

## EFFECT OF THREE QUINOA VARIETIES AND EDIBLE FLOWERS ON ANTIOXIDANT PROPERTIES OF ALE BEER

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Received 21 July 2023  
Accepted 31 August 2023

DOI: 10.59957/jctm.v59.i5.2024.15

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### ABSTRACT

Fermentation experiments were conducted with a top-fermenting brewing yeast strain and wort enriched with three varieties of quinoa (with white, red and black grain color), as well as edible flowers (marigold and dandelion) to study their influence on the antioxidant properties of ale beer. Plant additives led to a slight increase in pH and a lower alcohol content of the beer. By itself, quinoa did not improve the functional properties of the beverage, but the combination of black quinoa and edible flowers resulted in an increased content of polyphenols in beer; marigold showed a 48.7 % increase in this parameter compared to the control, and dandelion - 51.2 %. The content of flavonoids in beer was not affected by the addition of plant additives to the wort. A slight increase in anthocyanins (0.7 - 4.4 %) was reported for black quinoa beer as well as black quinoa with flowers.

A significant increase in antioxidant activity was reported for the variants with flowers (54 % for marigold and 56.6 % for dandelion). The increased antioxidant capacity combined with the reduced alcohol content of the beers with black quinoa and flowers suggests that a beverage with improved functional properties was obtained.

Black quinoa, combined with edible flowers, was more favorable for increasing the antioxidant capacity of beer than other pseudo-cereals (amaranth and chia).

Keywords: ale beer, antioxidant activity, quinoa, marigold, dandelion.

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### INTRODUCTION

Beer is the most popular low-alcohol beverage, and the brewing industry is an important global business with huge annual revenues [1]. Nowadays, the importance of the so-called craft breweries is increasing [2]. A huge variety of new beer varieties are developed, containing raw or malted cereals, with modified aroma and taste due to the enrichment of fruits, spices, etc. [3]. Besides barley [4] and other cereals (wheat, maize, rice, etc.) traditionally used as raw materials for brewing [5], there is currently a growing interest in the pseudo-cereals that are considered the “superfoods” of the 21st century [6].

Quinoa (*Chenopodium quinoa*) is a gluten-free crop [7]; compared to cereals, it has a higher protein, fat and fiber content [8]. Quinoa is one of the best vegetable sources of protein [9]; it is comparable to beef [10]. There is a good balance of amino acids and an excellent composition of essential amino acids; the amount of calcium, phosphorus, iron and B vitamins exceeds that of barley, rice, maize, wheat and oats [11, 12]. There are also polyphenols, phytosterols and flavonoids [11, 13],  $\omega$ -fatty acids [14, 15], etc. According to Asao and Watanabe [16], quinoa contains more phenolics than cereals. It is characterized by a high content of d-xylose and maltose, while the amount of glucose and fructose is low, suggesting that quinoa may be useful in

the preparation of malt beverages [17].

Quinoa is an excellent example of a functional food; it has a positive effect on health [13, 18]. There are about 250 varieties (seed color varies from white to black, with shades of yellow, pink, red, purple and violet) [9, 19]. The most widespread and mass cultivated are white, red and black quinoa. Tang et al. studied the content of substances with antioxidant activity (tocopherols, tocotrienols, carotenoids, fatty acids) in the three varieties; the amount of the listed components varied considerably, being higher in darker seeds [20].

Other plant additives suitable for putting into beer are edible flowers, e.g. - some members of Asteraceae. The positive effect of these plants on health is known; it is related to their antioxidant, anti-inflammatory, antimicrobial and prebiotic properties. The listed properties are a consequence of the bioactive components contained in the plants, incl. polyphenols, phenolic acids, flavonoids, triterpenes, polysaccharides, etc. [21]. Widespread and well-known representatives of the family are marigold (*Calendula officinalis*) and dandelion (*Taraxacum officinale*); in addition to cooking, they are used in perfumery, pharmacy and medicine.

The aim of the present study is to investigate how the enrichment of wort with quinoa (white, red and black varieties) and edible flowers (marigold and dandelion) affects the antioxidant properties of ale beer.

## EXPERIMENTAL

Experiments were conducted according to the methodology of the European Brewing Convention (EBC). The wort was obtained by the infusion method with a Bender & Hobein mash-apparatus. The control wort contained 100 % barley malt. In three of the variants, 20 % of the barley malt was replaced with unmalted quinoa (white, red and black grain varieties). According to literature data, black quinoa contains the highest number of substances with antioxidant activity [20]. Because of that, black quinoa was chosen as an ingredient for two additional variants: 20 % black quinoa and 8.5 g L<sup>-1</sup> edible flower (marigold and dandelion, respectively).

The hydromodulus of mixing was 1:3. There were 5 temperature pauses in the range from 40°C to 70°C for 120 minutes. The saccharification of the wort was checked using an iodine test. The wort was boiled

on a Kjeldahl apparatus (90 min). Hopping (90 mg L<sup>-1</sup>  $\alpha$ -acids) was carried out in two batches: I batch - bitter variety Galena - 70 %, added 10 min after the start of boiling; II batch - aromatic varieties Perla - 15 % and Hallertau Mittelfrueh - 15 %, added 30 min after the start of boiling. The flowers (as dry substances) were added 30 min before the end of boiling. After boiling, the hot sediments were removed, and the wort was cooled. The hopped wort was analyzed according to the following physico-chemical parameters: extract, pH, color, isohumulones, bitterness,  $\alpha$ -amino nitrogen, polyphenols, flavonoids [22].

The fermentation (duration of 7 days) was carried out in fermentation tubes with a volume of 0.5 L, at 15°C. A top-fermenting brewing yeast strain *Saccharomyces cerevisiae* Bry-97 West Coast Ale Yeast was used. The inoculation number of cells was 17 - 20.10<sup>6</sup> mL<sup>-1</sup>.

Young beer was examined for basic physico-chemical (initial, apparent and real extract, pH, alcohol,  $\alpha$ -amino nitrogen) [22] and microbiological parameters (culture uniformity, cell morphology, protoplasm condition, AP-test (fermentation activity), biomass increase, dead cells) [23]. The young beer was left for lagering (14 days at 4°C). Finished beer was examined for the following physico-chemical parameters: initial, apparent and real extract, apparent and real degree of fermentation, pH, alcohol, color, isohumulones, bitterness,  $\alpha$ -amino nitrogen, polyphenols, flavonoids, anthocyanins [22], radical scavenging capacity [24], and tasting evaluation.

## RESULTS AND DISCUSSION

Analyzes of the six wort variants were performed to determine how quinoa and edible flower enrichment affects physico-chemical parameters. The obtained results showed a change in some parameters for the wort with a changed composition compared to the classic wort containing 100 % barley malt.

All samples showed complete saccharification during mashing. The differences between the extracts of the wort variants were insignificant, which means that quinoa is a suitable substitute for barley malt. It was expected, as Kordialik-Bogacka et al. reported that unmalted quinoa is suitable for brewing; up to 30 % of barley malt can be replaced with quinoa [25].

pH also varied within narrow limits (it increased

Table 1. Physico-chemical parameters of hopped wort.

Parameters	100 % barley malt (control)	20 % white quinoa	20 % red quinoa	20 % black quinoa	20 % black quinoa with 8.5 g L <sup>-1</sup> marigold	20 % black quinoa with 8.5 g L <sup>-1</sup> dandelion
Extract, %	10.87	10.81	10.76	10.62	10.91	10.88
pH	5.41	5.43	5.45	5.53	5.50	5.52
Color, units EBC	14.2	15.1	15.3	15.5	26.5	28.8
Isohumulones, mg L <sup>-1</sup>	28.31	28.19	27.91	28.02	27.79	28.13
Bitterness, bitter units	29.9	29.8	29.55	29.65	29.45	29.75
α-amino nitrogen, mg L <sup>-1</sup>	180.91	163.72	153.4	147.5	155.13	158.47
Polyphenols, mg L <sup>-1</sup>	418.9 ± 2.5	323.2 ± 2.1	313.3 ± 1.9	309.2 ± 1.8	428.4 ± 2.9	450.5 ± 3.1
Flavonoids, mg L <sup>-1</sup>	25.8 ± 0.5	21.1 ± 0.3	19.2 ± 0.2	19.4 ± 0.2	19.1 ± 0.3	19.8 ± 0.1

Table 2. Physico-chemical and microbiological parameters of young beer.

Parameters	100 % barley malt (control)	20 % white quinoa	20 % red quinoa	20 % black quinoa	20 % black quinoa with 8.5 g L <sup>-1</sup> marigold	20 % black quinoa with 8.5 g L <sup>-1</sup> dandelion
Initial extract, %	10.65	10.48	10.54	10.41	10.57	10.29
Apparent extract, %	2.83	2.75	2.75	2.76	3.84	3.51
Real extract, %	4.32	4.22	4.22	4.21	5.12	4.80
Alcohol, %	3.24	3.21	3.23	3.17	2.79	2.81
pH	4.17	4.23	4.20	4.22	4.54	4.50
α-amino nitrogen - residual, mg L <sup>-1</sup>	71	45.43	44.8	44.5	68.62	60.7
- assimilated, %	60.75	72.25	70.80	69.83	55.77	61.70
AP-test (fermentation activity)	2.72	2.63	2.65	2.67	2.68	2.70
Biomass increase, times fold	2.56	2.41	2.4	2.42	2.17	2.21
Dead cells, %	9	10	12	13	10	11

slightly with quinoa and flower enrichment). Expectedly, a noticeable color change (toward a darker shade) occurred in variants with added flowers, as these plants contain dyes [26, 27]. The wort variants with altered composition did not differ from the control in their bitterness. No increased content of α-amino nitrogen and flavonoids was observed for the variants with plant additives, but worts containing flowers showed an increased content of polyphenols compared to the control.

After the end of the main fermentation, analyzes of the obtained young beer variants were carried out. The results are summarized in Table 2.

Apparent extract at the end of fermentation was identical for the three quinoa samples (2.75 - 2.76 %). For the samples with black quinoa and flowers, the apparent extract was higher (a greater part of the initial extract remained unfermented). The same trend was observed for the real extract.

The alcohol content of the young beer was highest for the control (3.24 %); a slight decrease was observed for the quinoa variants. For the beers with flowers, probably due to the incomplete fermentation of the wort extract, the alcohol content was lower (2.79 - 2.81 %).

pH of the young beer was lowest for the control. For the three quinoa variants, slight alkalinization

(4.20 - 4.23) was observed. Adding edible flowers to the wort resulted in a further increase in pH (about 4.50).

Assimilation of  $\alpha$ -amino nitrogen by yeasts was facilitated by the presence of quinoa in the wort. About 2/3 of the initial amount of  $\alpha$ -amino nitrogen was consumed by the yeasts during the fermentation with wort containing white, red and black quinoa. For the other three variants, the values were slightly lower; the most significant part of  $\alpha$ -amino nitrogen (about 1/2 of the initial amount) remained unabsorbed in the beer containing black quinoa and marigold.

The AP-test results varied within narrow limits (2.63 - 2.72) and proved the good fermentation ability of the yeast strain Bry-97 West Coast Ale Yeast. Similar results were obtained in previous fermentations with the same strain [28, 29].

For all samples, the biomass increase during the main fermentation was more than 2 times fold, being the highest for the control (2.56 times fold); for the

variants with plant additives, the values were slightly lower. For the three beers with quinoa (white, black and red) almost no difference was observed in this parameter (the biomass increase was about 2.4 times fold). A slight decrease was reported for the variants with edible flowers, for which there was also a more incomplete fermentation compared to the control. The yeast culture (observed under a microscope) was uniform; the cells were large, rounded, with clear protoplasm.

The yeast viability is one of the most important factors for manufacturing alcoholic beverages [30]. In our case, the content of dead cells at the end of main fermentation varied from 9 to 13 % (the yeasts showed good survival rate and immediately after the end of the process could be used as a second generation inoculum for subsequent fermentation).

The results of the analyzes of the finished beer are summarized in Table 3.

Table 3. Physico-chemical parameters of finished beer.

Parameters	100 % barley malt (control)	20 % white quinoa	20 % red quinoa	20 % black quinoa	20 % black quinoa with 8.5 g L <sup>-1</sup> marigold	20 % black quinoa with 8.5 g L <sup>-1</sup> dandelion
Initial extract, %	10.73	10.63	10.62	10.48	10.75	10.49
Apparent extract, %	2.75	2.68	2.69	2.75	3.61	3.42
Real extract, %	4.26	4.19	4.20	4.21	4.96	4.76
Alcohol, %	3.31	3.29	3.28	3.20	2.96	2.93
Apparent degree of fermentation, %	74.38	74.74	74.59	73.77	66.41	67.38
Real degree of fermentation, %	60.28	60.54	60.42	59.78	53.81	54.60
pH	4.20	4.29	4.23	4.26	4.65	4.62
Color, units EBC	8.2	9.35	9.47	9.81	21.63	22.56
Isohumulones, mg L <sup>-1</sup>	19.27	19.38	18.98	19.09	19.35	19.15
Bitterness, bitter units	22	22.1	21.75	21.85	22.05	21.9
Usability of bitter substances, %	24.4	24.6	24.2	24.3	24.5	24.3
$\alpha$ -amino nitrogen - residual, mg L <sup>-1</sup>	68.6	43.2	42.4	42.1	66.1	58.7
- assimilated, %	62	73.6	72.36	71.46	57.39	62.96
Polyphenols, mg L <sup>-1</sup>	254.1 ± 1.9	246.7 ± 1.8	244 ± 1.8	243.8 ± 1.6	377.7 ± 2.5	384.3 ± 2.8
Flavonoids, mg L <sup>-1</sup>	23.3 ± 0.5	20.2 ± 0.2	18.4 ± 0.1	18.6 ± 0.2	18.1 ± 0.3	18.3 ± 0.3
Anthocyanins, mg L <sup>-1</sup>	89.6 ± 1.4	89.3 ± 1.3	89.1 ± 1.5	90.2 ± 1.3	91.3 ± 1.2	93.5 ± 1.4
Antioxidant activity, equ. vit. C mmol L <sup>-1</sup>	1492.15	1484.18	1476.21	1468.23	2297.41	2337.28

The apparent extract decreased slightly after lagering. The lowest apparent extract was reported for beers with 20 % white quinoa and 20 % red quinoa (the apparent degree of fermentation was the highest for them, i.e. - the most complete fermentation of the initial extract was achieved). A slower fermentation was observed for the flower samples - their apparent extracts were the highest, and the achieved apparent degree of fermentation was 66 - 67 %.

Deželak et al. reported lower real fermentability of a beer-like quinoa beverage compared to a barley beverage [31]. In our case, probably due to the replacement of only 20 % of the barley malt with quinoa, there was not much difference in the real degree of fermentation values for the control and the three quinoa samples.

The alcohol content of the finished beer increased slightly after lagering. The highest alcohol content was recorded for the control (3.31 %); values for the quinoa beers were slightly lower. Similar results have been obtained by other researchers; according to Cela et al., there was a decrease in alcohol content of beer with 40 % quinoa compared to the classic version (100 % barley malt) [32]. As expected, given the incomplete fermentation, the alcohol content of the samples with added flowers was the lowest (below 3 %).

Lagering resulted in a slight increase of pH (due to the yeast lysis). The lowest remained the control value (4.20), and the highest values were for the beers with flowers (above 4.60). A similar trend has been observed by other authors, e.g. Yao et al. reported an increased pH value of craft beer with dandelion compared to a blank sample [33].

Regarding the beer color, the trend observed at the wort stage was maintained - the lightest was the control (8.2 units EBC). The replacement of part of the barley malt with quinoa resulted in slight darkening. Cela et al. reported a lighter color of beer with quinoa compared to the classic version, but this may be due to some specific features of the brewing process (used raw materials and their concentration in the wort, duration of boiling, etc.) [32]. Considering the color of the wort variants (Table 1), beers with flowers were, as expected, significantly darker than the control.

The amount of assimilated  $\alpha$ -amino nitrogen increased slightly after lagering. The best utilization of  $\alpha$ -amino nitrogen was found in the presence of quinoa

in the wort - for all three variants the value was over 70 %, i.e. - about 2/3 of the initial amount of nitrogen was assimilated by the cells. A similar trend (better assimilation of  $\alpha$ -amino nitrogen by yeast during fermentation of wort with altered composition) was also observed in our previous research with another pseudo-cereal (beer with 20 % amaranth) [28]. Adding flowers to the wort made nitrogen assimilation more difficult. While for dandelion the value was comparable to the control one, for marigold it was lower (this sample also recorded the slowest fermentation).

The content of isohumulones did not show significant differences in the six variants, i.e. - the addition of quinoa and flowers to the wort had no effect on this parameter. The usability of bitter substances was about 24 % for all samples. However, all beers containing quinoa and flowers showed an increased bitter taste, possibly due to saponins in the quinoa seeds pericarp [34, 35] and some bitter substances contained in the flowers (eg sesquiterpene lactones) [21].

The content of polyphenols in the control was 254 mg L<sup>-1</sup>. The replacement of part of the barley malt with quinoa did not lead to an increase in this parameter (for all three beers with quinoa the values were lower than the control). A possible reason is that raw quinoa was used; Carciochi et al. studied the polyphenolic content in raw and malted quinoa extracts and stated that it increased after malting process [36]. A significant increase in polyphenols was reported for the samples with black quinoa and flowers - 1.49 times for marigold and 1.51 times for dandelion. An increased polyphenolic content of beer with dandelion has also been observed by other researchers [33].

The enrichment of the wort with plant additives did not contribute to the enrichment of the final product with flavonoids. Again, this probably has to do with using unmalted quinoa (the flavonoid content of quinoa increases after malting process [36]). Although marigold and dandelion contain flavonoids [37, 38] and there are reports of increased flavonoid content in beer with flowers [33], in our case it was not found. Similarly, we did not register an increase in this parameter in previous research with the same flowers and other pseudo-cereals [28, 29].

The number of anthocyanins in the control was 89.6 mg L<sup>-1</sup>. The presence of white and red quinoa in the beer did not increase this parameter. A slight increase

was recorded for the three other samples (with black quinoa and flowers). This probably has to do with the fact that anthocyanins are present in the colored quinoa seeds [39, 40], as well as in the flowers [41, 42].

Durga Prasad et al. reported for enhanced antioxidant activity of quinoa-based beer fermented with *Pichia myanmarensis* [43]. In our case, a traditional brewing yeast strain (*Saccharomyces cerevisiae*) was used; the enrichment of wort with quinoa (regardless of the grain color) did not lead to increased antioxidant activity of the beverage. However, for beers containing a combination of black quinoa and flowers, there was a significant increase in this parameter compared to the control. The antioxidant activity of the beer with marigold showed 1.54 times increase compared to the control, and with dandelion - 1.57 times. This was expected given the antioxidant properties of the plants of the Asteraceae family [21] and confirmed earlier reports that dandelion craft beer had stronger antioxidant activity than commercial beer [33].

The increased antioxidant capacity combined with the reduced alcohol content of the beers with black quinoa and flowers suggests that a beverage with improved functional properties was obtained.

Our previous research conducted with other pseudo-cereals (amaranth [28] and chia [29]) showed similar results. A significant increase was reported for the polyphenols content and antioxidant activity of the beers. By themselves, pseudo-cereals did not contribute to improving these parameters, but the combination of pseudo-cereals and edible flowers was beneficial for better functional properties of beer. The most significant increase of polyphenols and antioxidant activity was reported when the flowers were combined with black quinoa.

Tasting evaluation of beers with quinoa and edible flowers: the control had good clarity; the other variants were slightly cloudy (due to replacing part of the barley malt with quinoa). The beers were light (a darker color was observed only when flowers were added). All samples containing plant additives had enhanced bitterness unrelated to isohumulones. Beers with flowers had a distinct grassy aroma. Kordialik-Bogacka et al. also reported that unmalted quinoa did not give unpleasant characteristics to the beer [25].

## CONCLUSIONS

Plant additives led to a slight increase in pH and a lower alcohol content of the beer. By itself, quinoa did not improve the functional properties of the beverage, but the combination of black quinoa and edible flowers resulted in an increased content of polyphenols in beer; marigold showed a 48.7 % increase in this parameter compared to the control, and dandelion - 51.2 %. The content of flavonoids in beer was not affected by the addition of plant additives to the wort. A slight increase in anthocyanins (0.7 - 4.4 %) was reported for black quinoa beer as well as black quinoa with flowers. A significant increase in antioxidant activity was reported for the variants with flowers (54 % for marigold and 56.6 % for dandelion). Black quinoa, combined with edible flowers, was more favorable for increasing the antioxidant capacity of beer than other pseudo-cereals (amaranth and chia).

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