The science behind beverage flavors: The role of pH and amylase enzyme in the human mouth

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Abstract. The chemical composition of alcoholic beverages plays a crucial role in their flavor, and the interaction with the chemistry of the mouth, particularly saliva, further shapes the sensory experience. Saliva's pH and enzyme activity can affect the chemical balance of the drink, and therefore, the taste and aroma perceived by the taster. This study examined the influence of saliva on the pH and α-amylase activity of alcoholic beverages, through a potentiometer and enzymatic kits respectively, and how this affected their sensory profile, through the performance of a Descriptive Analysis (DA) sensory test. The results showed that the pH values of the drinks were altered after contact with saliva, with brandies showing an increase in pH and wines showing a decrease. Additionally, the α-amylase activity was found to be influenced by the presence of acids, ethanol, and tannins in the drink. These observations suggest that the chemical composition of the drink and the saliva can impact the sensory experience. Further studies can help to elucidate the mechanisms underlying this interaction and how it varies across different types of beverages and individuals through sensitive enzyme kits.

1 Introduction

Many connoisseurs of alcoholic beverages are spellbound by the superb descriptions of the aromas and tastes of wine or beer, emerging into a plethora of sensations. So, what underlies these sensations? The flavor. The flavor is determined by the chemical balance of the drink, namely the tannins, acidity, enzymes, percentage of ethanol, fermentation nature, and how these chemical components interact with the mouth chemistry. An understanding of the factors that influence flavor perception can help guide food choices and promote a healthy nutritional status [1-3]. The main influence on the chemistry of the oral cavity is saliva. Saliva´s pH is between 6.2 and 7.4 and its constitution is approximately 99% water, the rest is inorganic and organic compounds of which an enzyme may contribute to flavor perception [1,4,5], called α-amylase, which is the main protein in human saliva. This enzyme catalyzes the hydrolysis of 1,4-glycosidic bonds in starch and other polysaccharides, resulting in the production of smaller sugars such as glucose and maltose, which can be detected by the sweet receptors in the mouth [4,6,7]. This protein is either secreted by the salivary glands or produced from the breakdown of shed epithelial cells. Some aroma compounds may adsorb onto the mucosal pellicle, and the aggregation of the mucosal pellicle by tannins may disrupt these interactions [7,8].

This research aimed to assess how the type of alcoholic drink, such as beer, still wine, port wine, and brandy, affects the pH and α-amylase activity in the oral cavity. The study involved two groups of tasters who participated in sensory evaluations and collected saliva and beverages.

2 Materials and methods

2.1 Characterisation of the panel and descriptive sensory analysis (DA)

For this study two panels of tasters participated, the first panel (P1) of tasters was composed of 11 individuals who had previously participated in the sensory assessment of food and beverages. The second panel (P2) of tasters was composed of 19 individuals and they were the untrained panel.

To conduct a Descriptive Analysis (DA) Sensory Test, namely a CATA (Check-All-That-Apply) test, P1

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was provided with a tasting sheet for each category of beverage, two brandies (Grappa and "Aguardente Velha" with 41 and 40% (v/v) of ethanol, respectively), two wines (one red and one white with an alcoholic degree of 13.5 and 13.0% (v/v), respectively), and one blonde beer, with an alcoholic degree of 4.9 (v/v). Panel two (P2) underwent a similar process for red wine (13.5% v/v) and port wine (19% v/v) with a new set of tasting sheets that included descriptors specifically chosen for the test. During both sessions with P1 and P2, tasters were instructed to mark the attributes they deemed appropriate to describe each beverage.

2.2 Methodology for Evaluating pH Changes and Enzyme Activity

The beverages were presented to the tasting panel in ISO glasses [10], at room temperature (20 ± 2.0 °C), with a sufficient amount for the taster to place in their mouth (20 mL). The tasters were instructed as follows – "Please place each of the presented solutions/beverages in your mouth, spread the liquid well through the oral cavity, and wait for 10 seconds. After 10 seconds, spit the solution into the respective glass." Next, the pH of each solution/beverage was measured using a potentiometer. Then, with the help of pipettes, saliva, and beverage samples were collected in 1.5 mL Eppendorf plastic tubes. All Eppendorfs were identified and stored in the freezer at -18 °C until they were used for enzymatic activity determinations.

The determination of α-amylase activity was performed using an enzymatic assay kit (Biovision α-amylase enzymatic kit, Milpitas, USA) and spectrophotometric methods. To determine whether saliva influences the pH values of different alcoholic beverages, a one-sample t-test was performed, and to verify whether there were statistically significant differences in the α-amylase enzymatic activity in alcoholic beverages, a univariate analysis of variance (ANOVA) was performed. The statistical analyses were carried out using SPSS Statistics software (version 27.0), and a significance level (α) of 0.05 was considered.

3 Results and discussion

3.1 Sensory profile of the alcoholic drinks deter mined by descriptive analysis (DA) sensory test

Through the use of the CATA test, it was possible to determine which attributes were the most salient for each type of beverage and to identify any potential differences in the perception of these attributes between tasters.

Regarding the brandies, although the alcoholic strength is similar for both beverages, the Grappa (colorless brandy) was perceived as having more "alcohol" and the aroma of varnish (ethyl acetate) was mentioned by twice as many tasters in the colorless brandy. The "Aguardente Velha" (color brandy) is characterized by the "spices/wood" aromas and was also perceived as being more "sweetness", Table 1.

Table 1. Attributes highlighted by the tasters (>50%) for each brandy. V.A-Visual Aspect; A-Aroma; T-Taste/Texture; F-Flavor.

	Brandies	
>50%	Grappa	"Aguardente Velha"
V.A- clarity	X	X
A-alcohol	X	X
A-spices/wood		X
A-roasted/burnt		X
A-ethyl acetate/varnish	X	
T-sweetness		X
T-bitterness	X	
T-burning	X	X
T-asperity	X	
T-astringency	X	X
T-persistence	X	X
F-spices		X

The tasters considered red wine to be more "fruity" and "sweeter" than white wine (Table 2). In red wine, the descriptors "astringent" and "body" had a citation percentage higher than 50%. On the other hand, white wine was perceived as having more "citrus", "mineral", "acid" and "floral" aromas and flavors.

Table 2. Attributes highlighted by the tasters (>50%) for each wine. V.A-Visual aspect; A-Aroma; T-Taste/Texture; F-Flavor.

	Wines	
>50%	Red wine	White wine
V.A-clarity	X	X
A-citrus fruit		X
A-red fruit	X	
A-black fruit	X	
A-mineral		X
T-acidity		X
T-astringent	X	X
T-alcohol	X	X
T-body	X	
F-floral		X
F-fruity	X	

For blonde beer, the descriptors with the highest percentage of citations are "foam" and "foam color", "malt aroma", "acidity", "bitterness", the feeling of bubbles in the mouth, and "malt flavour", Table 3.

Table 3. Attributes highlighted by the tasters (>50%) for beer. V.A-Visual aspect; A-Aroma; T-Taste/Texture; F-Flavor.

	Beer
>50%	Blond beer
V.A-foaming	X
A-foam color	X
A-malt	X
T-acidity	X
T-bitterness	X
T-sparkling	X
T-malt	X
T-alcohol	X
T-persistence	X

To better understand what the tasters' reaction was regarding the sensory analysis of alcoholic beverages, a more specific descriptive analysis was performed for red wine and another typical Portuguese beverage, Port Wine, Table 4.

Table 4. Attributes highlighted by the tasters (>25%) for red wine and port wine. V.A-Visual aspect; A-Aroma; T-Taste/Texture; F-Flavor.

>25%	
Red wine	Port Wine
V.A-Clarity	V.a-Brownish
V.A-Intensity	V.a-Persistent tear
V.A-Persistent tear	V.a-Clearness
V.A-Clearness	A-Caramel
A-Red berries	A-Dried fruit
T-Acidity	T-Acidity
T-Astringency	T-Alcohol
T-Full-bodied	T-Sweet
T-Persistence	F-Dried Fruits
T-Bitter taste	F-Wood
T-Tanicity	
F-Wood/Spices	

3.2 pH

pH variations showed statistically significant differences, Table 5. In Grappa (Colorless brand) and Aguardente Velha (Color brandy), pH values increased significantly after exposure to human saliva, ranging from 4.30 to 4.84 (p<0.001) in Grappa and 3.93 to 4.45 (p<0.001) in Aguardente Velha. Like other body fluids, saliva has a buffering capacity that allows it to absorb or release hydrogen ions (H^+) to reduce changes in their concentration, specifically concerning pH values [11]. In this case, the contact time of the drink with saliva and its buffering capacity was a limitation for a greater action of the pH of the saliva in these drinks.

Red wine exhibited a decrease in pH from 3.87 to 3.74 (p<0.05), while white wine showed a decrease from 3.37 to 3.22 (p<0.05). These findings can be rationalized by taking into account that wine is a drink containing a diverse array of acids in varying concentrations. The acids, notably tartaric, malic, and citric acids, function to restrict the pH level of wine and furnish it with a buffering capacity that is contingent upon the types of acids that are present [11]. As a result, this buffering capacity may serve to hinder the influence of wine on the pH of saliva.

In contrast, there was no significant pH change in blonde beer (p=0.941), which may be attributed to its higher pH level (4.34) compared to that of white and red wine (3.37 and 3.87, respectively). Due to the proximity of saliva's pH (usually around 6.2-7.6) to the pH of the beer, it is natural that the pH change would be more challenging to achieve within 10 seconds of contact.

Table 5. Descriptive measures (M ± SD) and univariate effects of pH of alcoholic beverages before (pH B) and after (pH A) contact with saliva.

Type of beverage	**pH B**	**pH A (M ± SD)**	***p***
"Grappa"	4.30	4.84±0.249	<0.001
"Aguardente Velha"	3.93	4.45±0.268	<0.001
White wine	3.37	3.22±0.123	0.004
Red wine	3.87	3.74±0.119	0.008
Blond beer	4.34	4.34±0.066	0.941

3.3 Enzymatic activity

Following saliva collection of the first panel (P1), enzymatic kits were used to determine enzymatic activity. Results analysis indicated a statistically significant impact of the α-amylase enzyme ($F(3, 5) = 56.59$; p<0.001), Table 6. Tamhane's multiple comparison tests showed that grappa led to significantly higher α-amylase enzyme activity (148.11 mU/mL) compared to other drinks (p<0.001). As suggested by various authors [12], α-amylase activity tends to increase in response to physical or psychological stress. The high alcohol content (41.0%, v/v) of grappa, coupled with a "burning sensation" in the mouth and sensations of "varnish," "glue," "asperity", and "bitterness" (CATA test) upon tasting, may indicate an increase in stress and anxiety in the taster as a physiological response. Red wine exhibited higher enzyme activity (13.84 mU/mL) compared to white wine [4.15 mU/mL (p=0.006)] and blonde beer [2.03 mU/mL (p<0.001)]. The α-amylase enzyme showed greater activity in red wine and Aguardente Velha aged in oak wood, possibly due to the vinification process of red wine, which increases reducing sugars, and the aging process of colored spirits, which mellows them, giving rise to more roasted and caramelized aromas, thus creating a positive synergistic effect between these drinks and the enzyme's activity.

Table 6. Means (M), standard deviations (SD), and univariate effects of enzymatic activity (lipase and α-amylase enzymes) in alcoholic beverages. Values with the same letter are not significantly different (post hoc HSD Tukey test). G -"Grappa"; A.V – "Aguardente Velha"; Ww – White wine; Rw- Red wine; Bb – Blond beer.

Enzyme	G M±SD	A.V M ± SD	Ww M ± SD	Rw M ± SD	Bb M ± SD	F	*p*
α-Amylase mU/mL	148.1±31.2 c	44.2±38.7 ab	4.2±4.78 a	13.84±4.85 b	2.03±1.61 a	56.59	<0.001

To validate the hypotheses and attain more dependable data, a second panel (P2) with a higher number of tasters was used. Furthermore, new tests and saliva samples were obtained to evaluate α-amylase enzyme activity, Table 7. The beverages under study were this time a red wine and a port wine and it was verified that the α-amylase enzyme activity was higher in the port wine (72.55 mU/mL).

The Port wine used was a Tawny Port. This style of wine has a sugar content of 40-65 g/L and is, therefore, a very sweet wine. Furthermore, its aroma varies between jams and nuts, such as hazelnuts and walnuts [13] and the wood aging of this type of wine enhances the caramel and woodiness resulting from the Maillard reactions [13,14]. All these descriptors were mentioned by the tasters (Table 4). These attributes, together with the alcohol percentage and the acidity, also detected by the panel, reinforce the idea of increased α-amylase activity in this type of beverage.

Table 7. Descriptive measurements and univariate effects of enzymatic activity (α-amylase) in alcoholic beverages (red wine and Port Wine) and t-Student test for independent samples.

	Red wine M ± SD	Port wine M ± SD	t	*p*
α – Amylase mU/mL	28.62±49.54	72.55±65.64	-2.90	0.005

4. Conclusion

Saliva plays a critical role in taste perception due to its composition and buffering capacity. However, the pH of alcoholic beverages remained closer to their initial values than the pH of human saliva, indicating that the buffering capacity of saliva is inadequate to maintain a stable pH after contact with these beverages. Generally, α-amylase enzyme activity increased significantly in the presence of acids and/or ethanol and decreased in the presence of tannin, possibly due to tannin-protein interactions which may have led to protein precipitation. Future studies involving the influence of enzymes in flavor perception may improve our understanding of the interaction between saliva and beverages. However, more sensitive kits or alternative analytical methods are needed to improve determinations of saliva enzyme activity.

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