-wine@t-online.de

Introduction

Bottle closures, like any other part of packaging, are subject to the legal requirement of uniform sensory neutrality. In order to meet this requirement, they must not release any substances into the product which could alter it. Natural cork, with its inconsistent potential influence on the product, is still permitted as a closure solely on the grounds of its biological variability and its tradition.

Natural and agglomerated corks vary in many aspects such as their oxygen permeability, their capability of flavor scalping, and their susceptibility to contributing taints of varying character to the wine. Flavor scalping was observed with regard to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) causing 'petrol flavor' in some white wines (Tarasov et al. 2019), methoxypyrazines responsible for green-vegetative aroma notes (Blake et al. 2009), volatile sulfur compounds involved in 'reduced off-flavors' (Silva et al. 2012), and esters and monoterpenes participating in the desired fruity aroma attributes of wine (Capone et al. 2003). Among the compounds accidentally released by natural corks in odor-active amounts, 2,4,6-trichloroanisole (TCA) is the most important one, because it elicits the so-called cork taint as one of the most serious off-flavors caused by corks. Additional taint compounds such as other chloroanisoles, 2,4,6-tribromoanisole, pentachlorophenol, geosmin, 2-methylisoborneol, 1-octen-3-one, 1-octen-3-ol, guaiacol (Sefton and Simpson 2005) and 2-methoxy-3,5-dimethylpyrazine (Simpson et al 2004) were detected in cork-tainted bottled wines and in extracts from the corresponding corks. With regard to agglomerated corks, there were reports on a poorly investigated glue-related flavor caused by 1,2,3,4-tetrahydronaphthalene and traced back to unknown precursors released by agglomerated corks (Diekmann 1997).

The perception of the sensory effects caused by the release of cork-derived compounds or by flavor scalping effects takes place primarily at the olfactory level. Hence, the focus in cork quality control has always been on olfactory perceptions caused by odor-active volatile compounds (Silva et al. 2011). Such compounds are quantified by gas chromatography (GC). In this way, the cork industry has succeeded in recent years in considerably reducing TCA release from corks and the risk of cork taints. However, this analytical approach does not detect non-volatile compounds that are not perceived by smell but only become active on the palate. Therefore, taste aspects of the use of corks have traditionally been neglected.

Anecdotal tasting results on originally identical wines sealed with both agglomerated corks and alternative closures gave rise to the assumption that the lots sealed with certain agglomerated stoppers exhibited a lingering irritation on the mouth and throat mucous membranes, which caused an alteration of the wines' gustative profile. The sensory term that best described this irritation was lingering astringency. This article reports on the astringent impact of odorless suberic acid released by some agglomerated corks and associated flavor changes.

Carry-over effects of astringency - a sensory challenge

Astringency is not a taste but a chemically induced tactile sensation due to shrinking, drawing, puckering, roughing, and drying of the mucous membranes. A disruption of oral moistening or lubricating coatings results in friction between oral surfaces and contributes to the sensation's development (Gawel 1998). Various wine components are known to elicit astringency. The astringency caused by wine tannins has been intensively investigated. Its duration has been shown to be able to amount to more than one minute after sipping or spitting out (Noble 1995). Upon repeated ingestion, total duration and maximum intensity of astringency increase. The reason for is that residual astringency of the previous wine is carried over to the next sample, to whose astringency it adds to if the residual astringency is not allowed to decay to zero before the next ingestion. As an example, when red wines are sipped several times at half-minute intervals between sips, the astringency intensity increases significantly with each sip, reaches a maximum intensity about 15-30 seconds after the sip was taken, and then decreases slowly until the next sip is taken and again astringency increases (Guinard et al. 1986, Lee and Lawless 1991). Hence, many assessments of red and other wines' astringency are rendered invalid.

The sensory bias caused by the convergence of the residual sensation of previously tasted samples is called the carry-over effect. It shows that saturation can be reached after a few ingestions of astringent beverages, so that past astringency becomes the limiting factor in its own discrimination during further evaluations. Additionally, it demonstrates the need for careful experimental design of trials involving astringency scorings, due to the strength and duration of the carry-over effect.

In the case of the astringency believed to be elicited by the agglomerated cork stoppers referred to in the introduction, preliminary tests demonstrated that it can persist for more than an hour, thus making the gustative evaluation of subsequent samples difficult if not impossible.

Sensory discrimination tests of cork-derived astringency

An initial investigation was performed to determine with what confidence level the change in the wine caused by the corks under study could be reproduced. For sensory difference testing, the triangle test is usually preferred to paired-comparison or other tests due to its higher statistical power. However, this test is not the correct choice when there are significant carry-over effects between samples owing to the presence of intrinsic physiological and psychological biases (Williams and Arnold 1991, Lau et al. 2004, Yang and Ng 2017).

Due to the long-lasting persistence of the astringency elicited by the corks used in this study and the carry-over effect resulting therefrom, it became apparent that samples could not be tasted in short intervals as it is required in triangle tests. Otherwise the cork-effect is transferred to the reference wine. As a result, the difference is perceived to be smaller than it actually is, and finally it is no longer perceptible at all. Therefore, paired-comparison tests were used as 'same/different' tests. In these tests, 11 experienced panelists tasted eight pairs of wine and one of water by mouth. Each pair comprised a commercial reference wine coded A and a cork treatment of that wine coded B. Tasters were asked to evaluate the A and B samples served in the order 'reference – treatment' without tasting back, and to indicate whether samples were the same or different and whether they varied in astringency. A waiting period of at least four hours was observed between each pairwise comparison. Table 1 reports the cork treatments and the results.

Table 1: Paired-comparison tests by mouth on the impact of three agglomerated corks from the same manufacturer on various wines and water.

Type of cork	Variety and origin of wine	Treatment	Panel-ists, to-tal	Panelists distin-guishing differ-ence
1	Müller-Thur-gau, Germany	2 corks soaked in 1500 mL wine during 48 hours	11	10**
1	Muscat blanc, Germany	Bottles sealed with cork and stored laid down during 37 days	11	11***
2	Syrah Rosé, Italy	2 corks soaked in 1500 mL wine during 72 hours	11	9*
2	Pinot blanc, Germany	Bottles sealed with cork and stored laid down during 41 days.	11	9**
3	Silvaner, Germany	2 cork soaked in 1500 mL wine dur-ing 32 hours	11	10**
3	Riesling, half-dry, Germany	2 corks soaked in 1500 mL wine during 72 hours	11	11***
3	Riesling, dry, Germany	2 corks soaked in 1500 mL wine during 72 hours	11	8
3	Pinot noir, Switzerland	Bottles sealed with cork and stored laid down during 94 days.	11	9*
Mix of corks 1 + 2 + 2 x 3	Water	4 corks soaked in 1500 mL water during 48 hours.	11	8
Significance levels: * p=0.05, ** p=0.01, *** p=0.001				

It can be seen in table 1 that most of the agglomerated corks used in this trial significantly changed the wine after a short period of one to three days when they were soaked in wine corresponding to the volume of a bottle, or in 37, 41 and 94 days when they were used as a closure for bottles stored laid down. It is worth mentioning in this context that many consumers, and also some producers, ship and store the bottles laid down.

Evidence of the sensory impact of agglomerated corks by an electronic tongue

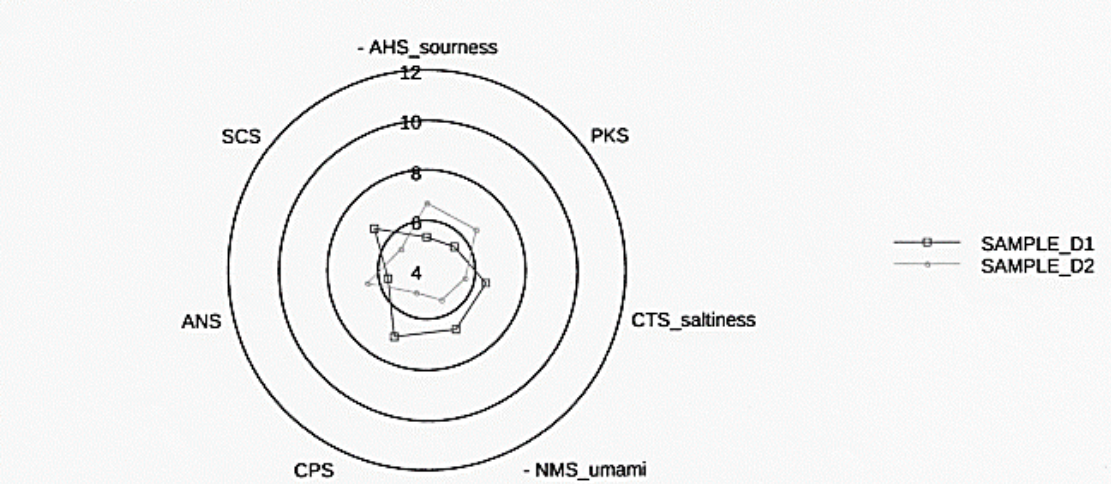
Sensory discrimination tests are prone to response bias as a result of the variation in tasters' criteria for assigning a sample as 'same' or 'different', raising the question of how different the two samples must be before the taster feels confident enough to report that they are different. Additionally, as the order of samples could not be randomized due to the carry-over effect of the sample matrices, the statistical power of the paired-comparison tests was reduced. Therefore, they were complemented by the use of an electronic tongue.

Electronic tongues are multisensory systems consisting of arrays of sensor with cross-sensitivity, combined with pattern recognition software that analyzes and translates the identified compounds into the quantifiable components of taste. Thus, they provide a fingerprint of the samples that can be used to discriminate or classify samples without the influence of human subjectivity. Principles and examples of practical application in the wine industry are given in Rodríguez-Méndez et al. 2016. For this study, the Astree (Alpha-MOS, Toulouse) electronic tongue was used on four wines. Its detection principle is based on potentiometric measurements with seven sensors.

Figure 1 shows results obtained on a Pinot wine sealed with an agglomerated cork (cork type 2 in table 1) in comparison with the same wine sealed with a screw cap. Both lots were stored laid down during four years at 14-16° C. Samples would be identical if the measurement points

of the seven sensors were identical, which was not the case. The electronic tongue confirmed the results obtained by sensory paired-comparison tests.

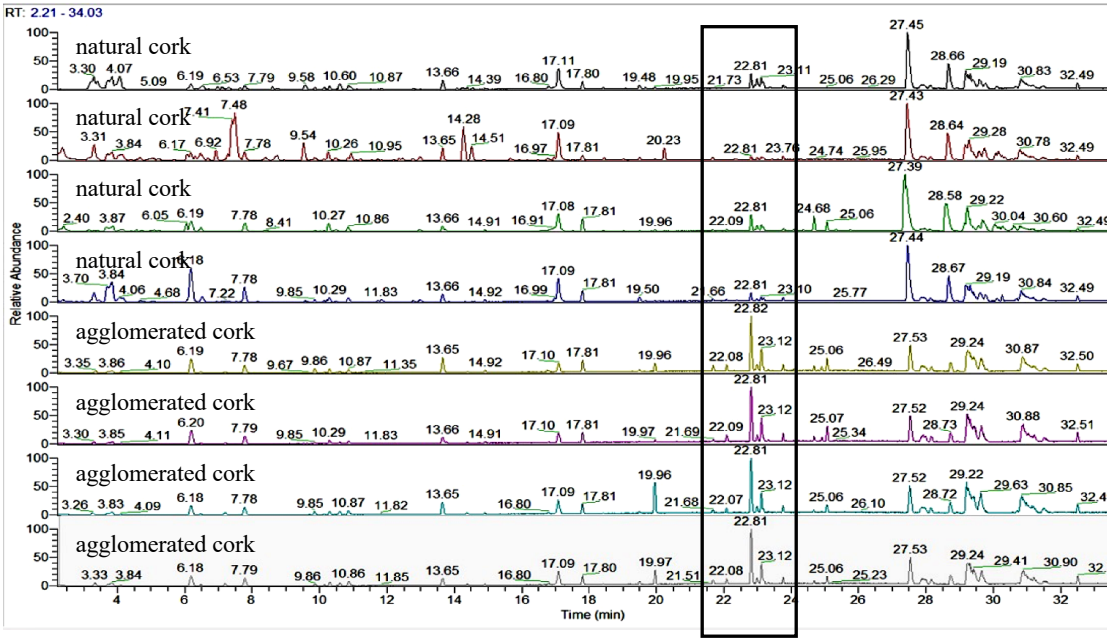
Figure 1: Taste fingerprint obtained by the Astree electronic tongue of two samples of Pinot blanc sealed with an agglomerated cork in comparison with a screw cap as reference.



Tentative identification of flavor-active compounds released by agglomerated corks

Slices of 1 mm thickness were cut from various natural corks and two agglomerated corks and extracted in 20 mL ethanol 10 % over night. The extracts obtained were filtered and analyzed by LC-HRMS. Differential analyses of the chromatograms at different retention times showed more than 40 detected compounds that with a significance level of p=0.05 only occurred in the agglomerated corks. Further examination of the chromatograms yielded specific compounds that were typical for the agglomerated corks and which did not, or only in substantially lower amounts, occur in natural corks (Figure 2). Thus, a search on database for the peak at 22.8 minutes with the mass of 346.2351, corresponding to the molecular formula C_{18}-H_{34}-O_6, proposed 9,10-dihydroxyoctadecanedioic acid (floionic acid) as the responsible compound. This acid is a component of polymeric suberin, the key component of cork (Marques and Perreira 2019). It is not commercially available in its pure form. Therefore, it could not be investigated whether it is responsible for the sensory effects observed.

Figure 2: LC-MS chromatograms of the extracts of four natural corks and four agglomerated corks.



The chromatographic differences between natural and agglomerated corks suggest that the manufacturing process of the latter leads to a change in the substances released. Therefore, the next step was to check which of the detected substances is sensorially active and, above all, migrates from the normal cork surface in contact with wine into the liquid. The indication of the involvement of suberin was very valuable in this regard, because natural cork consists of 45% suberin, whereas the agglomerated corks, according to the manufacturer's specifications, consist of 85% suberin. This means that suberin was concentrated during the manufacturing process of these corks.

Measurements of suberic acid

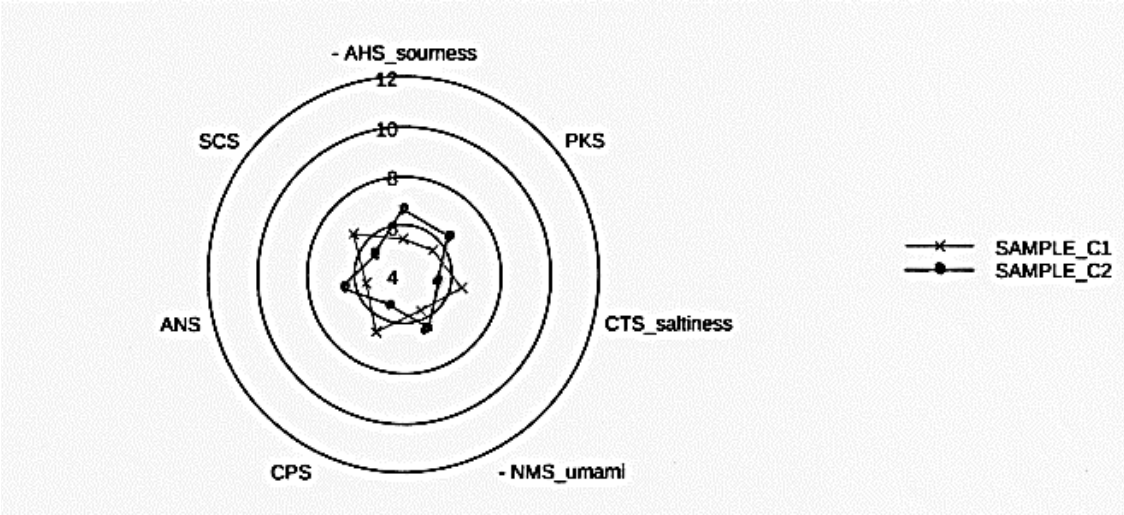
Floionic acid belongs to the group of the so-called cork acids making up the suberin polymer. Suberic acid, also designated as octanedioic acid, is another cork acid which was released in increased and consistent amounts from the agglomerated corks. It is an odorless dicarboxylic acid with the formula COOH-$(CH_2)_6$-COOH and a molar mass of 174.20. It is commercially available in pure form.

The release of suberic acid from the front side of seven agglomerated corks from two manufacturers was quantified. For that purpose, the front side of the corks was soaked in 15 mL of model wine consisting of 13 % ethanol, 4 g/L tartaric acid, and 3 g/L glucose during 7 days at 40° C. According to the Arrhenius equation, these extraction conditions correspond to approximately one month at ambient temperature. Subsequently suberic acid in the model wine was quantified using reversed phase UPLC-MS/MS on two extraction replicates. Concentrations in the 15-mL extracts ranged from 0.062 to 0.108 mg/L, corresponding to 1,240 to 2,160 ng/L in a standard wine bottle of 750 mL.

In order to approximately record the time course of suberic acid release, the same procedure was applied to agglomerated corks of another manufacturer after extraction periods of 7 and 14 days. Suberic acid concentrations measured were 0.062 mg/L in the extract (1,240 ng/L in a standard bottle) after 7 days and 0.087 mg/L in the extract (1,740 ng/L in a standard bottle) after 14 days.

In a subsequent step, wines were spiked with 1,200 to 2,200 ng/L suberic acid (Sigma Aldrich 60930) and submitted to analysis by the electronic tongue in comparison with the unspiked reference. As an example, figure 3 displays the sensory fingerprint obtained on a Cabernet Sauvignon. The two samples turned out not to be identical.

Figure 3: Impact of the addition of 1,600 ng/L suberic acid on the taste fingerprint of Cabernet Sauvignon obtained by an electronic tongue in comparison with the untreated sample.



Taste threshold of suberic acid

Informal tastings were performed after addition of increasing amounts of suberic acid to 40 white and red wine samples. Paired-comparison tests with the untreated reference tasted first showed that additions of 1,400 ng/L suberic acid caused a significant difference. Trained testers are able to respond to less than 200 ng/L. The treated samples displayed a lingering astringency similarly to the results obtained after treatment with agglomerated corks as reported in table 1. Threshold concentrations were more dependent on tasters than on wines.

Astringency elicited by acids

Wine samples treated with some agglomerated corks as referred to in table 1 diverged sensorially from the untreated references by their lingering astringency. In the wine industry, astringency is associated with the action of phenolic compounds, in particular tannins (Gawel 1998). The accepted mechanism evoking astringency is that proteins in saliva combine with vicinal hydroxyl groups of tannins and precipitate (McManus et al. 1981). Many organic acids such as tartaric or suberic acid contain vicinal hydroxyl groups and thus fit this theory. Thus, variable astringency intensities and sub-qualities have been shown for the acids contained in wine (Rubico and McDaniel 1992, Thomas and Lawless 1995), as well as their dependence on pH suggesting that the acidic properties of these acids are an additional cause of their concurrent astringency (Lawless et al. 1996). Suberic acid has similar properties, as can easily be understood when one tastes aqueous solutions containing as little as 1,000 ng/L of it. The widely held view that only phenolic hydroxyl groups elicit astringency is plainly inaccurate.

Whilst astringency is a well-defined tactile sensation, it would be an inadmissible simplification to reduce flavor changes caused by elevated concentrations of suberic acid to astringency alone. Rather, it causes additional changes in the mouthfeel, as can also be seen from the sensory fingerprints of the electronic tongue shown in figures 1 and 3.

Summary

Liquid chromatography-mass spectrometry revealed that agglomerated corks from one manufacturer released more than 40 odorless compounds into the wine, which were not, or only in substantially lower amounts, released by natural corks. One of these compounds has been identified as suberic acid, a key component of the cork suberin polymer. It has also been detected in agglomerated corks from a second manufacturer. This contamination generates sensory changes on the palate described as the tactile perception of lingering astringency. Additional gustative changes caused by suberic acid and agglomerated corks have been confirmed by the use of an electronic tongue. In contrast to cork taint, they are identical in all bottles sealed with the agglomerated corks concerned since their production process delivers product homogeneity. Hence, they do not attract sensory attention when all bottles are sealed with the same cork, although they compromise producers' efforts to create distinctive wines since the wines are uniformly affected by the bottle closure.

References

Blake A., Kotseridis Y., Brindle I.D, Inglis D., Sears M., Pickering G.J., 2009. Effect of closure and packaging type on 3-alkyl-2-methoxypyrazines and other impact odorants of Riesling and Cabernet Franc wines. J Agric Food Chem. 57(11):4680–4690.

Capone D., Sefton M., Pretorius I., Høj P., 2003. Flavour "scalping" by wine bottle closures. – The "winemaking" continues post vineyard and winery. Aust. N.Z. Wine Ind. J. 18 (5): 16-20.

Diekmann J., 1997. Korkgeschmack und Leimton. Agglomeratkorken unter der Lupe. Deut. Weinmagazin 16/17: 26-28.

Gawel R., 1998. Red wine astringency: a review. Aust. J. Grape Wine Res. 4 (2): 74-95.

Guinard J.-X., Pangborn R.M., Lewis M.J., 1986. The time-course of astringency in wine upon repeated ingestion. Am. J. Enol. Vitic. 37 (3): 184-189.

Lau S., O'Mahony M., Rousseau B., 2004. Are three-sample tasks less sensitive than two-sample tasks? Memory effects in the testing of taste discrimination. Percept. Psychophys. 66 (3): 464-474.

Lawless H.T., Horne J., Giasi P., 1996. Astringency of organic acids is related to pH. Chem. Senses 21 (4): 397-403.

Lee C.B. and Lawless H.T., 1991. Time-course of astringent sensations. Chem. Senses 16 (3): 225-238.

Marques A.V. and Perreira H., 2019. A methodological approach for the simultaneous quantification of glycerol and fatty acids from cork suberin in a single GC run. Phytochem. Anal. 30 (6): 687-699.

McManus J.P., Davis K.G., Lilley T.H., Haslam E., 1981. The association of proteins with polyphenols. J. Chem. Soc. Chem. Commun., 7, 309-311.

Noble A.C., 1995. Application of time-intensity procedures for the evaluation of taste and mouthfeel. Am. J. Enol. Vitic. 46 (1): 128-133.

Rodríguez-Méndez M.L., de Saja J.A., Gonzáles-Antón R., García-Hernandez C., Medina-Plaza C., García-Cabezón C., Martín-Pedrosas F., 2016. Electronic noses and tongues in wine industry. Front. Bioeng. Biotechnol. 4, 61. DOI: 10.3389/fbioe.2016.00081

Rubico S.M. and McDaniel M.R., 1992. Sensory evaluation of acids by free-choice profiling. Chem. Senses 17 (3): 273-289.

Simpson R.F., Capone D.L., Sefton M.A., 2004. Isolation and identification of 2-methoxy-3,5-dimethylpyrazine, a potent musty compound of wine corks. J. Agric. Food Chem. 52 (17): 5425-5430.

Sefton M.A. and Simpson R.F., 2005. Compounds causing cork taint and the factors affecting their transfer from natural cork closures to wine – a review. Aust. J. Grape Wine Res. 11 (2): 226-240.

Silva M.A., Julien M., Jourdes M., Teissedre P.-L., 2011. Impact of closures on wine post-bottling development. Eur. Food Res. Technol. 233: 905-914.

Silva M.A., Jourdes M., Darriet P., Teissedre P.-L., 2012. Scalping of light volatile sulfur compounds by wine closures. J. Agric. Food Chem. 60 (44): 10952-10956.

Tarasov A., Giuliani N., Dobrydnev A., Müller N., Volovenko Y., Rauhut D., Jung R., 2019. Absorbtion of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) from wine by bottle closures. Eur. Food Res. Technol. 245: 2343-2351.

Thomas C.J.C. and Lawless H.T., 1995. Astringent subqualities in acids. Chem. Senses 20 (6): 593-600.

Williams A.A. and Arnold G.A., 1991. The influence of presentation factors on the sensory assessment of beverages. Food Qual. Pref. 3 (2): 101-107.

Yang Q. and Ng M.L., 2017. Paired Comparison/Directional Difference Test/2-Alternative Forced Choice (2-AFC) test, simple difference test/same-different test. In: Discrimination Testing in Sensory Science, A Practical Handbook. Woodhead Publishing Series in Food Science, Technology and Nutrition, 2017, pp. 109-134.