

THE USE OF GASEOUS OZONE TO REDUCE THE MICROBIAL LOAD OF RHUBARB (*Rheum L.*) PETIOLES AS AN ADDITIVE TO CRAFT WHEAT BEERS

– Research paper –

Miłosz ZARDZEWIĄŁY¹, Justyna BELCAR^{1,2,*}, Józef GORZELANY¹

¹*Department of Food and Agriculture Production Engineering, University of Rzeszów, 4 Zelwerowicza Street, 35-601 Rzeszów, Poland*

²*Doctoral School of the University of Rzeszów, University of Rzeszów, st Rejtana 16C, 35-959 Rzeszów; Poland*

* Corresponding author; e-mail address: justyna.belcar@op.pl

Abstract: Rhubarb (*Rheum rhaponticum* L.) is a valuable source of active components responsible for laxative, antibacterial, diuretic, anti-inflammatory and anti-cancer properties. On the other hand, ozone being an allotropic form of oxygen, due to its potential is a powerful antimicrobial agent. Treatment of rhubarb petioles with gaseous ozone, especially at a concentration of 50 ppm, for 30 minutes had a positive result on the microbiological stability and quality of the finished material. Sensory evaluation of prepared beers enriched without ozonated and ozonated rhubarb pulp showed that wheat beer with the addition of ozonated (50 ppm) rhubarb pulp was characterized by a balanced aroma and taste. The physico-chemical analysis of wheat beers demonstrated that the increasing concentration of ozone had an impact on, among others, increased the degree of final real attenuation (by 21.97%), decreased pH (by 18.43%) and energy value (by 36.30%), but had a negative impact on the content of ethyl alcohol (by 17.32%) compared to the control beer. Among the analyzed beers enriched with rhubarb, the beer product with the addition of ozonated (50 ppm) rhubarb was the most microbiologically stable. The research shows that wheat beers enriched with fragmented rhubarb can be a new trend to expand the range of wheat beers, and the use of gaseous ozone, especially at a concentration of 50 ppm for 30 minutes, can be a trend to extend the microbiological stability of beers, especially craft beers.

Key words: rhubarb petioles, microbiological stability, wheat beers, beer quality

INTRODUCTION

Beer is a drink that is consumed in large quantities in many countries of the world (Callemien et al., 2005). It is a carbonated drink with nutritional properties, the production of which is widespread around the world. The Egyptians, Sumerians, and Babylonians were the first to prepare this drink in ancient times. The popularity of beer is related to the minerals and vitamins it contains.

Considering its nutritional value, we call it a functional drink (Wootton-Beard et al., 2011). Currently, beer is enriched with additives in the form of herbs, raw vegetables and fruits as well as amino acids, vitamins or minerals (Kausar et al., 2012). Beers with the addition of fruit are becoming more and more popular all over the world (Ritter et

al., 2016) and vegetables (Sánchez-Bravo et al., 2023). This direction of development of the brewing industry is a response to consumer demand for new taste, olfactory and visual stimuli. It is practiced to add whole fruit to beer, i.e. cherries or raspberries. The sugar contained in the fruit causes secondary fermentation, during which aromas and bioactive substances, especially carotenoids and polyphenols, are extracted from the fruit during the maturation of beers (Granato et al., 2011). In the case of vegetables, beers can be supplemented with pumpkin, beetroot, broccoli or rhubarb. Beers with vegetables are not as popular as fruit beers, but now this type of product is beginning to appear in the trade and is gaining more and more recognition among customers.

In addition, beer can be enriched with herbs or their extracts, which, as in the case of fruit or vegetables, change the intensity of flavor or aroma. Recently,

Received: 30.08.2023

Accepted in revised form: 04.11.2023

* Corresponding author.

E-Mail address: justyna.belcar@op.pl

additions to beers in the form of lavender, licorice, angelica, ginger, coriander, nettle, lemongrass and dandelion have been reported (Rošul et al., 2019; Hayward et al., 2019).

Beer additives have bactericidal, fungicidal, sedative, antidiabetic or antioxidant effects, taking into account the types of fruits, vegetables and herbs used in its production. It should be borne in mind that beer additives not only improve their health-promoting properties, but may also cause side effects. Their consumption in too much amounts causes psychoactive or abortifacient effects (Stachowiak & Bukowski, 2021; Rošul et al., 2019). There are many pro-health ingredients such as antioxidants, vitamins, antioxidants, polyphenols, microelements and fiber (Leitao et al., 2012; He et al., 2012). The antioxidants in beer provide protection the human organism against oxidative stress by capturing free radicals from the organism, which destroy the cellular structures (Belcar et al., 2022). It has been reported that the consumption of beer has an anticoagulant effect and a positively effect on the level of lipids in human plasma. Moderate beer consumption lowers the risk of cardiovascular diseases (Gorinstein et al., 2007).

Rhubarb, which was used as an addition to beers, includes 60 plant species of the genus *Rheum* L. of the *Polygonaceae* family (Xiang et al., 2020), and is classified as a perennial small-area crop. The plantation is used for 10-15 years. It is cultivated not only for direct consumption, but also as a raw material for industry, intended for the natural acidification of fruit and vegetable preserves and candy, it is also used in pharmacy (Matyjaszczyk et al., 2015). Rhubarb petioles are valuable source of substances with health-promoting effects responsible for laxative, weather antibacterial, and anticancer properties (Huang et al., 2019; Jintao et

al., 2018). Rhubarb is applicable when sick of ischemic stroke and limiting multiplication of hepatitis B virus (Xiong et al., 2019). Rhubarb petioles contain several natural bioactive components, including anthraquinones, dianthrone, flavonoids and stilbenes, which contribute to the improvement of human and animal health (Krafczyk et al., 2008). In addition, they also have a high fiber content and contain organic acids, including malic acid, as well as oxalic, citric and fumaric acid (Liudvytska & Kolodziejczyk-Czepas, 2022).

Soil, climatic and fertilization conditions are factors affecting the composition of bioactive substances in plant materials (Matłok et al., 2020). In addition, the electromagnetic field (Zhong et al., 2021) and the atmosphere of gaseous ozone (O₃) affect the quality of plant raw materials (Zapałowska et al., 2021). The latter method does not generate residues in plant materials and at the same time modifies their chemical composition. It has been reported that properly performed ozonation of plant materials (fruits, herbs and vegetables) increases the content of bioactive compounds (Matłok et al., 2020; Zardzewiały et al., 2020; Gorzelany et al., 2017). In addition, a properly selected dose of ozone reduces the microbiological load of substances (Xue et al., 2023; Zardzewiały et al., 2020). It is worth noting that despite the benefits of ozonation, its negative effects are also noted. Chronic exposure of plants to gaseous O₃ causes their damage in the form of chloroses and necroses on the surface of leaves or stems (Rai et al., 2012).

The objective of the study is to determine the effect of gaseous ozone on the microbiological stability of rhubarb petioles and the effect of the addition of ozonized rhubarb on the microbiological and physico-chemical quality of wheat beers.

MATERIAL AND METHODS

Material

The explore material consisted of rhubarb petioles of the "Malinowy" variety - a late variety with red flesh, from commercial cultivation in the Podkarpackie Province (Poland). Immediately after harvesting, the petioles were ozonated and to hold in a climatic chamber at 2°C and 95% relative humidity until physicochemical determinations were made.

For the production of craft wheat beers, winter wheat grain of the 'Elixer' variety was used from a field experience carried out in 2022 in Przeworsk (50°03'31"N 22°29'37"E), Podkarpackie Pvince (Poland). Common wheat grain was characterized

by total protein content - 11.4% d.m.; starch content - 68.7% d.m.; grain moisture - 14.1% d.m, grain uniformity - 87.0%.

The stuff material input for brewing craft wheat beers was 70% commercial ground barley malt and 30% ground common wheat grain. Malt from barley grain from the Viking Malt malthouse in Strzegom (Poland) was addition to brew the beer. The malt from barley had characterized: total protein content – 11.2% d.m., extract potential – 80.6% d.m., content of soluble protein – 3.71% d.m., degree of final attenuation – 82.1%, and diastatic power – 349 WK.

The process of ozonation of rhubarb leaf petioles

At once after harvesting, the rhubarb was randomly into three parties of 3,000 g each. The first part was constituted control sample (untreated). The remaining two parties were ozonated in an ozonation chamber with shapes L x W x H 0.6 x 0.5 x 0.5 m. Ozone in the form of gas was used at a strenght of 10 ppm and 50 ppm for 30 minutes (temp. 20°C, flow 40 g O₃·h⁻¹). Ozone was made 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 rhubarb petioles

Rhubarb petioles (24 hours after ozonation) were collected for microbiological determinations. Determination of the number of mesophilic aerobic bacteria, mesophilic lactic acid bacteria, yeasts and molds was performed in accordance with the research methodology described by Zardzewiały et al. (2020).

Beer production

The manufacture production of beer process was conducted using the infusion method in the laboratory of the Department of Agricultural and Food Production Engineering at the University of Rzeszów.

Common wheat grain weighing 1.5 kg and barley malt weighing 3.5 kg were ground in a ROYAL RCBM-40N mash kettle (Expondo; Poland; process efficiency of 80%) and 15.0 l of water was appended (3 l of water for each kg of malt). The mashing, the hop-cooking process and the cooling of the beer wort were conducted according to the methodology described by Gorzelany et al. (2022).

The beer worts was prepared so that its extract was at a level of 12.0°P. After the brewing process was completed, the wort was subjected to cooling and placed in fermenters and inoculated with *Saccharomyces cerevisiae* from company Fermentis Safale US-05 yeast (6 x 10⁹/g), which had previously been rehydrated, in accordance with the instructions (0.58 g d.m./L of wort). Wort fermentation lasted for 21 days at 21°C. After 7 days of fermentation process, 1 kg of rhubarb pulp (10% by volume of the wort) was appended to the fermenting wort and the process continued through for another 14 days. After the fermentation, the beer was poured into bottles, after adding a dilution of sucrose (0.3%) in water for obtaining the appropriate saturation of the beer. The beers were

hold at 20°C and then the finished beer product was subjected to physico-chemical and organoleptic assessment (1 month after bottling).

Wheat beers prepared without the addition of rhubarb pulp, with the insert of non-ozonated rhubarb pulp and with the addition of ozonated (10 ppm and 50 ppm) rhubarb pulp were marked: CB, RB0, RB10 and RB50, respectively. In total, 4 different of wheat beers were produced.

Analysis of beer quality

The content of alcohol (% m/m and % v/v), real extract (% m/m), apparent extract (% m/m), and original extract in beer (% m/m), total acidity (0.1M NaOH/100 mL), the degree of final apparent and real attenuation (%), pH, carbon dioxide content (%), color (EBC units), bitterness content (IBU units) and energy value of beer (kcal/100mL) were determined in accordance with the methodology defined by Belcar et al. (2022). The analyses were conducted in three replications.

The microbiological stability of wheat beers

Microbiological tests were performed in accordance with the following applicable standards:

-number of mesophilic aerobic bacteria: PB-77/LM ed. 5 of February 10, 2022

-number of mesophilic lactic acid bacteria - PN-ISO 15214:2002

-number of yeasts and molds - PN-ISO 21527-1:2009.

Microbiological analyzes of the beers were performed twice: on the 15th and 45th day from the bottling of the beers.

Organoleptic analysis

The organoleptic analysis was conducted by a team consisting of 11 people (7 women and 4 men aged 30-45) in the organoleptic evaluation laboratory in accordance with the EBC 13.13 method. Beer samples were giving chilled to 10°C, in plastic cups with a capacity of 250 mL in a random order early has coded. Water for mouthwash was given between each assessment. The organoleptic analysis of the beers was developed in accordance with the methodology described in Belcar & Gorzelany (2022).

Statistical analysis

Statistical evaluation of the results was performed using the Statistica 13.3. program (TIBCO Software Inc., Tulsa, OK, USA) which included ignificance LSD test ($\alpha = 0.05$), and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Rhubarb petioles were ozonized after harvesting. Then, after 24 hours, they were used as an addition to beers. The use of gaseous ozone, irrespective of the dose, reduced the number of molds and yeasts in the research plant material. Ozone in the form of gas at a concentration of 10 ppm decrease the number of tested microorganisms by 14.4% in comparison to the control. Ozonation with a strength of 50 ppm reduced in the number of molds and yeasts by 16% liken to the control sample (Table 1). The other tested parameters determining the microbiological quality of the substrate were below the quantification threshold for both the control sample and the ozonated variants.

Ozone (O₃), an allotropic form of oxygen, is a potent antimicrobial due to its potential (Guo et al., 2022). Increasingly, the ozonation process is used in the food industry due to its many advantages, in particular powerful oxidation, while causing it to decay into oxygen molecules without leaving residues in the ozonated material (Qi et al., 2016). It is worth noting that ozone is increasingly used to reduce mycotoxin contamination of plant materials such as fruits, vegetables or cereals (Ong and Ali, 2015). In addition, the bactericidal effect of O₃ has

been confirmed in studies that result in the reduction of gram-negative and gram-positive bacteria, as well as spores of bacteria (Brodowska et al., 2018). Pretell-Vázquez et al. (2022) used gaseous ozone in low concentrations on harvested asparagus and found that the gas used had an impact on reduction the number of microbes, taking into account the number of mesophilic aerobic bacteria and molds during the storage of the tested raw material. Yassen et al. (2012) applied gaseous ozone to strawberry fruit. The test results confirmed that, regardless of the storage temperature, ozone gas significantly reduced yeasts, molds and bacteria on the tested raw material.

The quality of beers enriched with rhubarb

The results concerning the assessment of physico-chemical indicators of beers product enriched with rhubarb pulp are presented in Table 2.

The apparent extract of the obtained wheat beers ranged from 2.03% m/m (for RB10 beer) to 5.88% m/m for the beer without rhubarb (CB), while the real extract was on average 7.65% higher than the of the obtained wheat beers.

Table 1. Evaluation of the microbiological load on rhubarb petioles 24 h after ozonation

Ozone dose	Tested parameters		
	The number of mesophilic lactic acid bacteria (log cfu g ⁻¹)	The number of aerobic mesophilic bacteria (log cfu g ⁻¹)	The number of yeasts and molds (log cfu g ⁻¹)
0 ppm	<1,0 ^a	<1,0 ^a	3,75 ^b
10 ppm	<1,0 ^a	<1,0 ^a	3,21 ^a
50 ppm	<1,0 ^a	<1,0 ^a	3,15 ^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. Results of physical and chemical analysis of beers with the addition of rhubarb pulp

	CB	RB0	RB10	RB50
Apparent extract (%; m/m)	5.88 ^d ±0.04	2.96 ^b ±0.06	2.03 ^a ±0.03	3.14 ^c ±0.04
Real extract (%; m/m)	6.02 ^d ±0.02	3.33 ^b ±0.03	3.94 ^c ±0.04	1.88 ^a ±0.02
Original extract (%; m/m)	17.52 ^d ±0.02	14.02 ^c ±0.01	13.91 ^b ±0.04	11.68 ^a ±0.02
Degree of final apparent attenuation (%)	66.44 ^a ±0.04	78.89 ^c ±0.01	85.41 ^d ±0.04	73.12 ^b ±0.03
Degree of final real attenuation (%)	65.64 ^a ±0.04	76.25 ^c ±0.05	71.68 ^b ±0.02	84.12 ^d ±0.03
Content of alcohol (%; v/v)	6.12 ^d ±0.02	5.58 ^c ±0.03	5.20 ^b ±0.10	5.06 ^a ±0.06
Content of alcohol (%; m/m)	4.89 ^d ±0.01	4.44 ^c ±0.04	4.14 ^b ±0.02	4.03 ^a ±0.03
Colour (EBC units)	19.4 ^a ±0.2	23.8 ^d ±0.2	21.0 ^b ±0.5	22.9 ^c ±0.2
Titrateable acidity (0.1 M NaOH/100 mL)	2.72 ^a ±0.02	3.06 ^b ±0.06	3.22 ^c ±0.03	4.94 ^d ±0.04
pH	4.83 ^d ±0.03	4.62 ^c ±0.02	4.54 ^b ±0.03	3.94 ^a ±0.02
Bitter substances (IBU)	15.1 ^a ±0.5	17.1 ^b ±0.2	18.7 ^c ±0.3	21.7 ^d ±0.2
Content of carbon dioxide (%)	0.47 ^a ±0.03	0.43 ^a ±0.03	0.44 ^a ±0.04	0.46 ^a ±0.04
Energy value (kcal/100 mL)	67.49 ^c ±0.04	52.55 ^b ±0.05	52.40 ^b ±0.10	42.99 ^a ±0.01

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 rhubarb; RB0 – wheat beer with the addition of non-ozonated rhubarb; RB10 – wheat beer with the addition of ozonated (10 ppm) rhubarb; RB50 – wheat beer with the addition of ozonized (50 ppm) rhubarb

The beer without the addition of rhubarb pulp (17.52% m/m) was characterized by a definitely higher original extract, while the lowest value was obtained for wheat beer enriched with ozonated (50 ppm) rhubarb pulp (11.68% m/m; Table 2). Wheat beers supplemented with crushed lemongrass were characterized by a mean apparent extract of 2.29% m/m, while the average original extract was at the level of 11.43% m/m (Belcar & Gorzelany, 2022), while the addition of sea buckthorn fruit juice ordinary extract resulted in a higher content of apparent extract and original extract of craft wheat beers in relation to wheat beers enriched with rhubarb pulp (Belcar & Gorzelany, 2022).

Fermentation of beers product affects not only the composition of flavor and aroma, but also the content of individual beer ingredients, including ethyl alcohol (Mascia et al., 2014). The highest grade of final real and apparent attenuation was found in wheat beers enriched with rhubarb pulp treated with ozone gas at a concentration of 10 ppm (apparent) and 50 ppm (final degree of real attenuation; Table 2). The grade of beer attenuation affects the content of alcohol in the finished beer product. The supreme ethanol content was found in the beer without rhubarb (CB), while the addition of non-ozonated rhubarb pulp reduced the concentration by an average of 8.82%, an average of 15.03% (ozone content of 10 ppm) and an average of 17.32% (ozone concentration of 50 ppm) for the addition of ozonized rhubarb pulp in relation to the CB beer (Table 2). In the studies of Baigts-Allende et al. (2021), beers with the addition of citrus were characterized by an ethyl alcohol content of 4.0-8.2% v/v, while in the studies of Patraşcu et al. (2018) the ethanol content in beers with lemon was 1.9 - 4.0% v/v, and in grapefruit beers 1.9 - 2.5% v/v. The content of ethyl alcohol in wheat beers and their extractability considerably affect the energy value of the beers product. Wheat beer without the addition of rhubarb (CB) was characterized by the energy value of the beer product at the level of 67.49 kcal/100 mL, while the

enrichment of wheat beer with rhubarb pulp reduced the energy value of the obtained beer products, especially for RB50 beer (Table 2).

The color of the obtained beer beverages was at a similar standard, the lowest color was found in wheat beer (CB), and the supplement of rhubarb pulp darkened the color of the finished product, and the greatest difference was observed for beer larded without ozone pulp (Table 2; Figure 1).

The lowest acidity of the beer was found in wheat beer without the addition of pulp, and the addition of rhubarb raised the acidity of the finished product, mainly the supplement of ozonized rhubarb tails. The average pH of wheat beers was 4.48, with the highest pH of the control beer, and the lowest value in the beer product enriched with ozonized (50 ppm) rhubarb pulp (Table 2). Beers with a low pH are the most microbiologically stable and quality due to the limit of the growth of undesirable microflora in the beer (Gasiński et al., 2020).

The bitterness of the obtained wheat beers with addition of rhubarb pulp was considerably higher than the control wheat beer (CB), on average by 11.70% for non-ozonated rhubarb and on average by 19.25% and 30.41% for ozonized (10 ppm and 50 ppm) rhubarb petioles (Table 2). Among vegetables, the addition of artichoke to beers caused appropriate sensory impressions, including the feeling of bitterness, while the addition of sweet purple potato and eggplant peel influenced the intensity of the overall color of the finished beer product (pink color) and the high content of anthocyanins (De Simone et al., 2021). The sense of aftertaste of bitterness in the beers comes from the substrate material used for beer production (hops), isomerization of α -acids at boiling point of the wort with hops, and reaction of polyphenols with proteins from malt, but also from the used supplements (Byeon et al., 2021; Habschied et al., 2021). The content of carbon dioxide in all beers product was at a similar level (0.43 - 0.47%; Table 2).



Figure 1. Appearance of the obtained wheat beers - from the left: control (CB), wheat beer with the addition of non-ozonated rhubarb (RB0), and with the addition of ozonated (10 ppm and 50 ppm) rhubarb (RB10 and RB50)

Microbiological quality of wheat beers with the addition of rhubarb

Table 3 presents the results of quality of the microbiological test of beers with the addition of pulp obtained from fragmented rhubarb. Other types of yeast (eg. *Saccharomyces cerevisiae*) than those added for a purpose for the fermentation should not be detected in the wort. The occurrence of other, wild yeasts contributes to formation of an phenolic lingering flavour in the finished beer, and also affects the beer cloudiness and its too much amount of carbon dioxide (Bokulich & Bamforth, 2013).

In the case of lactic fermentation bacteria, the addition of non-ozonated rhubarb in the beer production process resulted in an increase in the load with the tested bacteria compared to beer without this vegetable. The application of an ozone dose of 10 ppm to the raw material used as an addition to beer on the 7th day of fermentation did not reduce the number of analyzed bacteria in the beer after 15 days of storage. On the other hand, the ozone dose of 50 ppm effectively reduced the amount of tested microorganisms compared to other types of beer (Table 3). On the 45th day of storage of the tested beers, in the case of mesophilic lactic acid bacteria, it was observed that the beer with the addition of non-ozonated rhubarb was characterized by the highest number of tested bacteria with comparison to the another types of beer (Table 3). Lactic acid bacteria, in particular of the genus *Lactobacillus* and *Pediococcus*, cause beer spoilage to the greatest extent, because they are resistant to the temperatures of the wort preparation process with addition of hops and the high concentration of ethyl alcohol in the beer product (Silvetti et al., 2010).

In the case of beer without the addition of rhubarb, the highest amount of mesophilic aerobic bacteria

was found. The addition of ozonized rhubarb had a good effect on reducing the number of tested bacteria in the final product. On the 15th day of beer storage, it was noted that the use of ozone at a concentration of 10 ppm reduced the number of tested bacteria by 5.5% compared to beer with the addition of rhubarb without ozone. On the other hand, the ozone concentration of 50 ppm slightly decreased the number of tested bacteria in comparison to beer with the addition of non-ozonated rhubarb petioles (Table 3). The beneficial effect of ozonation was observed by examining the number of mesophilic aerobic bacteria after 45 days of beer storage. The addition of rhubarb ozonated with a dose of 10 ppm and a dose of 50 ppm reduced the microbiological load of the tested beers by 9.9% and 9.3%, respectively, compared to beer with the addition of rhubarb without ozone (Table 3). The most favorable effect of the ozonation process was noted for the number of yeasts and molds in finished beer product. The proposed addition of non-ozonated rhubarb to beer increased the number of yeasts and molds by 4% compared to beer without this addition. Ozonation of rhubarb with a dose of 50 ppm decreased the amount of tested microorganisms in the tested beer by 19.7% compared to beer with the addition of rhubarb not subjected to ozonation. In the case of yeast and mold on the 45th day of storage of the analyzed types of beer, a similar dependence was observed as for the analyzes performed on the 15th day (Table 3). The ozonation process of the raw material being an addition to beers decreased the number of yeasts and molds in the analyzed beer compared to other types of beers. The most favorable results in the lowering of the number of yeasts and molds were observed for beer supplemented with rhubarb petioles ozonated with a dose of 50 ppm (Table 3).

Table 3. Microbiological quality of wheat beers enriched with rhubarb

	Fermentation day after bottling the wheat beer	The number of yeasts and molds (log cfu mL ⁻¹)	The number of mesophilic aerobic bacteria (log cfu mL ⁻¹)	The number of mesophilic lactic acid bacteria (log cfu mL ⁻¹)
CB	15	3.23 ^b	3.63 ^b	3.40 ^a
	45	3.43 ^b	3.80 ^b	3.58 ^a
RB0	15	3.48 ^c	3.26 ^a	3.63 ^a
	45	3.64 ^c	3.45 ^b	3.74 ^a
RB10	15	3.11 ^a	3.08 ^a	3.81 ^b
	45	3.47 ^a	3.38 ^a	3.88 ^a
RB50	15	3.11 ^a	3.20 ^a	3.20 ^a
	45	3.28 ^a	3.30 ^a	3.43 ^a

Data are expressed as a mean values ($n = 3$) $\pm$ 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 rhubarb; RB0 – wheat beer with the addition of non-ozonated rhubarb; RB10 – wheat beer with the addition of ozonated (10 ppm) rhubarb; RB50 – wheat beer with the addition of ozonized (50 ppm) rhubarb

The quality of fruit or vegetable beers depends to a large degree on the quality of raw materials used during the production process (Gorzelay et al., 2022). The process of ozonation of plant raw materials used in food processing causes their disinfection and extends storage life (Zardzewiały et al., 2020). The results of this process are visible during the fermentation process, the addition of ozonated raw materials does not interfere with this process. In addition, the use of gaseous ozone improves the aroma and taste of the final beer product (Gorzelay et al., 2022). Gaseous ozone significantly extends the storage life of vegetables. In the case of carrots, its effect resulted in a decreasing of the number of tested microorganisms such as *E. coli* by 1.2 log CFU g⁻¹, *Listeria monocytogenes* by 0.8 log CFU g⁻¹ and *Salmonella* by 0.5 log CFU g⁻¹ (Bridges et al., 2018). The application of gaseous ozone at a dose of 1 ppm, 5 minutes reduced mesophilic aerobic bacteria by 0.4 log CFU g⁻¹ and the number of yeasts and molds by 0.6–0.7 log CFU g⁻¹ (Alegria et al., 2009). Ozonation of dates reduced the number of molds and yeasts in the tested samples. After the process of ozonation of fruits, their packaging and storage for 9 months at 25°C, the number of molds and yeasts in the tested samples decreased by about 94% compared to the number of molds and yeasts in non-ozonated samples (Farajzadeh et al., 2013). Gaseous ozone applied to the fruit of Saskatoon berries reduced the growth rate of microorganisms in comparison with non-ozonated fruit. The ozone dose of 10 ppm during 30 minutes had the most advantageous impact on reduction the number of aerobic bacteria, yeasts and molds throughout the storage time of the tested fruits (Matłok et al., 2022). In fruit beer produced in a craft brewery after bottling, the amount of yeast was at the level of 4.6 x 10⁴ cfu mL⁻¹ (Jakucs & Óré-Sütő, 2022). Craft beers contain live microorganisms due to the lack of pasteurization, which is why their direct

consumption is so important (Jagodziński et al., 2016).

Organoleptic analysis of wheat beers enriched with rhubarb

The sale of the finished beer product depends significantly on its attractiveness and acceptance by the final recipient, i.e. the consumer. Aroma and taste qualities of wheat beers supplemented with rhubarb pulp may influence consumers' preferences to buy a given beer or this purchase will be only a one-time purchase. The results of the organoleptic evaluation of beers carried out by a group of 11 people are presented in Table 4 and Figure 2. The aroma of wheat beers was characterized by a significantly more desirable intensity with the increase in the concentration of gaseous ozone, which was treated with rhubarb in relation to the control (CB; Table 4), moreover, wheat beer enriched with rhubarb pulp, regardless of the ozonation process, was characterized by a considerable higher taste than to the control beer product (CB; Table 4). In wheat beers aroma and taste are values derived from malt and cereals, as well as the very intensity and fullness of flavor and freshness were derived by from the addition of rhubarb (Figure 2.). Both raw materials and fermentation products (e.g. esters) significantly affect the organoleptic qualities of beer. Stability and durability of beer foam was at a similar level in wheat beers enriched with rhubarb, while wheat beer (CB) was characterized by less stable beer foam (Table 4). The feeling of bitterness in the analyzed wheat beers was at a such level, regardless of the addition of rhubarb. The most positive overall impression for the judges turned out to be beer supplemented with ozonated (ozone concentration 50 ppm) rhubarb pulp to improve the sensory qualities of craft wheat beers (Table 4; Figure 2).

Table 4. Organoleptic analysis of wheat beers enriched with rhubarb

	CB	RB0	RB10	RB50
Aroma	3.71 ^a ±0.11	3.93 ^a ±0.07	4.02 ^a ±0.30	4.33 ^a ±0.21
Taste	3.49 ^a ±0.24	4.14 ^b ±0.32	4.16 ^b ±0.16	4.17 ^b ±0.12
Foam stability	3.13 ^a ±0.17	3.42 ^b ±0.22	3.63 ^b ±0.18	3.57 ^b ±0.17
Bitterness	3.75 ^a ±0.25	3.61 ^a ±0.17	3.65 ^a ±0.11	3.69 ^a ±0.23
Saturation	4.12 ^b ±0.09	3.68 ^a ±0.15	3.68 ^a ±0.26	3.72 ^a ±0.33
Overall impression	3.53 ^a ±0.38	3.84 ^{ab} ±0.36	3.86 ^{ab} ±0.44	3.97 ^b ±0.32

Data are expressed as a mean values (n = 11) ± 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 rhubarb; RB0 – wheat beer with the addition of non-ozonated rhubarb; RB10 – wheat beer with the addition of ozonated (10 ppm) rhubarb; RB50 – wheat beer with the addition of ozonated (50 ppm) rhubarb

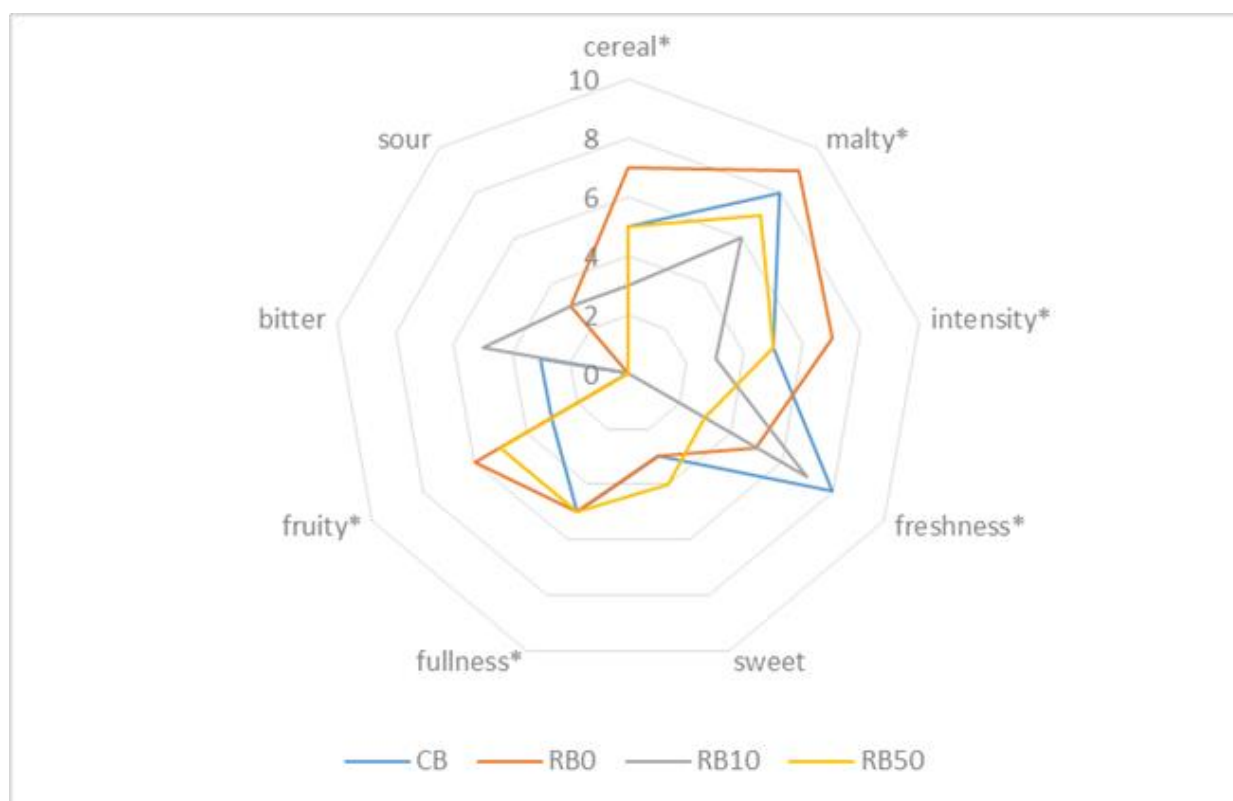


Figure 2. Organoleptic profile of wheat beers - control (CB) and with the addition of non-ozonated rhubarb (RB0) and ozonated (10 ppm; RB10 and 50 ppm; RB50) rhubarb (* indicates attributes which were statistically different at $p < 0.05$)

CONCLUSIONS

The conducted research on the possibility of using gaseous ozone to extend the microbiological stability of rhubarb petioles showed the highest limiting multiplication in the number of yeasts and molds when using ozone at a concentration of 50 ppm for 30 minutes. The use of ozonated rhubarb as an additive enriching wheat beers presented that the most sustainable flavor and aroma profile was found in beer enriched with ozonized (50 ppm) rhubarb pulp. In addition, this beer was characterized by the supreme degree of final real attenuation, the lowest

pH and energy value in relation to other wheat beers. The finished beer product marked as RB50 was characterized by the highest microbiological stability among the analyzed wheat beers. Enriching beers with fragmented rhubarb may be a new trend to increasing the range of new product of beers, and the use of gaseous ozone, especially at a concentration of 50 ppm for 30 minutes, may be a trend to extend the microbiological stability of beers, especially craft beers.

FUNDING

The project is financed by the program of the Minister of Education and Science named "Regional Initiative of Excellence" in the years 2019-2023, project number 026/RID/2018/19, the amount of financing PLN 9 542 500.00.

REFERENCES

1. Alegria C., Pinheiro J., Gonçalves E.M., Fernandes I., Moldão M., Abreu M. 2009. Quality attributes of shredded carrot (*Daucus carota* L. cv. Nantes) as affected by alternative decontamination processes to chlorine. *Innovative Food Science & Emerging Technologies*, 10, 61–69. DOI:10.1016/j.ifset.2008.08.006
2. Analytica EBC 13.13 - Sensory Analysis: Routine Descriptive Test Guideline. (2004). *European Brewery Convention*. Hans Carl Getränke-Fachverlag, Nürnberg.
3. Baigts-Allende D. K., Pérez-Alva A., Ramírez-Rodrigues M. A., Palacios A., Ramírez-Rodrigues M. M. 2021. A comparative study of polyphenolic and amino acids profiles of commercial fruit beers. *Journal of Food Composition and Analysis*, 100, 103921. DOI:10.1016/j.jfca.2021.103921
4. Belcar J., Buczek J., Kapusta I., Gorzelany J. 2022. Quality and Pro-Healthy Properties of Belgian Witbier-Style Beers Relative to the Cultivar of Winter Wheat and Raw Materials Used. *Foods*, 11, 1150. <https://doi.org/10.3390/foods11081150>
5. Belcar J., Gorzelany J. 2022. Feasibility of Defatted Juice from Sea-Buckthorn Berries (*Hippophae rhamnoides* L.) as a Wheat Beer Enhancer. *Molecules*, 27, 3916. <https://doi.org/10.3390/molecules27123916>
6. Belcar, J.; Gorzelany, J. 2022. Effect of the Addition of Lemongrass (*Cymbopogon citratus*) on the Quality and Microbiological Stability of Craft Wheat Beers. *Molecules*, 27, 9040. <https://doi.org/10.3390/molecules27249040>
7. Bokulich N.A., Bamforth Ch.W. 2013. The Microbiology of Malting and Brewing. *Microbiology and Molecular Biology Reviews*, 77(2), 157-172. doi: 10.1128/MMBR.00060-12
8. Bridges D.F., Rane B., Wu V.C.H. 2018. The effectiveness of closed-circulation gaseous chlorine dioxide or ozone treatment against bacterial pathogens on produce. *Food Control*, 91, 261–267. DOI:10.1016/j.foodcont.2018.04.004
9. Brodowska A.J., Nowak A., Śmigielski K. 2018. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. *Critical Reviews In Food Science And Nutrition*, 58(13), 2176-2201. doi: 10.1080/10408398.2017.1308313
10. Byeon Y. S., Lim S.-T., Kim H.-J., Kwak H. S., Kim S. S. 2021. Quality Characteristics of Wheat Malts with Different Country of Origin and Their Effect on Beer Brewing. *Journal of Food Quality*. ID:2146620. <https://doi.org/10.1155/2021/2146620>
11. Bokulich N.A., Bamforth Ch.W. 2013. The Microbiology of Malting and Brewing. *Microbiology and Molecular Biology Reviews*, 77(2), 157-172. doi: 10.1128/MMBR.00060-12
12. Callemien D., Jerkovic V., Rozenberg R., Collin S. 2005. Hop as an interesting source of resveratrol for brewers: Optimization of the extraction and quantitative study by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 53, 424–429. doi: 10.1021/jf040179n
13. De Simone N., Russo P., Tufariello M., Fragasso, M., Solimando M., Capozzi V., Grieco F., Spano G. 2021. Autochthonous Biological Resources for the Production of Regional Craft Beers: Exploring Possible Contributions of Cereals, Hops, Microbes, and Other Ingredients. *Foods*, 10, 1831. <https://doi.org/10.3390/foods10081831>
14. Farajzadeh D., Qorbanpoor A., Rafati H., Isfeedvajani M.S. 2013. Reduction of date microbial load with ozone. *Journal of Research in Medical Sciences*, 18(4), 330-334.
15. Gasiński A., Kawa-Rygielska J., Szumny A., Czubaszek A., Gąsior J., Pietrzak W. 2020. Volatile Compounds Content Physicochemical Parameters and Antioxidant Activity of Beers with Addition of Mango Fruit (*Mangifera Indica*). *Molecules*, 25(13), 3033. <https://doi.org/10.3390/molecules25133033>
16. Gorinstein S., Caspi A., Libman E., Leontowicz H., Leontowicz M., Tahsma Z., Trakhtenberg S. 2007. Bioactivity of beer and its influence on human metabolism. *International Journal of Food Science and Nutrition*, 58, 94–107.
17. Gorzelany J., Patyna M., Pluta S., Kapusta I., Balawejder M., Belcar J. 2022. The Effect of the Addition of Ozonated and Non-Ozonated Fruits of the Saskatoon Berry (*Amelanchier alnifolia* Nutt.) on the Quality and Pro-Healthy Profile of Craft Wheat Beers. *Molecules*, 27, 4544. <https://doi.org/10.3390/molecules27144544>
18. Gorzelany J., Michałowska D., Pluta S., Kapusta I., Belcar J. 2022. Effect of Ozone-Treated or Untreated Saskatoon Fruits (*Amelanchier alnifolia* Nutt.) Applied as an Additive on the Quality and Antioxidant Activity of Fruit Beers. *Molecules*, 27, 1976. <https://doi.org/10.3390/molecules27061976>

19. Gorzelany J., Migut D., Matłok N., Antos P., Kuźniar P., Balawejder M. 2017. Impact of Pre-Ozonation on Mechanical Properties of Selected Genotypes of Cucumber Fruits During the Souring Process. *Ozone Science & Engineering*, 39, 188–195. <https://doi.org/10.1080/01919512.2016.1273756>
20. Granato D., Branco G.F., Faria Jde A., Cruz A.G. 2011. Characterization of Brazilian lager and brown ale beers based on color, phenolic compounds, and antioxidant activity using chemometrics. *Journal of the Science of Food and Agriculture*, 91, 563–57. doi: 10.1002/jsfa.4222
21. Guo H., Wang Y., Yao K., Yang L., Cheng S. 2022. Comparisons of Combined Oxidant Capacity and Redox-Weighted Oxidant Capacity in Their Association with Increasing Levels of COVID-19 Infection. *Atmosphere*, 13, 569. <https://doi.org/10.3390/atmos13040569>
22. Habschied K., Košir I.J., Krstanović V., Kumrić G., Mastanjević K. 2021. Beer Polyphenols – Bitterness, Astrigency, and Off-Flavors. *Beverages*, 7, 38. <https://doi.org/10.3390/beverages7020038>
23. Hayward L., Wedel A., McSweeney M.B. 2019. Acceptability of beer produced with dandelion, nettle and sage. *International Journal of Gastronomy and Food Science*, 18, 100180. DOI:10.1016/j.ijgfs.2019.100180
24. He G., Du J., Zhang K., Wei G., Wang W. 2012. Antioxidant capability and potableness of fresh cloudy wheat beer stored at different temperatures. *Journal of the Institute of Brewing*, 118, 386–392. <https://doi.org/10.1002/jib.54>
25. Huang Z., Xu Y., Wang Q., Gao, X. 2019. Metabolism and mutual biotransformations of anthraquinones and anthrones in rhubarb by human intestinal flora using UPLC-QTOF/ MS. *Journal of Chromatography B*, 1104, 59–66. doi: 10.1016/j.jchromb.2018.10.008
26. Jagodziński J., Dziągowski S., Krzywonoś M. 2016. Microbiological stability of home unpasteurized beers. *Acta Sci. Pol., Biotechnology*. 15(3), 15–24. (in Polish)
27. Jakuos L., Óré-Sütő B.V. 2022. Initial microbiological experience in small-scale fruit beer product development. *Journal of Food Investigation*, 68(2), 3877–3887. DOI: <https://doi.org/10.52091/EVIK-2022/2-2-ENG>
28. Jintao X., Yongli S., Liming Y., Quanwei Y., Chunyan L., Xingyi C. 2018. Nearinfrared spectroscopy for rapid and simultaneous determination of five main active components in rhubarb of different geographical origins and processing. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 205, 419–427. doi: 10.1016/j.saa.2018.07.055
29. Kausar H., Saeed S., Ahmad M.M., Salam A. 2012. Studies on the development and storage stability of cucumber-melon functional drink. *Journal of Agriculture Research*, 50, 239–248.
30. Krafczyk N., Kötké M., Lehnert N., Glomb M.A. 2008. Phenolic composition of rhubarb. *European Food Research and Technology*, 228(2), 187–196. DOI:10.1007/s00217-008-0922-y
31. Leitao C., Marchioni E., Bergaentzlé M., Zhao M., Didierjean L., Miesch L., Holder E., Miesch M., Ennahar S. 2012. Fate of polyphenols and antioxidant activity of barley throughout malting and brewing. *Journal of Cereal Science*, 55, 318–322. doi:10.1016/j.jcs.2012.01.002
32. Liudvytska O., Kolodziejczyk-Czepas J. 2022. A Review on Rhubarb-Derived Substances as Modulators of Cardiovascular Risk Factors — A Special Emphasis on Anti-Obesity Action. *Nutrients*, 14, 2053. <https://doi.org/10.3390/nu14102053>
33. Mascia I., Fadda C., Dostálek P., Olšovská J., Del Caro A. 2014. Preliminary characterization of an Italian craft durum wheat beer. *Journal of the Institute of Brewing*, 120, 495–499. <https://doi.org/10.1002/jib.176>
34. Matłok N., Piechowiak T., Zardzewiały M., Balawejder M. 2022. Effects of Post-Harvest Ozone Treatment on Some Molecular Stability Markers of *Amelanchier alnifolia* Nutt. Fruit during Cold Storage. *International Journal of Molecular Sciences*, 23, 11152. <https://doi.org/10.3390/ijms231911152>
35. Matłok N., Stępień A.E., Gorzelany J., Wojnarowska-Nowak R., Balawejder M. 2020. Effects of Organic and Mineral Fertilization on Yield and Selected Quality Parameters for Dried Herbs of Two Varieties of Oregano (*Origanum vulgare* L.). *Applied Sciences*, 10, 5503. <https://doi.org/10.3390/app10165503>
36. Matłok N., Piechowiak T., Gorzelany J., Zardzewiały M., Balawejder M. 2020. Effect of Ozone Fumigation on Physiological Processes and Bioactive Compounds of Red-Veined Sorrel (*Rumex sanguineus* ssp. *sanguineus*). *Agronomy*, 10, 1726. <https://doi.org/10.3390/agronomy10111726>
37. Matyjaszczyk E., Dobrzański A. 2015. Weed management in rhubarb (*Rheum raponticum* L.) in Poland and other countries. *Progress in Plant Protection*, 55 (4), 466–471. <http://dx.doi.org/10.14199/ppp-2015-077>

38. Ong M.K., Ali A. 2015. Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. *Postharvest Biology and Technology*, 100, 113-119. DOI:10.1016/j.postharvbio.2014.09.023
39. Patraşcu L., Banu I., Bejan M., Aprodu I. 2018. Quality parameters of fruit beers available on Romanian market. *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, 19(3), 323-335.
40. PB-77/LM ed. 5. The number of mesophilic aerobic bacteria. 2022. Factory standards of Laboratory GBA POLSKA Sp. z o.o., Legionowo, Poland.
41. PN-ISO 15214:2002. 2002. Food and feed microbiology - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Plate method at 30 degrees C. Polish Committee for Standardization: Warsaw, Poland.
42. PN-ISO 21527:2009. 2009. Food and feed microbiology - Horizontal method for the enumeration of yeasts and molds - Part 1: Colony counting method in products with a water activity greater than 0.95. Polish Committee for Standardization: Warsaw, Poland.
43. Pretell-Vásquez C., Márquez-Villacorta L., Siche R., Hayayumi-Valdivia M. 2022. Optimization of ozone concentration and storage time in green asparagus (*Asparagus officinalis* L.) using response surface methodology. *Vitae*, 28, 3. <https://doi.org/10.17533/udea.vitae.v28n3a346752>
44. Qi L.J., Li Y.L., Luo X.H., Wang R., Zheng R., Wang L., Li Y.F., Yang D., Fang W.M., Chen Z.X. 2016. Detoxification of zearalenone and ochratoxin A by ozone and quality evaluation of ozonised corn. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 33(11), 1700-1710. doi: 10.1080/19440049.2016.1232863
45. Rai R., Agrawal M. 2012. Impact of Tropospheric Ozone on Crop Plants. *Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences*, 82(2), 241–257. DOI:10.1007/s40011-012-0032-2
46. Ritter S., Doelle K., Barga M., Piatkowski J. 2016. Fruits in Craft Beer: A Study to Evaluate the Impact of Fruits on the pH in the Brewing Process and the Breweries Waste Water. *Advances in Research*, 8, 1-8. DOI:10.9734/AIR/2016/30228.
47. Rošul M.D., Mandić A.I., Mišan A.Č., Derić N.R., Pejin J.D. 2019. Review of trends in formulation of functional beer. *Food Feed Research*, 46, 23–35. DOI: 10.5937/FFR1901023R
48. Sánchez-Bravo P., Ángel Abellán, P., J. Zapata J., García-Viguera C., Domínguez-Perles R., Giménez M.J. 2023. Broccoli products supplemented beers provide a sustainable source of dietary sulforaphane. *Food Bioscience*, 51, 102259, 2212-4292. <https://doi.org/10.1016/j.fbio.2022.102259>.
49. Silveti T., Brasca M., Lodi R., Vanoni L., Chiolerio F., de Groot M., Bravi A. 2010. Effect of Lysozyme on the Microbiological Stability and Organoleptic Properties of Unpasteurized Beer. *Journal of the Institute of Brewing*, 116(1), 33-40. <https://doi.org/10.1002/j.2050-0416.2010.tb00395.x>
50. Stachowiak B., Bukowski K. 2021. New trends in the brewing industry. Functional beers — Raw materials and technological aspects of their production. *FOOD Science Technology Quality*, 28, 5–27. DOI: 10.15193/zntj/2021/126/365 (In Polish)
51. Wootton-Beard P.C., Ryan L. 2011. Improving public health—The role of antioxidant-rich fruit and vegetable beverages. *Food Research International*, 44, 3135–3148. <https://doi.org/10.1016/j.foodres.2011.09.015>
52. Xiang H., Zuo J., Guo F., Dong D. 2020. What we already know about rhubarb: a comprehensive review. *Chinese Medicine*, 15,88.
53. Xiong F., Nie X., Zhao X., Yang L., Zhou G. 2019. Effects of different nitrogen fertilizer levels on growth and active compounds of rhubarb from Qinghai plateau. *Journal of the Science of Food and Agriculture*, 99(6), 2874–2882. doi: 10.1002/jsfa.9500
54. Xue W., Macleod J., Blaxland J. 2023. The Use of Ozone Technology to Control Microorganism Growth, Enhance Food Safety and Extend Shelf Life: A Promising Food Decontamination Technology. *Foods*, 12, 814. <https://doi.org/10.3390/foods12040814>
55. Yaseen T., D'Onghia A., Ricelli A. 2012. The use of ozone in strawberry post harvest conservation. Conference: *Biological Control of Fungal and Bacterial Plant Pathogens At: Reims, France*. Volume: IOBC-WPRS Bulletin 86.
56. Zapałowska A., Matłok N., Zardzewiały M., Piechowiak T., Balawejder M. 2021. Effect of Ozone Treatment on the Quality of Sea Buckthorn (*Hippophae rhamnoides* L.). *Plants*, 10, 847. <https://doi.org/10.3390/plants10050847>

57. Zardzewiały M., Matłok N., Piechowiak T., Gorzelany J., Balawejder M. 2020. Ozone Treatment as a Process of Quality Improvement Method of Rhubarb (*Rheum rhaponticum* L.) Petioles during Storage. *Applied Sciences*, 10, 8282. <https://doi.org/10.3390/app10228282>
58. Zhong Z., Wang X., Yin, X., Tian J., Komatsu S. 2021. Morphophysiological and Proteomic Responses on Plants of Irradiation with Electromagnetic Waves. *International Journal of Molecular Sciences*, 22, 12239. <https://doi.org/10.3390/ijms222212239>