UNIVERSITÀ DEGLI STUDI DI PADOVA

Administrative unit: **University of Padova**

Department: **Land, Environment, Agriculture and Forestry (LEAF)**

PhD Program: **Land, Environment, Resources and Health (LERH)**

Batch: **XXXIº**

UNRIPE GRAPE JUICE (VERJUICE): CHEMICAL AND SENSORIAL CHARACTERIZATION AND USE AS A NOVEL FOOD INGREDIENT

Thesis financially supported by **Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)** (grant number: 204483/2014-0)

PhD Program Coordinator: Prof. Davide Matteo Pettenella

Supervisor: Prof. Andrea Curioni

Co-Supervisor: Prof. Simone Vincenzi

PhD candidate: Amanda Dupas de Matos

Università degli Studi di Padova

Sede Amministrativa: **Università degli Studi di Padova**

Dipartimento: **Territorio e Sistemi Agro-Forestali (TESAF)**

CORSO DI DOTTORATO DI RICERCA: **Land, Environment, Resources, Health (LERH)**

Ciclo: **XXXIº**

SUCCO D'UVA ACERBA (VERJUICE): CARATTERIZZAZIONE CHIMICA E SENSORIALE ED USO COME NUOVO INGREDIENTE ALIMENTARE

Tesi redatta con il contributo finanziario del Nome Finanziatore **Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)** (grant number: 204483/2014-0)

Coordinatore: Prof. Davide Matteo Pettenella

Supervisore: Prof. Andrea Curioni

Co-Supervisore: Prof. Simone Vincenzi

Dottoranda: Amanda Dupas de Matos

Table of contents

Acknowledgements 7
Abstract 9
Riassunto 12
Resumo 15
List of abbreviations 18
Introduction 19
A brief story of verjuice 19
Chemical composition 20
Sugars and acids 20
Polyphenols 22
Antioxidant activity and health properties 23
Antimicrobial activity 25
Grape harvest for verjuice production 25
Verjuice production 27
Sensory analysis methods 28
References 30
Aim of the thesis 39

CHAPTER 1
CHEMICAL AND SENSORY ANALYSIS OF VERJUICE: AN ACIDIC FOOD INGREDIENT OBTAINED FROM UNRIPE GRAPE BERRIES 41
Abstract 42
1. Introduction 43
2. Materials and methods 44
2.1 Production of unripe grape juice 44
2.2 Chemical analysis 45
2.3 Sensory evaluation 45
2.4 Statistical analysis 46
3. Results and discussion 46
3.1 Yield of juice 46
3.2 Chemical composition of unripe grape juice 47
3.3 Color 49
3.4 Sensory analysis 50
3.4.1 Effect of harvest time 50
3.4.2 Effect of stabilization treatment 51
3.4.3 Effect of grape variety 52
4. Conclusions 53
References 54
Supplementary material 57

CHAPTER 2

USE OF VERJUICE AS AN ACIDIC SALAD SEASONING INGREDIENT: EVALUATION BY CONSUMERS' LIKING AND CHECK-ALL-THAT-APPLY

.... **60**
Abstract **61**
1. Introduction **62**
2. Materials and methods **63**
2.1 Production of verjuice *63*
2.2 Hedonic responses and sensory characterization of salad seasonings by CATA *63*
2.3 Statistical analyses *65*
3. Results **65**
3.1 Consumers' acceptability of different salad seasonings *65*
3.1.1 Overall liking assessment 65
3.1.2 Check-All-That-Apply (CATA) counts 67
3.1.3 Relating CATA with liking by PLSR analysis 68
3.1.4 Penalty analysis for verjuice 69
4. Discussion **70**
5. Conclusions **73**
References **74**

CHAPTER 3

SENSORY CHARACTERIZATION OF CUCUMBERS PICKLED WITH VERJUICE AS NOVEL ACIDIFYING AGENT **79**
Abstract **80**
1. Introduction **80**
2. Materials and methods **82**
2.1 Production of verjuice *82*
2.2 Production of pickled cucumber *82*
2.3 Analytical characterization *83*
2.3.1 Compositional data 83
2.3.2 HPLC analysis 83
2.3.3 Color measurement 83
2.3.4 Texture measurement 83
2.4 Sensory evaluation *84*
2.4.1 Sample preparation and presentation 84
2.4.2 Panel composition 84
2.4.3 Consumer test 85
2.5 Statistical analysis *86*
3. Results and discussion **86**
3.1 Product analytical characterization *86*
3.2 Sensory evaluation *88*

3.2.1 Descriptive analysis (DA) test 88
3.2.2 Temporal Dominance of Sensations (TDS) 89
3.2.3 Consumers: Liking and JAR scales 91
4. Discussion **92**
4.1 DA analysis *93*
4.2 TDS test *96*
5. Conclusions **99**
References **100**

CHAPTER 4
DEVELOPMENT OF A STABILIZATION PROCESS TO PROTECT VERJUICE AGAINST MICROBIAL SPOILAGE BY USING ONLY ORGANIC ACIDS **105**
Abstract **106**
1. Introduction **107**
2. Materials and Methods **108**
2.1 Yeast strains and media *108*
2.2 Yeast growth *108*
2.3 Model verjus *109*
2.4 Assessment of yeast survival in model verjus *109*
3. Results and discussion **109**
4. Conclusion **113**
References **114**
Supplementary Material **117**

General discussion and conclusions **119**
Appendix **123**

Acknowledgements

Firstly, I would like to thank the “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for the financial support through the Program called “Ciências sem Fronteiras” to my research activity in the “Dottorato di Ricerca in Territorio, Ambiente, Risorse e Salute” at Padova University.

To my supervisor, Prof Andrea Curioni, for giving me the opportunity to work with him on such innovative research project, and for his helpful considerations and discussions about the experiments during these three years. He always provided me encouragement, good ideas, and sound advices.

My special thanks goes to my co-supervisor, Prof Simone Vincenzi, for his constant presence, teaching and assistance in the laboratory. His way to explain things clearly and simply was fundamental in these years. Thanks in particular to Dr Matteo Marangon for his precious collaboration with my research activities. His priceless support was very important for the success of this thesis.

I also wish to thank Massimiliano Magli for the support in some experimental study designs related to the sensory aspects and statistical analyses. It was a pleasure to collaborate with him through the “Consiglio Nazionale delle Ricerche” (CNR) in Bologna.

My gratitude goes to Prof Alan Bakalinsky and the entire staff of Oregon State University, where I spent unforgettable six months. Thanks for his scientific support, weekly meetings, and the critical review of part of this manuscript.

My sincere thanks to my colleagues of Padova University especially from Conegliano campus for their hospitality and patience with me during the learning period of the Italian language. You all taught me a lot and I am very grateful for that. A special thanks to Milena Carlot, your friendship means so much to me.

Thanks to the undergraduate students for providing a stimulating and fun environment. I also want to thank all my friends to have shared good and bad moments with me. Your emotional support was essential during these years. Lastly, and most importantly, I would like to express my gratitude to my beloved parents, Armando de Matos e Maria Ivone Dupas de Matos, for always having put faith in my dreams. To them I dedicate this thesis.

"We have flown like birds and swum like fishes, but we have yet to learn walking like brothers"
- Martin Luther King

"Voamos como pássaros e nadamos como peixes, mas ainda precisamos aprender a caminhar como irmãos"
- Martin Luther King

Abstract

Unripe grapes can be used to produce an extract called verjuice, which is an unfermented high-acidity liquid. Despite a long history of use in ancient times, only recently verjuice has been rediscovered as a food ingredient to be used as an alternative to other acidic liquids such as vinegar and lemon juice in many food preparations. In addition, this product has received increasing attention because it is considered a versatile and healthy acidifying ingredient of great interest for the food industry and the grape growers as it can be made by thinned grapes, an unused by-product of viticulture.

This thesis is focused on the characterization of the chemical and sensorial properties of verjuice, and on its possible use as an alternative to lemon juice and vinegar as salad seasoning and as an alternative to vinegar for preservation of pickled vegetables.

Different issues have been faced during this three-year study.

Firstly, verjuice made from six different grape varieties, harvested at three different dates (from the bunch closure stage to the early veraison), and stabilized using two different preservatives (sulfur dioxide or sorbate) were subjected to compositional and sensory analyses in order to define the suitability of different grape varieties and the best conditions for verjuice production. The results showed that the harvest date, i.e. the degree of grape ripening, affected verjuice pH, organic acids and sugars contents. The harvest date influenced verjuice taste (acidity and sweetness) but did not affect its aroma (which was described as pear, cooked apple and herbaceous). The type of preservative used had no effect on taste, but affected the aroma, and also the color of verjuice at all harvest dates. Finally, the six grape varieties evaluated here made products of comparable sensorial quality.

The second part of the research focused on the identification of the drivers of consumers' liking of verjuice when used as salad seasoning in comparison to lemon juice and white wine vinegar. The Check-All-That-Apply (CATA) test was used in combination with statistical analyses of the data. This provided complementary results including a sensory map of the samples, the explanation of the variance studying the relationship between CATA question and liking data, information on the overall liking associated with a deviation from the ideal product for each attribute of the CATA question, and the maximum potential improvement of overall liking to be used for future reformulation of the product. The large majority of panelists described verjuice-based seasoning as appropriate for salads.

Verjuice and lemon juice were mainly described by lemon and herbaceous aroma, astringent, bitter, high acidity, and “aqueous” viscosity. In contrast, white wine vinegar was mainly described by vinegar and pear aroma. One key outcome is that the overall liking of verjuice- and lemon juice-seasoned salads were not significantly different, suggesting that verjuice can be an alternative to lemon juice as salad seasoning. Moreover, the lack of the pungent smell of acetic acid of verjuice could make it the preferred salad seasoning for those consumers disliking the smell of vinegar as well as a more suitable ingredient for seasoned foods-wine pairings.

The third part of the research investigated the suitability of verjuice as the acidifying agent in vegetable preserves. This was assessed by using as a model pickled cucumbers, which were industrially produced. The chemical and sensorial properties of the pickled cucumbers with verjuice were evaluated in comparison to samples pickled with vinegar, the acidifying agent normally used for their preservation. Several types of sensory analysis were employed to profile the different products and to get more information about the drivers of liking and reformulation priorities. Overall liking scores showed that pickles with verjuice were appreciated as the standard commercial product processed with vinegar. The descriptive analysis showed that appearance and texture of cucumbers pickled with verjuice and vinegar were similar, whereas smell (cucumber and vinegar smells), taste (acidity and saltiness), tactile (astringency and pungency), and aroma (vinegar and cooked vegetable aromas) were different among the samples. In addition, the verjuice concentration affected pickles’ acidity, pungency, and vinegar aroma. Pickles in verjuice showed less issues for reformulation than those in vinegar. Therefore, the possibility to obtain pickled vegetable products in which the absence of acetic acid would enhance their sensorial features, at least for some consumers, was assessed, thus opening the way for the production of a new type of preserved foods.

Despite the increasing interest in using verjuice in several food applications, this product does not have a recognized standard of identity nor is made by standardized methods. In general, verjuice has high levels of acidity, and low pH, but it also contains fermentable sugars which make this product microbiologically unstable because the relatively acid-tolerant wine yeast *Saccharomyces cerevisiae* can sometimes grow in it. Consequently, verjuice must be protected against the risk of fermentation occurring in the bottles. Therefore, the last part of the research focused on developing a stabilization process using some combinations of tartaric and malic acids to maximize the antimicrobial activity of the organic acids. The results showed that tartaric acid was more toxic to yeast than malic acid, but its toxicity towards wine yeast was potentiated by malic acid, leading to the disappearance viable cells in about 30 days. Thus, this study identified a simple and inexpensive approach to increase the microbiological stability of verjuice, avoiding the use of other chemical or physical treatments.

Although verjuice has a long history of use as a food ingredient, it is a relatively unknown product yet. The research reported in this thesis focused on some key features of verjuice, which at the moment does not have a regulation concerning its production. The fact that verjuice lacks acetic acid, but contains tartaric and malic acids, makes its acidity similar to that present in wine, and indicates its suitability as a substitute of vinegar as salad seasoning and as pickling liquid for vegetables when the sensorial impact of acetic acid is not desired, mainly in food-wine pairing. Indeed, food products containing vinegar seem to be losing popularity among consumers, as noted by some Italian producers of pickles, and therefore, as shown here, verjuice can be considered a valid substitute to vinegar for novel food preparation.

Riassunto

Le uve acerbe possono essere utilizzate per produrre un succo chiamato verjuice, che è un liquido non fermentato ad alta acidità. Nonostante ci sia una lunga storia di utilizzo fin dai tempi antichi, solo recentemente il verjuice è stato riscoperto come ingrediente alimentare da utilizzare come alternativa ad altri liquidi acidi come aceto e succo di limone in molte preparazioni alimentari. Inoltre, questo prodotto ha ricevuto crescente attenzione da parte dell'industria alimentare e dai viticoltori perché è considerato un ingrediente acidificante versatile e salutare, di interesse anche in quanto può essere prodotto da uve diradate, un sottoprodotto inutilizzato della viticoltura.

Questa tesi è incentrata sulla caratterizzazione chimica e sensoriale del verjuice e sul suo possibile uso come condimento per insalata e come alternativa all'aceto per la produzione di conserve vegetali.

In primo luogo, il verjuice ottenuto da sei diverse varietà di uva, raccolte in tre diversi momenti (dalla fase di chiusura del grappolo all'inizio dell'invaiatura) e stabilizzato con due diversi conservanti (anidride solforosa e sorbato) sono stati sottoposti ad analisi chimiche e sensoriali (analisi descrittiva) per definire l'idoneità di diversi vitigni e le migliori condizioni di produzione. I risultati hanno dimostrato che la data di raccolta, cioè il grado di maturazione dell'uva, determina il pH e il contenuto di acidi organici e zuccheri del verjuice. La data di raccolta influenza il gusto (acidità e dolcezza), ma non l'aroma (descritto come pera, mela cotta ed erbaceo). Il tipo di conservante usato non ha avuto alcun effetto sul gusto, ma ha influenzato l'aroma e anche il colore del verjuice in tutte le date di raccolta. Infine, le sei varietà di uva qui valutate hanno prodotto campioni di qualità sensoriale comparabile.

La seconda parte della ricerca si è concentrata sull'identificazione dei *drivers* per il gradimento del verjuice da parte dei consumatori quando viene usato come condimento per insalata rispetto al succo di limone e all'aceto di vino bianco. E' stato utilizzato Il test *Check-All-That-Apply (CATA)* in combinazione con diverse analisi statistiche dei dati. Ciò ha fornito risultati complementari tra cui una mappa sensoriale dei campioni, la spiegazione della varianza attraverso lo studio della relazione tra la domanda CATA e i dati del gradimento, informazioni su gradimento generale associato alla deviazione dal prodotto ideale per ogni attributo della domanda CATA, e le possibilità di miglioramento potenziale da utilizzare per la riformulazione futura del prodotto. La grande maggioranza dei membri del panel ha descritto il condimento a base di verjuice come appropriato per le insalate. Verjuice e il

succo di limone sono stati principalmente descritti dai descrittori aroma di limone ed aroma erbaceo, astringente, amaro, alta acidità e viscosità "acquosa". Al contrario, l'insalata condita con aceto di vino bianco è stata descritta principalmente dai descrittori aromatici aceto e pera. Un risultato chiave è che il gusto generale delle insalate condite con succo di limone e verjuice non sono stati significativamente diversi, suggerendo che il verjuice può essere un'alternativa al succo di limone come condimento per insalata. Inoltre, la mancanza dell'odore pungente dell'acido acetico nel verjuice potrebbe renderlo preferibile come condimento per l'insalata per quei consumatori che non gradiscono l'odore dell'aceto così come un ingrediente più adatto per gli abbinamenti cibo-vino.

La terza parte della ricerca ha riguardato l'idoneità del verjuice come agente acidificante nelle conserve vegetali. Questo tema è stato affrontato utilizzando come modello conserve di cetrioli, che sono stati prodotte industrialmente. Le proprietà chimiche e sensoriali dei cetrioli in conserva con verjuice sono state valutate in confronto ai campioni sotto aceto, l'agente acidificante normalmente utilizzato per la loro conservazione. Sono stati impiegati diversi tipi di analisi sensoriale per caratterizzare i diversi prodotti ed ottenere informazioni sul gradimento e le priorità per una eventuale riformulazione. I punteggi del gradimento hanno dimostrato che i cetriolini conservati con verjuice sono stati apprezzati come il prodotto commerciale standard elaborato con l'aceto. L'analisi descrittiva ha dimostrato che l'aspetto visivo e la consistenza dei cetrioli conservati con verjuice e con aceto sono simili, mentre l'odore (odori di cetriolo e di aceto), il sapore (acidità e sapidità), la sensazione tattile (astringenza e pungente), e l'aroma (aroma di aceto e di vegetale cotto) sono diversi tra i campioni. Inoltre, la concentrazione di verjuice ha influenzato l'acidità, la pungenza e l'aroma di aceto dei campioni. I cetriolini con verjuice hanno dimostrato meno problemi per la riformulazione rispetto a quelli con aceto. Pertanto, si è stabilita la possibilità di ottenere prodotti vegetali "sottaceti" in cui l'assenza di acido acetico potrebbe esaltare le caratteristiche sensoriali tipiche dellla materia prima utilizzata, aprendo così una nuova strada per la produzione di un nuovo tipo di alimento conservato.

Nonostante il crescente interesse nell'uso del verjuice in diverse applicazioni alimentari, questo ingrediente non ha metodi di produzione standardizzati. In generale, il verjuice contiene piccole quantità di zuccheri fermentescibili che rendono questo prodotto microbiologicamente instabile. In particolare il lievito *Saccharomyces cerevisiae*, relativamente acido tollerante, a volte può svilupparsi, provocando alterazioni dalle quali il verjuice deve essere protetto. Per risolvere questo problema, l'ultima parte della ricerca si è concentrata sul processo di stabilizzazione. A tal fine l'approccio utilizzato si è basato sull’uso di combinazioni di acidi tartarico e malico per massimizzare l'attività antimicrobica degli acidi organici. I risultati hanno dimostrato che l'acido tartarico è tossico per il lievito e che la sua tossicità è potenziata dalla combinazione con l'acido malico, portando alla scomparsa delle cellule vitali in circa 30 giorni. Pertanto, questo studio ha identificato un approccio semplice ed economico per aumentare la stabilità microbiologica del verjuice, evitando l'uso di altri trattamenti chimici o fisici.

Anche se il verjuice ha una lunga storia di uso come ingrediente alimentare, è ancora considerato un prodotto relativamente poco studiato. La ricerca riportata in questa tesi si è concentrata su alcune caratteristiche chiave del verjuice, che al momento non ha un metodo di produzione definito. Il fatto che il verjuice sia privo di acido acetico, ma contenga acidi tartarico e malico, fa sì che la sua acidità sia simile a quella presente nel vino ed indica la sua appropriatezza come sostituto dell'aceto nei condimenti e per la conservazione di verdure, soprattutto quando l'impatto sensoriale dell’acido acetico non è desiderato e principalmente negli abbinamenti cibo-vino. Infatti, i prodotti alimentari che contengono aceto sembrano perdere popolarità tra i consumatori, come notato da alcuni produttori Italiani di sottaceti, e quindi, come qui dimostrato, il verjuice ha la potenzialità per essere un valido sostituto dell'aceto per nuove preparazione alimentari.

Resumo

As uvas imaturas podem ser usadas para produzir o verjuice, um extrato líquido não fermentado com alta acidez. Apesar de existir uma longa história de uso desde os tempos antigos, somente recentemente o verjuice tem sido redescoberto como um ingrediente alternativo a outros líquidos ácidos como vinagre e suco de limão em muitas preparações alimentícias. Além disso, esse produto tem recebido cada vez mais atenção pois é considerado um ingrediente acidificante versátil e saudável, de grande interesse para a indústria alimentícia e para os produtores de uva, uma vez que pode ser feito a partir de uvas desbastadas, um subproduto não utilizado da viticultura.

Esta tese está focada na caracterização das propriedades químicas e sensoriais do verjuice e na sua possível utilização como alternativa ao suco de limão e vinagre como condimento de salada e também como alternativa ao vinagre para a preservação de vegetais em conserva.

Diferentes questões foram levantadas no decorrer desses três anos de pesquisa.

Em primeiro lugar, o verjuice feito a partir de seis castas diferentes, colhidas em três momentos distintos (da fase de fechamento do cacho de uva até o início do *veraison*) e estabilizadas com dois conservantes diferentes (dióxido de enxofre ou sorbato) foram submetidos a análises composicionais e sensoriais para definir a adequação de diferentes variedades de uvas e as melhores condições para sua produção. Os resultados mostraram que a data de colheita, ou seja, o grau de maturação da uva, afetou o pH do verjuice, os teores de ácidos orgânicos e açúcares. A data da colheita influenciou o gosto do verjuice (acidez e doçura), mas não afetou seu aroma (que foi descrito como pêra, maçã cozida e herbáceo). O tipo de conservante utilizado não teve efeito sobre o sabor, mas afetou o aroma e também a cor do verjuice independentemente da data de colheita. Por fim, as seis castas de uvas avaliadas nessa tese resultaram em produtos de qualidade sensorial comparável.

A segunda parte da pesquisa concentrou-se na identificação dos direcionadores de agradabilidade de verjuice pelos consumidores quando empregado como condimento de salada em comparação ao suco de limão e vinagre de vinho branco. O teste CATA (*Check-All-That-Apply*) foi usado em combinação com as análises estatísticas dos dados. Isso forneceu resultados complementares, incluindo um mapa sensorial das amostras, a explicação da variância estudando a relação entre a pergunta CATA e os dados de

agradabilidade, informações sobre a aceitação global associadas a um desvio do produto ideal para cada atributo da questão CATA e o máximo potencial de melhoria da agradabilidade geral a ser utilizado para uma futura reformulação do produto. A maioria dos painelistas descreveu o condimento a base de verjuice como apropriado para ser utilizado em saladas. O verjuice e o suco de limão foram descritos principalmente por aroma de limão e herbáceo, adstringente, amargo, altamente ácido e “aquoso” como viscosidade. Em contraste, o vinagre de vinho branco foi descrito principalmente por aroma de vinagre e de pêra. Um dos principais resultados é que a agradabilidade geral das saladas temperadas com suco de limão e verjuice não foi significativamente diferente, sugerindo que o verjuice pode ser uma alternativa ao suco de limão como condimento para saladas. Além disso, a ausência do odor pungente de ácido acético no verjuice pode torná-lo um condimento preferido pelos consumidores que não gostam do odor de vinagre, bem como um ingrediente mais adequado para ser utilizado em harmonizações entre vinho e comida.

A terceira parte da pesquisa investigou a adequação do verjuice como agente acidificante em conservas vegetais. Isto foi avaliado usando pepinos em conserva produzidos industrialmente como modelo. As propriedades químicas e sensoriais dos pepinos em conserva com verjuice foram avaliadas em comparação a amostras semelhantes em conserva com vinagre, o agente acidificante normalmente utilizado para preservação. Diversos tipos de análise sensorial foram empregados para definir o perfil dos diferentes produtos e obter mais informações sobre os direcionadores das prioridades de agradabilidade e de reformulação. No geral, os resultados mostraram que os pepinos em conserva com verjuice foram apreciados da mesma forma que o produto comercial conservado com vinagre. A análise descritiva mostrou que a aparência e a textura dos pepinos em conserva foram semelhantes, enquanto o olfato (cheiro de pepino e de vinagre), o sabor (ácido e salgado), a sensação tátil (adstringência e pungência) e o aroma (aroma de vinagre e de vegetal cozido) foram diferentes entre as amostras. Além disso, a concentração de verjuice utilizada na conserva de pepino afetou a acidez, a pungência e o aroma de vinagre. Conservas com verjuice mostraram menos problemas para reformulação do que aqueles preparados com vinagre. Portanto, foi avaliada a possibilidade de obter vegetais em conserva onde a ausência do ácido acético poderia realçar as características sensoriais do produto, pelo menos para alguns consumidores, abrindo assim um caminho para a produção de uma nova tipologia de alimentos em conserva.

Apesar do crescente interesse em usar o verjuice em várias aplicações alimentares, este produto não possui um padrão reconhecido de identidade nem é feito por métodos padronizados. Em geral, o verjuice tem altos níveis de acidez, baixo pH, e também contém açúcares fermentáveis tornando este produto microbiologicamente instável, uma vez que a levedura *Saccharomyces cerevisiae* é relativamente tolerante a ácidos, podendo se desenvolver neste meio. Consequentemente, o verjuice deve ser protegido contra o risco de fermentação que pode ocorrer uma vez engarrafado. Sendo assim, a última parte da pesquisa focou no desenvolvimento de um processo de estabilização utilizando algumas combinações de ácidos tartárico e málico para maximizar a atividade antimicrobiana dos ácidos orgânicos. Os resultados mostraram que o ácido tartárico foi mais tóxico do que o ácido málico, mas sua toxicidade em relação à levedura inoculada foi potencializada pelo ácido málico, levando ao desaparecimento de células viáveis presentes no verjuice após cerca de 30 dias. Assim, este estudo identificou uma abordagem simples e acessível para aumentar a estabilidade microbiológica do verjuice, evitando o uso de outros tratamentos químicos ou físicos.

Embora o verjuice possua uma longa história de uso como ingrediente alimentar, ainda é um produto relativamente desconhecido. A pesquisa reportada nesta tese focou em algumas características-chave do verjuice, que no momento não possui uma regulamentação relativa à sua produção. O fato do verjuice não conter ácido acético, mas conter ácidos tartárico e málico, faz com que a sua acidez seja semelhante à acidez do vinho e indica sua adequação como substituto do vinagre em condimento de saladas, assim como líquido de preservação em conservas vegetais, quando o impacto sensorial do ácido acético não é desejado, principalmente na harmonização de comida-vinho. De fato, os produtos alimentícios que contêm vinagre na sua formulação parecem estar perdendo popularidade entre os consumidores, como tem sido observado por alguns produtores Italianos de conservas vegetais. Dessa forma, como demonstrado neste trabalho, o verjuice pode ser considerado um substituto válido do vinagre para preparação de novos alimentos.

List of abbreviations

A_{420}: Absorbance reading at 420 nm

CA: Correspondence analysis

CATA: Check-All-That-Apply

DA: Descriptive Analysis

JAR: Just-About-Right

M: Malic acid

PLSR: Partial Least Square Regression

PM: Potassium Metabisulphite

PS: Potassium Sorbate

SCE: Specular Component Excluded

T: Tartaric acid

T_1: Grape bunch closure growth stage

T_2: 15 days after T_1

T_3: Grape early veraison growth stage

TDS: Temporal Dominance of Sensations

VER1:10: Pickled cucumber with verjuice diluted 1:10 (v/v) with water

VER1:2: Pickled cucumber with verjuice diluted 1:2 (v/v) with water

VIN: Pickled cucumber with vinegar

YEPD: Yeast Extract Peptone Dextrose

YNB: Yeast Nitrogen Base

Introduction

Grape is one of the world's largest fruit crops, being approximately 80% of its yield utilized for winemaking (Mildner-Szkudlarz et al., 2010). Grape growing and wine making produce of a large amount of wastes and by-products such as stalks, seeds, pomace, marc and lees. One undervalued by-product are also the unripe grapes derived from clusters thinning (Arvanitoyannis et al., 2006; Fia et al., 2018). Cluster thinning comprises the removal of grape clusters to improve the leaf area/fruit balance (Jackson, 2018). This widely accepted agronomic practice is performed for two main purposes: to achieve a yield balance in the vine and improve the quality of grapes (fruit set and composition) as well as to prevent overcropping (Dokoozlian & Hirschfelt, 1995; Ferree et al., 2003; Jackson, 2008; Valdés et al., 2008; Wang et al., 2018). The useful period of fruit thinning is normally between berry setting and veraison, which precedes the sugars accumulation phase.

In this context, the industrial wine sector has looked for alternatives to convert waste materials into food ingredients/products with high added value. One great example is the use of thinned unripe grapes, which are traditionally processed into two major products: verjuice and sour grape sauce (Dupas de Matos et al., 2017). The verjuice (or verjus) is then an unfermented grape juice obtained by pressing unripe berries, while sour grape sauce is derived from verjuice that undergoes an additional concentration step (Öncül & Karabiyikli, 2015). Thus, the utilization of thinned grapes represents an opportunity to produce a food product of great interest to grape growers and related industries besides to convert a waste into a product of value.

A brief story of verjuice

Verjuice is an unfermented juice made by pressing unripe grape berries whose culinary and medicinal uses date back to very ancient times (Setorki et al., 2010). The name verjuice derives from the French expression "vert jus" ("green juice"), and refers to a product characterized by high acidity and tart taste. It is mostly known as "verjuice" in English, but also called "agraz" in Spanish, and "agresto" in Italian (Nikfardjam, 2008; Hayoglu et al., 2009; Alipour et al., 2012). Derived from *Vitis vinifera* grapes, verjuice has a long history of use. With culinary and medicinal uses during Middle Age and Renaissance, verjus was used by the Romans before the discover of vinegar (Simone et al., 2013). Although abandoned in the in the west European countries, this ingredient is still used in the oriental cuisine, being regionally called “abe ghureh” (Persian: unripe grape water) and “koruk suyu” (Turkish: unripe grape juice) (Nikfardjam, 2008).

Verjuice has been used as digestive drink (Setorki et al., 2010) and is indicated as having some lipid lowering effects, as for example in the Iranian traditional medicine (ZibaeeNezhad et al., 2012). However, verjuice is consumed especially in the Mediterranean and southeastern regions of Turkey, to enhance the flavor of traditional meals as well as an ingredient of various drinks and mustard sauces (Hayoglu et al., 2009).

Indeed, verjuice is characterized by a unique flavor, sour taste, high acidity, and low sugar content (Freixa & Chaves, 2008; Hayoglu et al., 2009). Recently, this product has received more interest in Western countries and it is being currently rediscovered as flavoring condiment, mainly in Australia, United States, and Canada. Furthermore, it can be used as an alternative to vinegar and lemon juice as acidifying and flavoring agents for several meals, salads and appetizers (Setorki et al., 2010).

Chemical composition

Sugars and acids

The most important quality criteria for fruit products are obviously related to their chemical composition. In addition to the minor components deriving from the plant secondary metabolism, such as aroma compounds and polyphenols, which have important effects on fruit flavor and mouthfeel, organic acids and sugars play a major role in sensorial quality. In the case of fruit juices, such as verjuice, these major compounds derive directly from the fruit. In particular, the acid and sugar composition of grapes depends on many factors such as the variety, the soil climate conditions, the applied cultural practices, but mainly the maturity of the berries, which can be defined by using various maturity indices, which are normally based on the evaluation of the ratio between sugar and acid contents (Soyer et al., 2003). The ripe grape berry appropriate for winemaking contains large amounts of sugar, 20–25% of the berry weight and a lower quantity of organic acids (Cheynier et al., 2010). The two main sugars in grape are glucose and fructose, whose concentration change with a significant increase in the proportion of fructose during ripening to reach a ratio (glucose/fructose) close to one at ripeness (Ribéreau-Gayon et al., 2006). Instead, glucose accounts for 85% of the total sugar content of green berries sampled at an early developmental stage and also during veraison glucose predominates over fructose

(Seymor et al., 1993). So, in unripe grape juice, glucose was found to range from about 1 to 5% and fructose from about 0 to 4% (Dokoozlian et al 1996; Sabir et al., 2010).

Soluble sugar content can be determined by refractometer and expressed as degree Brix. According to Codex Alimentarius standard 247-2005 (CODEX, 2005), ripe grape juice must have the minimum concentration of 13.5 °Brix, whereas °Brix value for unripe grape juice was shown to range between 4 and 10 (Hayouglu et al., 2009; Öncül & Karabiyikli, 2015).

Next to sugars, organic acids are the second most abundant solids compounds present in the juice of grapes at the technological maturity for winemaking juice, having a direct effect on its pH and the organoleptic characteristics of the wine (Cheynier et al., 2010). The dominant organic acids in grapes are tartaric and malic acids, which account for 90% or more of the total acidity (Lamikanra et al., 1995; Granato et al., 2016) and are present in ripe berries in quantities of 4-8 and 2-7 g/L, respectively. In unripe grape juices, malic and tartaric acid concentrations are higher compared to those of ripe grapes and typically range between 10-30 and 10-20 g/L, respectively (Dokoozlian et al 1996; Sabir et al., 2010), and can vary according to the grape variety. Therefore, ripening involves a relevant diminution of the organic acid content of grapes. At an early ripening stage of grapes, the amount of tartaric acid is larger than that of malic acid. During the development, a progressive increase in tartaric and malic acid is expected until the veraison, in which their concentrations decrease with ripening. Tartaric acid reduction is usually more gradual and is converted to insoluble salts during the ripening process. In constrast, malic acid is degraded through the respiration process occurring in the berries. Therefore, the decrease in acidity during the ripening results from a number of factors such as increased respiration, reduced translocation of acids from leaves, transformation of acids to other compounds, dilution effect due to increased volume of fruit, and reduced ability of the fruit to synthesize acids with maturity (revised in Lamikanra et al., 1995).

The organic acid content (and their degree of salification) of the juice is related to its pH (Ribéreau-Gayon et al., 2006), which ultimately determines the acidity sensation in the mouth, in addition to many other technological and microbiological characteristics, making this parameter very important for quality. In general, fruit juices tend to have a low pH, which means they are acidic. The pH of lime and lemon juice are about 2.0-2.6, while ripe grape juice is usually below 4.0, depending on the grape variety. For verjuice, pH is much low ranging between 2.1 and 3.0 (Karapinar & Sengun, 2007; Nikfardjam, 2008; Hayoglu et al., 2009; Simone et al., 2013; Öncül & Karabiyikli, 2015).

Polyphenols

Phenolic compounds are plant secondary metabolites that play protective roles against biotic (fungi, pests, etc.) and abiotic (UV light exposure) stresses. Moreover, they affect the organoleptic properties of plant foods, including color and taste characteristics, such as astringency and bitterness. Finally, polyphenolic compounds generally show antioxidant properties, and for this reason they are considered to have health-promoting effects. Indeed, antioxidants have long been recognized to have protective functions against oxidative damages and are associated with a reduced risk of chronic diseases (Adom & Liu, 2002; Liu, 2007).

Grape polyphenols comprise both flavonoids and nonflavonoids compounds (Cheynier et al., 2010). Flavonoids are characterized by a C6-C3-C6 skeleton, consisting of two phenolic rings joined by a central pyran (oxygen-containing) ring, and comprise several groups such as flavones, flavonols, catechins, anthocyanidins, and tannins. Nonflavonoids (possessing a C6-C3 skeleton) are structurally simpler, and are represented in grapes mostly by hydroxybenzoic and hydroxycinnamic acids, including p-coumaric, caffeic, and ferulic acids (Jackson, 2008; Cheynier et al., 2010). In grape berries, the phenolic compounds are distributed in the different parts of the fruit (**Figure 1**).

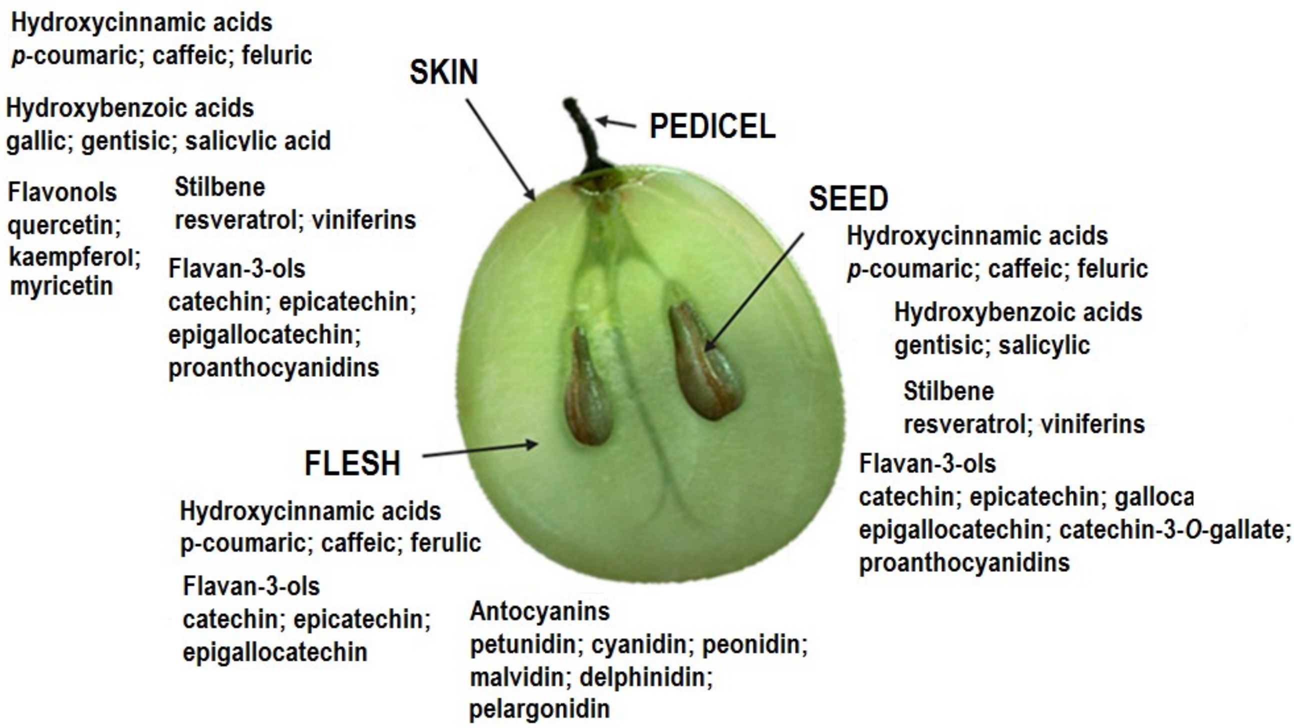

Fig. 1. Localization of the phenolic compounds in the ripe grape berry (Adapted Cosme et al., 2018).

Due to their relevance for wine quality, many studies have been performed on the phenols of ripe grapes (Pastrana-Bonilla et al., 2003; Lima et al., 2014; Granato et al., 2015; Granato et al., 2016; Gutiérrez-Gamboa et al., 2018). In contrast, currently available information on the composition and the characteristics of the phenolic compounds of unripe grapes is relatively scarce.

However, by studying verjuice it has been reported that this unripe grape extract is rich in bioactive polyphenols, which include both flavonoid and non-flavonoid compounds, which are well-known for their health benefits when consumed (Ahmadi & Roney, 2014). These polyphenols were found to include caffeic, caftaric, gallic, fertaric, p-coumaric, p-coutaric, and protocatechic acids and also catechin, epicatechin, quercetin-glucoside, and tyrosol (Nikfardjam, 2008; Bagheri & Esmaiili, 2017). Nevertheless, tyrosol is unexpected to be found in verjuice as this compound is derived from the metabolism of tyrosine by yeasts during fermentation (Sentheshanmuganathan & Elsden, 1957; Soejima et al., 2012), whereas verjuice is considered an unfermented product. Then, the presence of tyrosol can be considered as a marker of unwanted fermentation during the storage period. Even taking in account tyrosol, however, the main polyphenolic compound present in verjuice is caftaric acid, which may account for more than 60% of total polyphenols (Nikfardjam, 2008).

Antioxidant activity and health properties

Antioxidant activity is considered an important property of plant foods, due to its potential positive impact on consumer health (Vauzour et al., 2010; Krikorian et al., 2012). The antioxidant compounds are then considered to be bioactive in relation to their ability to sequester reactive oxygen species (ROS), such as hydroxyl radical and singlet oxygen (Marinova & Yanishlieva, 2003).

However, in most of the cases, this activity is measured only *in vitro* with several assays including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and the oxygen radical absorption capacity (ORAC). Nevertheless, these techniques have shown different results among crop species and across laboratories. For example, Ou et al. (2002) reported no correlation of the results obtained for antioxidant activity between the FRAP and ORAC techniques, whereas the same methods revealed high correlation according to Connor et al. (2002). Similarly, Awika et al. (2003) observed high correlation between ABTS, DPPH, and ORAC.

Due to the antioxidant activity, mainly attributed to stilbenes, phenolic acids and, principally, flavonoids such as anthocyanins, proanthocyanidins, flavanols and flavonols, grape juices (white, rosè and especially purple) have demonstrated beneficial effects to health (Wightman & Heuberger, 2015; Granato et al., 2016). However, Mulero et al. (2010) observed that the antioxidant activity of skin and pulp of organic grapes harvested 30 days before the normal date for wine production was higher than those harvested on that time. More recently, the evaluation of unripe grape juice as potential functional ingredient with antioxidant properties has been undertaken (Shojaee-Aliabadi et al., 2013; Öncül & Karabiyikli, 2015; Tinello & Lante, 2017), showing that the antioxidant activity of unripe grape juices was influenced by grape cultivar and harvest time. All these data indicate that verjuice can be considered as a potential source of natural antioxidants, thus reinforcing the interest for this food ingredient.

As reported above, the antioxidant activity is related to the positive properties that the fruit juices exert on the human health.

Many studies have investigated the positive effects of consuming grape juices, which include, among other, antiproliferative, antithrombotic, neuroprotective and anticonvulsant effects, improvement of Alzheimer's disease, hypocholesteremic properties, and anti-inflammatory (Granato et al., 2016). However, all these studies were performed with mature grapes, while, at the moment, the information about the effects of unripe grape juice (verjuice) for health promotion is scant. In Iranian folk medicine it is believed that verjuice (Ghure juice) has some lipid lowering effects and can control hypertension (ZibaeeNezhad et al., 2013). The possible effect of consuming verjuice on reducing the risk of atherosclerosis was mostly associated with inhibiting oxidative stress and lipid peroxidation by acting an antioxidant (Setorki et al., 2010). The effect on plasma lipids and blood pressure in humans after consuming verjuice was studied (Setorki et al., 2011), showing that verjuice consumption can decrease the negative effects of a cholesterol rich diet and reduce the mean arterial pressure (Mousa-Al-Reza et al., 2011; Zolfaghari et al., 2015).

Moreover, verjuice may have significant beneficial cardio-protective effects through normalization of blood lipids and other cardiovascular disease risk factors in hyperlipidemic patients (Alipour et al., 2012). However, in order to support these findings, further research on the effective dose is required to establish the maximum health benefit deriving from verjuice consumption. Indeed, in order to determine the appropriate quantity required to allow precise recommendations, future researches on humans or animals should include multiple groups receiving different amounts of verjuice with similar phytochemical profiles.

Antimicrobial activity

In recent years, the search for natural antimicrobial compounds has increased, because the food industry is looking for satisfying the consumer's demands for substitution of chemicals with natural means od preservation. Different fruit products, unfermented or fermented, produced from several raw materials such as herbs, crops, spices have been used as natural sanitizers. The antimicrobial effect of various fruit juices such as lemon or pomegranate on pathogenic and/or saprophytic microorganisms have been investigated in many studies. It was showed that the main compounds involved in microbial inhibition are the organic acids and the phenolic compounds in these materials, indicating their potential as natural antimicrobials to be used to prevent microbial spoilage (Saeed & Tariq, 2005; Aibinu et al., 2007; Öncül & Karabiyikli, 2016).

Karapinar and Sengun (2007) investigated the bactericidal activity of "koruk" (Turkish name used for verjuice) against *Salmonella typhimurium* added to vegetable salads. "Koruk" juice exerted an immediate antimicrobial effect on the test microorganisms causing reductions in initial populations, and this effect was dependent on the culture strains and products used. Then, author concluded that "koruk" may be considered a potential alternative to antimicrobial agents at household level for vegetable salads. Karabiyikli and Öncül (2016) have investigated unripe grape products (verjuice and sour grape sauce) as natural antimicrobials due to their organic acids and phenolic contents, and they found that its antimicrobial effects are mainly dependent on their acidity. In another research, the inhibitory effect of unripe grape products on foodborne pathogen have been studied (Oncül & Karabiyikli, 2016). The study concluded that unripe grape products are microbiologically safe and have a self-protection effect even when they were contaminated with pathogens at high levels. Although further studies are needed to determine more deeply the components and conditions involved in the antimicrobial effects, the use of unripe grape products seems to be a promising mean to be adopted for food preservation in substitution of chemicals that are considered "un-natural" by the consumer.

Grape harvest for verjuice production

Despite a long history of use as a food ingredient, verjuice does not have a recognized standard of identity nor the grape ripeness for its production is well-defined. This is a major point of interest, because the variations in the chemical composition of the grape berries during ripening obviously result in differences in the chemical and sensorial properties of the verjuice. For research purposes, picking of unripe grapes to make verjuice has been performed 45 days after flowering when the acidity was at its maximum (Hayoglu et al., 2009) or 30 days before the normal grape harvest date (Shojaee-Aliabadi et al., 2013).

Several studies reported the chemical composition of grapes during the fruit ripening (Dokoozlian et al., 1996; Coombe et al., 2000; Giribaldi et al 2007; Pinlati et al., 2007; Sabir et al., 2010). Taking in consideration the chemical aspects that verjuice, should have to be used as an acidic ingredient (i.e high acidity, very low pH and sugar content) and the chemical composition reported for grapes at different ripening stages, the most appropriated harvest date seems to fall in the range between stage 33 to 35 of the modified E-L phenological classification scheme (Coombe, 1995), in which the berries starts to soften and sugar starts increasing (**Figure 2**). In Italy, this period corresponds to dates between the end of July and beginning of August, which therefore can be expected to be the most suitable picking time for grapes to make verjuice.

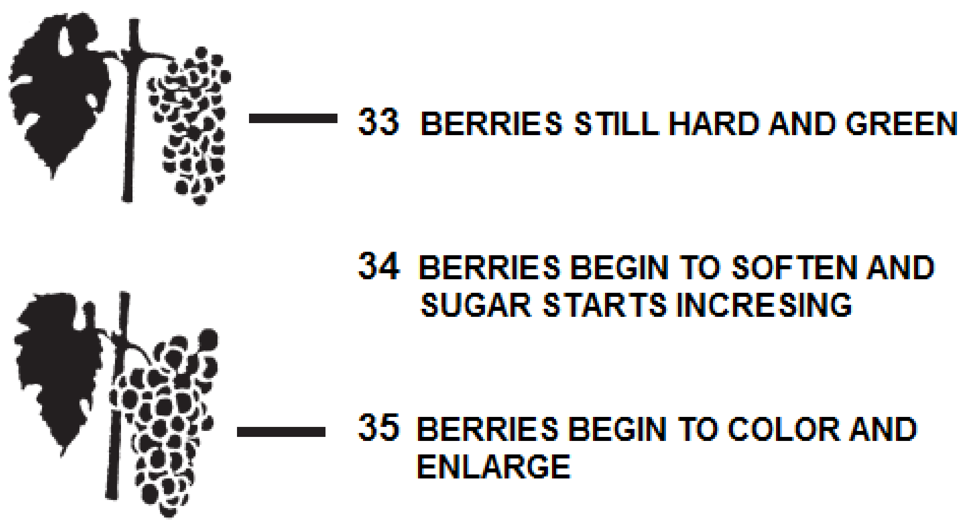

Fig. 2. Modified E-L system for identifying intermediate grapevine growth stages (Adapted Coombe, 1995).

While grape yield is generally seen as an important factor in wine production, for the verjuice production it is not established. However, taking in consideration that the grapes to make verjuice are harvested earlier than those to make wine, the juice yield is much lower. Depending on the grape variety and picking time, unripe grape juice yields is about 50% (Hayoglu et al., 2009). This low yield is consistent and the economic advantage of using grapes for verjuice production has to be established, taking into account all the aspects related to the costs (which are obviously lower compared to those for grapes at the commercial maturity) and the possible incomes deriving from verjuice commercialization.

Verjuice production

Verjuice is currently produced by small grape companies as a handcraft product by applying medieval recipes with the uncertainty of some variables such as grape ripening stage, picking time, and methods of production. In the traditional Turkish production method, unripe grapes are crushed, pressed, sieved and the juice is transferred into the bottles. This type of production is not standardized and needs to be improved to obtain a high quality product to be successfully put on the market. Only a few attempts have been made to achieve this goal. For example, in order to avoid haze formation during storage, verjuice has been clarified using gelatin, and in a previous study olive oil was added on top of the bottle before being stored at cold temperature to prevent oxidation (Hayoglu et al., 2009; Öncül & Karabiyikli, 2015).

A major problem for verjuice shelf-life is its microbial instability. While verjuice has high levels of acidity, due to the early grape harvest, it also contains fermentable sugars, whose concentration is obviously variable depending on the grape picking time (Hayoglu et al., 2009; Shojaee-Aliabadi et al., 2013; Öncül & Karabiyikli, 2015). The presence of sugar can induce fermentation by naturally present acid-tolerant yeasts making verjuice microbiologically unstable and, being easily fermented by naturally present yeasts, which resulting in the onset of turbidity an in a variation of the organoleptic properties. Thus, verjuice must be protected against the risk of fermentation that can occur during storage.

One approach to avoid this problem is the use of sulfites, which are normally used in winemaking to prevent oxidation and microbial spoilage, and also yeast development when used in high amounts. However, due to the very low pH of verjuice, the chemical equilibrium of sulfites would be in favor of the “molecular” SO_2, which is the active form (Stratford & Rose, 1986), thus allowing, in theory, to protect against yeast development also by using amounts of sulfites lower than those used to avoid fermentation in wine musts. In addition, other preservation techniques have been applied to increase the verjuice shelf life as, for example thermal treatments such as pasteurization and sterilization (Çevik et al., 2016). Due to the very low pH of verjuice, all these treatments are very effective in avoiding microbial growth during storage, although this is not guaranteed for the thermal treated verjuice after opening the bottle, which in this case must be stored in the fridge.

Sensory analysis methods

One of the most powerful and extensively applied tools to determine the organoleptic features of foods and beverages is their sensory characterization, which has been used for the development and marketing of new products, possible reformulations, monitoring of sensory characteristics, as well as for estimating shelf-life. Some techniques require a trained panel and the use of a rating system using unstructured scales such as the descriptive analysis.

The Quantitative Descriptive Analysis (QDA®) method is based on the panelist's ability to verbalize perceptions of a product in a consistent manner allowing the survey, description and quantification of sensory attributes (Alcantara & Freitas-Sá, 2018). Panelists are screened and trained for attribute recognition and scaling, in order to use a common and agreed sensory language. The products are scored on repeated trials to obtain a complete quantitative description (Moussaoui & Varela, 2010). Nevertheless, the sensory perception is a dynamic phenomenon, as the perceived intensity of the sensory attributes changes along with the in-mouth transformation of the food (Sudre et al., 2012). Thus, the temporal aspect is a crucial information for understanding consumer preferences. In this context, multiple methods have been developed to investigate the temporality of sensory perception such as Temporal Dominance of Sensations (TDS).

In the TDS method, trained assessors evaluate which sensation is dominant over the time and can score its intensity. Each time the panelist thinks the dominant sensory perception has changed (either in intensity or in quality) he/she has to score the new perception. For each run, this method enables to collect a sequence of sensory attributes (and their respective scoring) quoted at different times along the tasting time. This approach allows monitoring the behavior of the sample when it is broken down and physically transformed in the mouth, as well as the release of flavors and aromas during the time (Pineau et al., 2009; Varela & Ares, 2014). Finally, without asking panelists about intensity, TDS becomes feasible also for a consumer panel (Albert et al., 2012; Castura & Li, 2016).

Due to the cost and time needed for application of sensory tests using trained assessors, several alternative methods have been recently developed. Some tests do not require training and can be performed by consumers, semi-trained, or untrained assessors. It has been considered as a reliable option when quick information about the sensory characteristics of a set of products is needed (Varela & Ares, 2014; Alcantara & Freitas-Sá, 2018).

Information about consumers' perception on the sensory characteristics of food products is often collected with overall liking scores. Moreover, some sensorial methods have been combined to others in order to provide useful information about the sensory characterization of food products and to find key drivers for the development of new foods products as well as its future possible reformulation.

One typical example is the Penalty Analysis (or mean drop analysis), which is an emerging method in the food industry used to provide direction in product development and optimization. Penalty analysis have been combined to Just-About-Right (JAR) and overall liking tests to relate a decrease in consumer acceptance to attributes not at the JAR level (Lawless & Heymann, 2010). JAR scale is a well-established approach to identify the optimum intensity of sensory attributes in a food product (Popper & Kroll, 2005) and is considered as a valid method in market and product development research. Generally, consumers are asked to indicate whether the intensity of a sensory attribute is too strong, too weak or just-about-right using a 5-point bipolar scale.

Alternative approaches to study consumers' perception of the sensory characteristics of products have been recently proposed.

Check-all-that-apply (CATA) questions are currently one of the most popular approaches for getting consumer-based sensory characterizations. In this methodology, consumers are presented with a list of sensory terms (from 10 to 40 terms) and are asked to select all those they consider appropriate for describing the sample. This versatile methodology has been reported to be easy and intuitive for consumers, providing valid and repeatable product information (Ares & Jaeger, 2015) in both academic and industrial applications. Considering that CATA questions are usually included in hedonic tests, the usual number of consumers necessary for obtaining reliable overall liking scores is 100-120 participants. The Ideal Profile method has been developed for identifying the consumer ideal product (Van Trijp et al., 2007). Ares et al. (2014) suggested asking consumers to answer a CATA question to describe the sensory characteristics of their ideal product. When consumers are asked to describe the product and their ideal product, penalty analysis can be used to identify deviations from optimal product improvement when hedonic scores are concurrently collected (Ares & Jaeger, 2015).

These techniques for sensorial characterization have been applied in this thesis, not only to determine the characteristics of verjuice in relation to type of grape variety, degree of ripeness and preservatives used, but also the sensorial effects of using this new ingredient as a food ingredient. In particular, since verjuice can be a valid alternative to lemon juice or vinegar in different food preparations such as salad seasoning and has the chemical characteristics to be used as a preserving liquid in food preserves, our sensorial study evaluated the impact of its use in both salads and pickled cucumber conserves.

References

Adom, K. K., Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, 50:6182-6187.

Ahmadi, L., Roney, S. K. (2014) Pharmacological and Phytochemical Properties of Unripe Grape Juice (Verjuice): A Review. *Austin Journal of Nutrition & Metabolism*, 1(2): id1006.

Aibinu, I., Adenipekun, T., Adelowotan, T., Ogunsanya, T., Odugbemi, T. (2007). Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally. *African Journal of Traditional, Complementary, and Alternative Medicines*, 4(2):185.

Albert, A., Salvador, A., Schlich, P., Fiszman, S. (2012). Comparison between temporal dominance of sensations (TDS) and key-attribute sensory profiling for evaluating solid food with contrasting textural layers: Fish sticks. *Food Quality and Preference*, 24(1):111-118.

Alcantara, M. De, & Freitas-Sá, D. D. G. C. (2018). Metodologias sensoriais descritivas mais rápidas e versáteis – uma atualidade na ciência sensorial. Brazilian Journal Food Technology, 21, 1–12.

Alipour, M., Davoudi, P., Davoudi, Z. (2012). Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal, hyperlipidemic and hyperlipidemic with hypertensive human volunteers. *Journal of Medicinal Plants Research*, 6(45):5677-5683.

Ares, G., Etchemendy, E., Antúnez, L., Vidal, L., Giménez, A., & Jaeger, S. R. (2014). Visual attention by consumers to check-all-that-apply questions: Insights to support methodological development. Food Quality and Preference, 32, 210–220.

Ares, G., Jaeger, S.R. (2015). Check-all-that-apply (CATA) questions with consumers in practice: experimental considerations and impact on outcome. In: Delarue J, Lawlor B, Rogeaux M, editors. *Rapid Sensory Profiling Techniques*. Cambridge: Woodhead Publishing, 227–45.

Arvanitoyannis, S. I., Ladas, D., Mavromatis, A. (2006). Potential uses and applications of treated wine waste: a review. *International Journal of Food Science and Technology*, 41:475-487.

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., CisnerosZevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (Sorghum bicolour) and sorghum products. *Journal of Agricultural and Food Chemistry*, 51:6657-6662.

Bagheri, H., Esmaiili, M. (2017). Ultrasound-Assisted Extraction of Phenolic Compounds from Unripe Grape (Qora). Erwerbs-Obstbau.

Castura, J. C., Li, M. (2016). Using TDS dyads and other dominance sequences to characterize products and investigate liking changes. *Food Quality and Preference*, 47:109-121.

Çevik, M., Tezcan, D., Sabancı, S., İçie, F. (2016). Changes in Rheological Properties of Koruk (Unripe Grape) Juice Concentrates During Vacuum Evaporation. *Akademik Gıda*, 14(4):322-332.

Cheynier, V., Schneider, R., Salmon, J-M., Fulcrand, H. (2010). Chemistry of wine. Chapter 3.26 - Chemistry of Wine, In: *Comprehensive Natural Products II*, Elsevier, Oxford, p.1119-1172, ISBN 9780080453828.

Codex Alimentarius. CODEX general standard for fruit juices and nectars (CODEX STAN 247-2005). Available at http://www.codexalimentarius.org/input/

Connor, A. M., Luby, J. J., Tong, C. B. S. (2002). Variability in antioxidant activity in blueberry and correlations among different antioxidant activity assays. *Journal of the American Society for Horticultural and Science*, 127:238-244.

Coombe, B. G. (1995). Adoption of a system for identifying grapevine growth stages. *Australian Journal Grape Wine Research*, 1:100-110.

Coombe, B. G.; McCarthy, E. M. G. (2000). Dynamics of grape berry growth and physiology of ripening. *Australian Journal of Grape and Wine Research*, 6(2):131-135.

Cosme, F., Pinto, T., Vilela, A. (2018). Phenolic Compounds and Antioxidant Activity in Grape Juices: A Chemical and Sensory View. *Beverages*, 4:22.

Dokoozlian, N. K., Hirschfelt, D. J. (1995). The Influence of Cluster Thinning at Various Stages of Fruit Development on Flame Seedless Table Grapes. *American Journal of Enology and Viticulture*, 46:4.

Dokoozlian, N. K., Kliewer, W. M. (1996). Influence of Light on Grape Berry Growth and Composition Varies during Fruit Development. *Journal of the American Society for Horticultural Science*, 121:869-874.

Ferree, D. C., Cahoon, G. A., Scurlock, D. M., Brown, M. V. (2003). Effect of Time of Cluster Thinning Grapevines. *Small Fruits Review*. 2(1):1-14.

Fia, G., Gori, C., Bucalossi, G., Borghini, F., Zanoni, B. (2018). A Naturally Occurring Antioxidant Complex from Unripe Grapes: The Case of Sangiovese (v. *Vitis vinifera*). *Antioxidants*, 7:27.

Freixa, D., Chaves, G. (2008). Gastronomia no Brasil e no mundo. Rio de Janeiro: Senac Nacional, 304 p.

Giribaldi, M., Perugini, I., Sauvage, F., Schubert, A. (2007). Analysis of protein changes during grape berry ripening by 2-DE and MALDI-TOF. *Proteomics*, 7:3154-3170.

Granato, D., Margraf, T., Brotzakis, I., Capuano, E., Ruth, S. M. (2015). Characterization of Conventional, Biodynamic, and Organic Purple Grape Juices by Chemical Markers, Antioxidant Capacity, and Instrumental Taste Profile. *Journal of Food Science*, 80: C55-C65.

Granato, D., Carrapeiro, M. M., Fogliano, V., Ruth, S. M. V. (2016). Effects of geographical origin, varietal and farming system on the chemical composition and functional properties of purple grape juices: A review. *Trends in Food Science & Technology*, 52:31-48.

Gutiérrez-Gamboa, G., Carrasco-Quiroz, M., Verdugo-Vásquez, N., Díaz-Gálvez, I., Garde-Cerdán, T., Moreno-Simunovic, Y. (2018). Characterization of grape phenolic compounds of 'Carignan' grapevines grafted onto 'País' rootstock from Maule Valley (Chile): Implications of climate and soil conditions. *Chilean Journal of Agricultural Research*, 78(2):310-315.

Hayoglu, I., Kola, O., Kaya, C., Özer, S., Turkoglu, H. (2009). Chemical and sensory properties of verjuice, a traditional Turkish non-fermented beverage from kabarcik and yediveren grapes. *Journal of Food Processing and Preservation*, 33:252-263.

Jackson, R.S. (2008). Wine Science: Principles and Applications. Chapter 4, 3rd Edition, Elsevier, ISBN: 978-0-12-373646-8.

Karapinar, M., Sengun, I. Y. (2007). Antimicrobial effect of koruk (unripe grape - *Vitis vinifera*) juice against *Salmonella typhimuriumon* salad vegetables. *Food Control*, 18:702-706.

Krikorian, R., Boespflug, E.L., Fleck, D.E., Stein, A.L., Wightman, J.D., Shidler, M.D. et al. (2012). Concord grape juice supplementation and neurocognitive function in human aging. *Journal of Agricultural and Food Chemistry*, 60, 5736-5742.

Lamikanra, O., Inyang, I. D., & Leong, S. (1995). *Journal of Agricultural and Food Chemistry*, 43:3026-3028.

Lawless, H. T., & Heymann, H. (2010). Other acceptance scales and just-about right scales. In: *Sensory Evaluation of Food: Principles and Practices*, pp. 328–340, Springer Science + Business Media LLC, New York, NY.

Lima, M., Silani, I., Toaldo, I. M., Corr^ ea, L., Biasoto, A., Pereira, G., Bordignon-Luiz, M.T., Ninow, J.L. (2014). Phenolic compounds, organic acids, and antioxidant activity of grape juices produced from new Brazilian varieties planted in the Northeast region of Brazil. *Food Chemistry*, 161:94e103.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, 46:207-219.

Marinova, E. M., Yanishlieva, N. V. (2003). Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperatures. *Food Chemistry*, 81:189-197.

Mildner-Szkudlarz, S., Zawirska-Wojtasiak, R., Goslinski, M. (2010). Phenolic compounds from winemaking waste and its antioxidant activity towards oxidation of rapeseed oil. *International Journal of Food Science and Technology*, 45:2272-2280.

Mousa-Al-Reza, H., Ziba, R., Zakieh, K., rad Tania, S., Mohammad-Mahdi, S. (2011). The Effect of Verjuice on Serum Lipid Levels in Mice Rendered Atherosclerosis. *Journal of Biologically Active Products from Nature*, 1:229-235.

Moussaoui, K. A., Varela, p. (2010). Exploring consumer product profiling techniques and their linkage to a quantitative descriptive analysis. *Food Quality and Preference*, 21(8):1088-1099.

Murelo, J., Pardo, F., Zafrilla, P. (2010). Antioxidant activity and phenolic composition of organic and conventional grapes and wines. *Journal of Food Composition and Analysis*, 23:569–574.

Nikfardjam, M. S. P. (2008). General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers. *Mitteilungen Klosterneuburg*, 58:28-31.

Öncül, N. and Karabiyikli, S. (2016). Survival of foodborne pathogens in unripe grape products. *LWT - Food Science and Technology*, 74:168-175.

Öncül, N., Karabiyikli, S. (2015). Factors affecting the quality attributes of unripe grape functional food products. *Journal of Food Biochemistry*, ISSN 1745-4514.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, 50:3122-3128.

Pastrana-Bonilla, E., Akoh, C.C., Sellappan, S., Krewer, G. (2003). Phenolic Content and Antioxidant Capacity of Muscadine Grapes. *Journal of Agricultural and Food Chemistry*, 51, 5497−5503.

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., Rogeaux, M., Etiévant, P., & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time-intensity. Food Quality and Preference, 20(6), 450–455.

Pinlati, S., Perazzolli, M., Malossini, A., Cestaro, A., Demattè, L., Fontana, P., Dal Ri, A., Viola, R. Velasco, R., Moser, C. (2007). Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison. *BMC Genomics*, 8:428.

Popper, R., Kroll, J. J. (2005). Conducting sensory research with children. *Journal of Sensory Studies*, 20: 75-87.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). Handbook of Enology. The Microbiology of Wine and Vinifications. (2nd ed.). England: Chichester,

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2006). Handbook of Enology. The Chemistry of Wine Stabilization and Treatments. (2nd ed.). England: Chichester.

Sabir, A., Kafkas, E., Tangolar, S. (2010). Distribution of major sugars, acids and total phenols in juice of five grapevine (Vitis spp.) cultivars at different stages of berry development. *Spanish Journal of Agricultural Research*, 8:425-433.

Saeed, S., Tariq, P. (2005). Antibacterial activities of Mentha piperita, Pisum sativum and Momordica charantia. *Pakistan Journal of Botany*, 37(4):997.

Sentheshanmuganathan, S and Elsden, S. R. (1957). The Mechanism of the Formation of Tyrosol by *Saccharomyces cerevisiae*. *Biochemistry Journal*, 69(2): 210-218.

Setorki, M., Asgary, S., Eidi, A., Rohani, A. H. (2010). Effects of acute verjuice consumption with a high-cholesterol diet on some biochemical risk factors of atherosclerosis in rabbits. *Medical Science Monitor*, 16(4):124-130.

Setorki, M., Nazari, B., Asgary, S., Azadbakht, L., Rafieian-Kopaei, M. (2011). Anti atherosclerotic effects of verjuice on hypocholesterolemic rabbits. *African Journal of Pharmacy and Pharmacology*, 5(8):1038-1045.

Seymor, G. B., *Taylor*, J. E., Tucker, G. A. (1993). Biochemistry of fruit ripening (1st ed.). USA: Chapman and Hall NY.

Shojaee-Aliabadi, S., Hosseini, S. M., Tiwari, B., Hashemi, M., Fadavi, G., Khaksar, R. (2013). Polyphenols content and antioxidant activity of Ghure (unripe grape) marc extract: influence of extraction time, temperature and solvent type. *International Journal of Food Science and Technology*, 48:412-418.

Simone, G. V., Montevecchi, G., Masino, F., Matrella, V., Imazio, S. A., Antonelli, A., & Bignami, C. (2013). Ampelographic and chemical characterization of Reggio Emilia and Modena (northern Italy) grapes for two traditional seasonings: ‘Saba’ and ‘agresto’. *Journal of the Science of Food and Agriculture*, 93:3502–3511.

Soejima, H., Tsuge, K., Yoshimura, T., Sawada, K., Kitagaki, H. (2012). Breeding of a high tyrosol-producing sake yeast by isolation of an ethanol-resistant mutant from a trp3 mutant. *Journal of the Institute of Brewing*, 118:264-268.

Soyer, Y., Koca, N., Karadeniz, F. (2003). Organic acid profile of Turkish white grapes and grape juices. *Journal of Food Composition and Analysis*, 16:629-636.

Stratford, M., Rose, A.H. (1986). Transport of Sulphur Dioxide by *Saccharomyces cerevisiae. Journal of General Microbiology*, 132:1-6.

Sudre, J., Pineau, N., Loret, C., Martin, N. (2012). Comparison of methods to monitor liking of food during consumption. *Food Quality and Preference*, 24:179-189.

Tinello, F., Lante, A. (2017). Evaluation of antibrowning and antioxidant activities in unripe grapes recovered during bunch thinning. *Australian Society of Viticulture* and *Oenology*, 23:33-41.

Valdés, M. E., Moreno, D., Gamero, E., Uriarte, D., Prieto, M. H., Manzano, R., Picon, J., *Intrigliolo*, D.S. (2009). Effects of cluster thinning and irrigation amount on water relations, growth, yield and fruit and wine composition of tempranillo grapes in extremadura (Spain). *Journal International des Sciences de la Vigne et du Vin*, 43(2):67-76.

Van Trijp, H.C., Punter, P.H., Mickartz, F., Kruithof, L. (2007). The quest for the ideal product: Comparing different methods and approaches. *Food Quality and Preference*, 18:729–740.

Varela, P., Ares, G. (2014). Novel Techniques in Sensory Characterization and Consumer Profiling; CRC Press, Taylor & Francis Group; Boca Raton.

Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M.J., Spencer, J.E.P. (2010). Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients*, 2, 1106-1131.

Wang, Y., He, Y-N., Chen, W-K., He, F., Chen, W., Cai, X-D., Duan, C-Q., Wang, J. (2018). Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet Sauvignon. *Food Chemistry*, 248:101-110.

Wightman, J. D., Heuberger, R. A. (2015). Effect of grape and other berries on cardiovascular health. *Journal of the Science of Food and Agriculture*, 95(8):1584-97.

ZibaeeNezhad, M. J., Mohammadi, E., Beigi, M. A. B.; Mirzamohammadi, F., Salehi, O. (2012). The Effects of Unripe Grape Juice on Lipid Profile Improvement. *Cholesterol*, 1-3.

Zolfaghari, B., Kazemi, M., Nematbakhsh, M. (2015). The effects of unripe grape extract on systemic blood pressure and serum levels of superoxide dismutase, malondialdehyde and nitric oxide in rat. *Advanced Biomedical Research*.

Aim of the thesis

This thesis aims to improve the knowledge on verjuice by studying its chemical, microbiological and sensorial characteristics. Taking into account that, in general, verjuice is made from different grapes, harvested at different degree of ripeness, the first objective of the thesis was to investigate how picking grape time, grapevine variety and preserving additives affect the chemical and sensorial properties of verjuice. To date, these aspects have not been studied in detail and therefore more information is needed in order to define the best conditions for production of a high-quality verjuice.

While organoleptic properties play as a crucial role in determining the acceptance of consumers for new food products, the research on verjuice has overlooked this fundamental aspect and information on the sensory properties of foods seasoned with this acidic ingredient is lacking. Therefore, the second objective of the thesis was to gain new information on the sensorial properties of salads seasoned with verjuice and on its acceptability by consumers in comparison to other acidic seasonings, in particular vinegar and lemon juice.

Because of verjuice’s chemical characteristics, it is also interesting to explore the possibility of its use as an acidulant in pickled preserves, which are normally produced by using wine vinegar. Thus, the third objective of the thesis was to evaluate, through chemical and sensorial analyses, the applicability of verjuice as acidifying ingredient in vegetable preserves, by using industrially pickled cucumber conserves as a model for vegetable foods. In particular, the aim is to verify the impact on the sensorial characteristics of the typical absence of acetic acid in verjuice, which can potentially give an additional value as compared to its vinegar picked counterparts.

Finally, the last objective of this thesis was to evaluate alternative methods for preventing microbial spoilage and to prolong the shelf life of verjuice, which can be unstable due to the presence of residual fermentable sugars. Also this aspect, at the moment, is not completely clarified and needs to be investigated.

In conclusion, at the end of the research it is expected to achieve new information on i) the chemical and sensorial characterization of several verjuice products prepared on lab-scale, ii) the suitability of verjuice as a salad seasoning in comparison to the most common acidic seasonings used by consumers (wine vinegar and lemon juice), iii) the applicability of verjuice as a novel acidifying agent in vegetable conserves and its suitability for the creation of new pickled products, and iv) the possibility to enhance the shelf life using simple methods allowing to avoid sulfite or other chemical preservatives addition.

The following text introduces the topics of doctoral research that focuses mainly on the chemical and sensorial characterization of verjuice. A part of this study is focused on developing an effective stabilization process of verjuice from the microbiological point of view, whereas the other results are reported as scientific papers, published or under review.

CHAPTER 1

CHEMICAL AND SENSORY ANALYSIS OF VERJUICE: AN ACIDIC FOOD INGREDIENT OBTAINED FROM UNRIPE GRAPE BERRIES

Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries[1]

Abstract

Verjuice is the unfermented juice obtained from unripe grape berries, typically thinned fruit that is normally discarded. Despite a long history of use as a food ingredient, verjuice does not have a recognized standard of identity nor is made by standardized methods. To assess the potential for production in the Veneto region of Italy, verjuice produced from six different winegrape varieties harvested at three different dates from bunch closure through early veraison, and stabilized using either sulphite or sorbate, was subjected to compositional and sensory analyses. pH was found to range from 2.6 to 2.9 while soluble solids (°Brix) ranged from 3.8 to 9.9. Acidity (g/L) ranged from 17.4 to 40.5 while color (A_{420}) varied from 0.04 to 0.50. With respect to sensory character, harvest date had no influence on aroma, but affected taste, whereas the type of preservative used had no effect on taste, but did affect aroma.

Keywords

Aroma

Descriptive sensory analysis

Taste

Unripe grape juice

Verjuice

Verjus

[1]This chapter is an edited version of: Dupas de Matos, A., Curioni, A., Bakalinsky, A.T., Marangon, M., Pasini, G., Vincenzi, S. (2017). Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries, Innovative Food Science and Emerging Technologies, v. 44, p. 9-14, http://dx.doi.org/10.1016/j.ifset.2017.09.014, ISSN: 1466-8564. Copyright 2017 Elsevier.

1. Introduction

Unripe grapes can be processed into two major products: verjuice and sour grape sauce. The verjuice or “verjus” or “agraz”, hereafter referred to as “unripe grape juice,” is obtained by pressing unripe grapes, while sour grape sauce is derived from verjuice that undergoes an additional concentration step followed by salting (Öncül & Karabiyikli, 2015). For example, “agresto” is a type of traditional sour grape sauce from the Italian region of Tuscany (Simone et al., 2013).

Unripe grape juice is characterized by high acidity, low sugar content and a sour/tart taste (Nikfardjam, 2008; Hayoglu, Kola, Kaya, Özer, & Turkoglu, 2009). While known by the ancient Romans, widespread culinary and medicinal uses date to the Middle Ages and to the Renaissance (Simone et al., 2013). Unripe grape juice is still widely used in Iran and Turkey, where it is called “abe ghureh” (Persian: unripe grape water) and “koruk suyu” (Turkish: unripe grape juice), respectively (Nikfardjam, 2008; Alipour, Davoudi, & Davoudi, 2012). Uses in Iran include salad dressings and digestive drinks (Setorki, Asgary, Eidi, & Rohani, 2010). More recently, unripe grape juice has received increasing attention in Western cuisine as a re-discovered food ingredient to be used as an alternative to vinegar or lemon juice (Nikfardjam, 2008; Setorki et al., 2010; Öncül & Karabiyikli, 2015). Unripe grape juice has also been tested as a potential food preservative (Karapinar & Sengun, 2007; Karabiyikli & Öncül, 2016), due to its high organic acid content and elevated concentration of phenolic compounds (Saeed & Tariq, 2005; Aibinu, Adenipekun, Adelowotan, Ogunsanya, & Odugbemi, 2007; Öncül & Karabiyikli, 2016).

Because unripe grape juice has a history of use in Iranian folk medicine, it has been evaluated for lipid lowering activity and for the ability to control hypertension (ZibaeeNezhad, Mohammadi, Beigi, Mirzamohammadi, & Salehi, 2012), and to reduce risk of atherosclerosis (Setorki et al., 2010; Setorki, Nazari, Asgary, Azadbakht, & Rafieian-Kopaei, 2011; Mousa-Al-Reza, Ziba, Zakieh, Tania, & Mohammad-Mahdi, 2011; Alipour et al., 2012; Zolfaghari, Kazemi, & Nematbakhsh, 2015). These reported health benefits could relate to its known antioxidant activity and high content of polyphenolic compounds, the latter of which have an astringent character (Soares et al., 2011). The concentration of most polyphenolic compounds in general (Iland, Dry, Proffitt, & Tyerman, 2011), and astringent condensed tannins in particular (Dinnella, Recchia, Fia, Bertuccioli, & Monteleone, 2009), reaches a maximum around 45 days after flowering (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2005), a time approximately corresponding to the harvest date for producing unripe grape juice. To the best of our knowledge, descriptive evaluations of mouthfeel/astringency and aroma have not been reported for this product.

Because of increasing interest in using unripe grape juice in food applications in spite of a lack of a standard of identity and standardized methods of production, the present study was undertaken to evaluate basic production parameters to provide baseline data. Specifically, a number of winegrape varieties grown in the Veneto region of Italy were harvested at different stages of “unripeness,” processed into unripe grape juice, and subjected to both compositional and sensory analysis.

2. Materials and methods

2.1 Production of unripe grape juice

Six different grape varieties (Glera, Chardonnay, Sauvignon blanc, Merlot, Cabernet franc and Cabernet sauvignon) were used. Unripe grape clusters were harvested at three different dates (T_1: bunch closure, T_2: 15 days after T_1, and T_3: early *veraison*) which correspond to stages 32-34 of the modified E-L phenological classification scheme (Coombe, 1995). Clusters were destemmed, washed and pressed in the presence of either 0.5 g/Kg potassium metabisulphite (PM) or 0.125 g/Kg potassium sorbate (PS) (**Figure 1**), both added to prevent possible microbial spoilage and oxidations of this unfermented product.

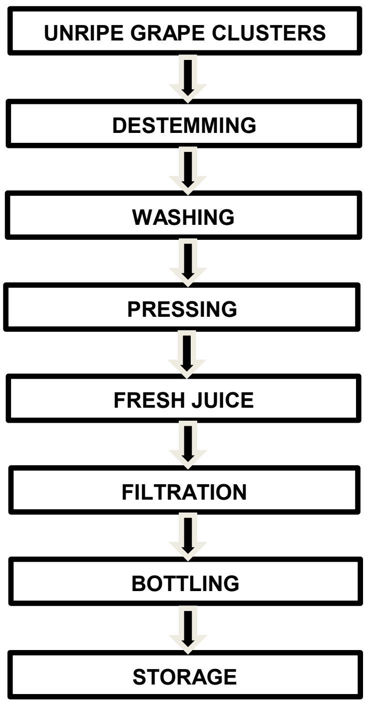

Fig. 1. Lab-scale processing of unripe grape juice.

Despite the low pH of the juice, these phenomena both occasionally occurred during preliminary trials. Addition rates were decided after assessing the residual free sulphur dioxide after PM additions, and accordingly to manufacturer instructions for PS. The juices were stored at 5°C for 5 days to promote tartrate precipitation, and were then filtered under vacuum through 1.6 µm glass fiber filters, bottled (200 mL dark bottles) and stored at 5 °C until sensory analysis.

2.2 Chemical analysis

Unripe grape juice samples were analyzed for pH (Hanna Instruments 8424, Padova, Italy), soluble solids (Atago PR-101, Tokyo, Japan) and A_{420} (Ultrospec 2100 pro UV-Vis spectrophotometer, Amersham Biosciences, Uppsala, Sweden). Organic acids (malic and tartaric) and sugars (glucose and fructose) were separated by HPLC using an Aminex 87H column (Biorad, California, USA) and detected by UV absorbance or refractive index (RI), respectively (Eyéghé-Bickong, Alexandersson, Gouws, Young, & Vivier, 2012).

2.3 Sensory evaluation

Twenty eight subjects (eleven females and seventeen males) aged from 20 to 25 years were recruited at the University of Padova. The subjects had no stated history of disorders in oral perception. A preliminary selection of candidates was carried out in order to eliminate subjects not suitable for tasting food products. After a full explanation of the experiment, a voluntary consent form was provided that included a questionnaire about food habits and intolerances, smoking, diet, and acceptability of relevant food products. The consent form was signed by suitable candidates who agreed to participate in the subsequent training and sensory analysis sessions.

An initial qualitative analysis phase consisted in presenting eight different experimental unripe grape juice samples selected among the total 36 samples to represent the range of grape varieties, harvest dates, and stabilization treatments in order to define a common vocabulary of both gustatory and olfactory sensory descriptors specific for unripe grape juice. Following the qualitative analysis, a specific training program developed in sessions of approximately 45 minutes each (two sessions per week for six weeks) was undertaken on the descriptors obtained in the first phase and panelists were regularly checked. The recognition and classification of standard samples were performed by asking subjects to identify the taste and to order the standard samples according to perceived intensity for each descriptor. The subjects were then instructed to use a continuous linear scale 15 cm long by placing a mark along its length to represent perceived intensity (Meilgaard, Civille, & Carr, 2007; Gasperi & Endrizzi, 2012) for taste descriptors including acidity, salty, and sweetness using standard solutions of malic acid (4, 8, 12, 16 g/L), sodium chloride (0.5, 1, 2, 3 g/L), and sucrose (10, 20, 40, 60 g/L) respectively, and tactile descriptor including astringency using standard solutions of tannic acid (0.5, 0.75, 1, 1.5 g/L).

Subjects were also trained to recognize and rate the perceived intensity of aromas (orthonasal perception) with a 9-point intensity scale (from 1 = extremely weak to 9 = extremely strong) using standard solutions prepared with lemon, pear, cooked apple, green apple, kiwi, pomegranate, dried prunes, honey and tomatoes, and solutions of isoamyl acetate, cis-3-hexenol and 2-phenylethanol (Sigma-Aldrich, St. Louis, USA) for banana, herbal and floral, respectively (Gasperi & Endrizzi, 2012). During training sessions, subjects were provided with plain crackers and water and were instructed to rinse between samples. Following completion of the training, all 36 samples of unripe grape juice were offered randomly to subjects for evaluation of intensity of taste and aroma for each descriptor. Each sample was evaluated at least five times in each session.

2.4 Statistical analysis

Chemical analyses were carried in duplicate. Significant differences between means was established using a paired t-test or one-way analysis of variance (ANOVA) followed by Tukey's test ($p<0.05$), combined with Principal Component Analysis (PCA). All results were analyzed using XLStat software.

3. Results and discussion

3.1 Yield of juice

Juice yield from the unripe grapes was about 56% (w/w) regardless of harvest date. This uniformity is consistent with the fact that the early harvest predated the normal expansion that subsequently occurs in berry volume. Depending on variety, yields ranged from 54 to 60% (**Supplementary Table 1**). Our yields were somewhat higher than reported by Hayoglu et al. (2009) who obtained a juice yield of 47% from "Kabarcık" and "Yediveren" grape varieties harvested 45 days after flowering.

3.2 Chemical composition of unripe grape juice

The chemical composition of the unripe grape juices is shown in **Table 1**. The pH ranged from 2.6 to 2.9, while the soluble solids (°Brix) varied between 3.8 and 9.9. Both pH and soluble solids increased significantly only at T_3. Similar results have been reported previously, pH: 2.4-2.8 (Karapinar & Sengun, 2007), 2.9-3.0 (Hayoglu et al., 2009), 2.8 (Simone et al., 2013), and 2.1-2.7 (Öncül & Karabiyikli, 2015); soluble solids (°Brix): 4.5-7.5 (Hayoglu et al., 2009) and 4.2-8.0 (Öncül & Karabiyikli, 2015). We presume that the higher values for soluble solids (17.3-26 °Brix) reported by Simone et al. (2013) were due to inclusion of a concentration step.

Table 1. pH and soluble solids of unripe grape juice[1].

Grape variety	pH			Soluble solids (°Brix)		
	T_1	T_2	T_3	T_1	T_2	T_3
Glera	2.62^B	2.64^B	2.91^A	3.81^B	4.35^B	9.65^A
Chardonnay	2.77^B	2.72^B	2.90^A	3.86^B	4.23^B	7.88^A
S. blanc	2.67^B	2.65^B	2.83^A	3.80^B	4.43^B	9.86^A
Merlot	2.62^B	2.58^B	2.73^A	3.86^B	4.43^B	7.29^A
C. franc	2.56^B	2.59^B	2.72^A	4.24^B	4.76^B	5.99^A
C. sauvignon	2.57^B	2.60^B	2.82^A	4.24^B	4.30^B	7.31^A

[1]Means followed by the same letter in the row do not differ significantly by one-way ANOVA followed Tukey's test ($p<0.05$). T_1: bunch closure; T_2: 15 days after T_1; T_3: early veraison.

Grapes typically exhibit quantitative and qualitative differences in chemical composition as a function of variety, maturity, growing region and year (Lamikanra, Inyang, & Leong, 1995). Malic and tartaric acids are the dominant organic acids in grapes, accounting for 90% of the total acids present. While the berry accumulates malic acid rapidly, the accumulation of tartaric acid is slower, although its concentration in unripe grapes can be as high as 15 g/L at the end of the vegetative growth phase (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). To the best of our knowledge, the individual malic and tartaric acid contents of unripe grape juice have not previously been reported.

The acid and sugar content of the unripe grape juices is shown in **Table 2**. As expected, malic acid concentrations were higher than tartaric acid in all samples, contrary to what is observed in ripe grapes.

Table 2. Acid and sugar levels in unripe grape juices[1].

White variety	Malic acid	Tartaric acid	Glucose	Fructose	Acidity[2]
Glera T_1 PM[3]	23.06	11.71	7.68	nd[4]	34.76
Glera T_1 PS[3]	21.59	11.78	7.82	nd	33.37
Glera T_2 PM	22.43	10.93	10.12	nd	33.36
Glera T_2 PS	22.93	11.37	11.43	nd	34.30
Glera T_3 PM	12.35	5.51	39.91	31.51	17.85
Glera T_3 PS	10.90	6.54	47.06	39.95	17.44
Chard T_1 PM	27.13	8.90	6.84	nd	36.03
Chard T_1 PS	27.13	9.66	6.79	nd	36.79
Chard T_2 PM	28.62	8.61	8.63	nd	37.23
Chard T_2 PS	27.60	8.79	8.11	nd	36.39
Chard T_3 PM	18.53	6.53	34.47	21.23	25.06
Chard T_3 PS	21.57	7.73	29.98	14.33	29.30
S. bianco T_1 PM	22.92	11.34	6.72	nd	34.26
S. bianco T_1 PS	22.12	11.55	5.91	nd	33.68
S. bianco T_2 PM	27.74	11.01	8.32	nd	38.75
S. bianco T_2 PS	27.13	10.86	7.68	nd	37.99
S. bianco T_3 PM	18.55	7.89	40.65	27.55	26.44
S. bianco T_3 PS	17.59	8.10	46.44	34.22	25.69
Red variety	**Malic acid**	**Tartaric acid**	**Glucose**	**Fructose**	**Acidity[2]**
Merlot T_1 PM	23.73	12.84	7.13	nd	36.57
Merlot T_1 PS	23.32	14.05	6.81	nd	37.37
Merlot T_2 PM	25.79	13.07	10.69	nd	38.86
Merlot T_2 PS	24.58	13.73	9.89	nd	38.31
Merlot T_3 PM	17.64	9.43	31.70	19.08	27.07
Merlot T_3 PS	18.58	9.87	27.36	13.91	28.45
C. franc T_1 PM	27.13	13.40	9.80	nd	40.53
C. franc T_1 PS	26.77	13.38	9.64	nd	40.15
C. franc T_2 PM	27.70	10.85	13.30	nd	38.55
C. franc T_2 PS	24.59	12.44	13.98	nd	37.03
C. franc T_3 PM	21.00	9.15	21.72	6.29	30.15
C. franc T_3 PS	22.38	11.35	19.74	3.21	33.73
C. sauv T_1 PM	29.95	10.45	6.76	nd	40.39
C. sauv T_1 PS	29.76	9.98	6.59	nd	39.75
C. sauv T_2 PM	30.38	8.96	9.54	nd	39.33
C. sauv T_2 PS	28.78	10.02	9.84	nd	38.80
C. sauv T_3 PM	18.48	6.77	28.01	14.66	25.24
C. sauv T_3 PS	21.58	6.25	28.52	12.81	27.84

[1]Values are expressed as g/L. [2]The sum of malic and tartaric acids. [3]PM is potassium metabisulphite; PS is potassium sorbate. [4]nd = not detected. Harvest dates (T) are the same as noted in the legend to Table 1.

The highest malic acid concentrations were found in the Chardonnay T_2 PM (28.6 g/L) and Cabernet sauvignon T_2 PM (30.4 g/L) juices, while tartaric acid levels were highest in Glera T_1 PS (11.8 g/L) and Merlot T_1 PS (14.0 g/L). Glera T_3 PS and Merlot T_3 PM juices had the highest content of both glucose (47.1 and 31.7 g/L, respectively) and fructose (40.0 and 19.1 g/L, respectively). In general, juices at T_1 and T_2 had similar levels of malic and tartaric acids and glucose, regardless of variety, but had no fructose. Fructose was only detected in the T_3 samples, consistent with this stage of ripeness. In previous work, glucose was found to account for 85% of the total sugar content of green berries sampled at an early developmental stage while during veraison, glucose was found to predominate over fructose (Seymor, Taylor, & Tucker, 1993).

In the present study, unripe grape juices had malic and tartaric acid values between 10.9 and 30.4 g/L, and 5.5 and 14.0 g/L, respectively. The total acidity calculated as malic plus tartaric acids ranged from 17.4-38.8 g/L for the white varieties and from 25.4-40.5 g/L for the red varieties.

In previous work, titratable acidity expressed in tartaric acid equivalents, unripe grape juices ranged from 19.6 to 39.6 g/L (Nikfardjam, 2008), 24.8 to 30.0 g/L (Hayoglu et al., 2009), 32.7 to 39.8 g/L (Simone et al., 2013), and 22.9 to 70.9 g/L (Öncül & Karabiyikli, 2015).

3.3 Color

Although color is one of the most important qualities of grape products, an agreed upon standard does not yet exist for defining the quality of unripe grape juice. The A_{420} value which is an index of yellow-orange color, varied according to the stabilization agent used (**Table 3**).

Table 3. A_{420} values of unripe grape juices[1].

Grape variety	T_1		T_2		T_3	
	PM	**PS**	**PM**	**PS**	**PM**	**PS**
Glera	0.093^B	0.444^A	0.065^B	0.346^A	0.046^B	0.407^A
Chardonnay	0.045^B	0.305^A	0.064^B	0.388^A	0.085^B	0.340^A
Sauvignon blanc	0.059^B	0.207^A	0.060^B	0.315^A	0.081^B	0.493^A
Merlot	0.049^B	0.116^A	0.053^B	0.235^A	0.053^B	0.369^A
Cabernet franc	0.096^B	0.318^A	0.087^B	0.471^A	0.123^B	0.460^A
Cabernet sauvignon	0.054^B	0.261^A	0.056^B	0.326^A	0.065^B	0.298^A

[1]Means followed by the same letter in a given row do not differ significantly according to stabilization treatment at the same harvest time by paired t-test ($p<0.05$). Harvest dates (T) are the same as noted in the legend to Table 1. PM and PS are the same as noted in Table 2.

The samples treated with potassium metabisulphite (PM) had lower values (0.06-0.08) independent of harvest date, indicating a lower level of oxidation relative to the potassium sorbate-treated samples (PS) (0.28-0.40). A great deal of variability regarding the color of unripe grape juice has been reported, depending largely on stabilization treatment. For example, in a study of seven unripe grape juice samples, Nikfardjam (2008) reported A_{420} values from 0.23-0.78, while Hayoglu et al. (2009) reported lower values, 0.20-0.19, which increased modestly to 0.23-0.22, after heating.

3.4 Sensory analysis

In the first qualitative phase of the study, the most frequently used descriptors perceived for taste were acid (74.19%), astringency (51.61%), salty (35.48%) and sweet (25.80%), while the most common aroma terms were herbaceous (50.00%), cooked apple (43.55%), pear (29.03%), floral (29.03%) and green apple (25.81%). After specific training on these selected descriptors, samples were presented to panelists in a randomized manner in a number of separate sessions for quantitative assessment. Only the T_1 and T_3 samples were subjected to sensory analysis because the T_1 and T_2 samples had been found to be chemically similar.

3.4.1 Effect of harvest time

PCA analysis performed on all the data while considering harvest date the discriminating parameter, showed that two principal components accounted for 66.74% of the total variance. From the loadings of the variables, acid and sweet were found to be in opposition. The T_1 samples were present in the second quadrant while the T_3 samples were found in the fourth (**Figure 2**). This distribution is entirely plausible knowing that during grape ripening these two parameters have reversed trends. Acid, salty and astringency are dominant features in the first principal component (F1), which account for 40.94% of the total variance. Sweet dominates in the second principal component (F2) representing 25.80% of the total variance (**Supplementary Table 2**) which is consistent with the chemical data. Hence, the harvest date had an effect on taste, independent of the stabilization treatment or variety (**Supplementary Table 3**).

Regarding aroma, the data are more uniformly distributed in the two-dimensional space delimited by the two main components, which, in this case, account for 66.2% of the total variability (**Supplementary Figure 1**). Cooked apple, pear and herbaceous are dominant features in the first principal component (F1), responsible for 37.04% of the total variance. Green apple, pear, floral and herbaceous dominate in the second principal component (F2) representing 29.17% of the total variance (**Supplementary Table 4**). The samples were not significantly different for aroma as a function of the harvest date (**Supplementary Table 5**).

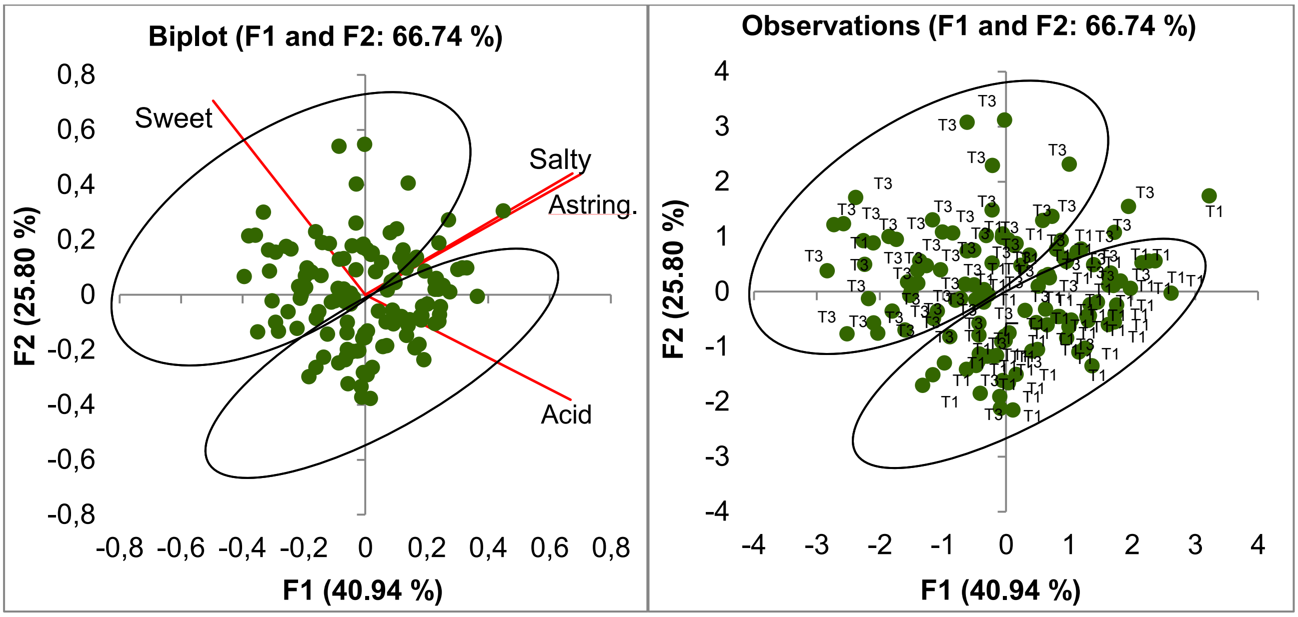

Fig. 2. Principal component analysis of the taste/tactile of the unripe grape juices.
T_1: bunch closure; T_3: early veraison.

3.4.2 Effect of stabilization treatment

Because harvest date exhibited significant differences among the samples for taste, a deeper analysis of the effect of the stabilization treatment was performed. For taste evaluation, the type of treatment had no effect regardless of the harvest time, consistent with the chemical analysis (**Supplementary Table 6**).

Regarding the aromatic evaluation of the samples containing PM and PS, the two main principal components accounted for 70.31% of total variance (**Figure 3**). Herbaceous, green apple, pear and cooked apple were the most important features in the first principal component (F1), which accounted for 42.11% of the total variability; while pear and floral dominate in the second principal component (F2), representing 28.20% of the total variance (Supplementary Table 7). Samples treated with PM are present mostly in the quadrants described by herbaceous, green apple and floral, while those treated with PS are present in the quadrants described by pear and cooked apple. Statistically, the stabilization treatment had an effect on aroma evaluation only at T_1, with cooked apple, pear and herbaceous being significantly different (**Supplementary Table 8**). This result is likely related to the ability of PM to minimize oxidation and to preserve fresh aromas better than PS. At T_3, the aromatic evaluation did not reveal any differences among the samples which had intensities rated weak/moderate to moderate (**Supplementary Table 8**).

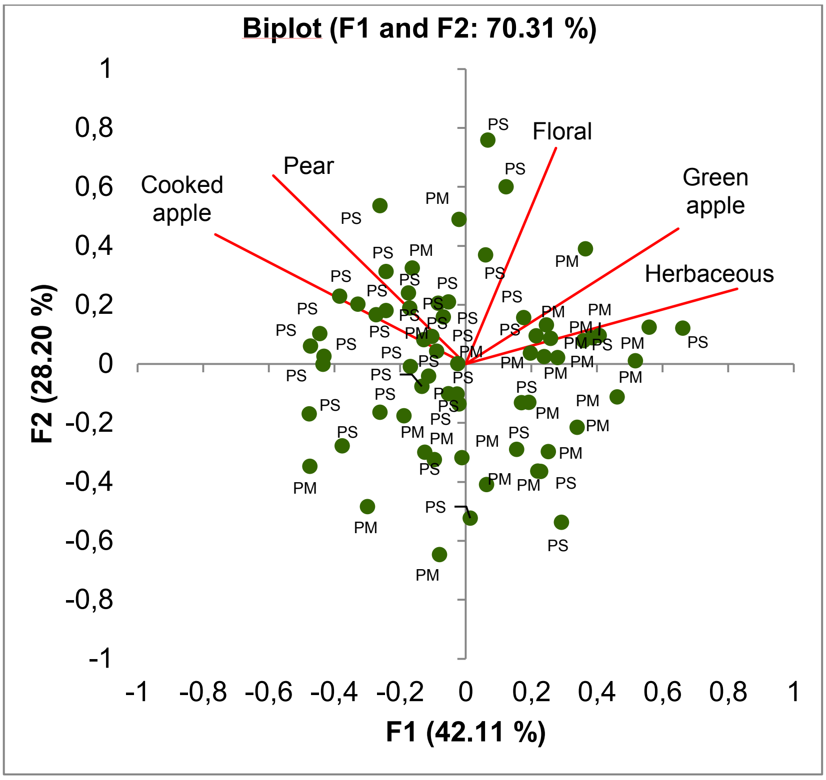

Fig. 3. Principal component analysis of aroma evaluation of unripe grape juices at T_1.
PM: potassium metabisulphite; PS: potassium sorbate.

3.4.3 Effect of grape variety

The effect of grape variety on sensory properties of the juices was examined. At T_1, the juices obtained from the different varieties (Glera, Chardonnay, Sauvignon blanc, Merlot, Cabernet franc and Cabernet Sauvignon) did not exhibit any difference in taste. However, the samples at T_3 differed for acidity and astringency (**Supplementary Table 9**). Specifically, the red varieties had the highest astringency and acidity, likely due to their delayed ripening period relative to the white varieties. These data are consistent with those reported in Table 2 for total acidity. Among the red varieties, Cabernet sauvignon had the lowest acidity while astringency did not differ. For the white varieties, no differences in acidity or astringency were observed.

Pear and floral aroma descriptors were the same at T_1, but differences in green apple, cooked apple and herbaceous aromas were detected among the grape varieties. Among the red varieties, Merlot was the most fresh variety having the highest intensity for green apple and herbaceous aromas both at T_1 and T_3. On the other hand, Cabernet sauvignon was the least fresh variety. Regarding the white varieties, Sauvignon blanc was the freshest at T_1 and T_3, while Glera was the least. At T_3, herbaceous aroma was the only one able to discriminate among the varieties (Supplementary Table 10). To our knowledge, the effect of grape variety on the sensory character of unripe grape juice has only been reported in a single study that compared two Turkish varieties (Yediveren and Kabarcik) (Hayoglu et al., 2009).

4. Conclusions

Although unripe grape juice has a long history of use as a food ingredient, it is a relatively unknown product. Because it can be made from winegrape thinnings that are normally discarded, it represents a potentially valuable product for grape growers. The present study evaluated key production variables because unripe grape juice has no agreed-upon standard of identity nor a standardized method of production. One promising application is use as a replacement for vinegar in salad dressings where acetic acid may be undesirable from a sensory point of view. The six grape varieties evaluated here made products of comparable sensory quality. Further evaluation of consumer preferences, and acceptable processing and compositional parameters will help to refine a product that has significant market potential.

Funding

Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) (grant number: 204483/2014-0) and the University of Padova (grant number: project BIRD165379 – 2016).

Acknowledgements

This study was undertaken as part of the PhD program of A. Dupas de Matos in the Department of Land, Environment, Resources, and Health at the University of Padova. The authors wish to thank oenologist Marco Lucchetta for donating grapes, Linda De Conti and the students in the enology program for their participation in the sensory analysis.

References

Aibinu, I., Adenipekun, T., Adelowotan, T., Ogunsanya, T., & Odugbemi, T. (2007). Evaluation of the antimicrobial properties of different parts of Citrus aurantifolia (lime fruit) as used locally. *African Journal of Traditional, Complementary, and Alternative Medicines*, 4, 185.

Alipour, M., Davoudi, P., & Davoudi, Z. (2012). Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal, hyperlipidemic and hyperlipidemic with hypertensive human volunteers. *Journal of Medicinal Plants Research*, 6, 5677-5683.

Coombe, B. G. (1995). Adoption of a system for identifying grapevine growth stages. *Australian Journal Grape Wine Research*, 1, 100-110.

Dinnella, C., Recchia, A., Fia, G., Bertuccioli, M. & Monteleone, E. (2009). Saliva Characteristics and Individual Sensitivity to Phenolic Astringent Stimuli. *Chemical Senses*, 34, 295-304.

Eyéghé-Bickong, H. A., Alexandersson, E. O., Gouws, L. M., Young, P. R., & Vivier, M. A. (2012). Optimisation of an HPLC method for the simultaneous quantification of the major sugars and organic acids in grapevine berries. *Journal of Chromatography B*, 885-886, 43-49.

Gasperi, F., & Endrizzi, I. (2012). L'analisi descrittiva. In: "*Atlante sensoriale dei prodotti alimentari"* (Chapter 2, 1st ed.), Società Italiana di Scienze Sensoriali. Italy: Milan.

Hayoglu, I., Kola, O., Kaya, C., Özer, S., Turkoglu, H. (2009). Chemical and sensory properties of verjuice, a traditional Turkish non-fermented beverage from kabarcik and yediveren grapes. *Journal of Food Processing and Preservation*, 33, 252-263.

Iland, P., Dry, P., Proffitt, T., & Tyerman, S. D. (2011). *The grapevine: from the science to the practice of growing vines for wine.* (1st ed.). Adelaide: Patrick Iland Wine Promotions Pty Ltd, (Chapter 8).

Karabiyikli, S., & Öncül, N. (2016). Inhibitory effect of unripe grape products on foodborne pathogens. *Journal of Food Processing and Preservation*, 1-7.

Karapinar, M., & Sengun, I. Y. (2007). Antimicrobial effect of koruk (unripe grape - Vitis vinifera) juice against Salmonella typhimuriumon salad vegetables. *Food Control*, 18, 702-706.

Lamikanra, O., Inyang, I. D., & Leong, S. (1995). *Journal of Agricultural and Food Chemistry*, 43, 3026-3028.

Meilgaard, M. C., Civille, G. V., & Carr, B. T. (2007). *Sensory Evaluation Techniques.* (4th ed.). New York: Boca Raton, (Chapter 5).

Mousa-Al-Reza, H., Ziba, R., Zakieh, K., Tania, S. R., & Mohammad-Mahdi, S. (2011). The Effect of Verjuice on Serum Lipid Levels in Mice Rendered. *Journal of Biologically Active Products from Nature*, 1, 229-235.

Nikfardjam, M. S. P. (2008). General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers. *Mitteilungen Klosterneuburg*, 58, 28-31.

Öncül, N., & Karabiyikli, S. (2015). Factors affecting the quality attributes of unripe grape functional food products. *Journal of Food Biochemistry*, 39, 689-695.

Öncül, N., & Karabiyikli, S. (2016). Survival of foodborne pathogens in unripe grape products. *LWT - Food Science and Technology*, 74, 168-175.

Ribéreau-Gayon, P., Dubourdieu, D., Donèche, B., & Lonvaud, A. (2005). *Trattato di Enologia 1. Microbiologia del vino e Vinificazioni.* (2nd ed.). Italy: Bologna, (Chapter 10).

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of Enology. The Chemistry of Wine Stabilization and Treatments.* (2nd ed.). England: Chichester, (Chapter 1).

Saeed, S., & Tariq, P. (2005). Antibacterial activities of Mentha piperita, Pisum sativum and Momordica charantia. *Pakistan Journal of Botany*, 37, 997.

Setorki, M., Asgary, S., Eidi, A., & Rohani, A. H. (2010). Effects of acute verjuice consumption with a high-cholesterol diet on some biochemical risk factors of atherosclerosis in rabbits. *Medical Science Monitor*, 16, 124-130.

Setorki, M., Nazari, B., Asgary, S., Azadbakht, L., & Rafieian-Kopaei, M. (2011). Anti atherosclerotic effects of verjuice on hypocholesterolemic rabbits. *African Journal of Pharmacy and Pharmacology*, 5, 1038-1045.

Seymor, G. B., Taylor, J. E., Tucker, G. A. (1993). *Biochemistry of Fruit Ripening*. (1st ed.). Chapman and Hall NY, USA, (Chapter 6).

Simone, G. V., Montevecchi, G., Masino, F., Matrella, V., Imazio, S. A., Antonelli, A., & Bignami, C. (2013). Ampelographic and chemical characterization of Reggio Emilia and Modena (northern Italy) grapes for two traditional seasonings: ‘saba’ and ‘agresto’. *Journal of the Science of Food and Agriculture*, 93, 3502-3511.

Soares, S., Vitorino, R., Osório, H., Fernandes, A., Venâncio, A., Mateus, N., Amado F, & de Freitas V. (2011). Reactivity of human salivary proteins families toward food polyphenols. *Journal of Agricultural and Food Chemistry*, 59, 5535-5547.

ZibaeeNezhad, M. J., Mohammadi, E., Beigi, M. A. B., Mirzamohammadi, F., & Salehi, O. (2012). The Effects of Unripe Grape Juice on Lipid Profile Improvement. *Cholesterol*, 1-3.

Zolfaghari, B., Kazemi, M., & Nematbakhsh, M. (2015). The effects of unripe grape extract on systemic blood pressure and serum levels of superoxide dismutase, malondialdehyde and nitric oxide in rat. *Advanced Biomedical Research*, 109, 1-4.

Supplementary material

Table 1S. Yield of unripe grape juice.

Grape variety	Yield (%(w/w)[1]			
	T_1	T_2	T_3	Mean
Glera	56.11	53.63	59.04	56.20
Chardonnay	60.81	62.95	57.61	60.46
Sauvignon blanc	55.76	58.69	54.40	56.29
Merlot	55.93	58.37	57.76	57.35
Cabernet franc	54.01	52.36	54.76	53.71
Cabernet sauvignon	55.56	53.02	54.83	54.47
Mean	56.36	56.51	56.40	

[1](Weight of juice/weight of bunch) x 100

T_1: bunch closure; T_2: 15 days after T_1; T_3: early veraison.

Table 2S. Loadings of the features for the two main principal components between T_1 and T_3.

	F1	F2
Acid	0.522	-0.376
Astringency	0.547	0.432
Salty	0.527	0.435
Sweet	-0.387	0.695
% Variance	40.944	25.797

Harvest dates (T) are the same as noted in the legend to Table 1S.

Table 3S. Intensity of four taste components[1] at different harvest dates.

	T_1	T_3
Acid	10.06^A	6.76^B
Astringency	7.85^A	6.73^A
Salty	4.23^A	3.82^A
Sweet	2.29^B	5.33^A

[1]Means expressed as cm using a 15 cm-long intensity scale.

Values followed by the same letter in a given row are not significantly different by paired t-test ($p<0.05$).

Harvest dates (T) are the same as noted in the legend to Table 1S.

Table 4S. Loadings of the features in the two main principal components between T_1 and T_3.

	F1	F2
Pear	0.520	0.410
Green apple	-0.234	0.584
Cooked apple	0.631	0.111
Floral	0.056	0.602
Herbaceous	-0.524	0.342
% Variance	37.039	29.165

Harvest dates (T) are the same as noted in the legend to Table 1S.

Table 5S. Intensity of five aroma components[1] in different harvest dates.

	T_1	T_3
Pear	4.26^A	4.38^A
Green apple	3.91^A	3.80^A
Cooked apple	4.97^A	5.40^A
Floral	4.40^A	4.15^A
Herbaceous	5.28^A	4.49^A

[1]Means expressed by 9-point intensity scale.
Values followed by the same letter in the same row are not significantly different by paired t-test ($p<0.05$).
Harvest dates (T) are the same as noted in the legend to Table 1S.

Table 6S. Intensity of four taste components[1] in different harvest dates according stabilization treatments.

	T_1		T_3	
	PM	PS	PM	PS
Acid	9.58^A	10.38^A	7.55^A	6.05^A
Astringency	7.69^A	7.96^A	6.69^A	6.77^A
Salty	3.76^A	4.55^A	3.66^A	3.97^A
Sweet	2.52^A	2.14^A	5.51^A	5.18^A

[1]Means expressed as cm on a 15-cm long intensity scale.
Values followed by the same letter in the same row do not differ significantly by paired t-test ($p<0.05$).
Harvest dates (T) are the same as noted in the legend to Table 1S. PM: potassium metabisulphite; PS: potassium sorbate.

Table 7S. Loadings of the features in the two main principal components between PM and PS.

	F1	F2
Pear	-0.404	0.538
Green apple	0.446	0.386
Cooked apple	-0.526	0.369
Floral	0.190	0.616
Herbaceous	0.570	0.214
% Variance	42.113	28.197

PM and PS are the same as noted in Table 6S.

Table 8S. Intensity of five aroma components[1] according to stabilization treatment at different harvest dates.

	T_1		T_3	
	PM	PS	PM	PS
Pear	3.50^B	4.77^A	4.42^A	4.35^A
Green apple	4.15^A	3.74^A	3.74^A	3.85^A
Cooked apple	3.58^B	5.90^A	5.23^A	5.56^A
Floral	4.38^A	4.41^A	4.00^A	4.29^A
Herbaceous	6.12^A	4.72^B	4.58^A	4.41^A

[1]Means expressed using a 9-point intensity scale. Values followed by the same letter on the same line do not differ significantly by paired t-test ($p<0.05$). Harvest dates (T) are the same as noted in the legend to Table 1S. PM and PS are the same as noted in Table 6S.

Table 9S. Intensity of taste components[1] of unripe grape juices at T_3.

Grape variety	Acid	Astringency
C. franc	9.51^{A}	9.45^{A}
Merlot	8.13^{AB}	8.34^{AB}
C. sauvignon	6.32^{BC}	6.23^{AB}
Chardonnay	5.79^{BC}	4.68^{B}
S. blanc	5.40^{BC}	4.68^{B}
Glera	4.36^{C}	5.51^{AB}

[1]Means expressed as cm on a 15-cm long intensity scale.
The same letter following the value in the same column do not differ significantly by one-way ANOVA followed Tukey's test ($p<0.05$). T_3: early veraison.

Table 10S. Intensity of aroma components[1] of unripe grape juices at T_1 and T_3.

Grape variety	T_1			T_3
	Green apple	Cooked apple	Herbaceous	Herbaceous
C. franc	3.78^{AB}	4.78^{AB}	6.11^{AB}	5.69^{AB}
Merlot	5.40^{A}	4.20^{AB}	8.20^{A}	6.91^{A}
C.sauvignon	3.14^{AB}	5.00^{AB}	3.43^{C}	3.40^{CD}
Chardonnay	2.88^{B}	6.35^{A}	4.06^{BC}	3.80^{BCD}
S. blanc	5.40^{A}	2.50^{B}	7.70^{A}	5.40^{ABC}
Glera	3.80^{AB}	7.00^{A}	2.40^{C}	2.36^{D}

[1]Values expressed as intensity of aromas using a 9-point intensity scale.
Means followed by the same letter in the same column do not differ significantly by one-way ANOVA followed Tukey's test ($p<0.05$). Harvest dates (T) are the same as noted in the legend to Table 1S.

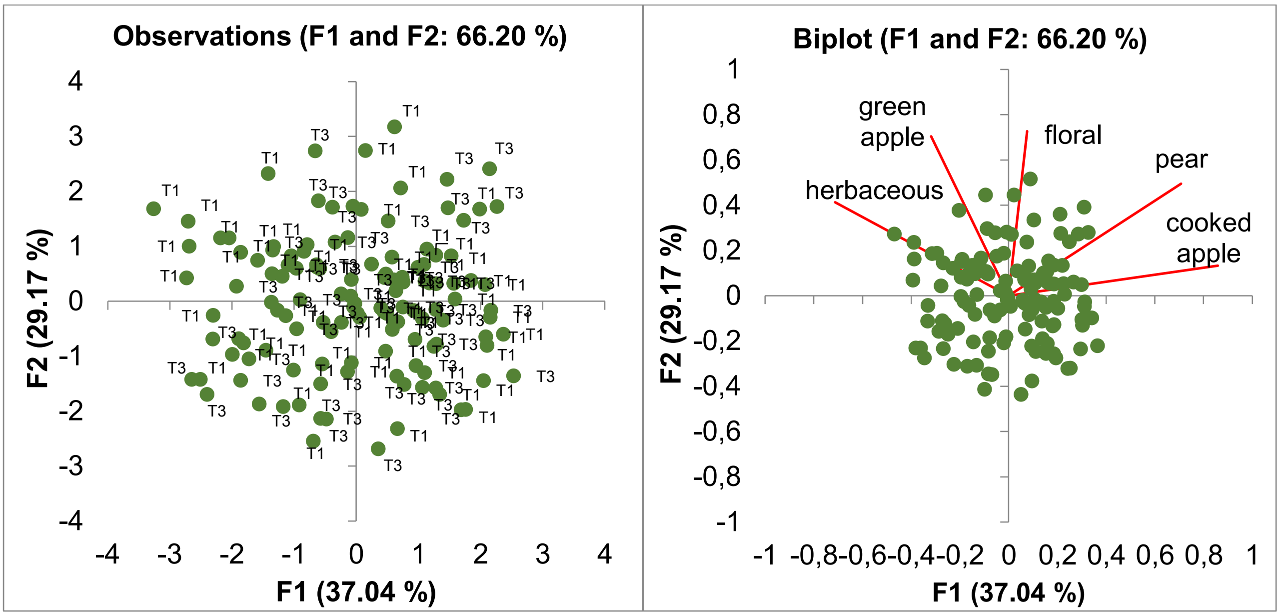

Fig. 1S. Principal component analysis of aroma evaluation of unripe grape juices.
T_1: bunch closure; T_3: early veraison.

CHAPTER 2

USE OF VERJUICE AS AN ACIDIC SALAD SEASONING INGREDIENT: EVALUATION BY CONSUMERS' LIKING AND CHECK-ALL-THAT-APPLY

Use of verjuice as an acidic salad seasoning ingredient: evaluation by consumers' liking and Check-All-That-Apply[2]

Abstract

Verjuice is an unfermented juice produced by pressing unripe grapes used as acidifying in food preparations. In this study, the drivers of consumers' liking of three acidic salad seasonings (verjuice, lemon juice and white wine vinegar) were compared by Check-All-That-Apply (CATA) test. Results showed that overall liking scores were not different for salads seasoned with verjuice and lemon juice, whereas they were significantly higher for those seasoned with vinegar. The liking was associated with moderate sweetness and vinegar aroma typical of vinegar, while it was opposed to lemon aroma, astringent, bitter, high acidity, aqueous viscosity, yellowish color of seasoning, herbaceous and vegetable aroma typical of lemon juice and verjuice. The approach used resulted appropriate to identify the drivers of liking. Results showed that verjuice can be a valid alternative to common acidic salad seasoning, and indicated the directions for future improvements of verjuice.

Keywords

Check-All-That-Apply

Consumer test

Salad seasoning

Sensory evaluation

Unripe grape juice

Verjuice

---

[2]This chapter is an edited version of: Dupas de Matos, A., Magli, M., Marangon, M., Curioni, A., Pasini, G., Vincenzi, S. (2018). Use of verjuice as an acidic salad seasoning ingredient: evaluation by consumers' liking and Check-All-That-Apply, European Food Research and Technology, v. 244, p. 2117-2125, https://doi.org/10.1007/s00217-018-3120-6, ISSN 1438-2377, Copyright 2018 Springer-Verlag GmbH Germany, part of Springer Nature.

1. Introduction

Acidic ingredients and dressings are commonly used to improve the taste of several dishes. In recent time, the demand for salads has been increasing given that consumers see it as a healthy food option [1]. In the past decades, consumers became increasingly aware of the nutritional and health-related aspects of the products they consume [2], and this is now considered a key driver for the development of new foods including sauces, dressings and condiments. To meet this demand, the food industry has been exploring and researching alternative salad dressings [1]. Salad dressings are widely consumed worldwide, and their nutritive significance and economic importance has been rising [3].

Vinegar, obtained from wine by the action of acetic acid bacteria, is produced and extensively used as a salad seasoning and as an acidifying and preserving agent especially in Mediterranean countries. Vinegar has been part of the human diet since ancient times and still plays an important culinary role [4, 5]. Vinegar contains polyphenols derived from grapes that are mostly responsible for its antioxidant activity [6], but they also account for its organoleptic properties as they are responsible for color and astringency [5].

A liquid food product derived from *Vitis vinifera* grapes and known as verjuice (or verjus) has been recently rediscovered for its use as acidic food condiment. Verjuice is the unfermented juice obtained from unripe grape berries. The name verjus derives from the French expression “vert jus” (“green juice”), and refers to a product characterized by high acidity and tart taste [7, 8]. Currently, verjuice is being used as a food preservative, flavoring and acidifying agent in several dishes, salads and appetizers [9, 10] being considered a good alternative to vinegar and lemon juice in many food preparations. Despite of the increasing interest in verjuice, only a couple of studies report on its sensorial properties [8, 11], but no information is currently available on the sensory analysis of salads seasoned with verjuice, nor its comparison to other acidic seasonings. One attractive characteristic of verjuice is that, similarly to vinegar, it contains phenolic compounds, and therefore has antioxidant properties.

Some studies linked the consumption of verjuice to positive effects on human health such as lipid lowering activity, ability to control hypertension [12], reduction of atherosclerosis risk, and a cardiotonification [13]. Moreover, verjuice possesses bactericidal activity, and consequently its utilization as a potential food preservative has been proposed [9, 10].

Besides the technological and nutritional value of using verjuice in food preparations, its possible success within the food industry will depend upon its organoleptical properties as they will play a crucial role in determining the acceptance of consumers for new food products as sauces, dressings and condiments. To date, research on verjuice has overlooked this fundamental aspect, and information on the sensory properties of verjuice used as a seasoning ingredient is lacking. In particular, data on the sensorial role of verjuice as an alternative to acidic salad seasoning such as wine vinegar and lemon juice are not available.

Check-All-That-Apply (CATA) question, a method firstly proposed for its use in food products by Adams et al. [14] is a type of sensory analysis in which a large number of consumers (50-100) are asked a certain question and respond by selecting one or more applicable answers from a list of words. This methodology has the advantage of allowing multiple options to be selected, and is considered to be a fast and easy method for the collection of information about consumer perception of the sensory characteristics of food products [15, 16]. The present study aims at elucidating by CATA test the drivers of consumers overall liking of one verjuice and two other common acidic salad seasonings: lemon juice and wine vinegar.

2. Materials and methods

2.1 Production of verjuice

Unripe grape clusters from Sauvignon blanc were harvested at stage 32 (bunch closure stage) of the modified E-L phenological classification scheme [17]. Clusters were destemmed by hand, washed with tap water, rinsed with distilled water, and then pressed by using a small-scale stainless-steel basket press until extracting at least 54% of juice (w/w). The basket press was loaded with berries in presence of either 0.5 g/Kg of potassium metabisulphite (PM). The obtained juice was stored at 5°C for 5 days to promote precipitation of bitartrate crystals, then vacuum filtered through 1.6 μm glass fiber filters (VWR, Milan, Italy), bottled in 200 mL glass amber bottles, closed with screw caps, and stored at 5°C for 90 days until sensory analysis.

2.2 Hedonic responses and sensory characterization of salad seasonings by CATA

Consumers (n= 135, 58 females and 77 males) aged from 19 to 63 years old (average age 29.17, standard deviation 12.16 years) were invited to participate in an unique tasting session and those consumers that accepted spontaneously were recruited (no cash incentive was provided). Before starting the session, the subjects were invited to sign a consent agreeing in participate voluntarily to the session and to answer a questionnaire on the manner that they normally season their salads (type of oil and of acidifying agent). The 135 consumers tested all declared to eat salad at least once a week and to make use of acidifying ingredients, so they were all deemed suitable to participate to the tasting session that was conducted in the Sensory Analysis Room at the Conegliano campus of Padova University. Before starting the session, the panelists received by the panel leader detailed instructions on how to answer the CATA question and about the meaning of the descriptors. They were informed that the attributes were related to both visual and flavor aspects, and that taste attributes were also provided with an intensity level (low, moderate, and high). Participants were asked to check all the terms that they considered appropriate to describe each portion of salad, without any constraint on the number of attributes they could select.

The session was organized in two steps. Participants were given three portions of salad and were first asked to rate their overall liking for each sample using a horizontal 9-point hedonic scale anchored at ‘dislike extremely’’ (1) and ‘like extremely’ (9). The three portions of lettuce (*Lactuca sativa*, 10 g each) were seasoned with a mixture of sunflower oil (2 mL), salt (NaCl, 0.1 g), and as acidic ingredient alternatively verjuice (2 mL), commercially available white wine vinegar (1 mL) or lemon juice (1 mL). The volume of vinegar and lemon juice was half (1 mL) of that of verjuice (2 mL) to reach the same titratable acidity (expressed as tartaric acid equivalents), which was originally 60 g L^{-1} for vinegar and lemon juice, and 30 g L^{-1} for verjuice. The three portions of salad were offered simultaneously to the participants. Unsalted crackers and water were also provided for participants to clean and rinse their mouth between tastings and breaks.

During the second step, the Check-All-That-Apply (CATA) question with 27 attributes was applied in order to describe the sensory characteristics of seasonings that maximize the consumer liking [18]. The sensory CATA terms used in this study were generated in a previous study by a focus group [11] composed of 28 students that were provided with salads seasoned with 8 different verjuice, and for each sample they were asked to pay attention to the appearance, aroma (retronasal perception), and taste. The terms considered in the CATA question included color: colorless, yellowish and greenish color of seasoning; viscosity: aqueous viscosity of seasoning; aroma: cooked apple, green apple, pear, raisin, lemon, vegetable, herbaceous, floral, vinegar, vinous, and honey; taste: bitter, acid, sweet, and salty; tactile sensation: astringent; trigeminal sensation: pungent. For each of the terms acidity, sweetness and saltiness three intensity adjectives were generated and used (low, moderate, high) [16, 19]. Then, consumers were asked to complete the CATA question to describe their ideal product [20, 21]. Presentation order of terms was counterbalanced between and within participants [22].

2.3 Statistical analyses

Statistical analysis was performed using SAS® software (SAS 9.4, SAS Institute Inc., Cary, NC, USA) with statistical significance determined using an alpha value of 0.05 unless otherwise stated. One-way analysis of variance (ANOVA) followed by post hoc Tukey test was performed on the overall liking scores of consumer test considering sample as fixed source of variation and consumer as random effect. Frequency of use of each sensory attribute was determined by counting the number of consumers that used that term to describe each sample. Cochran's Q test was carried out to identify significant differences between samples for each of the terms included on the CATA question [23]. Correspondence analysis (CA) was used to get a bi-dimensional representation of the samples and the relationship between samples and terms from the CATA question. This analysis was performed on the contingency table containing the samples in rows and the terms from the CATA question on the columns. The ideal product was considered as supplementary individual in the analysis [18]. Partial least square regression (PLSR) analysis were used to study the relationship between CATA questions and liking data as proposed by Meyners and colleagues [24]. PLSR analysis was performed considering CATA questions as X matrix (sensory profiling data) and average liking scores by product as Y matrix (hedonic data) [25]. Penalty analysis was carried out on consumer responses to determine the drop in overall liking associated with a deviation from the ideal for each attribute from the CATA question [21]. Moreover, PLSR was used to estimate the weight of the deviation from the ideal of each term from the CATA question. Only attributes which were considered as deviated from the ideal for at least 20% of the consumers were considered [26, 27].

3. Results

3.1 Consumers' acceptability of different salad seasonings

3.1.1 Overall liking assessment

Overall liking of three portions of salad seasoned with different acidifying was evaluated on a 9-point scale before consumer indicated the CATA terms that applied to each sample. Verjuice (5.81 ± 1.46) and lemon juice (5.93 ± 1.46) liking scores were not significantly different, whereas white wine vinegar-based salad overall liking score was significantly higher (6.36 ± 1.54, see **Table 1**).

Table 1. Frequency of mention (n) for Check-All-That-Apply evaluation and the hypothetical ideal product, and the average liking scores of three different seasonings-based salad (n = 135)

Attribute	Verjuice[a]	White wine vinegar	Lemon juice	Ideal product	Total
Lemon aroma***	68	28	54	43	193
Vinegar aroma***	38	75	34	42	189
Pear aroma**	2	11	13	13	39
Herbaceous aroma*	38	28	46	37	149
Vegetable aroma*	41	24	30	50	145
Vinous aroma[ns]	13	29	14	11	67
Honey aroma[ns]	3	8	7	12	30
Raisin aroma[ns]	4	7	8	4	23
Floral aroma[ns]	5	8	10	20	43
Cooked apple aroma[ns]	5	7	11	8	31
Green apple aroma[ns]	13	18	12	28	71
Astringent**	27	7	19	5	58
Pungent[ns]	39	33	29	11	112
Bitter*	19	8	20	8	55
High sweetness[ns]	1	5	5	0	11
Moderate sweetness*	27	39	22	55	143
Low sweetnees[ns]	21	10	19	27	77
High acidity*	56	21	37	4	118
Moderate acidity[ns]	47	60	43	81	231
Low acidity[ns]	12	23	20	43	98
High salt[ns]	27	23	37	0	87
Moderate salt[ns]	55	69	58	85	267
Low salt[ns]	17	19	17	47	100
Yellowish color***	21	1	1	14	37
Colorless[ns]	67	82	75	40	264
Greenish color[ns]	7	6	6	17	36
Aqueous viscosity*	40	22	31	6	99
Total	713	671	678	711	2773
Overall liking[b]	5.81 b	6.36 a	5.93 b		

[ns] not significantly different according to Cochran's Q test
Significant difference for * p<0.05; ** p<0.01; *** p<0.001
The ideal product was not included in Cochran's Q test
[a] Sauvignon blanc verjuice stabilized with potassium metabisulphite
[b] Means with different letters in the row are significantly different according to Tukey's test (p<0.05)

3.1.2 Check-All-That-Apply (CATA) counts

Regarding the total counts of CATA evaluation, the 27 attributes used to describe three portions of salad seasoned with different acidic ingredients, as well as the hypothetical ideal product/salad, are shown in **Table 1**. Eleven out of the 27 terms of the CATA question were found to be significantly different, indicating that consumers perceived differences in the sensory characteristics of the evaluated salads (**Table 1**). Given that a significant difference between the samples in the frequency of mentions for each term indicates its importance, all significant attributes were included in the further analysis as proposed by Varela & Ares [28].

Figure 1 shows samples and term configurations from the CA performed on the frequency table containing consumer responses to the CATA terms statistically different between the samples.

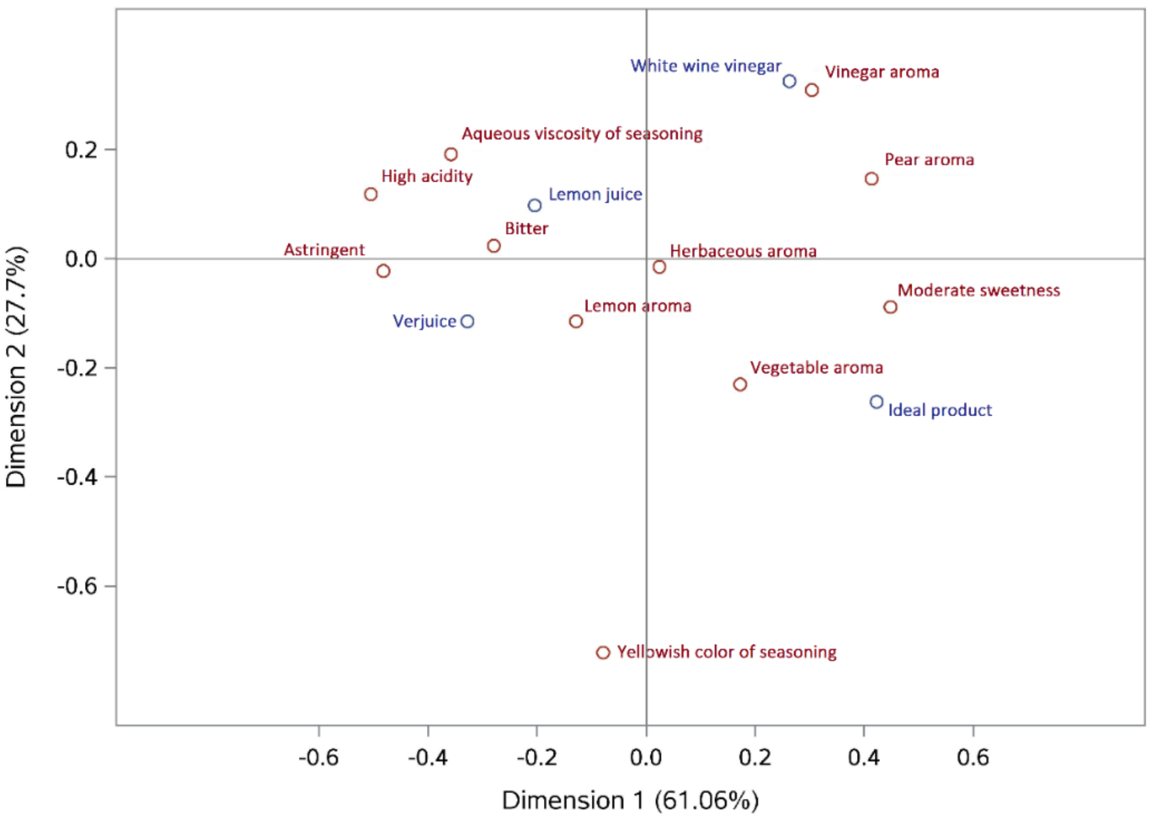

Fig. 1. Representation of the salad seasonings, the ideal product and the terms in the first and second dimensions of the correspondence analysis of the Check-All-That-Apply counts statistically different between the samples.

The first and second dimensions of the CA accounted for 61.06% and 27.70% of the inertia, respectively. Verjuice and lemon juice were located at negative values on the first dimension, being mainly described by lemon aroma, astringent, bitter, high acidity, herbaceous aroma and aqueous viscosity of seasoning. In contrast, white wine vinegar was located at positive values of the first and second dimension and was mainly described by vinegar aroma and pear aroma. Regarding the ideal product, it was characterized by moderate sweetness and vegetable aroma and, as expected, its behavior is analogous to that of the vinegar sample which showed the highest liking score (**Table 1**).

3.1.3 Relating CATA with liking by PLSR analysis

The relation between sensory terms and overall liking of the three samples is depicted in **Figure 2**, that shows that the PLS factor 1 explains most of the variation in X and Y (77.9 and 99%, respectively).The liking was associated with moderate sweetness and vinegar aroma, while it was opposed to lemon aroma, astringent, bitter, high acidity, aqueous viscosity of seasoning, and vegetable aroma attributes.

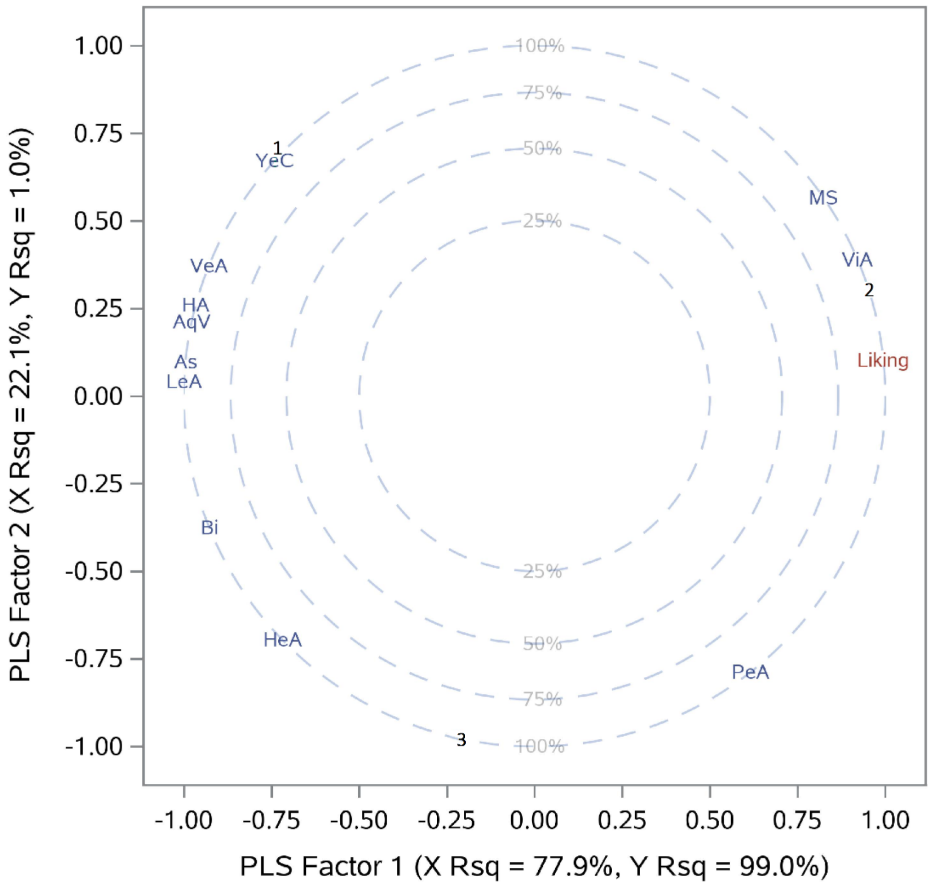

Fig. 2. Correlation Loadings Plot obtained by the PLS regression model of the three products based on CATA questions and hedonic data. Products: 1 verjuice, 2 white wine vinegar, 3 lemon juice. CATA terms: AqV aqueous viscosity of seasoning, As astringent, Bi bitter, HA high acidity, HeA herbaceous aroma, LeA lemon aroma, MS moderate sweetness, PeA pear aroma, VeA vegetable aroma, ViA vinegar aroma, YeC yellowish color of seasoning.

3.1.4 Penalty analysis for verjuice

Penalty analysis was applied to identify the average drop in overall liking associated with a deviation from the ideal product for each of the 28 attributes from CATA question. The mean drops in overall liking for verjuice (Figure 3) was calculated as a function of the proportion of consumers that checked an attribute differently than for the ideal product. The attributes high acidity, high salt, low acidity, astringent, pungent, herbaceous aroma, and aqueous viscosity of seasoning corresponded to those in which consumers considered that it deviated from the ideal and therefore caused a significant decrease in liking. The attribute with the highest mean drop and deviation from the ideal was high acidity (**Figure 3**).

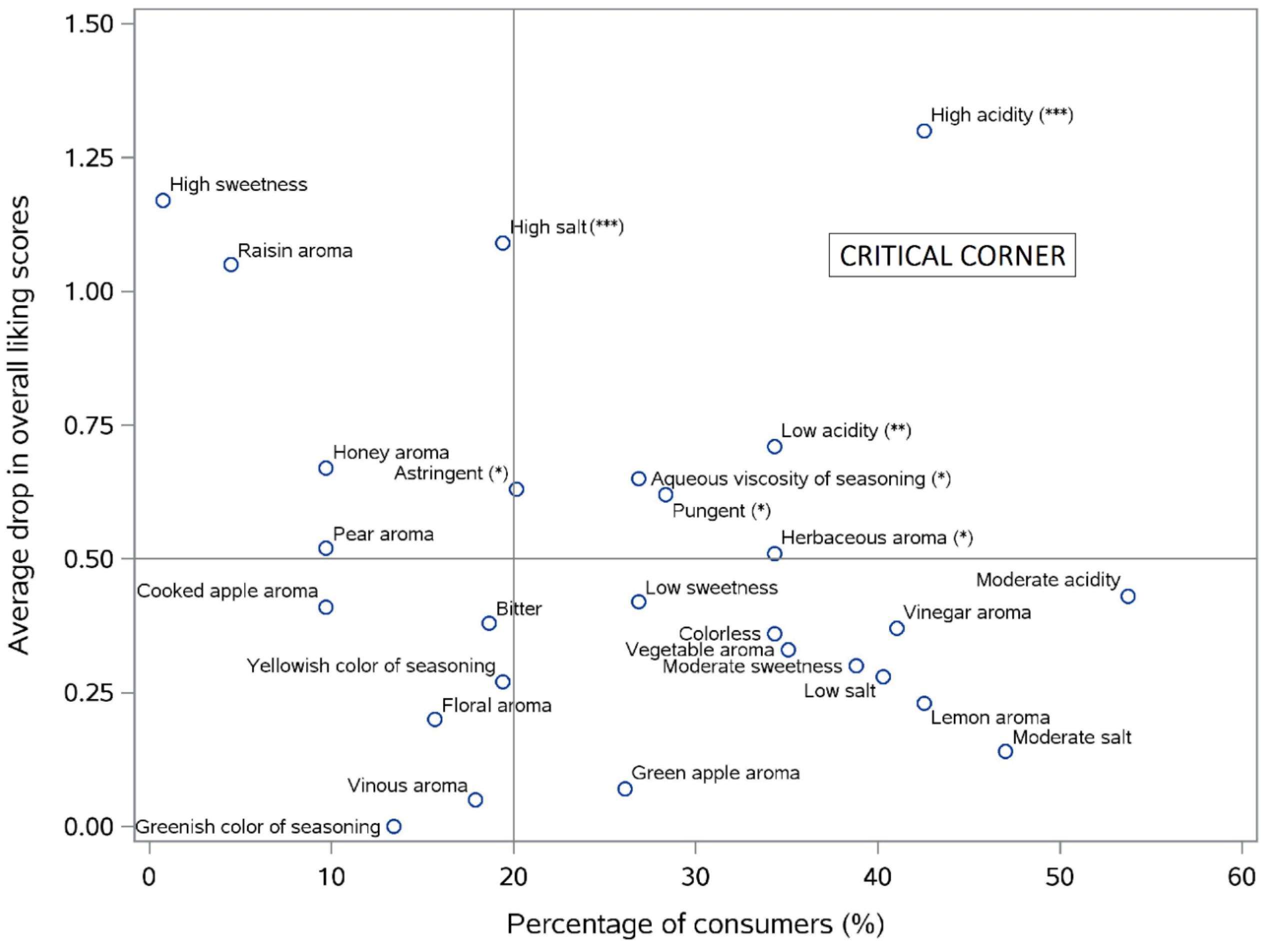

Fig. 3. Mean drops in overall liking as a function of the percentage of consumers that checked an attribute differently than for the ideal product for verjuice. *Significant differences in mean drop overall liking according to Kruskal–Wallis test at $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Regression coefficients of the partial least squares (PLS) regression model was used to estimate the weight of the deviation of verjuice from the ideal product of each term from the CATA question (**Figure 4**). The attributes that deviated the most were high and low acidity followed by moderate acidity, aqueous viscosity of seasoning, astringent, vegetable aroma and low sweetness. In this test, only the attributes considered as deviating from the ideal product for at least 20% of the consumers are taken into account [26, 27]. Deviation from the ideal product significantly affected the overall liking only for those attributes having a significant mean drop overall liking (**Figure 3**).

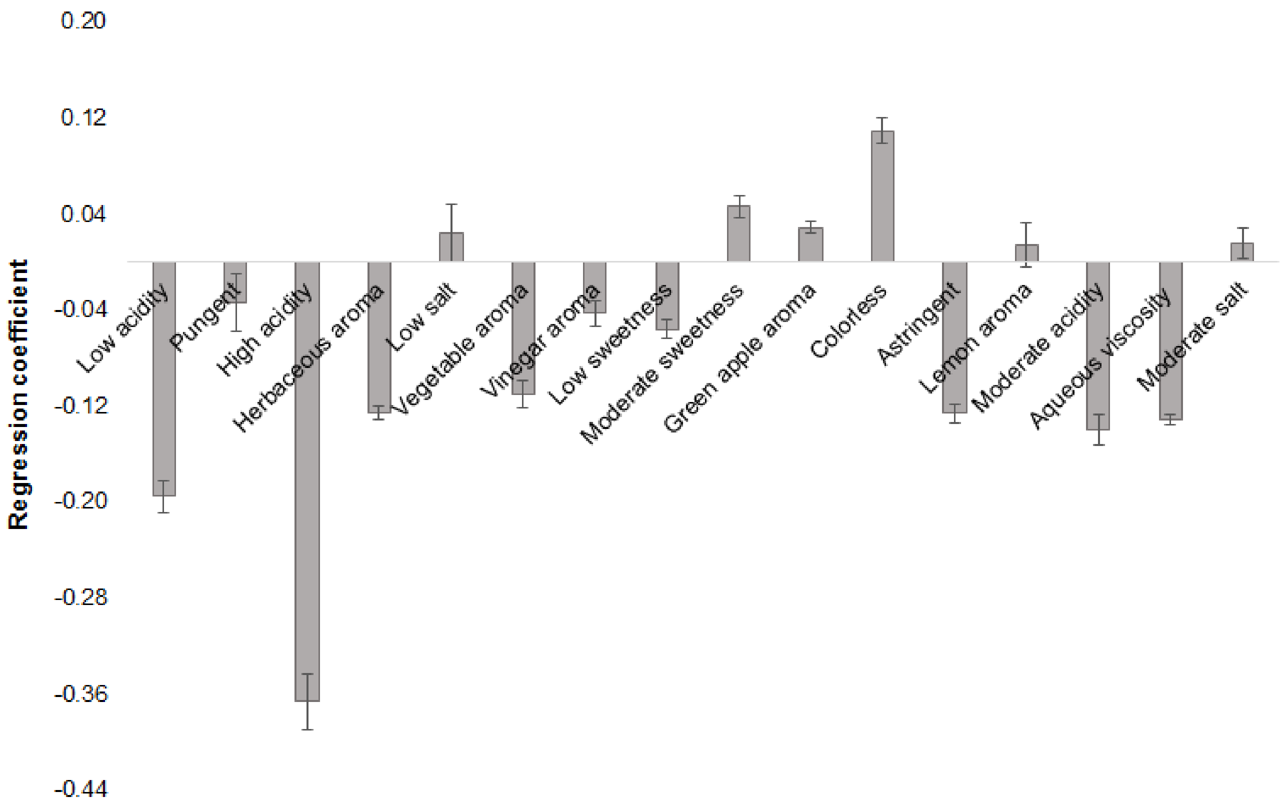

Fig. 4. Regression coefficients of the PLS model calculated considering overall liking and dummy variable indicating if each consumer used the term identically for describing the sample and their ideal product as independent variables, for verjuice-based salad.

4. Discussion

A novel product consisting of a verjuice made from Sauvignon blanc grapes and preserved with PM, previously screened for appropriateness (data not shown) among 8 other different verjuice samples [11], was benchmarked by CATA test with two other common acidic salad seasonings used in Italy, white wine vinegar and lemon juice in order to obtain information on the drivers of overall liking. To avoid possible interferences of the rather strong flavor of olive oil during the tasting sessions, this latter was replaced by the more neutral sunflower oil.

A growing trend in product development is to use consumer descriptions as a realistic tool to identify ideal products and possible deviations from ideal products, so to obtain indications on how a product could be improved [20, 21]. Among these, CATA question is a technique increasingly used for sensory characterization of food products [15], normally used in combination with several statistical analysis that can provide complementary information for the interpretation of the CATA results [18]. Among these, several authors commonly use Correspondence Analysis to obtain a sensory map of the samples and PLS model to find which are the significant variables able to explain over 50% of the variance studying the relationship between CATA questions and liking data. Additionally, penalty analysis is generally performed to provide information on the overall liking associated with a deviation from the ideal product for each attribute of the CATA question, and PLSR to provide information about the maximum potential improvement of overall liking to be used for product reformulation [18, 25, 29].

In this project, the application of the CATA question method revealed that wine vinegar was the preferred seasoning, while the liking for lemon juice and verjuice was at the same level (**Table 1**). Despite these differences, the three salad seasonings were all well accepted, with overall liking scores above the middle point of the scale (5.81-6.36). Consumers were able to perceive differences on 11 out of the 27 attributes (**Table 1**). This provides useful indications for the application of the CA aimed at visually identifying how the relevant attributes of the CATA question relate to both the products under investigation and the ideal product (**Figure 1**). CA clearly indicates that the each seasoning deviated from the ideal product, with each product located in a different quadrant of the CA. Consumers described verjuice and lemon juice as similar, as they are characterized by the same attributes (lemon aroma, astringent, bitter, high acidity, and aqueous viscosity of seasoning). On the other hand, white wine vinegar was better described by vinegar aroma and pear aroma, and is the seasoning most similar to the ideal product that, however, is also characterized by a moderate sweetness and vegetable aroma.

Results of **Figure 2** give some indications on the role of the drivers of consumers' liking. PLS regression analysis was performed to establish which sensory attributes were mainly related to the overall liking of the samples, and to obtain a perceptual map through the CATA method [25, 29]. Vinegar is the product that not only resulted closer to the ideal product in **Figure 1**, but it is also the one that is correlated with the liking by vinegar aroma and moderate sweetness attributes (**Figure 2**). On the other hand, both lemon juice and verjuice are positioned near the attributes that are driving negative liking from consumers.

Another powerful tool to shed light on the actions required to improve a product during its development is represented by the penalty analysis (**Figure 3**) [21].

The interpretation of the penalty analysis has been performed differently by different authors. There are discrepancies on the minimum percentages of consumers required to consider an attribute significant, generally between 20 and 30% [25, 26, 27, 30]. Additionally, the drop in overall liking varies between 0.5 and 1 point [21, 30]. The area comprises between these two parameters is considered the threshold for a meaningful decline in the liking of an attribute, which is also known as top right-hand quadrant or critical corner [31].

In this paper the critical corner was defined by at least 20% of consumers and a drop of at least 0.5 points. For verjuice, this analysis clearly indicated the attributes that consumers considered to differ from the ideal product, notably: high acidity, high salt, low acidity, astringent, pungent, herbaceous aroma, and aqueous viscosity of seasoning.

Some attributes showed discrepancies between the different methods of analysis adopted. Herbaceous received a number of mentions in the CATA question that was almost identical to those received by the ideal product (38 vs. 37, respectively, see **Table 1**), indicating that this was an attribute sought by consumers. However, by CA this attribute was positioned nearer to lemon juice and verjuice than to the ideal product, showing that this attribute better described these two products. In addition, the correlation loading plot analysis, the penalty analysis and the PLSR all confirmed the output of the CA, indicating that the attribute herbaceous was positioned opposite to the overall liking score (**Figure 2**) and was responsible for a significant drop in overall liking of 0.5 points (**Figure 3**). This showed that CATA output would have been misleading for this particular attribute, and therefore additional methods are required in order to fully understand the relationship between an attribute and the overall liking.

The attribute high salt was not significantly different between the three samples from the CATA question but showed that consumers do not look for this attribute in their ideal product (zero mention, see **Table 1**). However, by penalty analysis high salt was statistically different and responsible for over 1-point of drop in overall liking by 19% of consumers (**Figure 3**), showing that this attribute is lower than our cut-off and therefore not included in the PLSR regression analysis. The attribute high salt was not significantly different between the three samples from the CATA question but showed that consumers do not look for this attribute in their ideal product (zero mention, see **Table 1**). This finding is confirmed by penalty analysis where high salt was responsible for over 1-point of drop in overall liking by 19% of consumers (**Figure 3**). Considering that the cut off threshold adopted for the PLSR regression in this study was at least 20% of consumers (**Figure 4**), high salt was not included. Therefore, when looking at how to improve verjuice high salt could be considered as an additional priority for reformulation.

Both low and high acidity attributes affected the liking of verjuice. Some participants evaluated verjuice as having high acidity (43% of consumers), while others (34%) as having low acidity and penalized both for this (**Figure 3**). In fact, the PLSR model (**Figure 4**) confirmed that the weight of the deviation from the ideal product of both high and low acidity was important, a discrepancy that shows that consumers can have different perceptions and/or preferences of taste intensity for the term acidity.

These data are extremely useful as, alongside with indications provided by the CA (**Figure 1**) and the correlation loading plot (**Figure 2**), they provide clear indications on what characteristics of verjuice should be improved. From the consumers' description of verjuice and the ideal product (**Table 1**), and the percentage of consumers who considered that the verjuice deviated from the ideal product (**Figure 3**), the priority for verjuice reformulation would be increasing its sweetness, an action that would result in a decreased perception of acidity, astringency [32, 33] and saltiness. This could be achieved by simply adding a sweetener to the finished product, or by harvesting the grapes at a later ripening stage (e.g. at the beginning of veraison) [11, 34].

The selected approach, based on consumer-driven sensory characterization of salad seasonings, provides clear indications on the perception of specific attributes of a new product such as verjuice. This confirms that such a methodology, already successfully applied for the development of several other foods [15, 16, 35, 36], can have great external validity for product development within the food industry, and could be used to relate the attributes of a new product to those of both competitor's and the ideal product.

5. Conclusions

This study shows that verjuice, an acidic-food ingredient that is receiving increasing attention in cuisines of different countries, represents a viable alternative to lemon juice as salad seasoning as it confers to the salad similar sensorial characteristics. Given that verjuice produced from different grape varieties have all been evaluated appropriate for use in salads (data not shown), its production from single varieties or their blend could be considered for further experiments. In this perspective, further studies are also required to determine the health and antimicrobial properties of verjuice, its shelf-life and suitability for use in other food preparations.

An interesting feature of verjuice is its lack of the typical pungent smell of acetic acid, an aspect that could make it as the preferred salad seasoning for those consumers disliking the smell of vinegar. Additionally, verjuice could be a suitable ingredient for those food preparations where acetic acid may be undesirable. For example, verjuice could be a versatile ingredient for food and wine pairings given that it lacks volatile acidity and due to the affinity of its acidity with that of wine. This consideration might be valid also for food preparations other than salads, such as vinegar-preserved vegetables. Indeed, food products containing vinegar seem to be losing popularity among consumers, as noted by some Italian producers of pickles.

Given that verjuice can be produced from clusters thinning, that are normally early removed from the vines to favor the ripening of the remaining clusters, verjuice represents an opportunity for grapegrowers to increase their income by commercially exploiting a material that is normally discarded.

Acknowledgements

This work was support by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant number: 204483/2014-0) and University of Padova. This study was undertaken as part of the PhD program Land, Environment, Resources, and Health of Amanda Dupas de Matos. The authors wish to thank Marco Lucchetta for donating the grapes.

References

1. De Melo ANF, De Souza EL, Da Silva Araujo VB, Magnani M (2015) Stability, nutritional and sensory characteristics of French salad dressing made with mannoprotein from spent brewer's yeast. LWT - Food Sci Technol 62:771–4

2. Sirò I, Kápolna E, Kápolna B, Lugasi A (2008) Functional food. Product development, marketing and consumer acceptance - A review. Appetite 51:456–467

3. Sikora M, Badrie N, Deisingh AK, Kowalski S (2008) Sauces and Dressings: A Review of Properties and Applications. Crit Rev Food Sci Nutr 48:50–77

4. Tesfaye W, Morales ML, GarcÍa-Parrilla MC, Troncoso AM (2002) Wine vinegar: technology, authenticity and quality evaluation. Trends Food Sci Technol 13:12–21

5. Mas A, Torija MJ, García-Parrilla MC, Troncoso AM (2014) Acetic acid bacteria and the production and quality of wine vinegar. Sci World J 2014:394671

6. Ho CW, Lazim AM, Fazry S, Zaki UKHH, Lim SJ (2017) Varieties, production, composition and health benefits of vinegars: A review. Food Chem 221:1621–30

7. Nikfardjam MSP (2008) General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers. Mitteilungen Klosterneubg 58:28–31

8. Hayoglu I, Kola O, Kaya C, Özer S, Turkoglu H (2009) Chemical and sensory properties of verjuice, a traditional Turkish non-fermented beverage from kabarcik and yediveren grapes. J Food Process Preserv 33:252–63

9. Karapinar M, Sengun IY (2007) Antimicrobial effect of koruk (unripe grape - Vitis vinifera) juice against Salmonella typhimuriumon salad vegetables. Food Control 18:702–6

10. Karabiyikli S, Öncül N (2016) Inhibitory effect of unripe grape products on foodborne pathogens. J Food Process Preserv 40:1459–1465

11. Dupas de Matos A, Curioni A, Bakalinsky AT, Marangon M, Pasini G, Vincenzi S (2017) Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries. Innov Food Sci Emerg Technol 44:9–14

12. ZibaeeNezhad MJ, Mohammadi E, Beigi MAB, Mirzamohammadi F, Salehi O (2012) The Effects of Unripe Grape Juice on Lipid Profile Improvement. Cholesterol 2012:890262

13. Alipour M, Davoudi P, Davoudi Z (2012) Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal,

hyperlipidemic and hyperlipidemic with hypertensive human volunteers. J Med Plants Res 6:5677–83

14. Adams J, Williams A, Lancaster B, Foley M (2007) Advantages and uses of check-all-that-apply response compared to traditional scaling of attributes for salty snacks. In: 7th Pangborn Sensory Science Symposium Minneapolis, USA, 12–16 August, 2007

15. Varela P, Ares G (2012) Sensory profiling, the blurred line between sensory and consumer science. A review of novel methods for product characterization. Food Res Int 48:893–908

16. Bruzzone F, Vidal L, Antúnez L, Giménez A, Deliza R, Ares G (2015) Comparison of intensity scales and CATA questions in new product development: Sensory characterisation and directions for product reformulation of milk desserts. Food Qual Prefer 44:183–93

17. Coombe B (1995) Adoption of a system for identifying grapevine growth stages. Aust J Grape Wine Res 1:100–10.

18. Ares G, Jaeger SR (2015) Check-all-that-apply (CATA) questions with consumers in practice: experimental considerations and impact on outcome. In: Delarue J, Lawlor B, Rogeaux M, editors. Rapid Sensory Profiling Techniques. Cambridge: Woodhead Publishing 227–45

19. Alcaire F, Antúnez L, Vidal L, Giménez A, Ares G (2017) Aroma-related cross-modal interactions for sugar reduction in milk desserts: Influence on consumer perception. Food Res Int 97:45–50

20. Van Trijp HC, Punter PH, Mickartz F, Kruithof L (2007) The quest for the ideal product: Comparing different methods and approaches. Food Qual Prefer 18:729–740

21. Ares G, Dauber C, Fernández E, Giménez A, Varela P (2014) Penalty analysis based on CATA questions to identify drivers of liking and directions for product reformulation. Food Qual Prefer 32:65–76

22. Ares G, Etchemendy E, Antúnez L, Vidal L, Giménez A, Jaeger SR (2014). Visual attention by consumers to check-all-that-apply questions: Insights to support methodological development. Food Qual Prefer 32:210–20

23. Parente ME, Manzoni A V, Ares G (2011) External preference mapping of commercial antiaging creams based on consumers' responses to a check-all-that-apply question. J Sens Stud 26:158–66.

24. Meyners M, Castura JC, Carr T (2013) Existing and new approaches for the analysis of CATA data. Food Qual Prefer 30:309–19

25. Laureati M, Cattaneo C, Lavelli V, Bergamaschi V, Riso P, Pagliarini E (2017) Application of the check-all-that-apply method (CATA) to get insights on children's drivers of liking of fiber-enriched apple purees. J Sens Stud 32:e12253

26. Xiong R, Meullenet JF (2006) A PLS dummy variable approach to assess the impact of jar attributes on liking. Food Qual Prefer 17:188–98

27. Plaehn D (2012) CATA penalty/reward. Food Qual Prefer 24:141–52

28. Varela P., Ares G (2014) Novel Techniques in Sensory Characterization and Consumer Profiling; CRC Press, Taylor & Francis Group; Boca Raton

29. Martens H, Martens M (2000) Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). Food Qual Prefer 11:5–16

30. Cadot Y, Caillé S, Samsonc A, Barbeaua G, Cheynier V (2010) Sensory dimension of wine typicality related to a terroir by Quantitative Descriptive Analysis, Just About Right analysis and typicality assessment. Anal Chim Acta 660:53–62

31. Marcano J, Varela P, Cunha LM, S. Fiszman S. (2015) Relating dynamic perception of reformulated cheese pies to consumers' expectations of satiating ability. Food Res Int 78:369-377

32. Byrne B (2016) Interactions in chemesthesis: everything affects everything else. In: S. T. McDonald, Bolliet DA, Hayes JE, editors. Chemesthesis: Chemical Touch in Food and Eating. New Jersey: Wiley Blackwell 154–65.

33. Duffy VB, Rawal S, Park J, Brand MH, Sharafi M, Bolling BW (2016) Characterizing and improving the sensory and hedonic responses to polyphenol-rich aronia berry juice. Appetite 107:116–25

35. Iland PG, Dry PR, Proffit T, Tyerman S (2011) The grapevine: from the science to the practice of growing vines for wine. Adelaide (SA): Patrick Iland Wine Promotions

35. Ares G, Barreiro C, Deliza R, Giménez ANA, Gambaro A (2010) Application of a check-all-that-apply question to the development of chocolate milk desserts. J Sens Stud 25:67–86

36. Dooley L, Lee Y-S, Meullenet J-F (2010) The application of check-all-that-apply (CATA) consumer profiling to preference mapping of vanilla ice cream and its comparison to classical external preference mapping. Food Qual Prefer 21:394–401

CHAPTER 3

SENSORY CHARACTERIZATION OF CUCUMBERS PICKLED WITH VERJUICE AS NOVEL ACIDIFYING AGENT

Sensory characterization of cucumbers pickled with verjuice as novel acidifying agent[3]

Abstract

Verjuice is an acidic unfermented unripe grape juice that has recently been proposed as an alternative to vinegar in different food preparations. In this study, pickled cucumber preserves were industrially prepared using two dilutions of verjuice as acidifying agent and compared with the traditional vinegar-pickled samples. Samples were chemically characterized and subjected to sensory analyses using a trained panel and consumers. The different acidifying agents resulted in pickled cucumbers giving different olfactory and gustatory evaluations. No differences in appearance and texture were found. Despite showing chemical and sensory differences, pickles preserved with verjuice and vinegar had similar overall liking scores for visual, olfactory and gustatory aspects. A key feature of pickles prepared with verjuice is their lack of acetic acid potentially conferring them an additional value, suggesting that verjuice represents a viable alternative to vinegar for the production of pickles with distinctive sensorial properties.

Keywords

Acidifying

Cucumber

Pickling

Sensory Analysis

Verjuice

Vinegar

---

[3]This chapter is an edited version of: Dupas de Matos, A., Marangon, M., Magli, M., Cianciabella, M., Predieri, S., Curioni, S., Vincenzi, S. Sensory characterization of cucumbers pickled with verjuice as novel acidifying agent, Food Chemistry, ISSN: 0308-8146, **under review**.

1. Introduction

A widespread approach for preserving vegetables is pickling, a technique based on the addition of salt and/or an acidic liquid as primary means of preservation. Direct acidification, with or without pasteurization, is applied to vegetable foods to improve their shelf life and quality, as well as to enhance their palatability. In particular, pickling is generally performed by the addition of an organic acid, which can be coupled to pasteurization, addition of other preservatives (normally sulfur dioxide or potassium sorbate), refrigerated storage or a combination of these treatments, resulting in unfermented preserves with distinctive sensorial properties (Siddid & Uebersax, 2018). Indeed, in addition to the preserving effects against microbial spoilage obtained by reducing the pH, these acids can also affect also the organoleptic properties of the preserved food. Traditionally, this type of processing is obtained by using acid liquids based on vinegar, which is largely employed as preservative for several food preparations, providing them with a peculiar acetic flavor, a feature that can be appreciated or not by consumers.

However, other natural acidic liquids seem to have the potential to be used for direct acidification of vegetables. Among these, verjuice, the liquid with high acidity obtained from pressing unripe grape berries (Nikfardjam, 2008; Öncül & Karabiyikli, 2015), could be potentially used as an alternative to vinegar. Actually, verjuice has been shown to be a valid alternative to lemon juice in different food preparation such as salad seasoning, flavoring agent, digestive drinks and some cocktails (Nikfardjam, 2008; Setorki et al., 2010; Dupas de Matos et al., 2017; Dupas de Matos et al., 2018), although the sensorial consequences of its use as a preserving agent for direct acidification of vegetables has never been investigated. This is of particular interest because verjuice lacks acetic acid, its acidity being due to non-volatile tartaric and malic acids (Dupas de Matos et al., 2017). It is also noteworthy that unripe grape products (e.g. verjuice, sour grape sauce, etc.) have gained popularity among consumers due to their organoleptic, chemical and antioxidant properties (Öncül & Karabiyikli, 2015).

Because of this increasing interest in using verjuice in food applications, the present study was undertaken to evaluate the sensorial effects of its use as the substitute of vinegar in vegetable preserves by using pickled cucumbers as a model. Specifically, cucumber (*Cucumis sativus*) fruits, which are among the most popular vinegar-pickled vegetables, were submitted to direct acidification according to the industrial processing used in Italy for these products. This was performed with two concentration of verjuice and the obtained pickles were compared with the traditionally vinegar-pickled cucumbers by chemical characterization and sensory analysis performed by both a trained panel and consumers.

2. Materials and methods

2.1 Production of verjuice

Unripe grape clusters from Sauvignon blanc and Chardonnay varieties were harvested in 2016 season at stage 33 of the modified E-L phenological classification scheme (Coombe, 1995). Clusters were destemmed by hand, washed with tap water, and then pressed by using a small-scale stainless-steel basket press until extracting 51% of juice (w/w). The basket press was loaded with berries in presence of 40 mg/Kg of potassium metabisulphite. The obtained juices were stored at 0°C for 10 days to promote precipitation of potassium bitartrate crystals, then centrifuged (500 rpm, 10 minutes), vacuum filtered through 1.6 μm glass fiber filters (VWR, Milan, Italy), bottled in 500 mL glass green bottles, closed with crown cap, and stored at 5°C until being used for the production of pickled cucumbers.

2.2 Production of pickled cucumber

The pickled cucumber samples were prepared industrially by a food preserves company according to their standard processing system. The preserving liquids were prepared with citric and ascorbic acids, salt, water, and vinegar or verjuice as acidifying agents. The final pH of the preserving liquids were all adjusted to 2.8 before utilization. For the experimental samples vinegar was substituted by verjuice, prepared as described in the previous section (Sauvignon blanc and Chardonnay verjuice mixed 50:50, v/v). Two different concentrations of verjuice (1:2 and 1:10 (v/v) with water) were used for the preparation of the experimental samples. Cucumbers (original pH 6) were placed in hot water (90 °C) for 3 minutes, drained off and transferred to glass jars of 580 mL volume (300 g of cucumber/jar), filled with the three preserving liquids, closed with screw cap, pasteurized and stored for 8 months before being analyzed.

2.3 Analytical characterization

2.3.1 Compositional data

pH was measured using a portable pH meter (HACH, Colorado, United States) equipped with a 5050T pH electrode with temperature sensor. Sodium and potassium concentrations were measured by ICP-OES Arcos EOP (Spectro A. I. GmbH, Kleve, Germany), according to the AOAC Official method 2013.06 (2016).

2.3.2 HPLC analysis

Organic acids (tartaric, malic, citric, and acetic) as well as glucose and fructose contents were analyzed by high-performance liquid chromatography (HPLC) according to the method proposed by Coelho et al. (2018) with minor modifications. Liquid samples were prepared by diluting them with the mobile phase (5 mM H_2SO_4 in ultrapure water), before being syringe-filtered at 0.20 µm prior to injection. Solid samples (pickled cucumbers) were extracted in the mobile phase by homogenizing them with an ultraturrax homogeneiser (IKA, Staufen, Germany). Upon centrifugation, the supernatant was collected, adequately diluted with the mobile phase, and syringe-filtered at 0.20 µm before injection.

2.3.3 Color measurement

Color was measured with a portable spectrophotometer (Minolta CM-600d, Osaka, Japan) set on illuminant D65 (standard daylight) and with a 10° observer. Cucumbers were placed on an appropriate tray and the reflectance of the chromaticity coordinates was measured in triplicate (2 readings x 3 cucumbers = 6 readings for each jar) directly on their surface to a depth of 1 cm. Data were expressed according to the CIELab colorimetric system: “L” (brightness), “a” (red color coordinate) and “b” (yellow color coordinate) from the SCE (specular component excluded) measurement condition.

2.3.4 Texture measurement

The texture of the cucumbers, measured as maximum shear force (N/cm^2) required to cut the sample, was assessed using a LS5 dynamometer (Lloyd Instruments Ltd., AMETEK Measurement & Calibration Technologies, Florida, USA) equipped with a Warner-Bratzler probe, 500-N load cell, and crosshead speed of 2 mm/s, as reported by Segato et al. (2007).

The measurements were conducted in triplicate by placing the pickled cucumbers at room temperature on a non-lubricated flat platform.

2.4 Sensory evaluation

2.4.1 Sample preparation and presentation

The jars were opened and samples were prepared just before the analysis. One cucumber was sliced, placed in a cup with 10 mL of its own preserving liquid to keep the cucumber soaked. Pickles prepared with vinegar (VIN), verjuice diluted 1:2 (VER1:2) and 1:10 (VER1:10) were served at room temperature in white small plastic cups coded with 3-digit numbers. Presentation order of terms was counterbalanced between and within participants (Ares et al., 2014a). Mineral water was provided for the participants to rinse their mouths between samples.

2.4.2 Panel composition

Twelve trained judges (7 females and 5 males) with prior experience in sensory descriptive evaluation of fruit and vegetables, and familiarity with the sensory software used, were recruited from the IBIMET-CNR staff. The panel received a specific training on how to recognize and evaluate each descriptor based using intensity scales (ISO 8586:2012).

The same panel was used to perform both Descriptive Analysis (DA) and Temporal Dominance of Sensations (TDS) tests. Both tests were conducted in individual booths and carried out in duplicate with the samples' order randomized to prevent any statistical effects of order or carryover (Ares et al., 2014a). Data were recorded on computer notebooks equipped with a specific software for sensory data acquisition (FIZZ, Biosystemès, France).

2.4.2.1 Descriptive Analysis (DA)

Before starting the session, the judges received by the panel leader detailed instructions on the attributes to be evaluated and on how to answer the questions. They were informed that the attributes to be assessed would have been related to appearance (green color and brightness), orthonasal perception (vinegar and cucumber smells), retronasal perception (cucumber, vinegar, and cooked vegetable aromas), taste (acidity, sweetness, salt, bitterness), tactile sensation (crunchiness, firmness, astringency), and trigeminal sensation (pungency). Judges were given three slices of each pickled cucumber sample

(VIN, VER1:2, VER1:10) simultaneously and were asked to rate, according to their experience, all the attributes using a 9-point intensity scale. The evaluation of the samples was carried out under the conditions described in UNI 10957:2003.

2.4.2.2 Temporal Dominance of Sensations (TDS)

Prior to the evaluation sessions, a focus group conducted a pilot tasting aimed at determining the time required for all sensory sensations to end starting from the beginning of the mastication. This information was used to decide the duration of the test, which was set to 60 seconds. For each point of time, the proportion of evaluations (subject × replication) for which the given attribute was assessed as dominant is computed. These proportions were smoothed over time using a spline regression and displayed as curves of the evolution of the dominance rate for each attribute (Schlich & Pineau, 2017).

In the formal sensory session, the judges were asked to select all the in-mouth sensations they felt while tasting the three samples (VIN, VER1:2, VER1:10). One slice of pickled cucumber for each of the samples was given to them simultaneously. The attributes evaluated were crunchiness, firmness, acidity, sweetness, salt, bitterness, astringency, pungency, cucumber aroma, vinegar aroma, and cooked vegetable aroma. The evaluation of the samples was carried out under the conditions described in Pineau et al. (2009, 2012).

2.4.3 Consumer test

A total of 113 subjects participated in the consumer test, 42% were female and 58% were male, aged between 18 and 65 years, and living in both the Bologna (Emilia-Romagna, Italy) and Conegliano (Veneto, Italy) areas. Before starting the session, preliminary questions were asked regarding the frequency of consumption of vegetable preserves, the overall liking of different vegetables preserves if consumed, on their knowledge about the presence in the market of the “zero vinegar” vegetable preserves, and on their propensity to consume this category of products.

Participants were simultaneously given three samples of whole pickled cucumber and asked to rate, according to their preference, the visual, olfactory and gustatory overall liking for each sample using a horizontal 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). Only for the gustatory aspect, judges were also asked to assess the appropriateness of several attributes by using a 5-point just-about-right (JAR) scale (1 = too weak; 3 = just about right; 5 = too strong). JAR was applied to attributes such as firmness, acidity, sweetness, salt, bitterness, astringency, pungency, and cucumber aroma. All subjects received written information about the study before giving their consent as well as all sensorial experiments were performed in compliance with the institutional framework. Consumers spontaneously agreed in participating in a session without cash incentive.

The output from the JAR was submitted to penalty analysis to determine the drop in overall liking associated with each attribute evaluated (Ares, Dauber, Fernández, Giménez, & Varela, 2014b). The interpretation of the penalty analysis has been conducted by setting at 20% the minimum percentages of consumers and at 1 point of drop in overall liking required to consider an attribute significant, thus defining a top right-hand quadrant or critical corner (Marcano, Varela, Cunha, & Fiszman, 2015).

2.5 Statistical analysis

Statistical analysis was performed using SAS® software (SAS 9.4, SAS Institute Inc., Cary, NC, USA) with statistical significance determined using an alpha value of 0.05 unless otherwise stated. Physical and chemical characteristics, sensory profiles and consumer data were analyzed using ANOVA and Tukey's Honestly Significant Differences (HSD) for post hoc mean separation. TDS curves computation considers each attribute separately. For each point of time, the proportion of runs for which the given attribute was assessed as dominant is computed. These proportions, smoothed using the TRANSREG procedure of SAS®, are plotted against time and called TDS curves. Transformation used in the TRANSREG procedure is non-iterative smoothing spline. For each product, TDS curves of all the attributes are depicted on the same graph (Pineau et al., 2009).

3. Results and discussion

3.1 Product analytical characterization

Pickled cucumbers and their preserving liquids were characterized in order to obtain information about their chemical composition, color and texture (**Table 1**).

Table 1. Characteristics of the preserving liquid and of the pickled cucumbers.

		Pickled cucumber			Preserving liquid		
		VIN	VER1:2	VER1:10	VIN	VER1:2	VER1:10
pH		3.43 b	3.30 c	4.02 a	3.40 b	3.22 c	3.95 a
Organic acids [a]	Tartaric	0.05 c	0.80 a	0.19 b	0.17 c	1.40 a	0.40 b
	Malic	0.67 c	3.02 a	1.62 b	1.03 c	5.03 a	2.68 b
	Citric	0.51 b	0.77 a	0.69 a	0.21 b	0.78 a	0.79 a
	Acetic	7.50	n.d.	n.d.	10.44	n.d.	n.d.
Total Acids [a,b]		8.72 a	4.59 b	2.50 c	11.84 a	7.21 b	3.87 c
Sugars [a]	Glucose	2.40 c	5.90 a	3.66 b	3.71 c	8.88 a	5.16 b
	Fructose	2.77 b	3.80 a	3.14 ab	4.01 b	5.15 a	4.35 ab
K [a]		1.17 b	1.58 a	1.48 a	1.08 b	1.48 a	1.31 a
Na [a]		6.31 b	7.46 a	6.71 ab	6.16 a	6.71 a	6.17 a
Color	L*	32.51 a	32.20 a	33.74 a	-	-	-
	a*	3.46 a	1.90 b	2.26 b	-	-	-
	b*	28.91 a	25.87 a	27.55 a	-	-	-
Shear force (N/cm^2)		22.86 a	18.20 a	17.27 a	-	-	-

[a] Measured in mg/g for pickled cucumber, and in mg/mL for preserving liquid. [b] Sum of individual organic acid (tartaric, malic ,citric, acetic). n.d.: not detected. VIN: samples with vinegar; VER1:2: samples with verjuice 1:2; VER1:10: samples with verjuice 1:10. Means followed by the same letter in the row do not differ significantly by one-way ANOVA followed Tukey's test (p<0.05).

In general, the acidity of the pickles varied depending on the preserving liquid used. For cucumbers, whose original pH was around 6.0, the pH ranged between 3.30 to 4.02 after pickling, whereas it varied between 3.22 to 3.95 for the preserving liquids. For both cucumbers and preserving liquids, the most acidic were samples VER 1:2, followed by VIN and VER1:10. These results are in line with the quantity of the strongest organic acids present in the different samples. Malic and tartaric acid concentrations were significantly higher in VER1:2 for both samples, followed by VER1:10 and VIN. This is due to the high quantity of malic and tartaric acid present in unripe grapes (Iland, Dry, Proffitt, & Tyerman, 2011). Citric acid was added in the preserving liquid for all samples and its final content (added + contained in vinegar or verjuice) did not significantly differ among the two VER samples, both containing a significantly higher amount than VIN. As expected, acetic acid was the main organic acid in both VIN samples, while it was not detected in the VER samples as verjuice does not undergo fermentation.

The highest quantities of glucose and fructose were found in the VER1:2 sample, followed by VER1:10 and, as expected, in VIN in which reducing sugars are consumed during fermentation. The content of potassium and sodium varied only slightly among the samples. Regarding the instrumental color analysis, the values for all coordinates (L*, a*, b*) were positive, with a significant difference observed only for the intensity of the a* coordinate, which was however very low for all the samples. Luminosity (L*) showed values ranging from 33.20 to 33.74 and b* from 25.87 to 28.91 in pickled cucumber samples. The texture of the pickled cucumbers did not vary significantly between the samples.

3.2 Sensory evaluation

3.2.1 Descriptive analysis (DA) test

DA was performed to determine the flavor characteristics of the pickled cucumbers. Eight out of 15 terms of the DA were found to be significantly different among the evaluated samples (**Figure 1**).

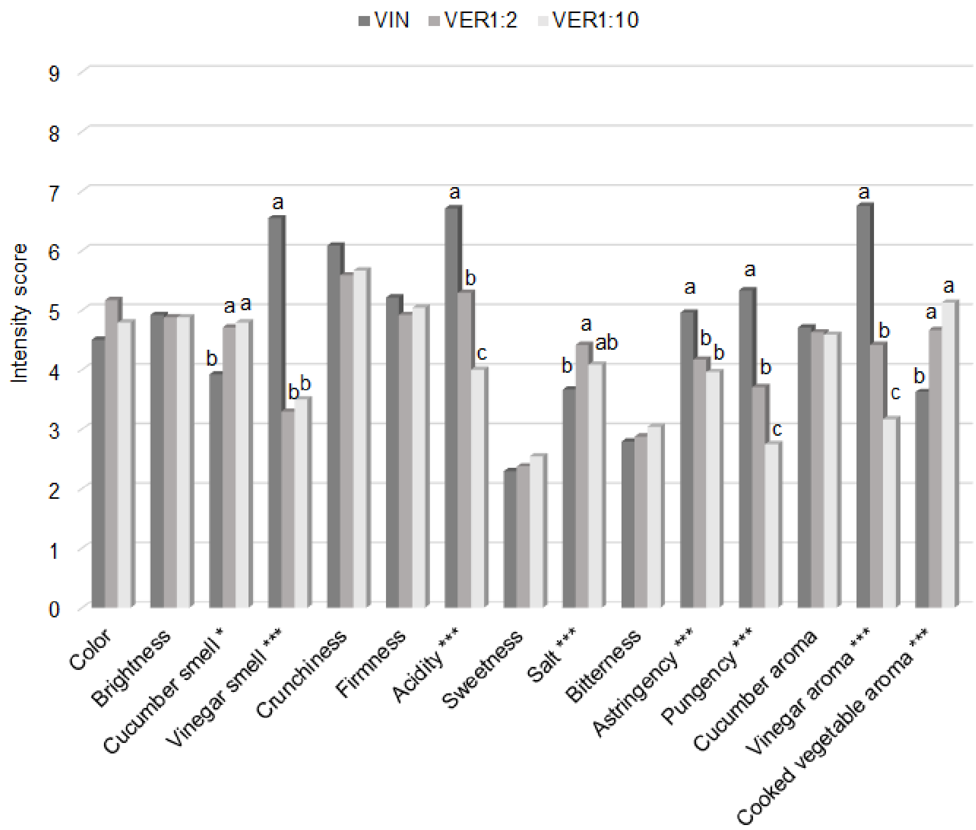

Fig. 1. Sensory profiles of three pickled cucumbers prepared with vinegar (VIN), verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based on DA test-fifteen attributes by twelve judges. * Indicates significant differences in mean according to Tukey's HSD test at $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Regarding the olfactory description, VIN was rated with a significantly higher intensity for "vinegar smell" than the two VER samples, as expected, while the "cucumber smell" attribute had significantly higher intensity for VER1:2 and VER1:10 than VIN. For "acidity", VIN was evaluated as the most acidic, followed by VER1:2 and VER1:10. Regarding the "salt" perception, judges evaluated VER1:2 and VER1:10 as more salty, but VER1:10 did not differ significantly from VIN. On the other hand, VIN was evaluated as the most "astringent" sample. Judges rated VIN as the sample with the highest "pungency" and "vinegar aroma", followed by VER1:2 and VER1:10. "Cooked vegetable aroma" was found to be more intense in VER1:2 and VER1:10 in comparison to VIN sample. Other attributes, including "color", "brightness", "crunchiness", "firmness", "bitterness" and "cucumber aroma" were not reported to be different among the different cucumbers.

3.2.2 Temporal Dominance of Sensations (TDS)

TDS was performed to evaluate which are the dominant in-mouth sensations for 11 predetermined attributes over 60 seconds. **Figure 2** shows the TDS graphs for the three pickled cucumber samples of the study, in which each curve represents the evolution of the dominance rate of an attribute over time. To assist with the interpretation of the results, a dashed line was added to the graph indicating the significance level (5%) on TDS curves (Pineau et al., 2009).

For the VIN sample five major sensations dominated over the evaluation time, with "crunchiness" being a significantly dominant sensation at the beginning, followed by "acidity" and "vinegar aroma", which dominated during almost the entire evaluation. The "pungent" sensation made a dominance for a short time after about 22 seconds. The "astringency" was significant towards the end of the evaluation time.

For the VER1:2 sample the TDS curve shows that "crunchiness" also was a significant dominant sensation at the beginning, along with "firmness". "Acidity" appeared a bit after "crunchiness" and was dominant until almost the middle of the evaluation time, alternating with "cucumber aroma" and a little peak of "salt" sensation. Differently to the VIN sample, here "cooked vegetable aroma" was dominant for a very short but significant at the middle of the consumption time. Also in this case "astringency" was the dominant sensation in the second half of the evaluation along with a discreet appearance of "salt" sensation.

The VER1:10 sample behaved quite differently from VIN and VER1:2. "Crunchiness" was also the dominant sensation at the beginning of the test, but lasted for longer than the other two samples. Differently from VIN and VER1:2, "cooked vegetable aroma" dominated during almost the entire evaluation being alternated with "salt" through two very short appearances (after 10 and 30 seconds of the evaluation time). Also in this case "astringency" was the dominant sensation at the end of the consumption time with but a lower dominance rate in comparison the other samples.

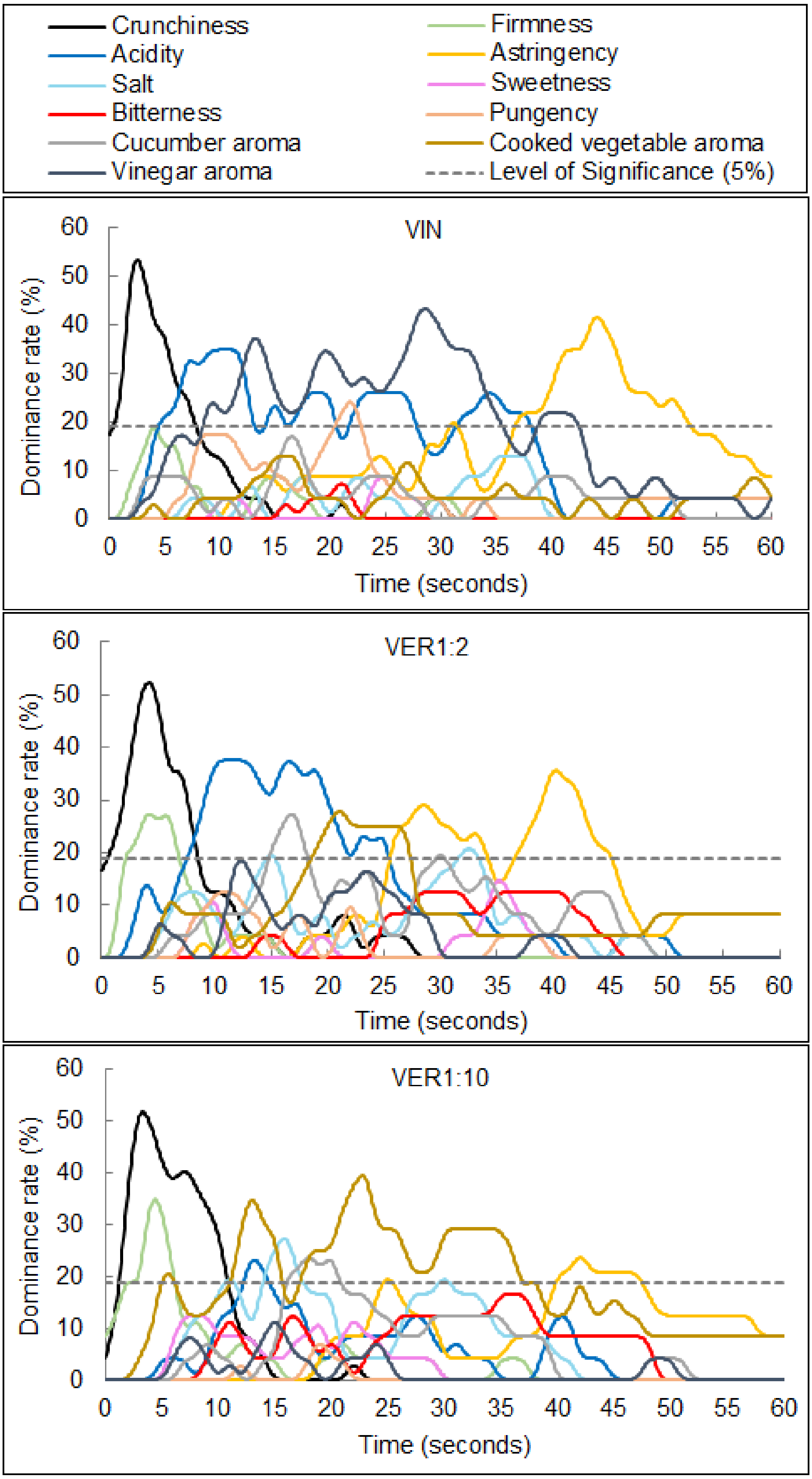

Fig. 2. Sensory profiles of three pickled cucumbers prepared with vinegar (VIN), verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based on TDS test-eleven attributes by twelve judges. Dashed line represents the level of significance (5%) on TDS curves.

3.2.3 Consumers: Liking and JAR scales

The overall liking of three pickled cucumbers with different acidifying agents was evaluated by a 9-point scale for visual, olfactory and gustatory aspects as showed in **Figure 3**. For visual and gustatory aspects, overall liking scores were not significantly different between the three samples. For the olfactory aspect VIN and VER1:2 liking scores were not significantly different, whereas a difference was reported for VER1:2 and VER1:10.

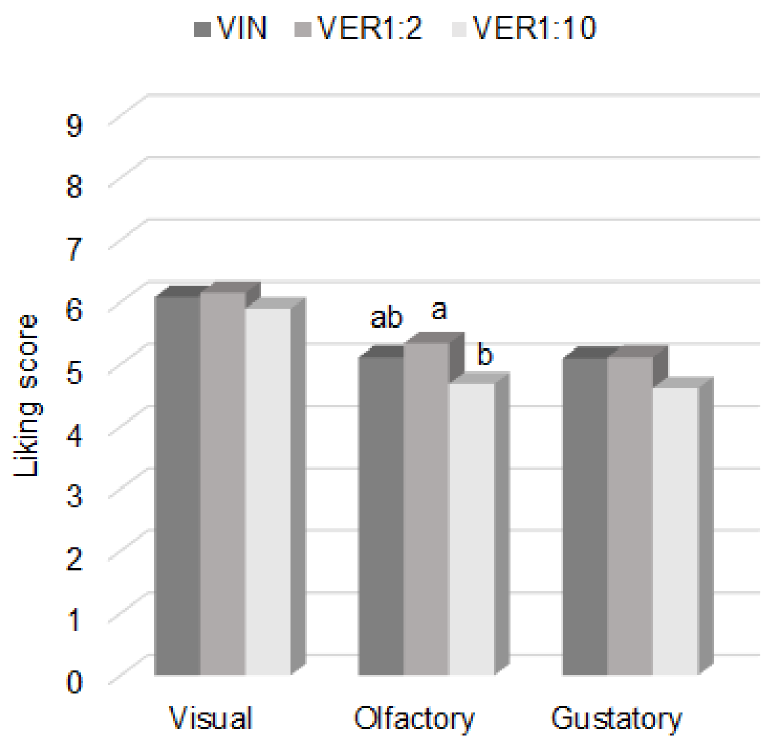

Fig. 3. Overall liking scores of three pickled cucumber prepared with vinegar (VIN), verjuice diluted 1:2 (VER1:2) and verjuice diluted 1:10 (VER1:10) based on consumer test by 113 consumers.

The appropriateness of eight attributes by JAR 5-point scale was evaluated and the penalties (or mean drops) were calculated for the gustatory aspect (**Figure 4**). The penalty analysis identified the following attributes as significant for the VIN sample: not enough cucumber aroma, not enough sweet, too acidic, too pungent, and too astringent; for VER1:2 sample: not enough cucumber aroma, not enough sweet, and not enough acidic; for VER1:10: not enough cucumber aroma, not enough sweet, not enough astringent, not enough bitter, not enough salt, and too sweet.

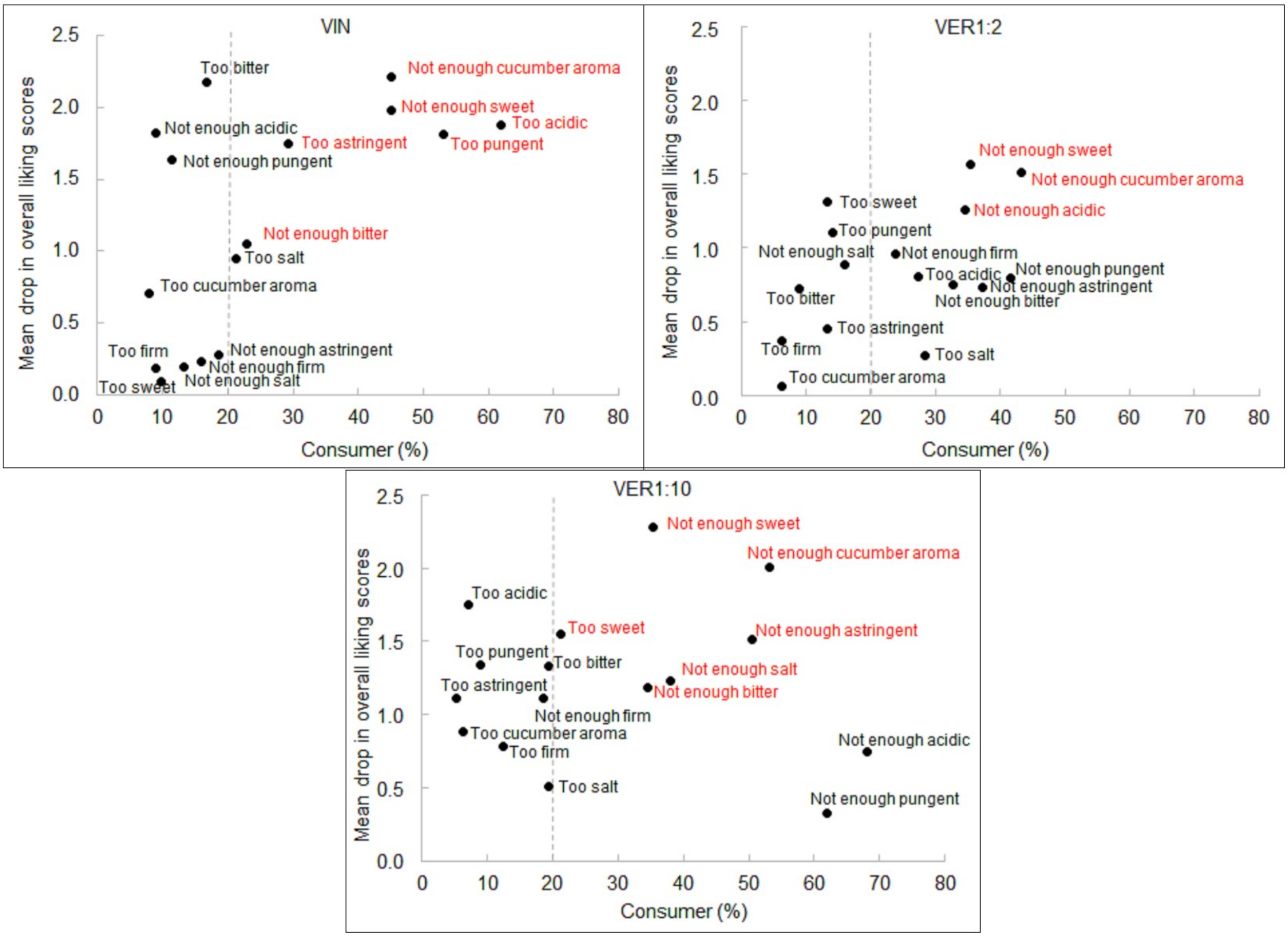

Fig. 4. Mean drops in overall liking as a function of the percentage of consumers. The vertical line represents 20% of consumers. Attributes in red indicate significant differences in mean drop overall liking at $p<0.05$.

4. Discussion

In this paper we explore the possibility to produce pickled vegetables by using verjuice, an acidic liquid obtained from unripe grape berries. The chemical characteristics of this product, and in particular its low pH, make verjuice a good candidate for the substitution of vinegar as a preserving agent for vegetable products. In addition, verjuice lacks in acetic acid (and therefore in volatile acidity), a feature that can be interesting from a sensory point of view. Therefore, the idea was to assess the possibility to obtain pickled vegetable products in which the absence of acetic acid would enhance their sensorial features at least for some consumers, thus opening the way for the production of a new type of preserved vegetables.

To compare the effects deriving from the substitution of vinegar with verjuice as the preserving agent, here the sensorial characteristics of cucumbers pickled with verjuice, used in two concentrations (VER 1:2 and VER 1:10), are reported and compared to those of cucumbers obtained by pickling with vinegar (VIN), which are the classical products that can be found on the Italian market. To this aim all the cucumber samples were prepared by following the procedure used for the industrial processing, thus yielding samples representative of commercial products. In addition to the sensorial characterization of the pickled cucumbers, also their basic chemical composition and some physical parameters were determined, along with those of the preserving liquids (**Table 1**). These analyses highlighted the compositional differences among products useful for the interpretation of the sensorial results, and provided also useful information to assess the suitability of verjuice for vegetable preservation.

4.1 DA analysis

The pickled cucumber samples were firstly studied by DA, a method based on the panelist’s ability to verbalize perceptions of a product in a consistent manner allowing the survey, description and quantification of sensory attributes (Alcantara & Freitas-Sá, 2018).

No significant differences in term of appearance (color and brightness, **Figure 1**) were found among the samples, indicating that no effects on color can be perceived when verjuice is used in place of vinegar. However, the instrumental color analysis (**Table 1**) indicated that the cucumbers pickled in vinegar had a significantly higher a* value then those pickled with verjuice. However, the a* value, which should be indicative of a reddish color, is very low for all the samples considered. Conversely, the b* value indicated that no differences in the typical yellowish color of the pickled cucumbers arise when verjuice is used in place of vinegar, confirming the results of the DA analysis. Also considering the color differences by calculating the ΔE, a parameter incorporating the three CIELab coordinates ($\Delta E = \sqrt{[(L1 - L2)^2 + (a1 - a2)^2 + (b1 - b2)^2]}$), the calculated values were: ΔE VINvsVER1:2 = 3.4, ΔE VINvsVER1:10 = 2.2, ΔE VER1:2vsVER1:10 = 2.3. Some authors report the concept of Just Noticeable Difference (JND) with ΔE values of around 2.3 (Sharma, 2003), thus justifying the DA results (**Figure 1**).

From the olfactory point of view, cucumbers pickled with vinegar, as expected, displayed the highest “vinegar smell” (**Figure 1**), indicating a main sensorial difference which is certainly of interest as a distinctive feature for verjuice pickled vegetables. This sensation is driven by the high acetic acid content of the VIN sample (**Table 1**). Moreover, the low "vinegar smell" could also be responsible for the enhancement of the "cucumber smell" detected in both samples picked with verjuice, where probably the lack of acetic acid did not interfere with the "cucumber smell" perception. This indicates that the typical aroma which is distinctive of the original plant food is likely to be better maintained when verjuice is used in place of vinegar. The same masking effect of the acetic acid was also probably present for the "cooked vegetable aroma" attribute, possibly arising from the initial heat treatment applied to the cucumbers, which was more prominent for the cucumbers pickled with verjuice (**Figure 1**).

Texture is an important characteristic for pickled vegetables. For this aspect, crunchiness and firmness did not differ among the samples, indicating that verjuice does not worsen the cucumber texture compared to vinegar. This is also supported by the analytical data that did not indicate any change in term of the shear force needed to cut the whole cucumber (**Table 1**).

Acidity is an important aspect for pickled vegetables to avoid microbial spoilage and because it contributes to their typical sensorial properties. According to this parameter, cucumbers pickled with verjuice showed pH values in the range of those typical of vinegar-pickled vegetables, which is generally between 3 and 4. However, the pH and the total acids content were dependent on the concentration of verjuice in the preserving liquid, as expected, with the dilution 1:2 giving the most acidic cucumbers, also in comparison with those pickled with vinegar (**Table 1**). Nevertheless, these latter had the highest perceived acidity in DA (**Figure 1**), a discrepancy between the chemical and sensorial values that seems again attributable to the confounding effect that acetic acid present in high quantities has for the perception of acidity by the judges. Moreover, to explain the difference in perceived acidity between the cucumbers pickled with the two verjuice the buffering activity of the saliva should be considered in relation to the different concentration of acids to be neutralized, which is clearly lower for the verjuice diluted 1:10.

In contrast with acidity, the "sweetness" attribute was evaluated at the same level for the three samples (**Figure 1**). Indeed, the total sugar content (glucose + fructose) measured in the pickles was very low and, according to the recognition threshold for sugars in water (Belitz, Grosch, & Schieberle, 2004), is not likely to contribute to the perceived sweetness of the products (**Table 1**), which can be further depressed by the presence of other attributes such as acidity and bitterness (Stampanoni, 1993; Brannap, Setser, & Kemp, 2001).

Concerning “salt” perception, despite the small variations in salt content, judges were able to perceive differences in saltiness, for which chemical and sensorial data are in agreement (**Table 1** and **Figure 1**).

Judges generally rated the attribute “bitterness” with low intensity scores, without any significant difference between the samples. Since bitterness is considered an important quality attribute for vegetables, this result again indicates the suitability of verjuice as a substitute of vinegar.

Concerning “astringency”, cucumbers that were rated as the most astringent were those pickled with vinegar, whereas pickling with verjuice had the same scores for this parameter, regardless of the verjuice dilution. Therefore, despite the fact that “astringency” is a descriptor for verjuice (Dupas de Matos et al., 2017), it seems that pickling with this liquid results in cucumbers less astringent than those in vinegar. The high astringency of the VIN sample is likely due to the acetic acid content as it is known that organic acids have themselves a direct effect on astringency perception. However, this effect is not linked to the type of acid but to the pH determined by the presence of a particular acid (Sowalski & Noble, 1998). When tasting the pickles it must be taken into account that the pH in the mouth is affected by the interaction between the bicarbonate ions of the saliva and the acids introduced with the food (Bardow, Moe, Nyvad, & Nauntofte, 2000). In turn, the pH affects the binding of the salivary proteins to the phenolics present in the food (Lawless, Horne, & Giasi, 1996), which is the mechanism responsible for the astringency sensation. Therefore, the presence of high quantity of acids, in particular acetic acid in the sample pickled with vinegar should maintain a lower pH upon placing the samples in the mouth, thus indirectly enhancing the astringency perception (**Figure 1**).

The samples reported to have the higher “pungency” (**Figure 1**) were the cucumbers pickled with vinegar, results clearly driven by their acetic acid content (**Table 1**). Interestingly, the two VER samples were rated differently despite not having acetic acid. This can possibly be the result of the lower buffering capacity of the more diluted preserving liquid (VER 1:10) when mixed with the saliva. However, this hypothesis does not explain the results observed for “vinegar aroma” which was found also in absence of acetic acid (**Figure 1** and **Table 1**). This is difficult to explain, but it might be the result of a psychological association between pickled cucumbers and vinegar aroma due to the fact that, at least in Italy, pickles always have vinegar taste. However, this result is also clearly related to the total acids content that seems to be the driver for the ability of judges in discriminating the two verjuice samples.

For the attribute "cucumber aroma", judges did not notice any differences, indicating that the preserving liquids did not interfere with its perceived intensity, again indicating the suitability of verjuice as pickling agent. Interestingly, the retronasal perception of the "cucumber aroma" does not show the differences that were seen for the "cucumber smell". This could be due to the physico-chemical conditions existing in the mouth which can modify the perception of this attribute.

4.2 TDS test

Given that the sensory perception is a dynamic phenomenon as the perceived intensity of the sensory attributes changes along with the in-mouth transformation of food (Sudre, Pineau, Loret, & Martin, 2012), the TDS test was developed to describe the sequence of the dominant sensory perceptions during the tasting of a product (Pineau et al., 2012). TDS experiments have been conducted to describe the temporal sensory patterns of different foods and beverages (Frost, Blackman, Ebeler, & Heymann, 2018; Charles et al., 2017; Rodrigues et al., 2016; Sokolowsky, Rosenberger, & Fischer, 2013; Ng et al., 2012). In this test, the dominant attribute in a given moment is not necessarily the one with the highest intensity (Pineau et al., 2009), being rated as the percentage of dominance among all attributes considered at a given time point (Di Monaco, Su, Masi, & Cavella, 2014).

The perceptions varied greatly for most attributes (**Figure 2**). All samples were dominated by a "crunchiness" sensation at the very beginning of the trial. In addition, in the two verjuice samples this was associated with "firmness". This might indicate that the replacement of vinegar with verjuice had an effect on the physical properties of the cucumbers. It might be that the structure of the cucumbers preserved in less acidic preserving liquids remain more intact, thus inducing a longer dominance for the parameters related to mastication.

The dominance's duration of the "acidity" sensation seemed linked to the total acids content (**Table 1**). Also according to the duration it seems that the more diluted verjuice sample did not yield the results that should be expected for pickles, while this is not the case for the more concentrated verjuice sample.

For the vinegar sample, the observed TDS profile seemed mostly driven by the "vinegar aroma" effect that was lacking in both verjuice samples (**Figure 2**).

By looking at “acidity”, “vinegar aroma” and “pungency” profiles together, it seems that these three attributes have a synergistic effect in the vinegar sample resulting in a longer dominant acid sensation when compared to the two verjuice samples.

The “cooked vegetable aroma” was never a dominant attribute for the vinegar sample, probably because of the concurrent and long-lasting dominance of the “vinegar aroma”. In contrast, the shorter period of dominance of the acidity in the two verjuice samples probably allowed for the dominance of this attribute that was, also in this case, linked to the acidity. Therefore, a certain level of acidity seems to be necessary to reduce the dominance of a negative aroma as the cooked vegetable one.

It is to be noted that the “cucumber aroma” did not emerge as dominant in the vinegar sample but dominated at the same evaluation time, although for short periods, for the verjuice samples. Again, this behavior can be due to the masking effect of the vinegar aroma absent in the verjuice samples. This confirms that verjuice can be a good choice for pickling cucumbers, if their native aroma has to be maintained.

“Astringency” was the last attribute to emerge as the dominant sensation towards the end of the evaluation time for all samples. Despite the time in which judges swallowed the samples was not recorded, it is almost certain that “astringency” became a dominant sensation after this moment and particularly after the acidity is no longer significantly dominant, a phenomenon that has already been described (Medel-Maraboli et al., 2017; Frost, Harbertson, & Heymann, 2017).

Adopting a TDS approach highlighted differences among the samples that could impact the appreciation of consumers, an aspect investigated in the next section.

4.3 Liking scores, JAR scale and penalty analysis

Traditional sensory methods to evaluate consumer acceptability such as hedonic scale (liking) in combination with JAR test have been widely used in food science, especially for new product development and understanding consumer needs (Viejo, Fuentes, Howell, Torrico, & Dunshea, 2018).

Normally, the 5-point JAR scale is reduced to three categories by combining the two categories on the “not enough” side (1 and 2) of the midpoint (3) and by combining the two categories on the “too much” side (4 and 5) of the midpoint (Rothman & Parker, 2009).

Then, the mean overall liking (rating) was calculated as the differences between the mean overall liking of each non-JAR category and the mean of the JAR category. Further, the penalties were plotted versus the percentage giving each response in a so-called mean drops (Gere, Sipos, & Héberger, 2015).

The three samples were all deemed similar for visual and gustative aspect, while the olfactory evaluation showed that VER1:2 was liked at the same level as that commercial standard VIN, and significantly more appreciated than VER1:10 (**Figure 3**). This indicates that cucumbers pickled with verjuice can represent a valid choice for the consumers alongside those pickled with vinegar.

The penalty analysis is used to obtain clear indications on the attributes to be considered for future reformulation as they are responsible for a drop in the overall liking score. This methodology determines if those consumers considering a particular attribute lower or higher than the midpoint (just-about-right) dropped the overall liking more than those consumers finding the same attribute just-about-right (Gere et al., 2015).

Penalty/mean drop analysis was then conducted on the JAR data obtained for the three cucumber samples. In all samples, the significant drop in overall liking was driven by the both the lack of some attributes (e.g. cucumber aroma, sweetness, bitterness, acidity, astringency and salt) and by the excess of others (e.g. acidity, astringency, pungency and sweetness, **Figure 4**).

Despite cucumbers pickled in vinegar are the commercial reference, consumers found several attributes responsible for a reduction in the overall liking of the product and that deserve to be considered to find an alternative without this drawbacks. Therefore, the search for an alternative preserving agent is justified. However, VER1:10 does not seem to be the right alternative as it also presents several penalizing attributes. Conversely VER1:2, having only 3 penalizing attributes ("not enough sweet", "not enough cucumber aroma", and "not enough acidic") against the six of the other two samples (**Figure 4**), seems to be a promising acidifying agent for the production of a novel style of pickled cucumbers with the potential to encounter the taste of consumers.

In particular, on the basis of the penalty analysis a few simple actions could be applied to improve the sensorial quality of VER1:2 by adjusting, for example, the sugar content and the acidity. It seems more complicated to improve the "cucumber aroma" attribute that, on the other hand, penalizes all the products examined here.

5. Conclusions

The use of different sensory methods (DA, TDS, Liking and Penalty analysis on JAR) allowed for a comprehensive investigation on the sensorial characteristics of cucumbers pickled a novel acidifying agent (verjuice) in comparison with a traditional one (vinegar). In general, this type of studies are essential for the development of a new food product. In our case, it was established that the use of verjuice as preserving liquid for the production of pickled cucumbers represents a viable alternative to vinegar conferring comparable characteristics to the reference in terms of visual and textural characteristics, sweetness, bitterness and cucumber aroma. However, some distinctive characteristics were detected in the cucumbers pickled with verjuice, indicating the possibility of developing a novel product different from the reference that, according to the penalty analysis reported here, could be further improved by some simple adjustments of the verjuice-containing preserving liquid. For example, the level of acidity, a very important factor as shown here, can be adjusted either by modulating the concentration of verjuice in the preserving liquid, or by using verjuice produced at different stages of ripening thus having a different sugar/acid balance. The absence of acetic acid seems to be the main distinguishing feature of pickles prepared with verjuice, potentially conferring them an additional value because acetic acid has a strong sensorial impact that can be disliked by some consumers.

The data obtained here for cucumbers are likely to be valid also for pickling other vegetables, and therefore this preserving liquid needs to be taken into consideration as an alternative to vinegar in other food preparations, for example to better maintain the smell of the original plant food more unaltered as demonstrated here for cucumbers. Verjuice presents the additional advantages of being produced from a byproduct of the grape and wine industries (e.g. thinned grapes), and for its simple method of production as it requires only a few processing steps.

Acknowledgements

The authors would like to thank Coelsanus spa (Sossano, Vicenza), and particularly Pierantonio Lando and Cristina Romani for supplying the commercial and experimental pickled cucumbers used in this study. IBIMET/CNR staff is thanked for their support with the sensory evaluation and the panelists for assessing the pickled cucumbers. Luciano Magro and Silvia Santagata of La.Chi/DAFNAE is thanked for carrying out some chemical analysis.

References

Alcantara, M. De, & Freitas-Sá, D. D. G. C. (2018). Metodologias sensoriais descritivas mais rápidas e versáteis – uma atualidade na ciência sensorial. Brazilian Journal Food Technology, 21, 1–12.

AOAC Official Method 2013.06. AOAC Official Methods of Analysis, 20th Edition, 2016.

Ares, G., Dauber, C., Fernández, E., Giménez, A., & Varela, P. (2014). Penalty analysis based on CATA questions to identify drivers of liking and directions for product reformulation. Food Quality and Preference, 32, 65–76.

Ares, G., Etchemendy, E., Antúnez, L., Vidal, L., Giménez, A., & Jaeger, S. R. (2014). Visual attention by consumers to check-all-that-apply questions: Insights to support methodological development. Food Quality and Preference, 32, 210–220.

Bardow, A., Moe, D., Nyvad, B., & Nauntofte, B. The buffer capacity and buffer systems of human whole saliva measured without loss of CO2. Archives of Oral Biology, 45, 1-12.

Belitz, H. D., Grosch, W., & Schieberle, P. (2004). Carbohydrates. In: H. D., Belitz, W.Grosch, & P. Schieberle (Eds.), Food chemistry (pp. 248-339). Springer, Berlin, Heidelberg.

Brannan, G. D., Setser, C. S., & Kemp, K. E. (2001). Effectiveness of rinses in alleviating bitterness and astringency residuals in model solutions. Journal of sensory studies, 16(3), 261-275.

Charles, M., Endrizzi, I., Aprea, E., Zambanini, J., Betta, E., & Gasperi, F. (2017). Dynamic and static sensory methods to study the role of aroma on taste and texture: A multisensory approach to apple perception. Food Quality and Preference, 62, 17–30.

Coelho, E.M., da Silva Padilha, C.V., Miskinis, G.A., de Sá, A.G.B., Pereira, G.E., de Azevêdo, L.C., & M. dos Santos Lima (2018). Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from Northeast Brazil. Journal of Food Composition and Analysis, 66, 160-167.

Coombe, B. (1995). Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research, 1, 100–110.

Di Monaco, R., Su, C., Masi, P., & Cavella, S. (2014). Temporal dominance of sensations: A review. Trends in Food Science and Technology, 38(2), 104–112.

Dupas de Matos, A., Curioni, A., Bakalinsky, A. T., Marangon, M., Pasini, G., & Vincenzi, S. (2017). Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries. Innovative Food Science and Emerging Technologies, 44, 9–14.

Dupas de Matos, Magli, M., Marangon, M., Curioni, A., Pasini, G., Vincenzi, S. (2018). Use of verjus as an acidic salad seasoning ingredient: evaluation by consumers' liking and Check-All-That-Apply. European Food Research and Technology, 244, 2117-2125.

Frost, S. C., Blackman, J. W., Ebeler, S. E., & Heymann, H. (2018). Analysis of temporal dominance of sensation data using correspondence analysis on Merlot wine with differing maceration and cap management regimes. Food Quality and Preference, 64, 245–252.

Frost, S. C., Harbertson, J. F., & Heymann, H. (2017). A full factorial study on the effect of tannins, acidity, and ethanol on the temporal perception of taste and mouthfeel in red wine. Food quality and preference, 62, 1-7.

Gere, A., Sipos, L., & Héberger, K. (2015). Generalized Pairwise Correlation and method comparison: Impact assessment for JAR attributes on overall liking. Food Quality and Preference, 43, 88–96.

Iland, P., Dry, P., Proffitt, T., & Tyerman, S. (2011). The grapevine: from the science to the practice of growing vines for wine. Adelaide: Patrick Iland Wine Promotions.

ISO 8586:2012. Sensory analysis. General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors.

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

Marcano, J., Varela, P., Cunha, L. M., & Fiszman, S. (2015). Relating dynamic perception of reformulated cheese pies to consumers' expectations of satiating ability. Food Research International, 78, 369–377.

Medel-Marabolí, M., Romero, J. L., Obreque-Slier, E., Contreras, A., & Peña-Neira, A. (2017). Effect of a commercial tannin on the sensorial temporality of astringency. Food Research International, 102, 341-347.

Ng, M., Lawlor, J. B., Chandra, S., Chaya, C., Hewson, L., & Hort, J. (2012). Using quantitative descriptive analysis and temporal dominance of sensations analysis as complementary methods for profiling commercial blackcurrant squashes. Food Quality and Preference, 25(2), 121–134.

Nikfardjam, M. S. P. (2008). General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers. Mitteilungen Klosterneubg, 58, 28–31.

Öncül, N., & Karabiyikli, Ş. (2015). Factors Affecting the Quality Attributes of Unripe Grape Functional Food Products. Journal of Food Biochemistry, 39(6), 689-695.

Pineau, N., de Bouillé, A. G., Lepage, M., Lenfant, F., Schlich, P., Martin, N., & Rytz, A. (2012). Temporal Dominance of Sensations: What is a good attribute list? Food Quality and Preference, 26(2), 159–165.

Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., Rogeaux, M., Etiévant, P., & Köster, E. (2009). Temporal Dominance of Sensations: Construction of the TDS curves and comparison with time-intensity. Food Quality and Preference, 20(6), 450–455.

Rodrigues, J. F., Souza, V. R. De, Lima, R. R., Carneiro, J. D. D. S., Nunes, C. A., & Pinheiro, A. C. M. (2016). Temporal dominance of sensations (TDS) panel behavior: A preliminary study with chocolate. Food Quality and Preference, 54, 51–57.

Rothman L., & Parker, M.J. (2009). Just-About-Right (JAR) Scales: Design, Usage, Benefits, and Risks. American Society for Testing & Materials, ISBN 978-0-8031-7010-0.

Schlich, P. & Pineau, N. (2017). Temporal Dominance of Sensations. In: Time-Dependent Measures of Perception in Sensory Evaluation. Edited by J. Hort, S. E. Kemp & T. Hollowood (Eds.), John Wiley & Sons Ltd.

Segato, S., Balzan, S., Elia, C. a., Lignitto, L., Granata, a., Magro, L., Contiero, B., Andrighetto, I., & Novelli, E. (2007). Effect of period of milk production and ripening on quality traits of Asiago cheese. Italian Journal of Animal Science, 6, 469–471.

Setorki, M., Asgary, S., Eidi, A., Rohani, A. H., & Khazaei, M. (2010). Acute effects of vinegar intake on some biochemical risk factors of atherosclerosis in hypercholesterolemic rabbits. Lipids in Health and Disease, 9(4), 10.

Sharma, G. (2003). Digital Color Imaging Handbook (1.7.2 ed.). CRC Press. ISBN 0-8493-0900-X.

Siddiq, M., & Uebersax, M. A. (2018). Vegetable Fermentation and Pickling. In: S. Ghnimi & N. Guizani, Handbook of Vegetables and Vegetable Processing, ISBN: 9781119098959, 2nd edition.

Sokolowsky, M., Rosenberger, A., & Fischer, U. (2013). Sensory impact of skin contact on white wines characterized by descriptive analysis, time-intensity analysis and temporal dominance of sensations analysis. Food Quality and Preference, 39, 1–13.

Sowalsky, R. A., & Noble, A. C. (1998). Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. Chemical senses, 23(3), 343-349.

Stampanoni, C. R. (1993). Influence of acid and sugar content on sweetness, sourness and the flavour profile of beverages and sherbets. Food quality and preference, 4(3), 169-176.

Sudre, J., Pineau, N., Loret, C., & Martin, N. (2012). Comparison of methods to monitor liking of food during consumption. Food Quality and Preference, 24(1), 179–189.

UNI 10957, 2003. Sensory analysis – method for establishing a sensory profile in foodstuffs and beverages.

Viejo, C. G., Fuentes, S., Howell, K., Torrico, D., & Dunshea, F. R. (2018). Robotics and computer vision techniques combined with non-invasive consumer biometrics to assess quality traits from beer foamability using machine learning: A potential for artificial intelligence applications. Food Control, 92, 72-79.

CHAPTER 4

DEVELOPMENT OF A STABILIZATION PROCESS TO PROTECT VERJUICE AGAINST MICROBIAL SPOILAGE BY USING ONLY ORGANIC ACIDS

Development of a stabilization process to protect verjuice against microbial spoilage by using only organic acids[4]

Abstract

Unripe grape juice (verjus or verjuice) is a potential byproduct that can be made from the thinned fruit that is normally removed from grapevines early in the growing season. Although most of the thinned fruit is discarded, verjus has a long history of use as a condiment and food seasoning in wine-producing regions of the world. This study focused on the microbial stability of verjus. Specifically, we tested the possibility of lowering pH moderately and increasing tartaric and malic acid concentrations moderately in model verjus as a means of preventing microbial spoilage without recourse to sulfite, other preservatives, pasteurization, or sterile filtration. Model verjus formulated in Yeast Nitrogen Base (YNB) to which 4% glucose and 2% malic acid were added was found either to be toxic to, or to inhibit the growth of a wine strain of *Saccharomyces cerevisiae* if it also contained at least 2% tartaric acid and had a pH no higher than 2.0. Because food-grade sources of tartaric and malic acids are available, use of this method does not require any artificial additives.

Keywords

pH

Malic acid

Tartaric acid

Spoilage

Verjus

Wine yeast

---

[4]This chapter represents the research period at Oregon State University (OSU) under the supervision of Prof Alan T. Bakalinsky at the Microbiology Laboratory of the Department of Food Science & Technology, academic year 2016/2017.

1. Introduction

In standard commercial practice, unripe wine grapes are typically thinned during the growing season to allow the grapes that remain on the vine to ripen optimally. Thinning refers to the removal of a portion of the fruit from the vine. Currently, the vast amount of thinned fruit is left to rot in the vineyard. Nonetheless, there is a history of use of thinned unripe fruit to produce a value-added food ingredient. Specifically, “verjus” is the unfermented juice obtained by pressing the unripe thinned fruit. When the juice is boiled and salted, the final product is known as “sour grape sauce” (Öncül & Karabiyikli, 2015). The Italian sweet-sour grape sauce is called “Agresto”, a traditional seasoning from the Tuscany region (Simone et al., 2013).

Verjus has a long history of use as a seasoning in foods dating back to medieval times, and is still used in oriental cuisine, regionally called “Abe ghureh” (Persian: unripe grape water) and “Koruk suyu” (Turkish: unripe grape juice) (Nikfardjam, 2008). While still a relatively unknown product in Western cuisine, verjus has recently been rediscovered as a food condiment where it serves as an alternative to vinegar and lemon juice for use in salad dressings and appetizers (Hayoglu et al., 2009; Setorki et al., 2010), especially when consumed with wine. Because verjus does not yet enjoy a mature market in Western cuisine, a standard of identity is lacking as are standardized methods of production. To address these deficiencies in light of growing interest, we recently undertook an evaluation of the major factors that determine quality for potential production in the Veneto region, namely: choice of grape variety, harvest date, and method of preservation (Dupas de Matos et al., 2017).

While verjus has been reported to have high levels of acidity, about 2 to 4% (w/v), and very low pH, 2.1 to 3.0 (Karapinar & Sengun, 2007; Nikfardjam, 2008; Hayoglu et al., 2009; Simone et al., 2013; Öncül & Karabiyikli, 2015), it also contains fermentable sugar, about 4 to 8% (w/w), depending on the date of harvest (Hayoglu et al., 2009; Shojaee-Aliabadi et al., 2013; Öncül & Karabiyikli, 2015; Dupas de Matos et al., 2017). The presence of the sugar makes verjus microbiologically unstable because it has the potential to support growth of the relatively acid-tolerant wine yeast *Saccharomyces cerevisiae*, and possibly other microbes commonly found in wine. Thus, the verjus must be processed to prevent fermentation from occurring in the bottled product. One approach is to use sulfites as is done in traditional winemaking to prevent microbial spoilage and oxidation. Alternatives include use of the preservative potassium sorbate, pasteurization, and sterile filtration. While all these approaches are effective, they are not consistent with the growing interest in minimally-processed organic food products, which comprise the high-value niche market we envision for verjus.

This study focused on developing an effective stabilization process which would protect verjus against microbial spoilage, but without requiring commonly used preservatives or use of pasteurization or sterile filtration. In general, the antimicrobial activity of organic acids is a function of pH. The lower the pH, the more toxic the acid because a greater fraction of it is present in the undissociated form that can enter and inhibit microbial cells (reviewed in Piper et al., 2001). Therefore, our approach exploited the natural high acidity and low pH of verjus to maximize the antimicrobial activity of the organic acids already present or which could be added.

2. Materials and Methods

2.1 Yeast strains and media

Saccharomyces cerevisiae EC1118 is from Lallemand. Yeast Extract Peptone Dextrose (YEPD) contains 1% yeast extract, 2% peptone (Sunrise Science Products), and 2% D-(+)-glucose (Sigma-Aldrich Chemical Company). YNB is Yeast Nitrogen Base without amino acids (Sunrise Science Products). YEPD plates were prepared by addition of 1.8% agar (Sunrise Science Products). All other chemicals were reagent grade and were purchased from Sigma-Aldrich Chemical Company.

2.2 Yeast growth

Yeast inocula were prepared by growing cells aerobically for 24-48 h in YEPD at 30°C and 200 rpm. Cells were harvested by centrifugation (3 min, 12,000 x *g*), washed once in sterile distilled water, resuspended in sterile distilled water at approximately 10^7 cells/mL, and stored at 4°C. The inocula stored at 4°C maintained ≥50% viability based on plate counts over the 4 weeks they were used. Fresh inocula were prepared as needed. Cell titers were estimated by counting cells under 400X magnification using a hemacytometer. Viability was assessed by plating on YEPD plates.

2.3 Model verjus

Filter-sterilized model verjus solutions were formulated in YNB + 4% glucose at pH 2-3 containing 1-2.5% tartaric acid, or 2-3% malic acid, or both acids in the above concentration range.

2.4 Assessment of yeast survival in model verjus

Solutions of model verjus were inoculated in 1 mL aliquots (n = 3) at an initial concentration of yeast that produced no visible turbidity, about 5 x 10^4 cells/mL. The inoculated model solutions were incubated statically at room temperature in 1.5 mL screw-capped microfuge tubes for up to 6 weeks. Aliquots (≥5μL) of appropriately diluted or undiluted model verjus solutions were plated (n = 4 for each of the 3 replicates) over the 6 week period and the YEPD plates were incubated at 30°C for about 24-48 h. Samples that contained 10-100 microcolonies within an approximate 1 cm square area were counted under a dissecting microscope at 1-3X magnification. Samples that contained >100 microcolonies were re-plated at a higher dilution to yield 10-100 colonies and were re-counted. Where possible, a larger volume was re-plated in cases where <10 colonies were observed.

3. Results and discussion

Model verjus solutions were formulated based on grape juice composition data reported for wine grape varieties harvested just before the period of development referred to as “véraison.” At such a time, glucose concentrations range from about 1-5%, malic and tartaric acid concentrations are typically 1-3 and 1-2%, respectively (Dokoozlian et al 1996; Sabir et al., 2010; Dupas de Matos et al., 2017), and potassium levels are about 0.2% (Davies et al., 2006).

Initially, unreplicated model solutions were formulated over a wide range of acid concentrations and pH values that approximated the above parameters, inoculated with yeast, and were assessed visually for growth (appearance of turbidity) in order to narrow the range of acid/pH combinations to evaluate (data not shown). Formulations that permitted growth were not pursued further, but rather, were modified by either increasing acid level (malic, tartaric or both), reducing pH, or adjusting both acid concentration and pH. Briefly, combinations of YNB + 4% glucose containing 1-2.5% tartaric acid alone, or 2-3% malic acid alone, or the combined acids, at pH 2-3 were tested. At pH ≥ 2.1, yeast growth was observed in all formulations (data not shown).

Addition of a maximum of 0.06% acetic acid to these formulations was tested because acetic acid is recognized as a one of the more toxic organic acids used in food processing (Ding et al., 2013; 2015). The relatively low threshold for the sensory perception of acetic acid limited the amount that could be used. Nonetheless, the addition of up to 0.06% acetic acid to the model verjus formulations was not found to prevent growth (data not shown). The only model verjus formulations that appeared to prevent growth of yeast contained 2 and 2.5% tartaric acid alone at pH 2. Based on these preliminary results, we narrowed the possible combinations for further evaluation to 2-2.5% tartaric acid, with or without 2% malic acid at pH 2. These further tests were done in triplicate and yeast growth was no longer assessed visually, but rather by plating.

Figure 1 illustrates the effect of 2-2.5% malic acid alone at pH 2 on yeast growth in model verjus. No inhibition of growth was evident in any of these model solutions as cell titers reached about 10^7 cells/mL by day 6 and therefore, no further evaluation of malic acid alone was performed.

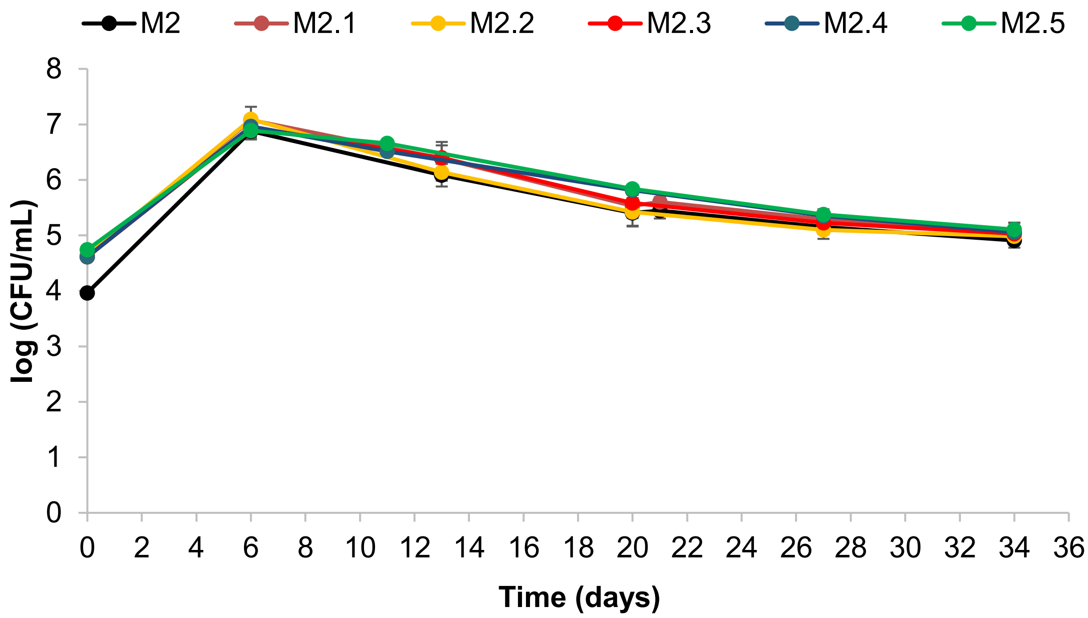

Fig. 1. Growth of *S. cerevisiae* EC1118 in model verjus at pH 2 as a function of malic acid. M, malic acid (%). Error bars are standard deviations (n = 3).

Figure 2 illustrates the effect of tartaric acid alone at pH 2 on yeast growth. Growth was evident in the 3 samples containing between 2 and 2.2% tartaric acid. Although the measured counts did not exceed 10^6 for these samples, a cell pellet was visible indicating that significant growth (≥ 10^7 cells/mL) must have occurred between sampling days 10 and 21, followed by a loss of viability upon further incubation of the presumably nutritionally-starved cells at room temperature. No growth was observed in the 3 samples that contained 2.3-2.5% tartaric acid. Indeed, a reduction in cell viability was already evident in these samples by day 7. By day 21, viability had dropped more than 100-fold. For the sample containing 2.5% tartaric acid, no viable cells were recovered after 27 days. For the samples containing 2.3 and 2.4% tartaric acid, cell viability stabilized after 27 days but no viable cells were recovered after 48 days. The high degree of variance observed in the model solutions containing 2.3 and 2.4% tartaric acid sampled on days 27-41 is due to the fact that fewer than 4 colonies were actually counted in these samples because a >100-fold reduction in cell viability had occurred by day 27.

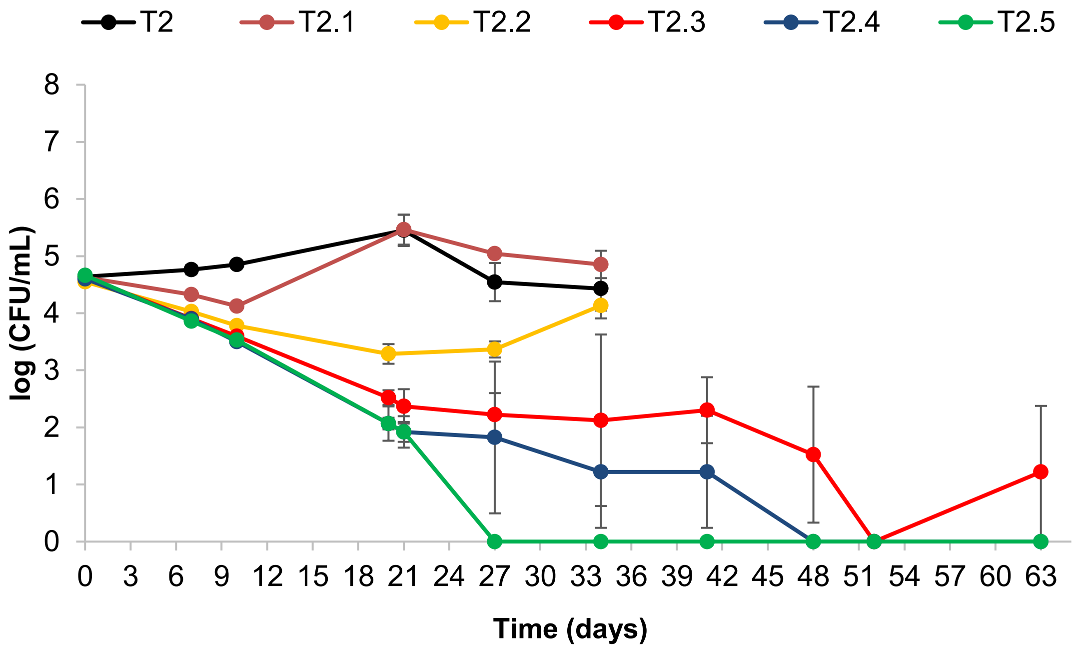

Fig. 2. Growth of *S. cerevisiae* EC1118 in model verjus at pH 2 as a function of tartaric acid. T, tartaric acid (%). Error bars are standard deviations (n = 3).

Comparison of **Figures 1** and **2** demonstrates that tartaric acid is more toxic to yeast than malic acid, even when the concentration of the presumed more toxic undissociated form of malic acid is higher than that of the undissociated form of tartaric acid (**Table 1**). This relative non-toxicity may be due to the fact that malic acid is a normal yeast metabolite (reviewed in Fraenkel, 1982) whereas tartaric acid is not. **Table 1** shows the predicted amounts of the undissociated forms of dicarboxylic malic and tartaric acids at pH 2 as a function of total acid in solution. Because malic acid has a higher pKa_1 value than tartaric acid, a greater fraction is always present in the undissociated form at the same pH. The table shows that on a weight basis at pH 2, the concentration of the undissociated form of malic acid is always about 15% higher than that of tartaric acid.

Table 1. Predicted concentrations of undissociated malic and tartaric acids at pH 2.

	Undissociated species (mM)	
Concentration of total acid (%)	Malic	Tartaric
2.0	143	122
2.1	151	128
2.2	158	134
2.3	165	140
2.4	172	146
2.5	179	152

Calculations are based on the following: the molecular weights of malic and tartaric acids are 134.1 and 150.1 grams, respectively; pKa_1 for malic and tartaric acids = 3.40 and 3.02, respectively; $pH = pKa + \log([A^-]/[HA])$.

Figure 3 illustrates the effect of tartaric acid in the presence of 2% malic acid at pH 2 on yeast growth. As noted below, unripe grape juice just prior to "véraison" contains about 1-3% malic acid (Dokoozlian et al., 1996; Sabir et al., 2010; Dupas de Matos et al., 2017). In order to formulate a more realistic model verjus and to assess potential synergy, we tested the effect of 2-2.4% tartaric acid in the presence of 2% malic acid. In all cases, an immediate and steady drop in cell viability was observed such that by about day 20, a greater than 100-fold reduction had occurred. In contrast to the formulations that contained 2-2.2% tartaric acid alone in which growth was observed (**Figure 2**), no growth was observed in the presence of these same tartaric acid concentrations when combined with 2% malic acid (**Figure 3**).

Thus, the toxicity of tartaric acid towards yeast appears to be potentiated by malic acid. By about day 30, no viable cells were recovered from any of the formulations. These promising results suggest that modest adjustment of tartaric acid concentration and pH of verjus may be sufficient to prevent growth of potential microbial contaminants without recourse to pasteurization, sterile filtration, or addition of sulfite or other preservatives.

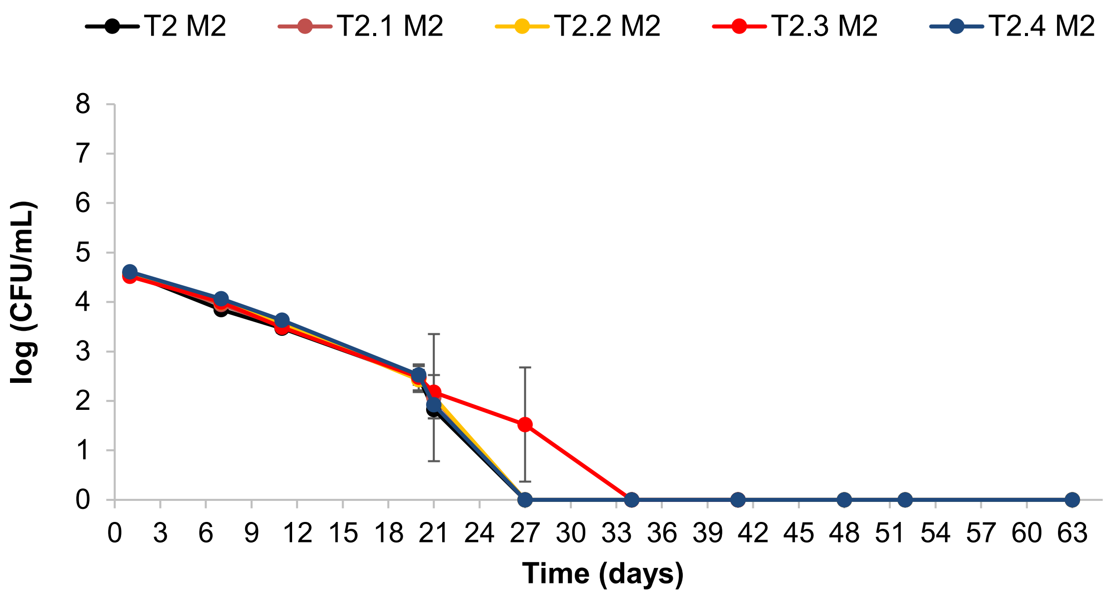

Fig. 3. Growth of *S. cerevisiae* EC1118 in model verjus at pH 2 as a function of tartaric acid in the presence of 2% malic acid. T, tartaric acid (%); M, malic acid (%). Error bars indicate the standard deviation (n = 3).

4. Conclusion

pH and organic acid adjustments of verjus made from Pinot noir cluster thinnings were tested as a sole means of inducing microbiological stability without addition of sulfite or potassium sorbate or use of pasteurization or sterile filtration. Model verjus samples containing at least 2% tartaric acid with a pH no higher than 2.0 were found to be toxic or to inhibit growth of a wine strain of the yeast *Saccharomyces cerevisiae*. These promising preliminary results need to be re-evaluated in model and in actual verjus, and in larger volumes over a narrower range of acid concentration and pH.

Because organic sources of both malic and tartaric acids are available, use of this method could increase the value of verjus for consumers interested in natural and organic food products. The data obtained in this study provide a preliminary and promising approach to producing microbiologically-stable verjus.

Funding

Financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) (grant number: 204483/2014-0), University of Padova (Resolution n. 435 of the Directors Board of 25/10/2016 - Call for University Cooperation

Initiatives 2017). In-kind support was provided by the Bakalinsky laboratory in the Department of Food Science and Technology at Oregon State University.

Acknowledgements

This study was undertaken in the Bakalinsky laboratory in the Department of Food Science and Technology at Oregon State University during the summer of 2017 as part of the PhD program of A. Dupas de Matos in the Department of Land, Environment, Agriculture and Forestry (LEAF) at Padova University. The authors wish to thank Sokol Blosser Winery for donating grapes, and especially their Executive Chef, Henry Kibit, for his help in the making of verjus. Garrett Holzwarth and Maria Del Pilar Alessandri Basaure are gratefully acknowledged for all their help, technical and otherwise.

References

Davies, C., Shin, R., Liu, W., Thomas, M.R., Schachtman, D.P. (2006). Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation. *J. Exp. Bot*., 57, 3209-3216.

Ding, J., Bierma, J., Smith, M. R., Poliner, E., Wolfe, C., Hadduck, A. N., Zara, S., Jirikovic, M., Zee, K. V., Penner, M. H., Patton-Vogt, J., & Bakalinsky, A. T. (2013). Acetic acid inhibits nutrient uptake in *Saccharomyces cerevisiae*: auxotrophy confounds the use of yeast deletion libraries for strain improvement. *Appl. Microbiol. Biotechnol*., 97, 7405-7416.

Ding, J. Holzwarth, G., Bradford, S., Cooley, B., Yoshinaga, A.S., Patton-Vogt, J., Abeliovich, H., Penner, M.H., & Bakalinsky, A.T. (2015). PEP3 overexpression shortens lag phase but does not alter growth rate in *Saccharomyces cerevisiae* exposed to acid stress. *Appl. Microbiol. Biotechnol*., 99, 8667-8680.

Dokoozlian, N.K., Kliewer, W. M. (1996). Influence of Light on Grape Berry Growth and Composition Varies during Fruit Development. *J. Am. Soc. Hort. Sci*., 121, 869-874.

Dupas de Matos, A., Curioni, A., Bakalinsky, A. T., Marangon, M., Pasini, G., & Vincenzi, S. (2017). Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries. *Innovative Food Sci. Emer. Technol.,* 44, 9-14.

Fraenkel, D.G. (1982). Carbohydrate metabolism, p. 1-37, In: The molecular biology of the yeast *Saccharomyces*, metabolism and gene expression, eds. Strathern, J.N., Jones, E.W., Broach, J.R. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hayoglu, I., Kola, O., Kaya, C., Özer, S., & Turkoglu, H. (2009). Chemical and sensory properties of verjus, a traditional Turkish non-fermented beverage from kabarcik and yediveren grapes. *J. Food Proc. Preserv.,* 33, 252-263.

Karapinar, M., & Sengun, I. Y. (2007). Antimicrobial effect of koruk (unripe grape - *Vitis vinifera*) juice against *Salmonella typhimuriumon* salad vegetables. *Food Control*, 18, 702-706.

Nikfardjam, M. S. P. (2008). General and polyphenolic composition of unripe grape juice (verjus/verjus) from various producers. *Mitteilungen Klosterneuburg*, 58, p. 28-31.

Öncül, N., & Karabiyikli, S. (2015). Factors affecting the quality attributes of unripe grape functional food products. *J. Food Biochem.,* 39, 689–695

Piper, P., Calderon, C.O., Hatzixanthis, K., Mollapour, M. (2001). Weak acid adaptation: the stress response that confers yeasts with resistance to organic acid food preservatives. *Microbiol.*, 147, 2635-2642.

Sabir, A., Kafkas, E., Tangolar, S. (2010). Distribution of major sugars, acids and total phenols in juice of five grapevine (*Vitis* spp.) cultivars at different stages of berry development. *Span. J. Agric. Res*., 8, 425-433.

Setorki, M., Asgary, S., Eidi, A., & Rohani, A. H. (2010). Effects of acute verjus consumption with a high-cholesterol diet on some biochemical risk factors of atherosclerosis in rabbits. *Med. Sci. Monit*., 16, 124-130.

Shojaee-Aliabadi, S., Hosseini, S. M., Tiwari, B., Hashemi, M., Fadavi, G., & Khaksar, R. (2013). Polyphenols content and antioxidant activity of Ghure (unripe grape) marc extract: influence of extraction time, temperature and solvent type. *Int. J. Food Sci. Tech.,* 48, 412-418.

Simone, G. V., Montevecchi, G., Masino, F., Matrella, V., Imazio, S. A., Antonelli, A., & Bignami, C. (2013). Ampelographic and chemical characterization of Reggio Emilia and Modena (northern Italy) grapes for two traditional seasonings: ‘saba’ and ‘agresto’. *J. Sci. Food Agric*., 93, 3502-3511.

Supplementary Material

Verjus was made from thinned estate-grown Pinot noir grapes obtained from Sokol Blosser Winery (Dayton, Oregon) (**Supplementary Figure 1**).

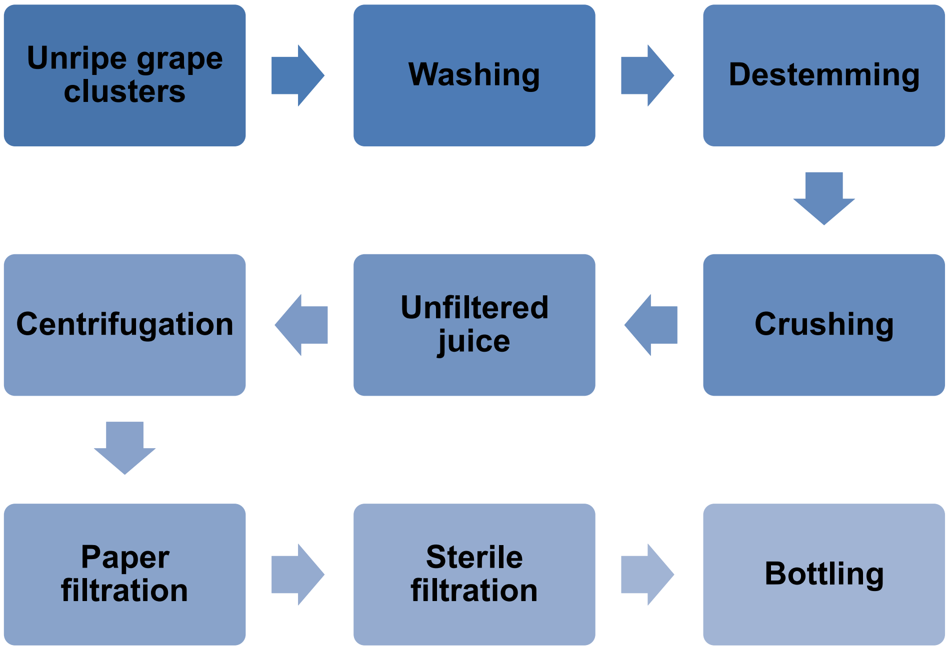

Fig. 1S. Flow diagram for lab-scale processing of verjus made from unripe Pinot noir grapes.

The thinned fruit was harvested by 9 am on August 14, 2017, rinsed with tap water, and destemmed by hand. The berries were then crushed using food mills which excluded the seeds. The resulting unfiltered juice was bottled in 750-mL glass bottles and stored on ice until transfer to 4°C. The chilled juice was clarified initially by centrifugation (10 min, 900 x *g*) followed by paper filtration using diatomaceous earth prior to sterile filtration (0.45 μm PES filter media, Whatman). The sterile-filtered verjus was then stored in the dark at room temperature in 125-mL glass bottles. **Supplementary Table 1** shows the chemical composition of the verjus.

Table 1S. Composition of verjus made from unripe Pinot noir grapes[1].

Analyte	**Value**
pH	2.37
Titratable acidity	35.5 g/L
L-malic acid	23.8 g/L
Tartaric acid	11.5 g/L
Solid soluble	5.2 °Brix
Glucose + Fructose	19.8 gL
Potassium	930 mg/L
Ammonia	56 mg/L
Alpha-amino compounds[2]	19 mg/L
Yeast assimilable nitrogen[2]	65 mg/L
Gallic acid	<0.2 mg/L
Catechin	14.5 mg/L
Astilbin	8.1 mg/L
Tannin	96.4 mg/L
Grape reaction product	1.9 mg/L
Caftaric acid	68.8 mg/L
Caffeic acid	6.2 mg/L
Quercetin glycosides	3.4 mg/L
Quercetin aglycone	<0.2 mg/L

[1] Analysis performed by ETS Laboratories, St. Helena, CA.
[2] As nitrogen.

General discussion and conclusions

This thesis aimed to improve the comprehension of some important aspects concerning a product called verjuice, an unfermented grape juice with a unique and sour flavor made by pressing un-ripened grape berries. Normally, verjuice is produced from the grapes that are thinned from the vineyard (a viticultural practice known as "green harvest" or "grape thinning"). Actually, early in the grape growing season, some grape growers thin the clusters of unripe grapes in order to allow the grapes that remain on the vine to ripen optimally producing a better crop and ultimately a wine of high quality. Currently, the vast amount of thinned grapes left in the rows of the vineyard represents a by-product in viticulture. Thus, the production of verjuice can represent a profitable conversion of discarded grapes into a high value-added food product of interest for viticulture and wine industry.

Verjuice is an ingredient that is both ancient and modern. Some food historians trace its use back to the Roman times but verjuice is also actively used by many chefs and barmen (Nikfardjam, 2008; Woolgar, 2010; Brigand & Nahon, 2016). In this context, some grape growers recently have started taking in consideration the production of verjuice from vineyards by harvesting unripe grapes. This is due to the fact that verjuice is slowly gaining a considerable popularity mainly by those consumers who appreciate wines. In fact, this shows that the market has reacted for this rediscovered acidic ingredient. In this context, verjuice has been strategically positioned on the market as the “ultimate in vineyard recycling” being suggested to consumers as a “friendly” food product, as can be seen on the labels of several verjuice bottles produced around the world. In addition, it has become popular and gourmet also because famous chefs started to cook using verjuice instead of vinegar or lemon juice suggesting its versatility in different food applications such as to marinate meat, to be added in sauces, also in different cocktails (alcoholic or not), spritzers, and so on (Hildebrandt & Matchuk, 2002; Hayoglu et al., 2009; Woolgar, 2010; Bagheri & Esmaiili, 2017).

Despite of the increasing use of verjuice, it still seems to be a relatively unknown product mainly because the information about its chemical and sensorial characteristics is scarce. Therefore, it is necessary to investigate different aspects concerning this ingredient in order to find optimized methods of production and utilization. This is general objective of this thesis.

Initially, some key production variables were evaluated because verjuice has no agreed-upon standard of identity nor a standardized method of production. The first study aspired to give some parameters to provide baseline data for verjuice production. Therefore, the evaluation of the major factors that determine the verjuice quality for potential production in the Veneto region was undertook. In particular, the choice of grape variety, harvest date, and method of preservation were studied. From the evaluation of chemical and sensorial characteristics, the results showed that verjuice produced from different varieties has comparable sensory quality suggesting the possibility of using any grape variety for its production. This is likely to be a consequence of the fact that the grapes harvested before commercial maturation do not have fully expressed their distinctive sensorial features yet. Therefore, the verjuice production from single varieties or their blend could be considered in order to increase the diversification of the verjuice options on the market, even though the sensorial differences are slight.

Taking in consideration the multiple possibilities of using verjuice as acidic ingredient, the second approach here was that to evaluate the sensorial characteristics of salads seasoned with verjuice in comparison to white wine vinegar and lemon juice, which are the mostly seasonings used. The results led to the conclusion that verjuice can be considered as a valid alternative to common acidic salad seasonings. In addition, compared to vinegar, a typical feature of verjuice is its lack of the pungent smell of acetic acid, an aspect that could make verjuice the preferred salad seasoning for those consumers disliking the smell of vinegar. For example, wine consumers usually have a particular attention to the interference of the acetic note in the wine perception during a meal. Thus, verjuice can have the advantage of being more harmonious and delicate from the sensory point of view in food-wine pairings. Indeed, this approach is already established in some countries, especially in the wineries that also have a part dedicated to restaurant/catering, where they use verjuice as the ingredient for replacing vinegar in several food preparations.

Thirdly, an innovative proposal for using verjuice as a food ingredient was assessed in this thesis staring from the idea that this acidic ingredient can be used not only for its taste properties but also as preservative in food conserves. In this context, one category of food product that is largely consumed in Italy as well as in other countries is the "sottaceto", which is produced using vegetables preserved with the classic wine vinegar as acidulant. Then, this aspect was studied by using cucumbers as a model for pickled vegetable foods. To this aim, the chemical and sensorial properties of cucumbers pickled with verjuice were evaluated in comparison to the same samples pickled with vinegar. The results led to the conclusion that the new pickled product can be produced by using verjuice instead of vinegar. Indeed, verjuice pickled cucumbers were well appreciated as the standard commercial pickles in vinegar. However, the typical aroma of cucumber was better maintained when verjuice was used in place of vinegar. Therefore, since the data obtained here for cucumbers are likely to be valid also for pickling other vegetables and fruits, this preserving liquid needs to be taken into consideration from the food industry as an alternative to vinegar when the valorization of the smell of the original plant food is desired. Moreover, pickled products that are incorrectly paired to wines during aperitifs could favorably be replaced by verjuice for the same products. Actually, there are some companies that started to produce a new category of “sottaceto” with the claim label “zero-vinegar" because it seems to be a trend in the market of pickled products. Therefore, verjuice should be promoted within the food industries in order to communicate the possibility of using verjuice in alternative to vinegar or other acidulants classified as food additives. Therefore, in addition to deepen flavors and add a delicate tartness to different plant products and a wide range of dishes, the use of verjuice can also support health clean labels for a given product.t.

For all the purposes described above, however it is necessary to have a verjuice with a prolonged shelf life, at least for the industrial production, as the low quantity of fermentable sugar present in verjuice can support growth of the relatively acid-tolerant wine yeast *Saccharomyces cerevisiae*. Therefore, a system to produce microbiologically-stable verjuice was studied for solving the question of whether verjuice could be protected from microbiological spoilage. In this context, the last study evaluated this question by simply increasing tartaric and/or malic acid levels and reducing pH, without adding sulfite or other chemical preservatives, or resorting to pasteurization or sterile filtration, which are not consistent with the growing interest in minimally-processed organic food products. The results led to conclude that it is possible to avoid microbial spoilage by a modest increase in tartaric acid and reduction in pH relative to a typical verjuice. Because tartaric acid is naturally present in grapes, the use of this system does not require any artificial additives, improving the clean image of the product, nor additional production steps.

To conclude, based on the results here reported, it can be stated that verjuice has a great potentiality to be used in several food preparations. However, more studies about the costs of verjuice production in relation to the potential incomes deriving from the market are necessary in order to establish the economic interest for its production at the industrial level. To address this question, preliminary studies performed during these 3-years research period have indicated the economic feasibility of verjuice production (see in the Appendix I the article “Il verjuice: dal vigneto al piatto”). At this point, a marketing strategy should be defined and applied promoting verjuice at the consumer level. To do that, the focus on education and information about verjuice, rather than conventional advertising, can definitely help to divulgate verjuice as an invitation to reap the benefits of using verjuice including sensorial, health, and recycling aspects. The consolidation of the verjuice’s market can help to legitimize verjuice as a grape product allowing wineries and grape growers to differentiate their products with more revenue both in local and international markets.

References

Bagheri, H., Esmaiili, M. (2017). Ultrasound-Assisted Extraction of Phenolic Compounds from Unripe Grape (Qora). Erwerbs-Obstbau.

Brigand, J-P., Nahon, p. (2016). Gastronomy and the citron tree (*Citrus medica* L.). *International Journal of Gastronomy and Food Science*, 3: 12-16.

Hayoglu, I., Kola, O., Kaya, C., Özer, S., Turkoglu, H. (2009). Chemical and sensory properties of verjuice, a traditional Turkish non-fermented beverage from kabarcik and yediveren grapes. *Journal of Food Processing and Preservation*, 33:252-263.

Hildebrandt, A., Matchuk, J. (2002). More than ordinary gring. Food Product Design, Weeks Publishing Co., Northbrook, IL.

Nikfardjam, M. S. P. (2008). General and polyphenolic composition of unripe grape juice (verjus/verjuice) from various producers. *Mitteilungen Klosterneuburg*, 58:28-31.

Woolgar, C.M. (2010). Food and the middle ages. *Journal of Medieval History,* 36:1-19.

Appendix

VERJUICE, DA UNO SCARTO VITICOLO UN PRODOTTO AD ALTO VALORE AGGIUNTO[5]

Origine

Il verjuice è un succo di uva acerba non fermentato che viene prodotto con uva raccolta durante il diradamento oppure da vigneti specializzati per la sua produzione. Già nell'antichità, al tempo dei Greci e dei Romani, il verjuice fu usato prima dell'avvento dell'aceto e successivamente fu diffuso in epoca medievale e nella cucina rinascimentale principalmente come condimento utilizzato nei banchetti e nelle tavole dei nobili. Esistono anche degli scritti di autori latini che lo citano, ad esempio Platina, che nel suo libro di ricette "De honesta Voluptate", facendo riferimento ai primi gastronomi della storia Plinio e Macrobio che ne descrivono le caratteristiche organolettiche.

Il verjuice ha molteplici utilizzi e dunque può essere impiegato per svariati scopi, alimentare come condimento per pietanze, nella preparazione di diversi cocktails, acidificante nell'industria enologica e come antiossidante naturale nel settore farmaceutico. Nel mercato mondiale si possono trovare diverse tipologie di verjuice in quanto non esiste ancora una precisa normativa di riferimento.

Questo prodotto alimentare prende diversi nomi a seconda del luogo d'origine. Verjuice è il termine in inglese, ma in Francese è conosciuto come "verjus", in Spagnolo come "agraz", in Turco come "koruk", in Persiano come "abe ghureh". Nel caso in cui questo succo venga riscaldato e concentrato, prende il nome di salsa d'uva acerba ("sour grape sauce", in inglese), che in Italia è chiamato "agresto".

Il verjuice è utilizzato particolarmente nelle regioni della Turchia e Iran per migliorare il sapore di pietanze tradizionali ed entra a far parte delle ricette di alcuni drink, soprattutto digestivi e salse di senape. Dalla produzione totale di uva in Turchia, 39% viene essiccato per la produzione di uva secca, 3% viene trasformato in vino, 40% viene consumato come uve da tavola e 18% viene trasformato in pekmez (succo d'uva concentrato). Tuttavia, le varietà sono utilizzate anche per la produzione di bevande alcoliche come per esempio il cognac, il raki ed alcuni liquori e anche bevande non alcoliche come per esempio i succhi di uva non fermentati ed anche verjuice. Solo recentemente tale succo ha ricevuto

[5] This is the edited version of: Scaggiante, S., Galletto, L., Ferresi, B., Dupas de Matos, A. (2018). Verjuice, da uno scarto viticolo un prodotto ad alto valore aggiunto, Informatore Agrario, n. 36, p. 39-41, ISSN:0020-0689, Copyright 2018 Edizioni L'Informatore Agrario S.r.l.

attenzione nel mondo occidentale, che lo ha riscoperto come condimento alimentare vista la possibilità di sostituirlo ai condimenti più usuali, quali l'aceto e il succo di limone, essendo più delicato e meno aggressivo nell'abbinamento cibo-vino.

Raccolta dell'uva

Nonostante la lunga storia di utilizzo, questo ingrediente alimentare, verjuice non ha un riconoscimento standard dell'identità nè una fase di maturazione dell'uva ben definita. Un gruppo di ricerca turco ha studiato le proprietà chimiche e sensoriali del verjuice ottenuto dalle varietà turche "Kabarcık" e "Yediveren" raccogliendo le uve 45 giorni dopo la fioritura, periodo in cui l'acidità totale è massima negli acini. Invece, in Iran, la data di raccolta indicata per la varietà iraniana "Asgari" si situa circa 30 giorni prima di quella della vendemmia tradizionale, che quindi corrisponderebbe al periodo tra la fine di luglio e inizio agosto, nel Nord Italia. Recentemente è stato pubblicato un articolo in cui il verjuice è stato prodotto da diverse varietà di uve (Chardonnay, Sauvignon bianco, Glera, Cabernet franc, Cabernet sauvignon e Merlot) raccolte in tre momenti diversi: dalla chiusura del grappolo all'inizio dell'invaiatura (Dupas de Matos et al., 2017). Questo studio è stato realizzato dalla dott.ssa Amanda Dupas de Matos insieme al Prof. Andrea Curioni e Simone Vincenzi, presso l'Università degli studi di Padova in collaborazione con il Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia (CIRVE), nella sede di Conegliano.

Caratteristiche chimiche

Il verjuice è prodotto mediante la pressatura delle uve acerbe, ed è caratterizzato da un'elevata acidità e da un basso contenuto di zuccheri. L'acidità è rappresentata da un alto contenuto di acido malico e tartarico rispettivamente con valori tra 10,9 e 30,4 g/L, e tra 5,5 e 14,0 g/L. L'acidità totale calcolata, data la sommatoria di acido malico più acido tartarico, varia da 17,4 a 38,8 g/L per le varietà di uve bianche e da 25,4 a 40,5 g/L per le varietà di uve rosse (Dupas de Matos et al., 2017). Mentre l'acido ascorbico risulta essere alto, visto il periodo di raccolta anticipato; infatti raggiunge valori di 20 mg/L che permettono al succo di conservarsi più facilmente perchè i batteri hanno difficoltà di svilupparsi in ambiente molto acido.

I composti aromatici, tipici delle uve, come terpenoli (geraniolo, nerolo, citronellolo e linalolo), norisprenoidi (beta-ionone, beta-damascone) e mercaptani, non hanno il tempo sufficiente per formarsi, ma si verifica un grande accumulo di pirazine, aromi varietali tipici del Sauvignon.

Altra caratteristica fondamentale del verjuice è il suo alto contenuto in polifenoli, i quali conferiscono un alto potenziale antiossidante; per questo motivo è ritenuto un prodotto salutare che, se assunto in grandi quantità e continuamente, potrebbe contribuire a ridurre l'invecchiamento delle cellule. Tra le sostanze polifenoliche, le più importanti sono: acido caftarico, caffeico, acido, fertarico, gallico, para-cumarico, para-cutarico, catechine, epicatechine e quercitina, ossia molecole che vanno a costituire nel vino il cosiddetto "corpo". È importante dire che, proprio come il vino, il verjuice assume diverse sfumature in relazione al clima, varietà e tecnologia di produzione nei vari paesi di origine.

Caratteristiche organolettiche

Pochi sono gli studi che riguardano l'aspetto organolettico del verjuice. Nello studio di Dupas de Matos et al. (2017), la caratterizzazione sensoriale è stata condotta attraverso l'analisi descrittiva per trovare i descrittori sensoriali contenuti nel prodotto. Successivamente, è stato effettuato il profilo sensoriale del verjuice con i soggetti che avevano seguito un programma di addestramento specifico. I principali descrittori percepiti per il gusto erano "acido", "astringente", "salato" e "dolce", mentre l'aroma più comune erano "erbaceo", "mela cotta", "pera", "mela verde" e "floreale". Questo studio ha valutato il verjuice tal quale per descriverlo solo dal punto di vista sensoriale. Successivamente si sono valutate l'appropriatezza e piacevolezza del verjuice come condimento per l'insalata in confronto con l'aceto e il succo di limone: ne è emerso che il verjuice è ritenuto un condimento appropriato dal 60-91% del campione ed ha avuto lo stesso punteggio del succo di limone per la piacevolezza.

Tecnica produttiva

Le uve vengono versate in una vasca in acciaio inox, dotata di inverter, e pressate in un ambiente protetto dall'ossidazione, con una resa di estrazione compresa tra il 54 e il 60%. Tramite un'elettropompa passano attraverso uno scambiatore refrigerante a tubi, per abbatterne la temperatura da 25 a 15°C. Successivamente passano in una pressa pneumatica a tank chiuso, alimentata da una pompa che in due ore riesce a caricare tutta l'uva, così da evitare l'ossidazione del prodotto. Quindi tramite un'elettropompa centrifuga utilizzabile anche per eventuali travasi, il succo ottenuto viene trasferito nel flottatore, dove avviene la precipitazione di sostanze presenti all'interno del succo torbido. Immediatamente viene eseguita anche la chiarificazione, atta a raggiungere la stabilità proteica. Una volta chiarificato, il succo viene stoccato all'interno di serbatoi di varia capienza, dotati di una tasca per il liquido refrigerante, la cui temperatura interna è di 2°C. Per una decina di giorni viene poi lasciato a basse temperature affinché avvenga la precipitazione tartarica.

Per la refrigerazione totale e il controllo della temperatura del verjuice è necessario installare una centrale di refrigerazione che verifichi l'abbattimento della temperatura che avviene nello scambiatore a tubi da 25°C a 15°C. Successivamente il succo subisce un'ulteriore refrigerazione per flottazione ed il mantenimento della temperatura costante a 2°C. La filtrazione finale, è strettamente necessaria al fine di eliminare, prima con un filtro tangenziale ceramico, i residui con dimensioni più grandi, quali proteine, sali e polisaccaridi; poi, con un impianto di micro-filtrazione tangenziale con sistema di lavaggio, sono eliminati possibili residui di batteri e lieviti che potrebbero abbassare la *shelf-life* del prodotto.

Successivamente si passa alla fase dell'imbottigliamento. Per imbottigliare il verjuice si potrebbe considerare una linea di imbottigliamento completa come quella del vino dotata di etichettatrice autoadesiva e di tappatrice. Considerando le tendenze di mercato, un tappo a vite di allumino rispetto ad uno di sughero potrebbe essere preferito. L'etichettatrice andrà ad applicare una contro-etichetta ed un'etichetta frontale sulla bottiglia, sulla quale saranno riportati i fattori nutrizionali del prodotto ed una sua breve descrizione.

Mercato

Tra i principali studi sul mercato del verjuice vi è quello effettuato in Cile dove è stata analizzata la fattibilità economica della produzione di verjuice ottenuto da uve Pais, attraverso interviste in un supermercato su diverse classi sociali al fine di valutare l'accettabilità del prodotto da parte dei consumatori. I risultati dimostrano che le fasce sociali medio alte hanno accettato di buon grado il succo, vista la particolarità del gusto e il suo prezzo. Quindi i viticoltori potrebbero produrre il verjuice ed ottenerne un reddito visti i vantaggi produttivi con la possibilità di utilizzare gli stessi macchinari e strumenti che già utilizzano per la produzione del vino, sia per l'incremento del prezzo delle uve rimaste in vigneto dopo il diradamento.

Un approccio interessante per il marketing potrebbe essere quello di associare il verjuice alle sue riconosciute proprietà benefiche, come ad esempio l'effetto ipolipemizzante, il controllo dell'ipertensione, effetti antitrombotici, neuroprotettivi e anticonvulsivanti e soprattutto antinfiammatori.

In Italia alcuni ricercatori dell'Università di Padova hanno valutato il potenziale antiossidante delle bacche immature, al fine di trovare un possibile utilizzo di questi sottoprodotti come un potenziale ingrediente funzionale. Come oggetto della ricerca, sono state considerate le uve Merlot e Barbera raccolte durante il diradamento, da cui è stato estratto il succo acerbo nelle annate 2013 e 2014. Ne è emerso che l'attività antiossidante di tutte le bacche era rilevante in misura variabile a seconda della varietà, ma non della stagione. In particolare, il succo di Merlot ha avuto un'attività antiossidante di due volte superiore a quello di Barbera (Tinello e Lante, 2016).

A livello internazionale negli ultimi anni questo succo è tornato di moda grazie alla chef Australiana Maggie Beer, che lo ha introdotto nella sua cucina come base acida in diversi piatti. In Europa, è molto utilizzato in Francia, e diffuso anche in Spagna, Austria, Inghilterra e Germania. Invece in America, viene consumato soprattutto in Oregon, New York e California. La previsione di consumo in Italia non è ancora stata definita poiché l'aceto è il condimento più utilizzato e quello preferito dalla maggior parte degli Italiani.

Esistono nel nostro paese delle alternative al verjuice, ad esempio l'agresto, diffuso in Toscana, che è un condimento preparato da uva non completamente matura, che viene poi riscaldato fino a che si addensa e prevede infine l'aggiunta di alcune spezie, tra cui dragoncello, cannella, cipolla, aglio e miele. Nella zona Romagnola invece, è presente il saba, un prodotto simile all'agresto, ma che si distingue per una diversa varietà di spezie utilizzate e per una maggiore dolcezza. Questo è dunque un concentrato di mosto cotto, aggiunto di frutta cotogna, cannella e chiodi di garofano. Secondo diversi studi, oltre l'aspetto sensoriale, il verjuice può essere impiegato come un'alternativa agli antimicrobici tradizionalmente utilizzati grazie alla sua attività battericida.

Packaging

Per la commercializzazione del verjuice vengono oggi utilizzati diversi formati di bottiglie che si distinguono per volume, colore e tipologia come si evince dalla **Tabella 1**, poiché fino ad oggi non è stata redatta una normativa per la produzione del succo. Alcune bottiglie sono verdi, come per un normale vino, per evitare l'ossidazione del prodotto; altre invece sono trasparenti, per favorire la visualizzazione del liquido, come è d'uso per gli aceti. Inoltre, sono presenti sul mercato anche bottiglie di colori molto scuri, quasi neri.

Tabella 1. Packaging e prezzo di verjuice presenti in alcuni mercati.

Azienda	Paese	Volume (ml)	Bottiglia	Tappo	Prezzo
Fusion	Stati Uniti	750	Bordolese	A vite	19,99$
Navarro	Stati Uniti	750	Bordolese	Sintetico	13$
Wofflër Estate	Stati Uniti	750	Alsaziana	Sintetico	12$
Maggie Beer	Australia	375, 750	Bordolese	A vite	10$, 29$
Saint Aidan Wines	Australia	250	Bordolese	A vite	15$
The Verjuice Co.	Sud africa	500	Bordolese	A vite	49,99R
Domaine du Siorac	Francia	330	Da birra	A vite	5,50€
Nathan Outlaw	Inghilterra	375	Borgognona	A vite	7,99£
Westmeston Estate	Inghliterra	375	Bordolese	A vite	7,95£
Au Fil du Vent	Canada	500	Da liquore	A vite	19,95$
Minus 8	Canada	500	Da liquore	Sughero	26.95$
Neudorf Vineyards	N. Zelanda	750	Alsaziana	A vite	17$
Jenny Dewars	N. Zelanda	750	Bordolese	A vite	22$
Sadaf ®	Iran	946	Da succo	A vite	5,99$

I volumi delle bottiglie, passano dai 250 ml fino ad arrivare ai 2 lt, bottiglie che risultano però eccessive per un condimento alimentare usato in casa. Tuttavia, le bottiglie di grande formato potrebbero essere interessanti per la vendita ai ristoranti, dove le quantità utilizzate sono assai maggiori.

Per un corretto posizionamento sul mercato sarebbe importante creare una bottiglia esclusiva, con una forma diversa rispetto alla classica bottiglia di vino che permetterebbe una migliore identificabilità del prodotto ed una maggiore visibilità sugli scaffali di vendita. Generalmente le bottiglie presentano sia un'etichetta, in cui è presente il logo dell'azienda produttrice e l'annata di produzione che una contro-etichetta dove si può trovare una breve descrizione del prodotto e le informazioni nutrizionali.

Per pubblicizzare ulteriormente la rinascita di questo antico prodotto, alcuni cuochi, come ad esempio Maggie Beer, hanno pubblicato svariati libri riguardanti tale argomento. La principale bibliografia della cuoca australiana è "Maggie's verjuice cookbook", che racchiude molte ricette da lei stessa sperimentate e spiega come questo ingrediente acidulo possa aiutare ad avere una cucina più saporita, delicata e armonica dal punto di vista dell'abbinamento cibo-vino.

Oltre a svariate aziende australiane, sono attive anche imprese viticole Neozelandesi, che dedicano la loro produzione al verjuice, come ad esempio quella di Jenny Dewar, situata a sud-ovest del paese.

Passando al continente americano, più precisamente nella Napa Valley, in California, è nota un'azienda produttrice, chiamata Fusion, che commercializza i prodotti derivati da varietà a bacca bianca e rossa. L'azienda Wofflër Estate, localizzata nel cuore di Hamptons (New York), ha un mercato affermato del verjuice e utilizza frequentemente i social media come instagram per pubblicizzare il loro prodotto in modo particolare.

In Europa, sono ancora poche le aziende che hanno deciso di intraprendere questa scelta produttiva. Due di queste si trovano in Francia, più precisamente una Domaine du Siorac in Dordogna, l'altra di Nathan Outlaw, chef stellato Michelin inglese, nella Valle della Loira, Domaine Levin. Entrambe le aziende sono viti-vinicole, cioè dedicano solo una parte della loro uva alla produzione di verjuice. Nel secondo caso invece, lo chef ha saputo abilmente sfruttare sia i social media (instagram, facebook e twitter) che il website aziendale per pubblicizzare il proprio prodotto e le sue possibilità di utilizzo. Lo chef é stato più volte nominato in riviste di cucina inglese, come ad esempio Imbibe UK 2016 e Olive Magazine Food & Trends, dove ne viene elogiata l'abilità culinaria e la capacità di portare un prodotto antico, ed ancora oggi sconosciuto, ma con notevoli potenzialità di sviluppo.

Un altro esempio che si può prendere in considerazione è l'azienda araba Sadaf, che produce un verjuice diverso rispetto a quelli sopra elencati. Infatti nella descrizione che si trova sul sito internet, il succo è utilizzato in alcuni piatti tipici persiani come l'Aash, una zuppa di verdure tipica Iraniana, a base di spezie e lenticchie, per insaporirli ulteriormente o anche nel brodo. Le caratteristiche organolettiche si discostano da quello che normalmente ci si aspetta da un succo acido perché ha molte note di verdura cotta ed è molto più amaro rispetto agli altri esempi presenti sul mercato, dovuto alla pastorizzazione utilizzata per una maggiore conservazione del prodotto.

Anche in Sud Africa, il verjuice si sta diffondendo, sono presenti nel paese alcune aziende specializzate per la sua produzione, come ad esempio l'azienda The Verjuice & Co, situata nella capitale, che ha creato un concorso a premi che mette in palio delle bottiglie di verjuice, per attirare i clienti.

Il consumatore

Nell'ultimo anno si è assistito a un grande boom del numero dei consumatori vegetariani e vegani in Italia, in cui il 6,2% della popolazione si dichiara vegetariana e lo 0,9% vegana (Rapporto Eurispes, 2018). Tenendo in considerazione che il verjuice, è un prodotto al 100% vegetale (ottenuto da uva), esso potrebbe incontrare l'interesse di questo gruppo di consumatori e raggiungere questo mercato di nicchia. Un aspetto molto importante dal punto di vista della sostenibilità è il fatto che il verjuice normalmente viene ottenuto dalle uve rimaste in vigneto dopo il diradamento e quindi questo rappresenta una possibilità di approfittare di uno scarto vitivinicolo e di convertirlo in un prodotto alimentare ad alto valore aggiunto. Inoltre il verjuice è un prodotto senza glutine (gluten free).

I consumatori che apprezzano il vino, hanno di solito anche una buona cultura del cibo e hanno una maggiore sensibilità nell'abbinamento. Pertanto, considerato che il verjuice non è fermentato e non ha l'acidità volatile presente nell'aceto, dal punto di vista dell'abbinamento cibo e vino, esso possiede il vantaggio di essere più armonioso e delicato. In alcuni paesi questa realtà è già affermata, soprattutto dove le cantine dispongono anche di una parte dedicata alla ristorazione, in cui utilizzano il verjuice come ingrediente in sostituzione dell'aceto in diverse preparazioni alimentari, proprio perchè è considerato da Sommelier e Chef un condimento più versatile, armonico ed equilibrato dal punto di vista sensoriale.

Una tipologia di prodotto che viene consumata molto in Italia è il "sottaceto", che usa normalmente come acidificante il classico aceto di vino bianco. Però, esiste già sul mercato la tipologia "zero-aceto" per i prodotti come insalatine di verdure, giardiniere e cetriolini. Ci sono alcune aziende italiane che producono il "zero-aceto", evidenziando come il mercato sia in constante evoluzione. Pertanto, il verjuice potrebbe essere utilizzato come acidificante alternativo all'aceto ed essere la base acida a costituire il liquido di governo nei prodotti vegetali.

Anche se il verjuice possiede una lunga storia d'uso come ingrediente alimentare, è un prodotto relativamente sconosciuto in Italia. È importante notare che la sua produzione può rappresentare un incremento economico ai viticoltori oltre alla diversificazione aziendale. Per quanto concerne il consumo sarebbe utile approfondire lo studio con una ricerca di mercato sulle preferenze del consumatore e la disponibilità all'acquisto per conoscerne appieno le potenzialità ancora inespresse.

Bibliografia

Dupas de Matos, A; Curioni, A., Bakalinsky, A. T., Marangon, M., Pasini, G., Vincenzi, S. (2017). Chemical and sensory analysis of verjuice: an acidic food ingredient obtained from unripe grape berries. *Innovative Food Science & Emerging Technologies*, 44, 9-14.

Tinello, F., Lante, A. (2017). Evaluation of antibrowning and antioxidant activities in unripe grapes recovered during bunch thinning. *Australian Journal of Grape and Wine Research*, 23, 33-41.

Rapporto Eurispes (2018). Rapporto Italia: Documento di Sintesi, Scheda-sondaggio 15 - Le alternative alimentari: vegetariani e vegani, Percorsi di Ricerca nella Società Italiana, Istituto di Studi Politici Economici e Sociali (Eurispes), Roma, 27p.

ALLA RISCOPERTA DEL VERJUICE[6]

Un prodotto che risale a tempi antichi ma che solo recentemente è stato proposto dall'alta gastronomia come ingrediente versatile nel settore di Ristorazione e Sommelierie

I grappoli d'uva acerbi possono essere utilizzati per dare origine a un ingrediente alimentare chiamato, in inglese, "verjuice", che viene definito come un succo d'uva fresco prodotto mediante la pressatura dell'uva acerba. Nel mercato mondiale si possono trovare diverse tipologie di verjuice e esso prende diversi nomi a seconda del luogo d'origine. Verjuice è il termine in inglese, ma in Francese è conosciuto come "verjus", in Spagnolo come "agraz", in Turco come "koruk", in Persiano come "abe ghureh". Nel caso in cui questo succo venga riscaldato e concentrato, prende il nome di salsa d'uva acerba ("sour grape sauce", in inglese), che in Italia è conosciuto come "agresto".

Il verjuice è caratterizzato da un sapore unico, una elevata acidità - paragonabile a quella dell'aceto - e basso contenuto di zucchero. Già nell'antichità, al tempo dei Greci e dei Romani, il verjuice fu usato prima dell'avvento dell'aceto e successivamente fu diffuso in epoca medievale e nella cucina rinascimentale principalmente come condimento utilizzato nei banchetti e nelle tavole dei nobili. Tuttavia, solo recentemente tale ingrediente non alcolico ha ricevuto attenzione nel mondo occidentale, che lo ha riscoperto come condimento alimentare vista la possibilità di usarlo come sostituto dei condimenti più usuali, quali l'aceto e il succo di limone, essendo più delicato e meno aggressivo in bocca anche dal punto di vista dell'abbinamento cibo-vino.

Avendo molteplici utilizzi e dunque potendo essere impiegato per svariati scopi alimentari, il verjuice viene comunente utilizzato come condimento per pietanze, nella preparazione di diversi cocktails, drinks, e salse di senape, oltre come acidificante nell'industria enologica. Considerato che il verjuice non ha l'acidità volatile presente nell'aceto, ma solo quella fissa presente naturalmente anche nel vino, dal punto di vista dell'abbinamento cibo e vino esso possiede il vantaggio di non apportare note acetiche percepibili al naso oltre ad essere più armonioso e delicato al gusto rispetto all'aceto. In alcuni paesi questo condimento è già affermato, soprattutto dove le cantine dispongono anche di una parte dedicata alla ristorazione, in cui utilizzano il verjuice come ingrediente in

[6] This is the edited version of: Dupas de Matos, A., Vincenzi, S., Curioni, A. (2018). Alla riscoperta del verjuice, Il Sommelier, n. 3, p. 61-62, anno XXXVI, https://issuu.com/gipi/docs/il_sommelier_3-18/63, ISSN: 1826-6533, Editore Federazione Italiana Sommelier Albergatori Ristoratori.

sostituzione dell'aceto in diverse preparazioni alimentari, proprio perché è considerato da Sommelier e Chef un condimento più versatile, armonico ed equilibrato dal punto di vista sensoriale.

Abbinare un piatto ed un vino significa ottenere un'armonia ovvero un equilibrio tra le sensazioni percepite facendo in modo che si completino a vicenda generando migliori, diverse o nuove sensazioni di piacevolezza in bocca. Si sa che non è sempre facile creare il giusto abbinamento tra vini e pietanze, visto che debbono essere l'uno al servizio dell'altro senza sovrastarsi. Quindi, quando l'acidità dell'aceto presente nel cibo, che può essere un'insalata o un sottaceto, è forte al punto di interferire negativamente sulla degustazione del vino, si crea uno squilibrio dal punto di vista sensoriale e forse anche una distorsione nel riconoscimento della qualità del vino che si consuma. In questo senso, per esempio, i prodotti sottaceti che vengono erroneamente abbinati ai vini durante gli aperitivi potrebbero felicemente essere sostituiti dagli stessi prodotti conservati sotto verjuice.

Tenendo in considerazione che i consumatori che apprezzano il vino hanno di solito anche una buona cultura del cibo e una maggiore sensibilità nell'abbinamento, il verjuice può essere considerato un prodotto enologico di importanza nel settore gastronomico e una bella alternativa alimentare da inserire nel contesto della ristorazione. Tra l'altro questo ingrediente potrebbe essere ricavato anche dall'uva acerba derivata dall'operazione di diradamento dei grappoli, una operazione che si effettua in vigneto prima della maturazione dell'uva per migliorare la qualità del raccolto. Poiché l'uva staccata precocemente dalla vite non viene normalmente utilizzata, ecco che la sua conversione in verjuice potrebbe suscitare l'interesse di aziende vitivinicole e di altre industrie connesse al settore vitivinicolo.

Verjuice mocktails
(Fonte: www.maggiebeer.com.au)