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EFFECT OF OZONE GAS ON SELECTED MICROBIOLOGICAL, CHEMICAL, ELECTRICAL AND ORGANOLEPTIC PROPERTIES OF CRAFT WHEAT BEERS ENRICHED JAPANESE QUINCE FRUIT (*Chaenomeles* L.)

– Research paper –

Józef GORZELANY[1], Miłosz ZARDZEWIAŁY[1], Pavol FINDURA[2], Ireneusz KAPUSTA[3], Katarzyna PENTOŚ[4,*], Justyna BELCAR[1,5,*]

[1]*Department of Food and Agriculture Production Engineering, University of Rzeszow, 4 Zelwerowicza Street, 35-601 Rzeszów, Poland*
[2] *Department of Machines and Production Biosystems, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovakia*
[3]*Department of Food Technology and Human Nutrition, University of Rzeszow, Zelwerowicza 4 St., 35-601 Rzeszów, Poland*
[4]*Institute of Agricultural Engineering, Wroclaw University of Environmental and Life Sciences, 37b Chelmonskiego Street, 51-630 Wroclaw, Poland*
[5]*Doctoral School of the University of Rzeszów, University of Rzeszów, st Rejtana 16C, 35-959 Rzeszów; Poland*

Abstract: Japanese quince fruits (*Chaenomeles* L.) are a valuable source of bioactive compounds, including polyphenolic compounds, vitamins, macro- and microelements. By using Japanese quince fruit, you can fortify wheat beers and influence the qualitative and sensory values as well as the content of bioactive compounds in the finished product. Sensory evaluation showed that wheat beers with the addition of nonozonated Japanese quince fruits were characterized by a balanced taste and aroma (overall impression), but all wheat beers obtained were characterized by high sensory values, and the ozonation process had a positive effect on the microbiological stability of both fruits and beers with the addition of these fruit. Physico-chemical analysis of wheat beers showed that beers enriched with Japanese quince fruit were characterized by high total acidity, respectively: average values of 4.95 (fruit-free) and 6.66 (0.1 M NaOH/100 mL), higher energy value, and, especially beers enriched with ozonated Japanese quince fruit and high total polyphenol content. It was shown that wheat beers enriched with quince fruit contained mainly chlorogenic acid at an average level of 4.35 and 5.42 mg/L (for ozonated and nonozonated fruit, respectively). Electrical properties (impedance and capacity) allowed the differentiation of the analyzed wheat beers. Based on the conducted research, it appears that wheat beers enriched with Japanese quince fruit may constitute a new trend in the brewing industry.

Keywords: ozone, Japanese quince fruit, microbiological stability, beer quality, polyphenols, impedance, organoleptic analysis

INTRODUCTION

The Japanese quince (*Chaenorneles*) is included in the rose family (*Roseacae*) and the apple subfamily (*Pornoideae*) (Rumpunen, 2002; Zhang and Li, 2009). Japanese quince plants were imported to European countries from Japan in the second half of the 18th century. The Japanese quince was initially considered an ornamental plant. When its fruits were found to be edible, it became a plant cultivated for culinary purposes (Zhang et al.., 2019). Japanese quince is a mostly thorny shrub, very spreading with a height of up to 1.2 m (Byczkiewicz et al., 2019). Japanese quince plants are recommended for cultivation in organic systems because they have low soil and climate requirements and high disease and pest resistance (Tarko et al., 2010).

The cultivation of Japanese quince is also supported by the rich chemical composition of the fruit, which contains nutritional and bioactive compounds that include pectin, vitamin C, catechin, epicatechin and triterpenes. Japanese quince fruits are characterised by a high vitamin C content of 55-92 mg·100 g^{-1},

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[*] Corresponding authors.
E-Mail addresses: katarzyna.pentos@upwr.edu.pl
justyna.belcar@op.pl

which is comparable to citrus fruits. Furthermore, the low acidity of the fruit and the high amount of polyphenolic compounds (about 20 have been isolated) support the use of Japanese quince fruit for pharmaceutical and food purposes (Du et al., 2013; Nahorska et al., 2014). In addition, Japanese quince seeds contain saturated and unsaturated omega 3, 6 and 9 fatty acids and sterols. Freeze-dried Japanese quince fruits are characterised by high antioxidant activity (Zhang et al., 2018).

Beer has been brewed for thousands of years. It is a beverage whose production has changed over the centuries (Ritter et al., 2017). Beer as the third most popular beverage after water and tea; has a huge demand in markets around the world (Baigts-Allende et al., 2021). As a result of changing consumer demands, breweries are modifying their beer production processes (Dugulin et al., 2020). Currently, we can distinguish two main directions of beer production. The first is the production of functional beers, such as low-calorie, low-alcohol and non-alcoholic beers (Martinez et al., 2017). The second is the production of beer with special aroma and flavour, which is produced by the addition of fruits, herbs and spices. Fruity beers are now readily available and have a wide audience, and are therefore favoured by producers and occupy a significant portion of the market (Zhao et al., 2023).

There are many factors that influence the content of bioactive compounds in plant raw materials. Abiotic and biotic environmental factors such as growing position, lighting, temperature, drought, soil salinity and pathogens significantly modify the chemical composition of the grown fruit (Cirak and Radusiene, 2019). One abiotic factor strongly affecting fruit quality is ozone gas. Ozonation is a non-thermal technology and leaves no residues in the products (Cao et al., 2022). Gaseous ozone has a positive effect on the organic matter of fruit and vegetables (Xu et al., 2019), with the ability to oxidise proteins, lipids, enzymes, nucleic acids, cell membranes and other cell components. Appropriate ozonation parameters cause physiological changes in fruits and vegetables (Pinto et al., 2020). Studies have shown that ozonated water extends the storage life of fresh celery, improving its sensory quality compared to the control sample (Zhang et al., 2005). It has been reported that ozone is a disinfectant and, when supported by ultrasound, increases the content of phenolic compounds in fresh lettuce (Wang et al., 2021). In addition, ozone has a bactericidal effect. Ozone gas reduces the number of yeasts and moulds, the number of mesophilic lactic fermentation bacteria or the number of mesophilic aerobic bacteria (Zardzewiały et al., 2023). Ozonation is an effective method for preserving fresh vegetables and fruits (Gibson et al., 2019) such as kiwifruit (Cao et al., 2022), mushroom (Wang et al., 2021), fennel (Lin et al., 2023) or fresh parsley (Gutarowska et al., 2023).

The aim of this study was to determine the effect of gaseous ozone on the microbial stability of Japanese quince fruit and the effect of the addition of ozonated quince fruit on the quality of the physicochemical quality, electrical properties and microbiological stability of wheat beers.

MATERIAL AND METHODS

Material

Winter wheat grain of the 'Gimantis' variety from a field experiment conducted in 2021 at the experimental station in Jelcz-Laskowice, Lower Silesia Province (Poland) was used to produce wheat beers. Five-day wheat malts were prepared from the grain (the malting process methodology is described in Belcar et al. (2021)). Commercial barley malt from the Viking Malt malting plant in Strzegom (Poland) was also used for brewing. Wheat and barley malts were grinded to appropriate particle size on a Cemotec disc mill from FOSS. Raw material charge for brewing was 50% commercial barley malt and 50% wheat malt.

For the enrichment of wheat beers, quince fruit cuttings of the 'North Lemon' cultivar (*Chaenomeles japonica*) produced in 2023 on the commercial farm of Japanese quince grower Mr Dariusz Murawski, Stoczek Łukowski, (Lublin Province, Poland) were used.

Ozone treatment of Japanese quince

Immediately after harvesting, the fruit was randomized into two batches of 2,000 g each. The first batch was left untreated (control sample). The remaining batch was subjected to the ozonation in a plastic container, with L x W x H dimensions of 0.6 x 0.4 x 0.4 m. Gaseous ozone was used at a concentration of 10 ppm for 30 minutes (flow 40 g $O_3 \cdot h^{-1,}$ temperature 20°C). The ozone dose was determined based on our own previous research on the effect of ozone on the quality of Japanese quince fruit, which has not yet been published. Ozone was produced with an ozone generator KORONA A 40 Standard (Laboratorium naukowo-wdrożeniowe Korona, Piotrków Trybunalski, Poland) with 106 M

UV Ozone Solution detector (Ozone Solution, Hull, MA, USA).

Microbiological stability of Japanese quince fruit

Japanese quince fruits (24 hours after ozonation) and non-ozonated fruits were taken for microbiological determinations. The number of aerobic mesophilic bacteria, mesophilic lactic fermentation bacteria and yeasts and moulds was determined according to the methodology described by Zardzewiały et al. (2023).

Beer production

The production process was carried out using the infusion method in the laboratory of the Department of Agricultural and Food Production Engineering at the University of Rzeszow. Barley malt of 1.0 kg and 1.0 kg of wheat malt were mashed and placed in a ROYAL RCBM-30CK mashing and brewing kettle (Expondo; Poland; assuming a process efficiency of 80%) and 6.0 l of water was added (3 l of water for each kilogram of malt). The mashing, boiling process with hops and cooling of the beer wort were carried out according to the methodology described in Gorzelany et al. (2022).

Each of the three produced beer worts was characterized by an extract at a temperature of 12.0°P. After cooling, the wort was inoculated with *Saccharomyces cerevisae* Fermentis Safale US-05 yeast (6 x 10^9/g), which had been previously hydrated according to the manufacturer's instructions (0.58 g d.m./L wort). The fermentation process was carried out at 21°C for 7 days. After this time, Japanese quince fruits in the form of slices were added to the fermenting beer in a specific amount (10% of wort volume; the dose of fruit added to beer was determined based on research by Zapata et al. (2019), who found that beer enriched with 10% of quince fruit had the best quality values) and left to ferment for another 14 days, and the control beer was bottled after 14 days. After 21 days, the beers were poured into bottles to which a solution of sucrose (0.3%) in water was previously added for refermentation and to obtain the appropriate saturation of the beer. The resulting beers were kept at 20°C. Organoleptic and physico-chemical tests were performed one month after bottling.

Wheat beers obtained without added fruit were designated control (CB), those enriched with ozonated Japanese quince fruit were designated PO, while those enriched with non-ozonated Japanese quince fruit were designated PN.

Microbiological stability of wheat beers

In table 2 were presented the number of mesophilic aerobic bacteria, the number of mesophilic lactic fermentation bacteria and the number of yeasts and moulds (methodology given in Zardzewiały et al. (2023)) 30 days after bottling of wheat beers.

Analysis of the quality indicators of wheat beers

In table 3 were presented quality indicators of wheat beers (methodology given in Gorzelany et al. (2022)). The analyses were performed in three replications.

Content of bioactive compounds in fruit beers

In table 4 were presented total polyphenol content using the Folin-Ciocalteu method (methodology given in Gorzelany et al. (2022)), and the polyphenol profile (methodology given in Żurek et al. (2021)).

Antioxidant activity

The antioxidant activity of fruit beers presented in Table 5 was determined according to the methodology given in Gorzelany et al. (2022). The analyses were performed in three replications.

Measurement of electrical parameters

The beer impedance measurements were conducted using the ATLAS 0441 HIA apparatus, an impedance analyzer, and a cylindrical electrode system. The electrode was composed of copper and coated with gold. The measuring cell's dimensions are provided in Figure 1.

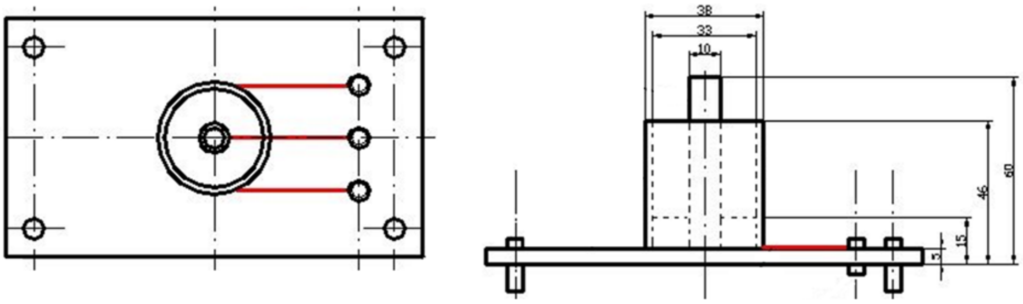

Figure 1. The dimensions of measuring cell

The measurement was carried out in a frequency range of 10 Hz to 1 MHz, with a voltage of 100 mV, and at the temperature of 23°C. The measurements were taken in triplicate, and the arithmetic mean was calculated as the results, represented in terms of real and imaginary components:

$$Z = Re(Z) + jIm(Z) \quad (1)$$

where Z - is complex impedance, Re(Z) – is the real part of impedance, Im(Z) - is the imaginary part of impedance, and $j = \sqrt{-1}$.

Based on results the impedance modulus of beer |Z| and capacitance C of cylindrical capacitor with beer sample were calculated according to the f equations:

$$|Z| = \sqrt{Re(Z)^2 + Im(Z)^2} \quad (2)$$

$$C = \frac{B}{2 \cdot \pi \cdot f} = \frac{\frac{-Im(Z)}{Re(z)^2 + Im(Z)^2}}{2 \cdot \pi \cdot f} \quad (3)$$

where B - is susceptance (S), f - is frequency (Hz).

Organoleptic analysis

The organoleptic analysis was carried out by a team of 11 people (four women and seven men, aged 30-40) in the organoleptic evaluation laboratory according to the methodology given in the work of Zordzewiały et al. (2023), by administering beer samples cooled to a temperature of 10 in random order. Water was administered to rinse the mouth between subsequent beer samples.

Statistical analysis

Statistical analysis of the results was performed using Statistica 13.3 statistical software (TIBCO Software Inc., Tulsa, OK, USA). Analyses used a two-factor ANOVA with a significance level of α = 0.05 for the individual analysed results of the fruit beers. Comparisons of mean values were made using the HSD-Tukey test.

RESULTS AND DISCUSSION

Microbiological stability of wheat beers and Japanese quince added to them

Ozone gas is widely used in the food industry as a means of reducing microbial contaminants in fruit and vegetables (Ong and Ali, 2015). The current state of scientific knowledge reports that mesophilic aerobic bacteria are reduced by the application of gaseous ozone. Beneficial results from the application of different concentrations of ozone have been reported in scientific papers on asparagus (Pretell-Vásquez et al., 2022), blueberry (Piechowiak et al., 2020), date palm fruit (Khalil 2019) or fresh parsley (Gutarowska et al., 2023). Yeasts and moulds are sensitive to the effects of ozone gas. The effect of gaseous ozone on reducing this type of microbial load in strawberries (Yaseen et al., 2013; Palou et al., 2001), carrot roots (Paulikienė et al., 2020) or figs (Zorlugenç et al., 2008) has been reported by other scientists, too.

After harvesting, the Japanese quince fruits were divided into two batches and one was ozonised. After 24 hours, the next step was to use them (both batches) as an additive for wheat beers. Application of the ozonation process at a dose of 10 ppm for 30 minutes reduced the number of mesophilic lactic fermentation bacteria and the number of mesophilic aerobic bacteria in a statistically significant manner. A similar trend was observed for the number of yeasts and moulds. In the plant material studied, the application of gaseous ozone resulted in a 39.4% reduction in the number of microorganisms tested compared to the control sample variant, i.e. fruit not subjected to ozone treatment (Table 1).

In order to determine the effect of the addition of Japanese quince fruit and the ozonation process on the microbiological quality of the beers produced, analyses were performed 30 days after bottling. A microbial load was recorded in each type of beer analysed, which varied according to the type of beer tested. In the case of yeasts and moulds, the addition of non-ozonated fruit to the beer increased the value of the tested parameter compared to wheat beer without the addition of the tested fruit. On the other hand, the addition of Japanese quince fruit previously ozonised reduced the number of yeasts and moulds by 23.5% compared to wheat beer without any additives and by 41.5% for beer with the addition of non-ozonised fruit. A similar trend to that for yeast and mould was recorded for mesophilic aerobic bacteria and mesophilic lactic fermentation bacteria (Table 2).

Table 1. Assessment of the microbiological load of Japanese quince fruit 24 h after the ozonation process

Type of fruit	Tested parameter		
	The number of mesophilic lactic acid bacteria (log cfu g^{-1})	Number of aerobic mesophilic bacteria (log cfu g^{-1})	Number of yeasts and molds (log cfu g^{-1})
Nonozonated fruits	$2,46^{b}$	$2,51^{b}$	$4,63^{b}$
Ozonated fruits	$<1,0^{a}$	$<1,0^{a}$	$2,57^{a}$

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$)

Table 2. Microbiological stability of wheat beers enriched with Japanese quince fruit

Type of beer	Number of yeasts and molds (log cfu mL^{-1})	Number of aerobic mesophilic bacteria (log cfu mL^{-1})	The number of mesophilic lactic acid bacteria (log cfu mL^{-1})
CB	3,32^{b}	5,96^{b}	5,46^{b}
PN	4,34^{c}	6,65^{c}	5,59^{b}
PO	2,54^{a}	5,09^{a}	4,19^{a}

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$); CB – wheat beer without the addition of Japanese quince fruit; PO – wheat beer with the addition of ozonated Japanese quince fruit; PN – wheat beer with the addition of nonozonated Japanese quince fruit

The quality of fruit beers depends primarily on the quality of the raw materials used during their production process (Nardini and Garaguso, 2020). Gaseous ozone as an abiotic agent applied under controlled conditions is able to extend the storage life of plant raw materials (Susan et al., 2018). Numerous scientific studies report that gaseous ozone reduced the microbial load on citrus fruit (Palou et al. 2001), carrot roots (Paulikienė et al. 2020), saskatoon berry (Gorzelany et al., 2022), raspberries (Piechowiak et al., 2019), mangoes (Nonjabulo et al., 2023) or strawberries (Panou et al., 2021). The metabolic processes that change under the influence of ozone during beer maturation affect the content of biologically active compounds and the antioxidant value of the finished product. As shown by Zhang et al. (2005); Lin et al. (2023) and Piechowiak et al. (2019), the stress factor of ozone application causes the production and accumulation of reactive oxygen species (ROS) in fruits and vegetables. This induces the synthesis of antioxidant and antimicrobial systems, including phytoalexins (isoflavonoids, stilbenephytoalexins). There is little research on explaining the effect of ozone gas on Japanese quince.

Studies on the quality of beers with rhubarb have shown that the use of previously ozonised raw material during their production has a beneficial effect on reducing the microbial load of the finished product. Within 45 days of beer production, a significant decrease in the number of microorganisms tested was recorded compared to the control sample (Zardzewiały et al., 2023). The consumption time of the finished beer should not be too long, as craft beers are often not pasteurised and therefore contain live microorganisms (Jagodziński et al., 2016). Scientific studies report that fruit beers during production that did not use microbial reduction methods contain a high yeast count of 4.6×10^{4} cfu mL^{-1} (Jakuos et al., 2022). Scientific studies report that ozone is an alternative to high-temperature pasteurisation. The use of gaseous ozone has been shown to be an effective method for reducing by 5 log^{-1} the number of *Escherichia coli* in apple cider and *Salmonella* in orange juice (Angelino et al., 2003).

Physico-chemical analysis of wheat beers

The results of the basic quality indicators of artisanal wheat beers enriched with ozonated and non-ozonated Japanese quince fruit are shown in Table 3. The control wheat beer (CB) had the lowest apparent extract value, while the addition of ozonated Japanese quince fruit increased the parameter in question. The smallest difference between apparent and real extract value was obtained for wheat beer enriched with non-ozonated Japanese quince fruit (by 18.62% on average). Also this beer (PN) was characterised by the lowest basic wort extract value (on average by 24.46% lower than PO beer and on average by 28.24% lower than CB beer), which significantly affected other quality characteristics such as attenuation (Table 3.).

The highest value of the degree of apparent and real attenuation was recorded for the control wheat beer (CB), but the differences between the quality characteristics discussed were the highest (by 11.43). Comparing wheat beers enriched with Japanese quince fruit, it was found that the ozonation process had a positive effect on the value of apparent attenuation (difference by 8.70%) and on actual attenuation (difference by 5.65%; Table 3.). The higher the attenuation value of the beers, the higher the ethanol content, while in our study the opposite results were obtained, because for wheat beer enriched with nonozonated Japanese quince fruit characterised by the lowest actual attenuation, the highest ethanol content was obtained, which was at the level of 4.78% v/v and was on average 36.40% higher than the control wheat beer (CB) and on average 6.69% higher than wheat beer enriched with ozonated Japanese quince fruit (Table 3.). In a study by Zapata et al. (2019) beers enriched with quince fruit (*Cydonia oblonga*) were characterised by an ethanol content of 5.51 - 5.62% v/v, while in the study by Baigts-Allende et al. (2021) apple beers were characterised by an alcohol content of 2.5 - 3.5% v/v. Yang et al. (2021) analysed apple beers, which were characterised by an ethanol content of 3.5% v/v.

In a study by Nordini and Garaguso (2020), apple beers were characterised by an alcohol content of 5.2% v/v. The control wheat beer was characterised

by the lowest energy value of the obtained beer product, mainly due to the relatively low proportion of ethanol in the beer's chemical composition, while no differences in the obtained energy value were found when comparing wheat beers enriched with ozonised and non-ozonised Japanese quince fruit.
The differences in the colour of the obtained wheat beers were not significant, while the addition of Japanese quince fruit resulted in a slight brightening of the colour of the finished beer product (from 21.8 EBC to 20.8 EBC; Figure. 2; Table 3.). In a study by Baigts-Allende et al. (2021) apple beers were characterised by a colour in the range of 6.34 - 9.81 EBC. In a study by Zapata et al. (2019) beers enriched with quince fruit (*Cydonia oblonga*) were characterised by a colour in the range of 37.38 - 41.38 EBC, depending on the cultivar or clone of quince fruit added.
The pH values of the wheat beers obtained did not vary according to the additive used, while clear differences were obtained for the acidity parameter. The control wheat beer (CB) was characterised by the lowest acidity sensation, which significantly influenced the sensory experience of the finished beer product. Comparing the fermentation products enriched with Japanese quince fruit, it was found that the addition of ozonated fruit significantly increased the acidity of the wheat beers compared to the beer enriched with non-ozonated fruit (by 25.68% on average; Table 3.). The lower the pH of the beer, the higher the effect on limiting the growth of undesirable microflora causing microbiological stability of the finished beer product (Gasiński et al., 2020). In a study by Nordini and Garaguso (2020), the apple beers analysed were characterised by a pH of 4.42. In a study by Zapata et al. (2019), beers enriched with quince fruit (*Cydonia oblonga*) were characterised by a pH value of 4.28 - 4.33, while the acidity of the resulting beers was 3.8 - 3.9, depending on the cultivar or clone of quince fruit used.

Table 3. Results of physico-chemical analysis of the analyzed wheat beers

	CB	**PO**	**PN**
Apparent extract (%; m/m)	2.83^{a}±0.06	3.21^{b}±0.08	3.06^{b}±0.06
Real extract (%; m/m)	4.38^{b}±0.07	4.28^{b}±0.05	3.76^{a}±0.01
Original extract (%; m/m)	13.56^{c}±0.08	12.88^{b}±0.10	9.73^{a}±0.06
Degree of final apparent attenuation (%)	79.13^{c}±0.10	75.08^{b}±0.06	68.55^{a}±0.09
Degree of final real attenuation (%)	67.70^{b}±0.04	66.77^{b}±0.05	63.00^{a}±0.04
Content of alcohol (%; m/m)	3.04^{a}±0.05	4.46^{b}±0.05	4.78^{c}±0.08
Content of alcohol (%; v/v)	2.41^{a}±0.05	3.55^{b}±0.10	3.80^{c}±0.05
Colour (EBC units)	21.8^{a}±0.6	21.4^{a}±0.5	20.8^{a}±0.2
Titratable acidity (0.1M NaOH/100 mL)	3.88^{a}±0.05	6.66^{c}±0.06	4.95^{b}±0.02
pH	3.35^{a}±0.04	3.32^{a}±0.03	3.49^{a}±0.03
Bitter substances (IBU)	14.4^{a}±0.3	16.8^{b}±0.1	17.1^{b}±0.4
Content of carbon dioxide (%)	0.43^{a}±0.02	0.47^{a}±0.07	0.45^{a}±0.04
Energy value (kcal/100 mL)	39.10^{a}±0.07	48.63^{b}±0.07	48.72^{b}±0.04

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$). CB – wheat beer without the addition of Japanese quince fruit; PO – wheat beer with the addition of ozonated Japanese quince fruit; PN – wheat beer with the addition of nonozonated Japanese quince fruit

Figure 2. Appearance of wheat beers - wheat beer without added fruit (from the left, CB beer), wheat beer with the addition of ozonated (in the middle - PO beer) and non-ozonated (PN beer) Japanese quince fruit

The average bitterness sensation in the control wheat beer (CB) was at 14.4 IBU and was on average 14.29% lower than the PO beer and on average 15.79% lower than the PN beer. The differences in bitterness content in wheat beers enriched with ozonated and non-ozonated Japanese quince fruit were not significant (Table 3.). In a study by Zapata et al. (2019) beers enriched with quince fruit (*Cydonia oblonga*) were characterised by a bitterness content of 8.70 - 9.52 IBU, depending on the cultivar or clone of quince fruit used and added to the beer. The degree of bitterness in the analysed beers comes from the freeze-dried hop cones added during the boiling process (the cultivar and dosage of hops used and the degree of α-acid isomerisation, as well as the degree of reaction of the proteins with the polyphenols in the malt, are important) (Byeon et al., 2021; Habschied et al., 2021). The carbon dioxide content of the wheat beers in question was at the same level regardless of the fruit additive used.

Content of bioactive compounds in beers

The main proportion of polyphenolic compounds that are contained in beers comes from and passes through the production process mainly from the malt used (70 - 80%) and hops (Habschied et al., 2021; Martínez et al., 2017). Bioactive compounds, which are polyphenols, are substances with a diverse chemical structure, which has a significant impact on their antioxidant or anti-radical activity (Mikyška et al., 2019). The polyphenols contained in beers have a significant impact on the sensory experience felt by consumers, including the sensation of body, bitterness, acidity or the sensation of fullness of taste. Wheat beer enriched with ozonated Japanese quince fruit had the highest content of total polyphenols, at 187.8 mg/L, and was significantly higher than both wheat beer with the addition of non-ozonated Japanese quince fruit and in the control beer without the fruit addition (Table 4.). The addition to beers of plant raw material, characterised by a high proportion of polyphenolic compounds, influences their content in the finished beer product, but also the process of fruit fragmentation (disruption of the cell wall results in faster extraction of polyphenolic compounds contained in the added raw material into the fermenting beer) (Gasiński et al., 2020). In Nardini and Garaguso's study (2020), apple beers were characterised by a total polyphenol content of 399 mg GAE/L. In a study by Zapata et al. (2019), as in the present study, a positive correlation was observed between the content of total polyphenols in beers and their antioxidant activity as determined by the ABTS method. The content of total polyphenols in quince-infused beers was between 159.0 and 175.5 mg GAE/L (Nardini, 2023; Zapata et al., 2019).

Table 4. Polyphenol content and polyphenol profile identified by UPLC-PDA-TQD-MS

						CB	**PO**	**PN**
Total polyphenols content (mg GAE/L)						160.9^a±0.34	187.8^b±0.18	164.3^a±0.32
Compound (mg/L)		Rt (min)	λ_{max} (nm)	(M-H) m/z MS (m/z)	(M-H) m/z MS/MS (m/z)			
1.	Chlorogenic acid	2.96	299sh, 327	353	191	<LOQ	4.35^a±0.09	5.42^b±0.05
2.	Kaempferol 3-*O*-glucoside	3.33	264, 352	447	285	<LOQ	0.22^a±0.00	0.31^b±0.00
3.	(+)catechin	3.48	277	289	141	<LOQ	0.24^a±0.01	0.30^b±0.00
4.	Kaempferol rhamnoside-pentoside	3.69	264, 351	563	285	0.13^a±0.01	0.62^c±0.01	0.53^b±0.00
5.	(+)catechin 3-*O*-glucoside	3.71	277	451	289	0.06^a±0.00	0.36^c±0.00	0.29^b±0.01
6.	Kaempferol rhamnoside-penroside-pentoside	3.85	264, 350	695	563, 285	0.03^a±0.00	0.14^b±0.00	0.14^b±0.01
7.	Kaempferol rhamnoside-pentoside	3.91	264, 350	563	285	0.15^a±0.00	0.71^c±0.01	0.63^b±0.00
8.	Kaempferol 3-*O*-rutinoside	4.91	264, 352	593	285	<LOQ	0.34^a±0.00	0.81^b±0.00
Total						0.37^a±0.00	6.98^b±0.15	8.43^c±0.08

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$). CB – wheat beer without the addition of Japanese quince fruit; PO – wheat beer with the addition of ozonated Japanese quince fruit; PN – wheat beer with the addition of nonozonated Japanese quince fruit. <LOQ - below limit of quantification

The identification of polyphenolic compounds in wheat beers enriched with ozonated and non-ozonated Japanese quince fruits was based on the analysis of characteristic spectral data: mass-to-charge m/z ratio and maximum of radiation absorption. Seven polyphenolic compounds were identified, the spectral properties of which are shown in Table 4. Chlorogenic acid belongs to the group of hydroxycinnamic acid derivatives, and the other identified compounds belonged to the flavonols group, whose representatives were (+)catechin and its derivative, as well as kempferol derivatives (in glycosidic form). In the wheat beer constituting the control sample, 3 polyphenolic compounds were identified: kaempferol rhamnoside-pentoside, kaempferol rhamnoside-penroside-pentoside and (+)catechin 3-*O*-glucoside, and the former compound accounted for 75.76% of the total sum of identified polyphenolic compounds in the analysed beer and most likely originated from hops added during boiling (Table 4.). The glycosidic derivatives (*O*-glycosides) of kempferol contained in hops during boiling pass from the hop cones into the boiling wort after about 30 minutes (depending on the dose) (Mikyška and Dušek 2019). In barley beers, the average kempferol content is between 0.10 and 1.64 mg/L (Radonjič et al., 2020; Almaguer et al., 2014). In wheat beers enriched with Japanese quince fruit, in addition to the above-mentioned flavonols, which had a higher proportion, chlorogenic acid, (+)catechin, kaempferol 3-*O*-glucoside and kaempferol 3-*O*-rutinoside were also identified, with the highest proportion being characterised by the former compound (Table 4). The polyphenolic compounds mentioned above were derived from quince fruit added during fermentation (Carbonell-Barrachina et al., 2015). The ozonation process generally negatively affected the content of the identified polyphenolic compounds, including chlorogenic acid by an average of 19.74%, and the total polyphenolic compounds were on average 17.20% lower in wheat beer enriched with ozonated Japanese quince fruit compared to wheat beer with fruit added without ozonation (Table 4.). The increase in the content of phenolic compounds in plants depends on phenylalanine ammonia lyase (PAL). The influence of abiotic stress, which is the dose of ozone, affects the increased catalysis of the transformation of l-phenylalanine into trans-cinnamic acid and further into various substances, including flavonoids, phenolic acids or anthocyanins, and therefore the higher the activity of PAL, the higher the content of phenolic compounds in the raw material (Gutarowska et al., 2023; Piechowiak et al., 2020; Paulikienė et al. 2020). In a study by Zapata et al. (2019) beers enriched with quince fruit (*Cydonia oblonga*) were characterised by a chlorogenic acid content of 6.5 - 9.7 mg/L. depending on the cultivar or clone of quince fruit added to the beer, while neochlorogenic acid (3.9 - 5.1 mg/L and 3,5-Dicaffeoylquinic acid (3.0 - 3.2 mg/L) were also identified. Kempferol glycosides affect the mouthfeel of astringency and, to a lesser extent, the sensation of bitterness, which is perceptible during consumption of the beer product (Ma et al., 2021). The identified flavonoid compounds have antioxidant properties, may have anticancer effects, and are helpful in the treatment of cardiovascular disease and autoimmune diseases and for transplant patients (Ma et al., 2021).

The content of biologically active compounds in beers, such as vitamins, bitter compounds or polyphenols, affects the antioxidant potential of the finished beer product (Bogdan and Kordialik-Bogacka, 2016; Ditrych et al., 2015). The control wheat beer (CB) had the highest antioxidant activity determined by the DPPH method and was at 1.14 mM TE/L, while wheat beers enriched with Japanese quince fruit had significantly lower activity; by 90.35% on average for PO beer and 57.02% on average for PN beer compared to the control wheat beer. In addition, wheat beer enriched with non-ozonated Japanese quince fruit was characterised by higher antioxidant activity (by 77.55% on average) compared to beer with ozonated fruit (Table 5). The results of antioxidant activity determined by the ABTS method were more varied; the highest value was obtained for wheat beer enriched with ozonated quince fruit and amounted on average to 1.05 mm TE/L and was on average 17.14% higher than wheat beer with the addition of non-ozonated Japanese quince fruit and definitely higher (on average by 78.10%) than control wheat beer.

Table 5. Antioxidant activity of the discussed wheat beers

	CB	PO	PN
$DPPH^{\cdot}$ (mM TE/L)	$1.14^{c}\pm0.08$	$0.11^{a}\pm0.06$	$0.49^{b}\pm0.05$
FRAP (mM Fe^{2+}/L)	$1.07^{a}\pm0.08$	$3.05^{b}\pm0.10$	$3.77^{c}\pm0.08$
$ABTS^{+\cdot}$ (mM TE/L)	$0.23^{a}\pm0.10$	$1.05^{c}\pm0.06$	$0.87^{b}\pm0.01$

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$). CB – wheat beer without the addition of quince fruit; PO – wheat beer with the addition of ozonated quince fruit; PN – wheat beer with the addition of nonozonated quince fruit

The antioxidant capacity of wheat beers determined by the FRAP method showed that wheat beer enriched with non-ozonated Japanese quince fruit was characterised by the highest value, while the addition of ozonated Japanese quince fruit to the finished beer product lowered the analysed parameter by an average of 19.20%, while a much lower value was obtained for the control wheat beer (CB; Table 5.). In a study by Nordini and Garaguso (2020), apple beers were characterised by antioxidant activity determined by FRAP and ABTS methods at levels of, respectively: 3.08 mM Fe^{2+}/L and 1.62 mM TE/L. In a study by Zapata et al. (2019), the antioxidant activity determined by the ABTS method of quince fruit (*Cydonia oblonga*)-enriched beers was between 7.2 and 7.3 mM TE/L, and the antioxidant activity of quince fruit-enriched beers was largely influenced by the contribution of chlorogenic acid; a similar relationship was also observed in this study. Furthermore, the antioxidant activity of the beers determined by the ABTS method (control and beers with quince fruit added) did not show significant differences. During beer storage, oxidative degradation of beer ingredients occurs by oxygen radicals. The antioxidant activity of beers is generally determined using the DPPH and FRAP methods. In general, the results of beers without fruit additives determined by the DPPH method are higher compared to the results determined by the FRAP method, but in beers without fruit additives there is a strong relationship between DPPH, FRAP and the polyphenol content (Bogdan and Kordialik-Bogacka, 2016; Ditrych et al., 2015). In fruit beers, there are additional, separate reaction mechanisms of substances with anti-oxidant properties in beer, i.e. they correspond to other anti-oxidant properties. Japanese quince fruits contain large amounts of falavan-3-ols, organic acids and ascorbic acid, which influence the overall content of polyphenols, as well as individual polyphenols, e.g. chlorogenic acid or the acidity of beers. Due to the very rapid decomposition of ascorbic acid in fruit beers (including lemon, grapefruit, as well as those with the addition of black currant or strawberry), the presence of this compound is not observed, even though the fruit added to beer has a relatively high ascorbic acid content. the degree of grinding of the added fruit (the smaller the fruit parts, the better, but do not forget about the addition of cell juice that flows out during fruit grinding) affects the degree of their penetration into the fruit beer.

Impedance and capacitance of wheat beers

Figure 3 depicts the frequency dependence of the impedance modulus for the analysed wheat beers enriched with Japanese quince fruit. The impedance values varied, with the highest value obtained for the control wheat beer (CB), a slightly lower value for the beer enriched with ozonated Japanese quince fruit (PO), and a significantly lower value for the beer with non-ozonated fruit (PN). In a study by Gorzelany et al. (2023) the impedance values for wheat beers enriched with lemongrass ranged from 140 to 170 kΩ, while those enriched with berry extract showed greater variation. For beers enriched with haskap (*Lonicera caerulea* var. *emphyllocalyx*) fruit, the impedance was in the range of 155-170 kΩ. However, for beers enriched with kamchatka berry (*Lonicera caerulea* var. *kamtschatica*), the impedance was significantly lower, at 25-30 kΩ. The highest impedance value in the present study but also in the study of Gorzelany et al. (2023) was obtained for a control beer product not subjected to enrichment with plant raw material. The addition of raw materials to wheat beers results in the delivery of new chemical compounds to the finished product. These compounds significantly affect the impedance value, allowing for differentiation between various types of beers. Additionally, this process can be used to monitor the increase in the number of yeasts during fermentation (Wyk and Silva, 2016).

Figure 4 displays the frequency dependence of capacitance for the wheat beers enriched with Japanese quince fruit that were analysed. To enhance legibility, the frequency range is limited to 5-100 kHz. Capacitance values for all samples decrease significantly for frequencies above 100 kHz. The capacitance value of the control wheat beer (CB) was the lowest, while the addition of Japanese quince fruit had a positive effect, resulting in a higher capacitance value for the beer enriched with non-ozoned Japanese quince fruit. Gorzelany et al. (2023) also observed similar relationships in their study, where wheat beers enriched with kamchatka berry (*Lonicera caerulea* var. *kamtschatica*) or haskap (*Lonicera caerulea* var. *emphyllocalyx*) fruit and lemongrass had higher capacities compared to wheat beers without added fruit or herbs.

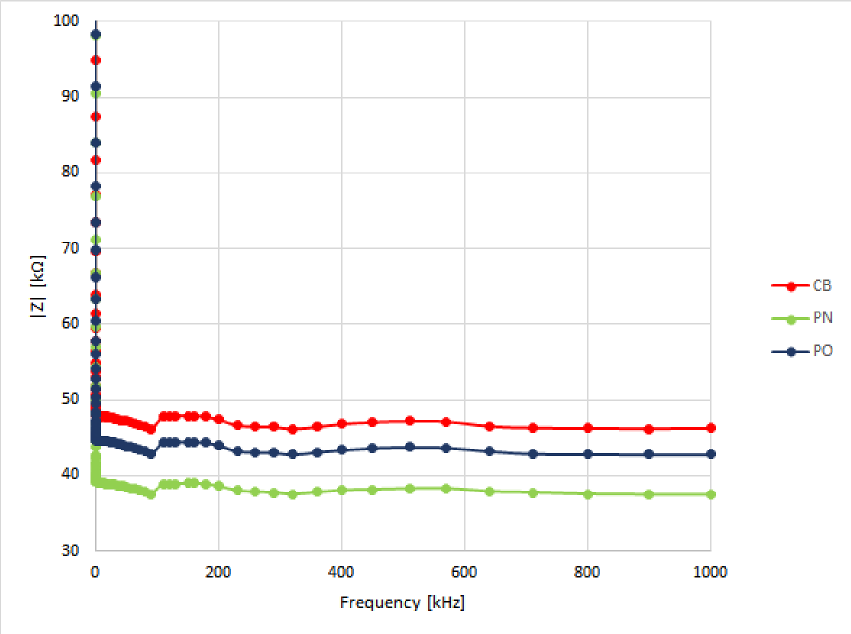

Figure 3. The frequency dependence of the impedance modulus of wheat beers

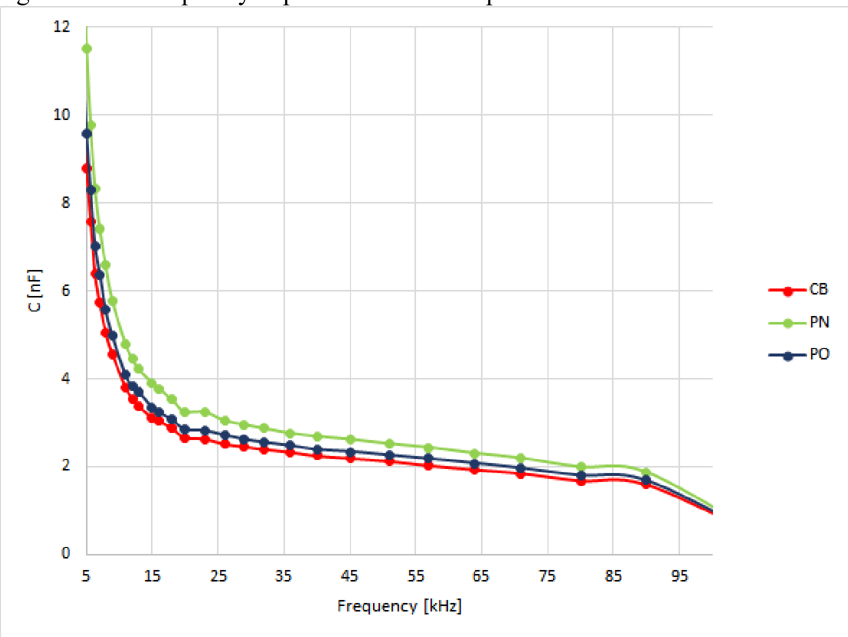

Figure 4. The frequency dependence of the capacitance of wheat beers

Organoleptic analysis of wheat beers

The addition of vegetable raw materials to wheat beers significantly affects their organoleptic characteristics, which determine a particular beer style and shape the appeal and acceptance of a particular beer among consumers. The results of the organoleptic evaluation of wheat beers carried out by an 11-member panel are presented in Table 6 and Figure 5. Wheat beers enriched with Japanese quince fruit were characterised by the highest sensation of palatability (combination of taste and aroma), however, the finished product enriched with non-ozoned fruit had a slightly higher sensation. Beer head stability, bitterness sensation and saturation of all analysed wheat beers were at similar levels (Table 6.). The control wheat beer had the lowest overall impression value, while the wheat beer enriched with non-ozonated Japanese quince fruit had the highest overall impression value, but these differences were not significant. Of the assessed quality attributes of wheat beers, the stability of beer head was rated lowest, and to a lesser extent the carbonation of wheat beers regardless of whether Japanese quince fruit was used as an additive (Table 6.). The palatability of the finished product of the fermentation process is influenced not only by the raw materials used, but also by the appropriate conditions of the process carried out and the products of the fermentation process (such as aldehydes, phenols or esters) affecting the taste profile of the beer in question.

The sensory profile of the wheat beers analysed varied; the control wheat beer (CB) was mainly characterised by cereal notes and, to a lesser extent, malty notes evoked by maltol or furaneol, among others (Faltermaier et al., 2014), and to a lesser extent by a sensation of fullness. The organoleptic evaluation of beers enriched with Japanese quince fruit showed an intensity of sensations of freshness as well as sweetness and intensity of flavour but also acid notes, especially for wheat beer enriched with ozonated Japanese quince fruit, which may affect the perception of acceptance by consumers (Figure 5.). In a study by Zapata et al. (2019) beers enriched with quince fruit (*Cydonia oblonga*) were characterised by floral and fruity notes. The interactions formed during the fermentation process, but also during the maturation of the beer between the chemical compounds in the finished beer product have a significant impact on the palatability of the beer produced (Faltermaier et al., 2014). Beers, especially wheat beers, which are characterised by fruity notes, sweet taste and pleasant aroma gain greater acceptance by consumers compared to traditional types of beers (Viejo et al., 2019; Adadi et al., 2017).

Table 6. Organoleptic analysis of wheat beers

	CB	PO	PN
Aroma	3.93^{a}±0.21	4.08^{a}±0.35	4.12^{a}±0.30
Taste	3.85^{a}±0.34	3.87^{a}±0.14	4.13^{b}±0.18
Foam stability	3.55^{a}±0.13	3.72^{a}±0.30	3.71^{a}±0.17
Bitterness	3.90^{a}±0.27	4.00^{a}±0.14	4.00^{a}±0.15
Saturation	3.73^{a}±0.34	3.77^{a}±0.17	3.84^{a}±0.27
Overall impression	3.96^{a}±0.27	4.03^{a}±0.27	4.11^{a}±0.36

Data are expressed as a mean values (n = 3) ± SD; SD – standard deviation. Mean values within a rows with different letters are significantly different ($p < 0.05$). CB – wheat beer without the addition of Japanese quince fruit; PO – wheat beer with the addition of ozonated Japanese quince fruit; PN – wheat beer with the addition of nonozonated Japanese quince fruit

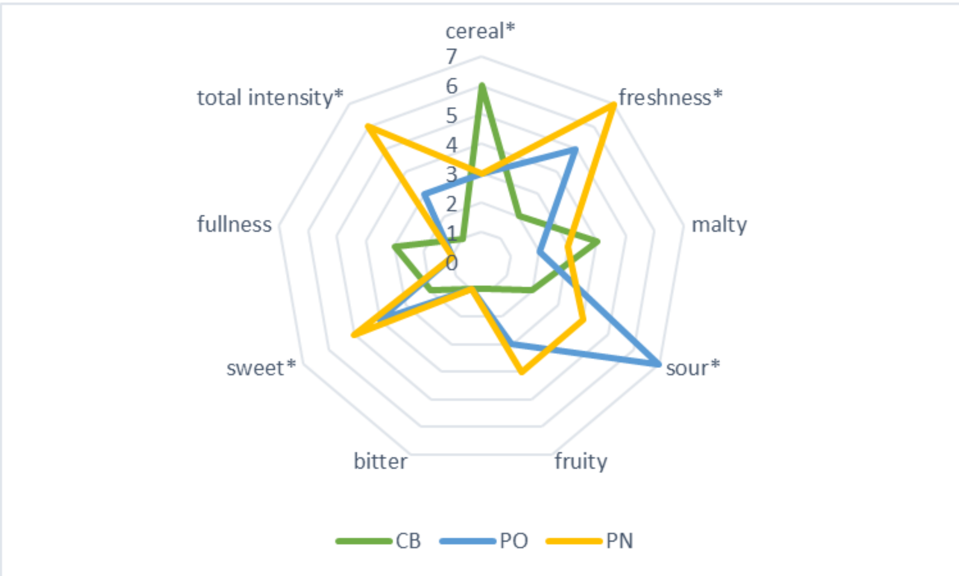

Figure 5. Sensory profile of wheat beers - control (CB) and with the addition of ozonated (PO) and non-ozonated (PN) Japanese quince berries (* indicates attributes which were statistically different at $p < 0.05$)

CONCLUSIONS

The research carried out on the possibility of using Japanese quince fruit for the production of fruit wheat beers showed that the most balanced flavor profile (intensity, feeling of bitterness giving a bitter aftertaste and fruity taste and aroma) was characteristic of beers enriched with nonozonated Japanese quince fruit. In addition, these beers were characterized by better color, lower acidity and antioxidant activity. The polyphenol profile of the analyzed wheat beers showed a high content of chlorogenic acid, especially for beer with the addition of nonozonated Japanese quince berries. Electrical properties (impedance and capacitance) allowed the differentiation of wheat beers. The ozonation process increased the microbiological stability of fruit and wheat beers. Due to the lack of literature information on changes in the chemical composition of Japanese quince fruits subjected to the ozonation process and being an addition to wheat beers, this research is innovative. Enriching wheat beers with Japanese quince fruit may be a new direction for expanding the range of fruit wheat beers.

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