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Influence of winemaking by-products on the phenolic activity of beer

This research explores a novel approach in beer production by incorporating waste materials such as grape seeds and skins, derived from wine processing, into the brewing process. This initiative holds promise for effective waste utilization, advancing craft beer, and utilizing cost-effective raw materials. Experiments explore the integration of grape by-products into beer production, evaluating their impact on polyphenol levels and sensory characteristics. The laboratory and pilot brewhouse trial results revealed a significant increase in the total polyphenol content when 0.6 % grape seeds were added during the boiling process. Specifically, the total polyphenol value doubled, reaching up to 500 mg/l compared to the 250 mg/l found in classical pilsner-type beer. The study indicates that grape seed addition is significantly more effective than grape skin addition under the same conditions, yielding around 1.5 times or more polyphenols. Sensory evaluations revealed a preference for beers supplemented with white grape seeds compared to red grape seeds, providing a unique taste experience with a pleasant grape aroma. Moreover, beers with grape seed additions exhibited elevated levels of specific polyphenolic compounds, particularly catechin and epicatechin. Additionally, the antioxidant capacity of the beer slightly increased with grape seed incorporation and colloidal stability was not negatively affected.

Descriptors: polyphenols, grape seed, value-added beer, epicatechin, grape skin

1 Introduction

Beer is one of the foremost globally consumed beverages, ranking fifth in popularity [1]. Traditionally, beer production relies on four primary constituents: malt, hops, yeast, and water. These elements not only contribute to the flavor and aroma profile of beer but also render it a potentially healthful beverage due to the presence of various beneficial compounds such as antioxidants, certain minerals, select vitamins, fiber, and low levels of ethanol [2] Beer derived from traditional ingredients exhibits antioxidant properties owing to its phenolic composition, sourced predominantly from malt (70 %) and hops (30 %) [3]. Consequently, polyphenols, known for their stability, are present in varied concentrations across different beer types [4]. Responding to heightened consumer interest, the brewing industry has invested considerable research endeavors into developing innovative technologies to expand the spectrum of specialty beers, including functional variants [5]. This pursuit involves incorporating diverse therapeutic and aromatic plants into brewing, offering a plethora of options. Achieving a harmonious blend of maximal functional attributes and pleasing sensory qualities hinges upon selecting the optimal plant composition [6]. Grapes emerge as phenol-rich botanicals, with grape seeds containing the highest extractable phenolic content at 70 %, followed by grape skins at 28 – 35 % [7]. Scientific studies underscore various pharmacological effects of grape seeds, spanning antioxidative, anti-inflammatory, anticancer, neuroprotective, lipid-lowering, bacteriostatic, and other properties [8–10]. Moreover, the application of grape seed extract in the food industry holds significant promise as food additives and preservatives [11–13]. Winemaking grape pomace also represents a valuable reservoir of nutrients, notably polyphenols [14]. The management of winemaking by-products is crucial [15], due to concerns regarding environmental impact, with winery residues deemed potential resources for valorization and the production of value-added goods [16]. A ton of winemaking pomace typically comprises 225 kg of grape seeds, 425 kg of grape skins, and 229 kg of stalks [17]. The principal objective of this research endeavor was to enhance the polyphenolic attributes of beer, introduce novel taste profiles, and advocate for environmental sustainability.

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2 Materials and methods

2.1 Brewing materials

For the study, Sebastian variety pilsner-type barley malt, hop pellets of Magnum variety (type 45), and the cultural yeast *Saccharomyces pastorianus* ssp. *carsbergenisis* strain W 34/70 were used.

The grape seed and skin powder used in beer production were sourced from Georgia's most prominent grape varieties, namely Rkatsiteli white and Saperavi red grapes, harvested in September 2022. The powder was produced from grape pomace by EcoGeorgianGroup LLC, situated in Velistsikhe village, Kakheti Region, Georgia. White grape pomace was obtained directly from wineries after pressing, while red grape pomace underwent a 7-day fermentation period. Subsequently, both types of pomaces were separated and milled to achieve a powder with a particle size of 100 microns.

2.2 Experimental Design:

2.2.1 Laboratory condition samples

Beer samples were acquired from wort obtained in commercial production at JSC „Georgian beer company" -Brewery Zedazeni, located in Georgia, Mtskheta region, village Saguramo.

Analysis conditions involved the addition of 2 grams of centrifuged yeast to 500 ml of wort. One sample served as the blank, while the other was supplemented with white grape seed and skin powder in different dosages such as 0.60 %, 0.76 %, and 1.20 %. In certain trials, grape seeds were introduced to cold wort without any heating, while in others, they were added to boiling wort and allowed to boil for 5 minutes. Following the addition of grape seeds, the samples were subjected to 48 hours of shaking in a shaker with orbital motion to maintain fermentation processes. Shaking the samples supports fermentation, as in the pilot brewhouse, they are held in fermenters.

2.2.2 Beer samples brewed in small-scale brewhouse.

Beer samples were brewed using a 100-liter small-scale pilot brewhouse manufactured by Shandong ZhongPi Machinery Equipment Co. Ltd. All essential procedures for pilot brewing, from milling to fermentation and maturation, were conducted at Zedazeni Brewery - JSC "Georgian Beer Company". The trial brewhouse comprises equipment such as a two-roller miller, mashing vessel, lauter tank, boiling tank, and whirlpool, with a working capacity of 100 liters for wort and fermentation tanks of the same volume. The beer was produced with a 0.6 % addition of grape seeds at various stages of brewing – during mashing, beginning of boiling, and fermentation – with different dosages. For brewing 100 liters of wort, 17 kg malt, 70-liter water, and 600-gram liquid yeast were used. The mashing regimes were selected based on their alignment with commercial brewing practices, as shown in figure 1

The sequence of processes	Duration(min.)
Mashing in 52 °C	25
Heating up to 62 °C and rest	35
Heating up to 65 °C and rest	30
Heating up to 72 °C and rest	35
Heating up to 78 °C and transfer to Lauter tun	
Filtration (Lauter tun)	
Boiling	40
Whirlpooling	20
Cooling at 12 °C	

Fig. 1 Steps of the brewing process

Fermentation in the trail brewhouse was done in 100 l working capacity CCT (cylindrical-conical tank) at a temperature of 15 °C. Maturation was achieved within two weeks at 0 °C.

For all analyses beer samples were obtained directly from fermenters. Only to assess shelf life, beer samples before analysis were manually filtered using fine filter aids, specifically Celatom FP2.

2.2.3 Analysis methods

All analyses were done in triplicate.

1. Total polyphenols analyses were done according to Analytica-EBC, Method 9.11 [18] which involves a reaction between polyphenols and iron (III) in an alkaline environment, resulting in colored complexes, notably a distinctive brown coloration. These complexes are quantified using a spectrophotometer, specifically the Hach Lange DR 5000 model. The reagents utilized in this method are carboxymethylcellulose-ethylenediaminetetraacetic acid (CMC-EDTA-Na) and ammonium iron (III) citrate (3.5 %), supplied by Merck. For the analyses, decarbonized samples were used. Results expressed in mg\L, measurements were done with a rectangular cuvette at 600 nm wavelength against the blank sample, blank sample consisted of the identical solution as the tested sample, with the exception that distilled water was substituted for ammonium iron.

2. MEBAK method 2.18.1 [19] for bitter unit measurement was used. The bitter substances, mainly iso-a-acids, are extracted from the acidified sample with iso-octane, and the concentration in the extract was determined with a spectrophotometer Hach Lange DR 5000. The sample preparation method involved utilizing a mechanical shaker and centrifuge to enable efficient phase separation, operating at a speed of 3000 rpm. The procedure utilized Hydrochloric acid with a concentration of 6N and spectroscopically pure iso-octane chemicals sourced from Merck, measurements were conducted against water at a wavelength of 275 nm.

3. Beer color analysis was performed using a spectrophotometer, Hach Lange DR 5000, with a 10 mm length rectangular cuvette at a wavelength of 430 nm. The analysis was conducted according to Analytica-EBC Method 9.6 [18].

4. Beer pH analyses were conducted using a Sartorius pH meter by EBC method 9.35. [18]

5. The alcohol content, apparent extract, real extract, fermentation degree, and original extract analyses were conducted using an Anton Paar DMA 4501 and Alcolyser 3001. These analyses were performed according to the methods outlined in the EBC standard chapters 8.2.2, 9.43.2, and 9.2.6 [18].

6. The following analyses: concentrations of Catechin, Epicatechin, Epigallocatechin, Epigallocatechin gallate (HPLC), Resveratrol LC/MS, and Proanthocyanidins (Bate Smith Reaction) were conducted by an external laboratory, Laboratoire EXCELL, located in Floriac, France.

7. The sensory analysis was conducted by a trained panel of 7 beer experts who evaluated various sensory attributes including aroma, taste, drinkability, body, mouthfeel, bitterness, and acid-

Table 1 Chemical Parameters of Laboratory Beer Samples

parameters	units	0.6 % GSB (C)	0.76 % GSB (C)	0.6 % GSB (H)	0.76 % GSB (H)	CB
Alcohol	% v/v	5.4 ± 0.05	5.52 ± 0.04	5.6 ± 0.04	5.5 ± 0.08	5.29 ± 0.05
Real Extract	% w/w	4.08 ± 0.06	4.07 ± 0.06	4.22 ± 0.07	4.12 ± 0.08	4.2 ± 0.07
Apparent Extract	% w/w	2.13 ± 0.04	2.07 ± 0.05	2.19 ± 0.06	2.15 ±0.05	2.25 ± 0.07
Original Extract	° Plato	12.26 ± 0.07	12.45 ± 0.06	12.53 ± 0.08	12.41± 0.08	12.37 ±0.06
Fer. Deg.	% w/w	82.59 ± 0.4	83.33 ± 0.5	82.79 ± 0.5	82.66 ± 0.6	81.77 ± 0.6
Color	EBC	5.87 ± 0.5	6.12 ± 0.4	8.34 ± 0.4	7.76 ± 0.3	7± 0.6
Bitterness	BU	12 ± 0.8	12.1 ± 0.7	15 ± 0.8	11.5 ± 0.7	20.5 ± 0.8
Polyphenols	mg/l	385 ± 17	420 ± 11	510 ± 15	530 ± 13	250 ± 18

0.6 % GSB/0.76 % GSB (Grape seed beer) beer obtained with addition of 0.6 %/0.76 % grape seed (C) – grape seed dissolving in cold wort, (H) – grape seed dissolving in hot wort, CB-control beer, beer without grape seed addition

ity. The evaluation involved grading taste, aroma, and others on a scale from 0 (unpleasant) to 6 (very pleasant). the body was also assessed using the same principle, with a score of 0 representing a thin body and 6 indicating full-bodied characteristics. Likewise, for mouthfeel, a score of 6 denoted a full mouthfeel. The sample preparation and processing were conducted by established sensory methods [20].

8. The antioxidant capacity was determined following the procedure described by Benzie et al. (1996) [21] with modifications. The FRAP reagent was freshly prepared by adding 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) (dissolved in 40 mM of Hydrochloric acid), 20 mM of Iron (III) chloride in water, and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. The FRAP reagent was warmed to 37 °C for 15 min. Then, 100 mL of the sample was added to a 3.0 mL reagent blank. The absorbance was recorded at 593 nm. The reaction was monitored for 4 min. FRAP values of samples were compared to that of ascorbic acid and expressed as mg of ascorbic acid equivalents (AAE) per 1 L of beer samples.

9. Evaluation of the inclination towards colloidal stability of beer. Chill haze assessments were performed using a tannometer device, using 95 – 96 % ethyl alcohol, following MEBAK-method 2.19.2.3 [19], the temperature of the beer was decreased to – 8 °C. Also, beer underwent an aging treatment involving alternating heat and chill cycles at 60 °C for 24 hours followed by 0 °C for 24 hours, as per Analytica-EBC method 9.30. [18]. The stability and quality retention of the samples were assessed using the Forcing Tester - Julabo and the Hazy Meter Vos-rota. This experimental setup investigated the influence of temperature fluctuations on the beer's stability over time.

3 Results and Discussion

The study encompassed experimental trials to assess the influence of altering quantities of grape seeds and their introduction at different stages of the brewing process. Additionally, it aimed to compare the effects of grape seeds and skins on the levels of polyphenols and phenolic compounds in beers brewed using seeds from different grape varieties. The taste characteristics of the beers brewed in a pilot brewery were also evaluated as part of the research.

3.1 Comparison of Laboratory Analysis Samples

Initially, laboratory analyses revealed that adding white grape seeds led to an increase in polyphenols in beer samples with 0.6 % and 0.76 % grape seed content. The rise in polyphenol levels stemmed from the incorporation of grape seed into the cold wort. Specifically, beer samples containing 0.6 % grape seed content yielded a polyphenol concentration of 387 mg/l, while those with 0.76 % content showed a concentration of 420 mg/l. These values surpass the polyphenol content of the control beer without any additives, which stood at 250 mg/l. However, they are lower than the polyphenol concentrations observed in beer produced with grape seed addition in hot wort. Notably, the addition of grape seeds during the boiling of wort resulted in a substantial increase in polyphenols content up to 510 – 530 mg/l compared with blank samples of 250 mg/l, during the boiling process. The addition of grape seeds in hot conditions resulted in a slight increase in color intensity. The data presented in table 1 illustrates the impact of grape seed addition on beer parameters. Additionally, grape seed addition was found to decrease bitterness level, probably due to that an increase in polyphenols might lead to more protein precipitation, which in turn could result in the removal of iso-alpha acids during the process. Polyphenols level was expected to increase. While there's limited research specifically delving into the use of winemaking byproducts in beer production [22–24] means there's a shortage of studies confirming the influence of grape materials on the increase of polyphenols in beer [25], but numerous studies have showcased the enhancement of polyphenol and antioxidant properties in food through the use of grape byproducts [26,27]

Further trials in laboratory conditions were conducted using grape seed and grape skin powders with 0.6 % and 1.2 % content added to the wort, both in powdered form with particles of uniform size. As previously mentioned, the process involved adding the powder to the cold wort and comparing it with a dissolving powder in the hot wort during boiling. At a concentration of 0.6 %, polyphenol levels reached 368 mg/l, while at 1.2 %, a higher concentration of 498 mg/l was observed. When grape skins were used, polyphenol levels were comparatively lower: 266 mg/l and 298 mg/l for 0.6 % and 1.2 %, respectively. Similar trends were observed in hot wort, where polyphenol concentrations substantially increased with 1.2 % grape seed addition, reaching 582 mg/l compared to the grape skin effect - 316 mg/l. Concurrently, the polyphenol content in beer

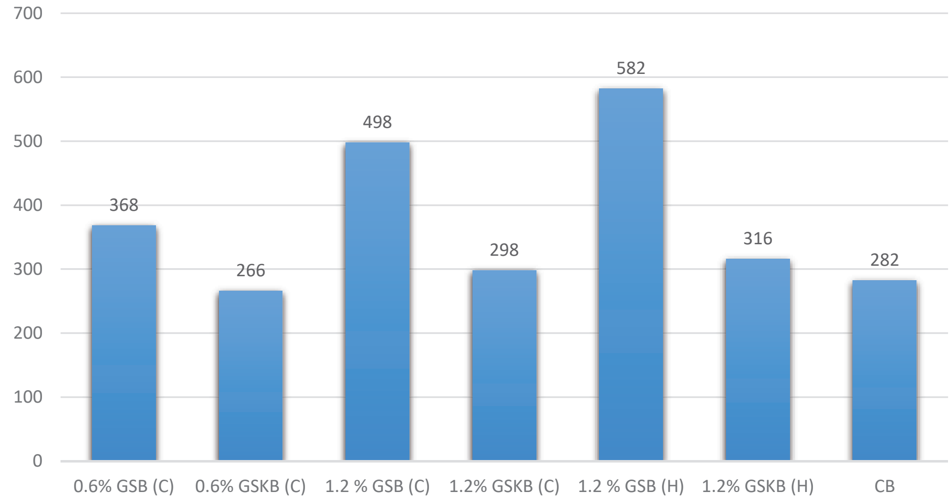

Fig. 2 Comparative polyphenol analysis: grape seed versus grape skin addition (0.6 % / 1.2 % GSB – Grape seed added beer, 0.6 % / 1.2 % GSKB – grape skin added beer, (C)–addition in cold wort, (H) – addition in hot wort, CB – Control beer without grape materials)

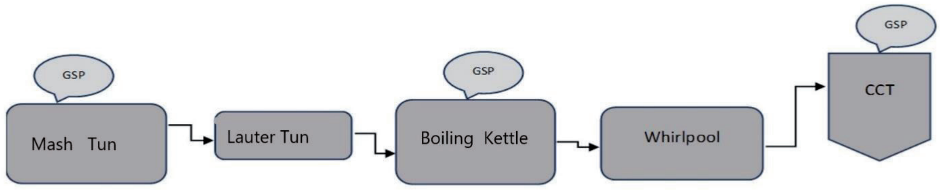

Fig. 3 Grape Seed Incorporation Steps

without additives resulted in a polyphenol concentration of 282 mg/l. Results are shown in figure 2, this finding is consistent with expectations based on previous studies comparing the polyphenolic content of grape skin and seed extracts. These studies have demonstrated that grape seeds are much richer in polyphenolic content compared to grape skins. [28, 29]. Consequently, all subsequent analyses in the pilot brewhouse utilized grape seed powder.

3.2 Comparison of Pilot Brewhouse Samples

In the pilot brewhouse trials, grape seeds were added at the beginning of different stages: mashing, boiling, and fermentation, as shown in figure 3.

The most significant results were observed when white grape seeds were added during boiling. Seeds added during mashing likely became lost in the filtration process, resulting in a lesser impact on polyphenol levels (207 mg/l). Introducing grape seed powder at the initiation of fermentation in the CCT (cylindrical-conical tank) demonstrated a marginal increase in polyphenol content (292 mg/l) compared to adding it during mashing. However, the polyphenol levels were still lower than those achieved through addition in hot conditions. The data presented in table 2 supports the conclusion that hot brewing conditions are more effective at extracting polyphenols. Specifically, when grape seeds were added at the beginning of boiling, it resulted in the highest total polyphenol concentration (347 mg/l), which is consistent with previous laboratory findings [30]. Notably, the addition of grape seeds had minimal impact on color parameters, while bitterness decreased as observed in the earlier analysis.

3.3 Analytical Results from Outsourced Laboratory

Beer samples collected from a pilot brewhouse, where 0.6 % white and red grape seeds were incorporated into the brewing process, in particular, at the beginning of boiling, were subjected to analysis for various polyphenolic compounds. Catechin, epicatechin, epigallocatechin, epigallocatechin gallate, resveratrol, and proanthocyanidins were among the compounds investigated. These analyses were conducted in an external laboratory.

Samples enriched with grape seeds, notably those derived from the white grape variety Rkatsiteli obtained immediately after the pressing of grapes in the winery, displayed elevated levels of specific compounds (Epicatechins – 11.02 mg/l, catechin 4.32 mg/l) compared to regular beer (lees then 1 mg/l) even beer with red grape seeds variety Saperavi subjected to a 7-day fermentation process in the winery exhibited heightened levels of epicatechins

Table 2 Chemical Parameters of Pilot Brewhouse Beer Samples

parameters	units	0.6 % GSB – MT	0.6 % GSB – BK	0.6 % GSB – CCT	CB
Alcohol	% v/v	4.63 ± 0.06	4.57 ± 0.03	4.45 ± 0.05	4.68 ± 0.05
Real Extract	% w/w	3.9 ± 0.05	3.8 ± 0.05	3.75 ± 0.05	4.12 ± 0.04
Apparent Extract	% w/w	2.21 ± 0.04	2.27 ± 0.06	2.21 ± 0.04	2.42 ± 0.05
Original Extract	° Plato	10.97 ± 0.08	10.8 ± 0.05	10.5 ± 0.03	11.27 ± 0.06
Fer. Deg.	% w/w	79.88 ± 0.3	79 ± 0.5	79.52 ± 0.4	78.52 ± 0.5
pH		4.69 ± 0.07	4.55 ± 0.05	4.31±0.08	4.67 ± 0.08
Color	EBC	7.12 ± 0.4	7.5 ± 0.4	6.76 ± 0.3	5.75 ± 0.5
Bitterness	BU	13.1 ± 0.7	7.85 ± 0.8	7.28 ± 0.9	17.7 ± 0.7
polyphenols	mg/l	207 ± 15	347 ± 18	292 ± 20	153 ± 13

0.6 % GBS – beer with 0.6 % grape seed added in mashing tun, GSB- BK – beer with 0.6 % grape seed added in boiling kettle, CCT – beer with 0.6 % grape seed added in cylindrical-conical tank, CB – Control beer – without grape seed

Table 3 Effect of Addition of Georgian white and red grape seeds on beer phenolic compounds

`parameters	units	0.6 % WGSB	0.6 % RGSB	CB
Resveratrol	mg/l	< LQ	< LQ	0.0003
Total anthocyanins	mg/l eq. malvidin	< LQ	< LQ	< LQ
Tannins proanthocyanidins	g/l	0.6	0.7	0.7
(–) Epicatechins	mg/l	11.02	9.63	0.97
(+) Catechin (catechin, epicatechin gallate, epicatechin, epigallocatechin gallate, epigallocatechin)	mg/l	4.32	6.95	< LQ
(–) epicatechin gallate	mg/l	ND	ND	ND
(–) epigallocatechin	mg/l	ND	ND	ND
(–) epigallocatechin gallate	mg/l	ND	ND	ND

0.6 % WGSB – 0.6 % white grape seed beer, 0.6 % RGSB – 0.6 % red grape seed beer, CB – beer without grape seed

(9.63 mg/l) and catechins (6.75 mg/l), table 3 shows the results. The presence of phenolic groups, including catechin [30], in food and beverage products can provide various health benefits [32, 33]. Resveratrol content was not detected, as expected, given the typical phenolic profile found in Georgian grape varieties such as Rkatsiteli and Saperavi. This aligns with findings from previous studies indicating that the resveratrol content in both grape varieties is significantly lower compared to catechins. Consequently, the analytical focus shifted towards the anticipated detection of catechin and epicatechin, compounds known to be abundant in various world-renowned grape varieties, as supported by previous studies, increasing the seed content in brewing could indeed lead to the detection of resveratrol in beer, However, this alteration may have critical consequences for the beer's flavor profile [34–36].

3.4 Sensory evaluation

The overall rating for the beer supplemented with white grape seeds was higher compared to that with red grape seeds. It's worth noting that the red grape seeds were obtained after wine fermentation,

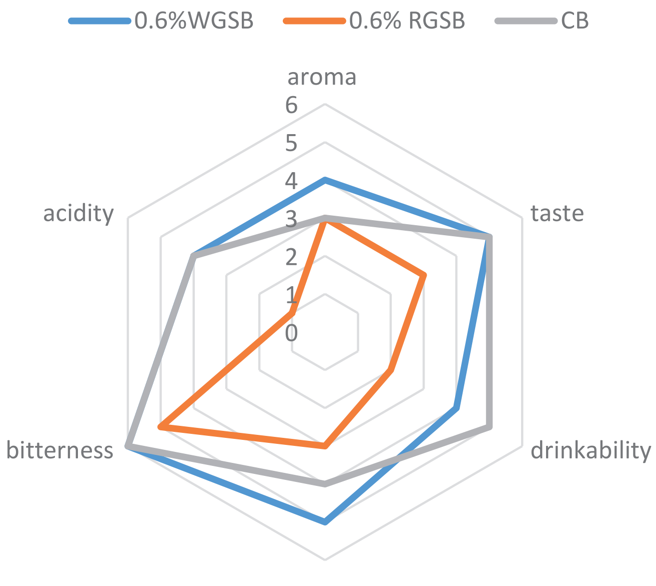

Fig. 4 Spider chart of the test panel (0.6 % WGSB – 0.6 % white grape seed added beer, 0.6 % RGSB – 0.6 % red grape seed added beer, CB – Control beer, without adjuncts)

approximately 7 days later, which likely contributed to sour notes in taste. Rkatsiteli grape pomace without fermentation directly after pressing, exhibited higher ratings in aroma, taste, drinkability, mouthfeel, and acidity compared to those supplemented with 0.6 % red grape seeds (0.6 % RGSB) and the control beer (B). Specifically, the white grape seed beer (0.6 % WGSB) had a superior aroma, taste, and drinkability, scoring 4, 5, and 4 respectively, compared to 3, 3, and 2 for the red grape seed beer (0.6 % RGSB) and 3, 5, and 5 for the control beer (B). Beer with white grape seed indicated slight, pleasant grape tones in the taste likely contributed to the overall flavor experience, adding depth and complexity. This highlights the potential for utilizing grape pomace. The findings from the tasting are illustrated in figure 4.

3.5 Antioxidant power

The FRAP assay was employed to assess the antioxidant capacity of beer, comparing samples without adjuncts to those with the addition of 0.6 % white grape seed during the boiling stage in the pilot brewhouse. The incorporation of grape seed led to increased antioxidant activity at 22.83 mg/l compared to the regular beer at 18.04 mg/l, aligning with expectations from prior studies showing higher antioxidant activity in beers with various adjuncts [37]. Furthermore, this outcome was anticipated due to the inherent antioxidant activity of grape seed [33].

3.6 Colloidal stability of beer

A comparative analysis was performed between the beer brewed with 0.6% white grape seeds and the beer brewed without any adjuncts. During the forced aging test, both beer samples yielded consistently similar results in every cycle, with a difference not exceeding 0.5 EBC. However, it's worth noting that the forced aging test may have been weakened due to the high initial haze in the samples, which could be attributed to the manual filtration of the beer. The addition of grape seeds increases the polyphenol content in beer, which could potentially have a negative effect on non-biological stability. However, the same addition of grape seeds also results in protein precipitation, which can be beneficial for tests like forced aging. This phenomenon is similar to observations where corn beer exhibits better colloidal stability than all-malt beer [38]. Therefore, while the increased polyphenol content from grape seeds might initially raise concerns about non-biological stability, the concurrent protein precipitation could counteract this effect and potentially improve overall stability.

4 Conclusion

The findings of this study underscore the difference between all-malt wort production and wort made with winemaking residuals. Specifically, the addition of grape seeds significantly increased polyphenol content in beer. The research indicates that the choice of grape varieties and the timing of adding seeds during brewing play crucial roles in shaping the polyphenol profile and sensory qualities of beer. Utilizing winemaking residuals in beer brewing not only adds unique flavors and aromas derived from grapes but also contributes to the sustainability of both the wine and beer industries. Beers with grape seed additions also exhibited elevated levels of specific polyphenolic compounds, particularly catechin and epicatechin, contributing to their antioxidant capacity. Interestingly, the addition of grape seeds did not negatively affect colloidal stability, suggesting potential improvements in beer stability despite increased polyphenol content. It minimizes waste and maximizes resource efficiency by transforming byproducts into valuable components of the brewing process. In summary, integrating winemaking residuals into beer production offers breweries opportunities to innovate, differentiate their products, and meet consumer demands for diverse and high-quality beverages.

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