*Article*

# Generation of Hydrogen Peroxide in Beer and Selected Strong Alcoholic Beverages

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**Abstract:** The generation of hydrogen peroxide has been documented in various plant-based beverages, such as coffee, tea, herbal infusions and wine, as well in energy drinks containing ascorbate and in plant-based food. There are no data in the literature on the presence and generation of hydrogen peroxide in beer and strong alcoholic beverages containing plant material. This study aimed to examine whether beer and selected strong alcoholic beverages (brandy, whisky and fruit liqueurs) contain hydrogen peroxide. The presence of hydrogen peroxide was found in freshly opened brandy, whisky, liqueurs and most diluted beers; subsequent incubation in an air atmosphere led to the generation of hydrogen peroxide. The presence of the electron paramagnetic resonance (EPR) signal of the semiquinone radical and the generation of the superoxide radical demonstrated in selected alcoholic beverages by the superoxide dismutase-inhibitable reduction of Nitrotetrazolium Blue and oxidation of dihydroethidium are in agreement with the two-step mechanism of generation of hydrogen peroxide by the autoxidation of phenolics. These results broaden the list of beverages containing and producing hydrogen peroxide.

**Keywords:** beer; brandy; hydrogen peroxide; polyphenols; semiquinone radical; superoxide; liqueurs; whisky

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## 1. Introduction

Hydrogen peroxide ($H_2O_2$) is an oxidant of considerable biological significance. Concentrated hydrogen peroxide is a strong oxidant, used for bleaching and as a bactericidal agent [1,2]. Concentrations of $H_2O_2$ ranging from 3 to 30% (0.8 to 8 M) are commonly used for disinfection and sanitization of medical equipment [3]. At the other extreme of the concentrations, nano- to low-micromolar concentrations of $H_2O_2$ play a role in intra- and intercellular signaling [4–7]. Hydrogen peroxide is present in beverages in micromolar amounts. Reports demonstrating the presence of hydrogen peroxide in beverages and food point to another source of hydrogen peroxide in the organism. They proved that polyphenol-rich beverages, especially tea and coffee, produce $H_2O_2$ upon brewing and subsequent standing [8–14]. Hydrogen peroxide was also found in infusions of medicinal herbs [15], hydrated protein drink mixes [16], formulated beverages [17,18], cooked vegetables and spices [19–21].

It is of interest whether $H_2O_2$ is also produced in alcoholic beverages. Generation of $H_2O_2$ was demonstrated during accelerated oxidation of wine [22]. Neither of the two red wines studied by Chai et al. was found to contain $H_2O_2$, nor did $H_2O_2$ appear on incubation of the red wines at room temperature, although hydrogen peroxide was generated in the wine samples diluted with a cell culture medium [23]. In a study of 40 samples of wines, we found that $H_2O_2$ can be detected in some but not all the wine samples; moreover, it can be both produced and scavenged by wines due to the sulfite present in the wine [24]. To our best knowledge, there are no data on the presence of hydrogen peroxide in other alcoholic beverages except for wine, including both beer and stronger alcoholic beverages. The present study aimed at examining if hydrogen peroxide can be present and generated in other alcoholic beverages, such as beer, brandy, whisky and liqueurs. These beverages also contain polyphenols and other components of plant origin, which may be subject to autoxidation, so the generation of hydrogen peroxide in these beverages could be expected.

In order to elucidate whether the mechanism of hydrogen peroxide generation, based on the two-step polyphenol autoxidation demonstrated to operate in other beverages, can also be valid for alcoholic beverages, the total phenolic content (TPC) was estimated and correlated with the hydrogen peroxide content and generation in these beverages. The generation of the semiquinone free radical, an intermediate product of polyphenol autoxidation, was demonstrated by electron spin resonance (ESR) spectroscopy. The generation of another intermediate, the superoxide radical anion, was checked using superoxide dismutase (SOD)-inhibitable reduction of Nitrotetrazolium Blue (NBT) and SOD-inhibitable oxidation of dihydroethidium (DHE).

## 2. Materials and Methods

### 2.1. Materials

Hydrochloric acid (CAS no. 7647-01-0, cat. no. 115752837, 35–38%), hydrogen peroxide (CAS no. 7722-84-1, cat. no. 118851934, 30%) and perchloric acid (CAS no. 7601-90-3, cat. no. 115649402, 60%) were obtained from Chempur (Piekary Slaskie, Poland). Xylenol Orange (CAS no 3618-43-7, cat. no. 704590231, purity $\leq$ 100%) was obtained from Avantor Performance Materials (Gliwice, Poland). Gallic acid (CAS no. 149-91-7; cat. no. G7384; purity of 97.5–102.5%), Mohr salt (CAS no. 7783-85-9, cat. no. 203505, purity $\geq$ 99.997), Folin–Ciocalteu phenol reagent (cat. no. F9252), sodium carbonate (CAS no. 497-19-8, cat. no. 106392), dimethylsulfoxide (DMSO; CAS no. 67-68-5, cat. no. 472301), catalase (CAS no. 9001-05-2, cat. no. C40; $\geq$10,000 units/mg protein), superoxide dismutase (SOD; CAS no. 9054-89-1, cat. no. S9697, Nitrotetrazolium Blue (NBT; CAS no. 298-83-9, cat. no. N6876) and dihydroethidium (CAS no. 104821-25-2, cat. no. 309800) were purchased from Merck (Poznan, Poland).

Bottled beer, whisky and most of the liqueurs (strong alcoholic drinks containing fruit or herbal extracts) were bought from local shops. Their characteristics are given in Tables S1 and S2. Moreover, home-made cherry (Cherry 2), dogwood and curcuma liqueurs (Table S2) were studied.

Distilled water was deionized with a Milli-Q system (Millipore, Bedford, MA, USA). The transparent (cat. no. 655101) and black (cat. no. 655076) flat-bottom 96-well plates used for the assays were provided by Greiner (Kremsmunster, Austria). Absorbance and fluorescence measurements performed done in a Spark multimode plate reader (Tecan Group Ltd., Männedorf, Switzerland).

### 2.2. Determination of the Polyphenol Concentration

The concentration of polyphenols in the beverages was estimated with the Folin–Ciocalteu reagent [25]. Briefly, 20 μL of a beverage (diluted if necessary) was added to

a well of a 96-well plate containing 100 μL of 10-fold diluted Folin–Ciocalteu reagent. After 4 min, 80 μL of saturated sodium carbonate solution (ca 75 g/L) was added. After a 60 min incubation at ambient temperature, the absorbance was measured at 750 nm. Gallic acid was used as a standard. The results were expressed in mmoles of gallic acid equivalents (GAE)/L.

### 2.3. Determination of Hydrogen Peroxide Concentration

The concentration of $H_2O_2$ was determined using a "reagent blank" modification [15] of the ferrous oxidation–xylenol orange (FOX) method [26]. Briefly, two 180 μL aliquots of each beverage were pipetted into two wells of a transparent 96-well plate. To one well was added 20 μL of the xylenol orange reagent (2.5 mM xylenol orange/2.5 mM Mohr salt in 1.1 M perchloric acid) and 20 μL of a blank reagent containing the Mohr salt and perchloric acid, but no xylenol orange was added to the other well. In parallel, a blank for xylenol orange was prepared: to two wells with 180 μL of water was added 20 μL of either the xylenol orange reagent or the blank reagent (a blank for the xylenol orange reagent). The plate was incubated for 30 min and the absorbance of the samples was measured at 560 nm. The concentration of hydrogen peroxide was calculated from the corrected absorbance $A_{corr}$:

$$A_{corr} = A_{S,XO} - A_{S,B} - (A_{W,XO} - A_{W,B})$$

where $A_{S,XO}$—absorbance of a sample with the xylenol orange reagent added;
$A_{S,B}$—absorbance of a sample with the blank reagent added;
$A_{W,XO}$—absorbance of water with the xylenol orange reagent added;
$A_{W,B}$—absorbance of water with the blank reagent added.

The hydrogen peroxide concentration was calculated from a standard curve obtained with pure hydrogen peroxide. The concentration of hydrogen peroxide solution was determined on the basis of the absorbance at 240 nm, using a molar absorption coefficient of 43.6 $M^{-1}cm^{-1}$ [27].

The generation of hydrogen peroxide in the beverages was estimated by comparing the alcohol concentration in the beverages at "0" time and after 1 h and 3 h of incubation at ambient temperature, with ai access. Strong alcoholic beverages were used without dilution. As no $H_2O_2$ generation was detected in the majority of the undiluted beers, the beers were studied after 4-fold dilution with deionized water.

### 2.4. Superoxide Dismutase-Inhibitable Reduction of Nitrotetrazolium Blue (NBT)

Aliquots (180 μL) of the beverages were added to 20 μL of 2.5 mM NBT and 2 μL of 1 mg/mL solution of SOD in phosphate-buffered saline (PBS) or 2 μL of PBS. The samples were incubated in the dark at room temperature for 180 min and their absorbance was measured at 530 nm. The increase in the absorbance of the aliquots containing SOD was subtracted from that without SOD. The concentration of the generated formazan was calculated using the millimolar absorption coefficient of 25.4 $mM^{-1}$ $cm^{-1}$ [28].

### 2.5. Superoxide Dismutase-Inhibitable Oxidation of Dihydroethidium (DHE)

Aliquots of the beverages (180 μL) were added to of 1 mM dihydroethidium solution in dimethylsulfoxide (DMSO) (20 μL). The samples were incubated in the dark at room temperature for 180 min, and their fluorescence was measured (excitation/emission wavelengths of 405 nm/570 nm, respectively). The fluorescence of the aliquots containing SOD was subtracted from that without SOD. Control samples containing pure alcohol solutions were also measured and the SOD-inhibitable DHE oxidation in these samples was subtracted from that obtained in the beverages.

### 2.6. Electron Paramagnetic Resonance Measurements

Electron paramagnetic resonance (EPR) measurements were performed using a Bruker multifrequency and multiresonance FT-EPR ELEXSYS E580 spectrometer (Bruker Analytische Messtechnik, Rheinstetten, Germany) operating at the X-band (9.378989 GHz). The following settings were used: modulation amplitude, 0.4 G; modulation frequency, 100 kHz; microwave power, 94.64 mW; power attenuation, 2 dB; conversion time, 25 ms; sweep time, 102.4 s; central field, 3353.15 G; scan range, 100 G; accumulation, 10.

The samples (about 30 µL) were measured in glass hematocrit capillary tubes, internal diameter 1 mm (Medlab Products, Raszyn, Poland).

### 2.7. Statistics

The significance of the differences with respect to the zero content of $H_2O_2$ was determined using the two-tailed Student's *t*-test as we were not interested in the differences between the various beverages but in the statistical confirmation of a non-zero concentration of hydrogen peroxide in the analyzed material. The dependence of the initial hydrogen concentration and the $H_2O_2$ generation in the alcoholic beverages on the measured and declared parameters of these beverages was evaluated by multiple regression analysis. Statistical calculations were performed using the STATISTICA software package (version 13.1, StatSoft Inc. 2016, Tulsa, OK, USA).

## 3. Results

### 3.1. Hydrogen Peroxide in Beer and Strong Beverages

Testing the $H_2O_2$ concentration in samples of ten brands of beer brought variable results. Most beers did not contain a measurable level of $H_2O_2$ after opening a bottle, but hydrogen peroxide was present in the majority of samples of beers diluted 4-fold with water, except for the Rauchdoppelbock, Kozel Cerny, Porter Bałtycki and Okocim beers. The average $H_2O_2$ concentration in the samples was 0.33 ± 0.56 (mean ± SD) µM (0–1.8 µM). The results for individual beer samples are shown in Figure 1.

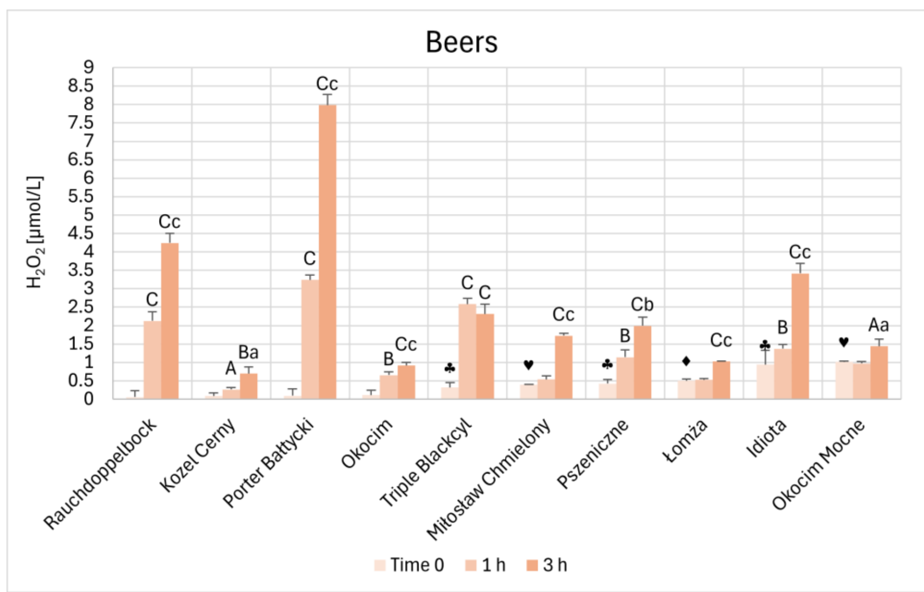


**Figure 1.** Concentration of hydrogen peroxide in various kinds of beer immediately after bottle opening and after 1 and 3 h of incubation at room temperature with air access. Beer samples were diluted 4-fold with deionized water. ♣, $p < 0.05$; ♦, $p < 0.01$; ♥, $p < 0.001$ (with respect to zero concentration); A, $p < 0.05$; B, $p < 0.01$; C, $p < 0.001$ (with respect to 0 time); a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$ (with respect to 1 h of incubation).

The presence and generation of $H_2O_2$ were also checked in strong alcoholic beverages: brandy, whisky as well as commercially available and home-made liqueurs. Low amounts of hydrogen peroxide were found in all the beverages (2.2 ± 0.1 µM in brandy, 21.7 ± 1.0 µM in whisky and 14.3 ± 16.1 µM (0.7–48.3 µM) in various liqueurs). The results for the individual beverages are presented in Figure 2.

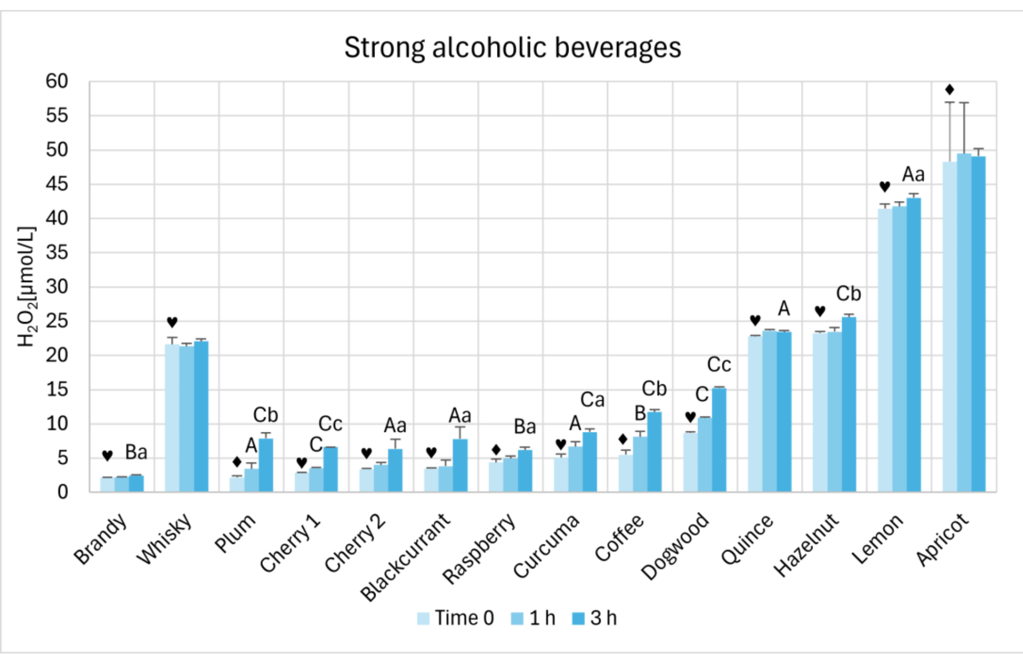


**Figure 2.** Concentration of hydrogen peroxide in brandy, whisky and various commercial and home-made liqueurs, immediately after bottle opening and after 1 and 3 h of incubation at room temperature with air access. ♦, $p < 0.01$; ♥, $p < 0.001$ (with respect to zero concentration); A, $p < 0.05$; B, $p < 0.01$; C, $p < 0.001$ (with respect to 0 time); a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$ (with respect to 1 h incubation).

No general pattern appeared from the measurements of the $H_2O_2$ concentration in the undiluted beer samples exposed to air for different times, but if the beer was diluted 4-fold with water, incubation for 1 and 3 h generally increased the $H_2O_2$ content, although a regular time-dependent increase was not observed in all the samples. On average, the increase in the $H_2O_2$ concentration for the 4-fold diluted beer samples was 0.86 ± 1.10 µM (−0.04 to 3.15 µM) after 1 h of incubation at ambient temperature and 2.18 ± 2.34 µM (0.43 to 7.89 µM).

Upon incubation at ambient temperature with air access, the $H_2O_2$ concentration increased slightly in the brandy (by 0.04 ± 0.10 µM after 1 h incubation and 0.29 ± 0.09 µM after 3 h incubation) and did not change significantly in the whisky (a decrease by 0.96 ± 0.80 µM after 1 h incubation and an increase by 0.4 ± 1.0 µM after 3 h incubation). Apparently, the level of hydrogen peroxide became equilibrated during their maturation. Small increases in the hydrogen peroxide concentration were found after incubation of the opened liqueurs: 0.7 ± 0.8 µM (0.2–2.6 µM) after 1 h of incubation and 3.11 ± 2.17 µM (0.6–6.6 µM) after 3 h of incubation (Figure 3).

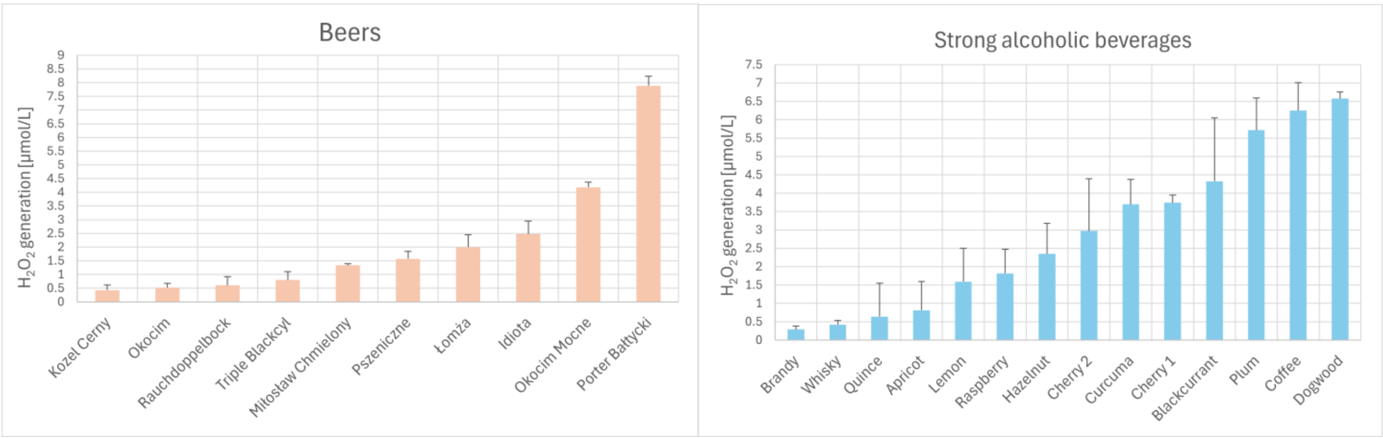


**Figure 3.** Generation of hydrogen peroxide during incubation of 4-fold diluted beers (**left**) and of strong alcoholic beverages (**right**) at room temperature, with air access, for 3 h.

Both the beers and the strong alcoholic beverages studied presented a broad variety of concentrations of polyphenols (Figure 4).

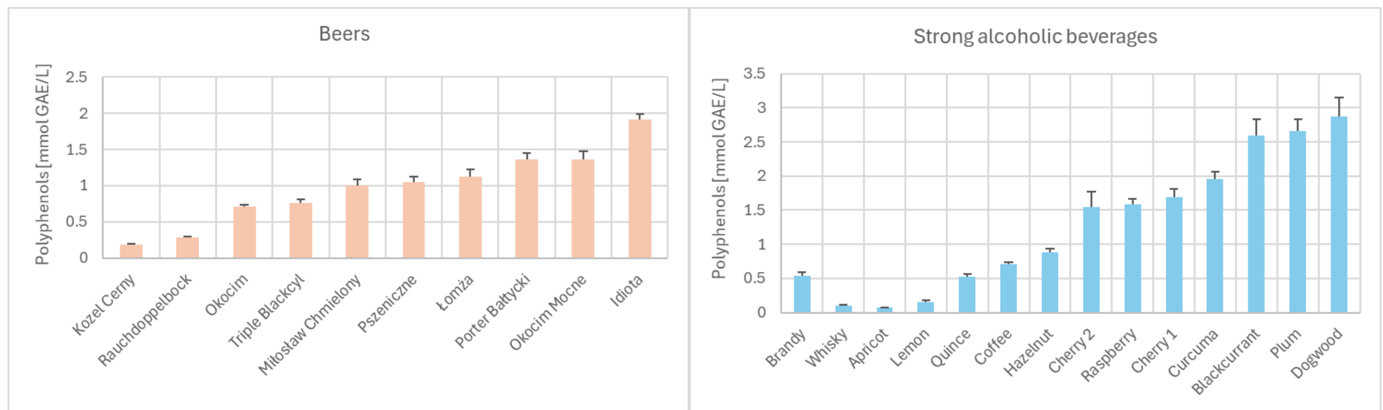


**Figure 4.** Polyphenol content of the beers and stronger alcoholic beverages studied. GAE, gallic acid equivalent.

The dependence of the initial concentration of hydrogen peroxide and the generation of hydrogen peroxide on the measured and declared alcoholic parameters was evaluated by multiple regression. For the beers, the initial concentration of hydrogen peroxide in the 4-fold diluted beers was significantly correlated with the TPC ($p < 0.001$), with the partial correlation coefficients with the extract content and alcohol content not being significant ($p > 0.05$). For the strong alcoholic beverages, the initial $H_2O_2$ concentration was surprisingly negative, with the correlation with the alcohol content being not significant.

The generation of hydrogen peroxide in the beers was significantly correlated with the TPC and the initial hydrogen peroxide concentration ($p < 0.01$), while the partial correlation coefficients with the extract concentration and alcohol concentration were not significant. The correlation of the $H_2O_2$ generation in the strong alcohols with the TPC was close to statistical significance ($p = 0.06$) and the correlation with the alcohol content and initial $H_2O_2$ concentration was not significant (Table S3).

### 3.2. Generation of Superoxide in Alcoholic Beverages

In order to demonstrate the generation of superoxide in alcoholic beverages, tests of the NBT reduction inhibitable by SOD and the DHE oxidation inhibitable by SOD were performed in selected beverages. In our opinion, the fractions of the NBT reduction and

DHE oxidation that are inhibited by SOD are more reliable than the total NBT reduction or DHE oxidation, which can be not specific for superoxide. Both tests revealed the generation of superoxide in one beer studied, the whisky and two liqueurs studied (Table 1).

**Table 1.** SOD-inhibitable NBT reduction and DHE oxidation in a beer and selected strong beverages (mean ± SD).

| Beverage | SOD-Inhibitable NBT Reduction (μM) | SOD-Inhibitable DHE Oxidation (Fluorescence Intensity, a.u.) |
|---|---|---|
| Beer Idiota | 1.8 ± 0.9 | 3868 ± 487 |
| Whisky | 9.1 ± 2.2 | 5772 ± 671 |
| Plum liqueur | 13.6 ± 4.0 | 12,896 ± 893 |
| Dogwood liqueur | 22.2 ± 4.9 | 19,375 ± 1294 |

*3.3. Detection of the Semiquinone Radical in Alcoholic Beverages*

Selected beverages were checked for the presence of the semiquinone radical. The EPR signal of this radical was found, in the background of a broader iron signal, in the Okocim Mocne beer, the brandy, and the lemon liqueur (Figure 5). No characteristic signal of the ascorbyl radical was seen.

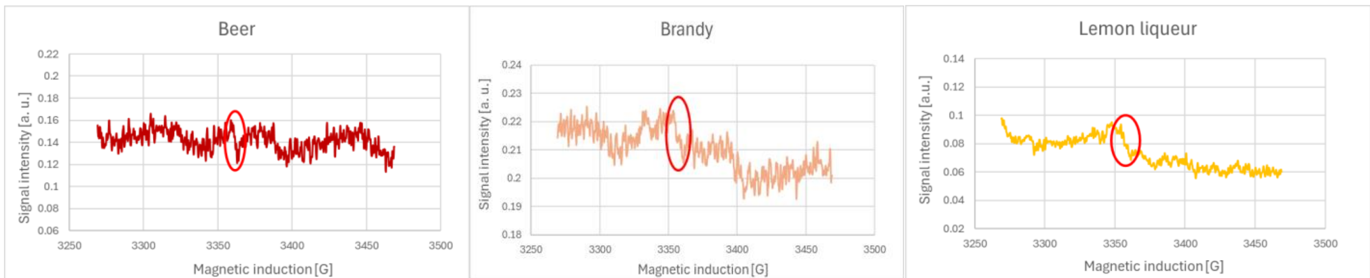


**Figure 5.** The presence of the signal of the semiquinone radical in the EPR spectra of the Okocim Mocne beer, brandy and lemon liqueur. The red oval encircles the signal of the semiquinone radical.

## 4. Discussion

Numerous studies have revealed that hydrogen peroxide is produced in many beverages, such as milk [29,30], honey [31–33], tea and coffee [8,9,12,13,34], infusions of medicinal herbs and spices [15,21] as well as formulated beverages and energy drinks [25,26] and hydrated protein drink mixes [16]. Even rainwater contains measurable amounts of hydrogen peroxide [35,36] and physical factors such as heating or oscillations may generate submicromolar concentrations of $H_2O_2$ in pure water [37,38].

The sources of hydrogen peroxide can be different in various beverages, but the most common source in beverages based on plant products is the autoxidation of polyphenols. The antioxidants present in plants, especially phenolics and ascorbate, protect against undesired oxidation but, when present in plant-derived beverages and food, may react with oxygen, ultimately producing hydrogen peroxide [8,9,39,40].

There are many methods to estimate the hydrogen peroxide concentration in the examined material, many of them employing the peroxidase reaction. We found that they may be not reliable when applied to materials containing polyphenols since many polyphenols inhibit peroxidase, leading to artefactually low results [41]. Therefore, in the present study, the classical ferrous oxidation−xylenol orange (FOX) method was used to determine the concentration of hydrogen peroxide. This method also has its drawbacks. It is a colorimetric method, so the color of the solutions may also introduce artefacts. To eliminate the interference, the "reagent blank" modification of the FOX method was

used, based on subtraction of the absorbance of a sample not containing xylenol orange from the results obtained for a sample reacting with complete xylenol orange reagent [15]. This method has its limitations as well, mainly the possibility of the reduction of $Fe^{3+}$ by ascorbate, polyphenols and other reducing compounds if present in the sample. However, such reactions should lead to a diminution in the yield of detection of hydrogen peroxide rather than an artefactual augmentation of the measured concentration of $H_2O_2$.

Although no hydrogen peroxide was detected in the not diluted beers, most of 4-fold diluted beers showed the presence of low concentrations of $H_2O_2$. Hydrogen peroxide was also detected in the brandy, whisky and fruit liqueurs immediately after bottle opening. The polyphenols present in these beverages (in the cases) were apparently the main source of hydrogen peroxide, although other compounds present could have made a significant contribution.

The lack of positive correlation between the TPC and the hydrogen peroxide content in the strong alcoholic beverages is surprising and can be explained by the dominant role of other factors not analyzed in this study, like the content of ascorbic acid and other compounds able to produce or react with hydrogen peroxide. The lemon, apricot and quince liqueurs were characterized by high hydrogen peroxide levels and low polyphenol levels. All these fruits are rich in ascorbic acid [42–44], which can also autoxidize, producing hydrogen peroxide [45]. Moreover, the content and composition of the additives, air exposure during production and bottling, the amount of air confined in the bottle, and the time and conditions of storage, all hard to control when buying commercial products, may affect the autoxidation of the components of the beverages and hydrogen peroxide production, thus complicating the relationship between the polyphenol content and the concentration of $H_2O_2$ in alcoholic beverages.

The generation of $H_2O_2$ upon opening the bottles generally correlated with the polyphenol content, both for the beers and for the stronger alcoholic beverages.

The generation of hydrogen peroxide during the oxidation of polyphenols is known to proceed in two steps. In the first step, a phenolic compound $H_2Q$ is oxidized to a semiquinone free radical $HQ^\bullet$. This oxidation is coupled to the reduction of molecular oxygen to the superoxide anion radical $O_2^{\bullet-}$

$$H_2Q + O_2 \rightarrow HQ^\bullet + O_2^{\bullet-} + H^+ \quad (1)$$

In the next step, the semiquinone free radical is oxidized to quinone Q, and either the next superoxide radical anion is produced (2) or the superoxide radical anion is reduced to hydrogen peroxide (3):

$$HQ^\bullet + O_2 \rightarrow Q + O_2^{\bullet-} + H^+ \quad (2)$$

$$HQ^\bullet + O_2^{\bullet-} + H^+ \rightarrow Q + H_2O_2 \quad (3)$$

The dismutation of superoxide radicals generates hydrogen peroxide:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (4)$$

Transition metal ions accelerate this reaction [40,46].

The autoxidation of ascorbic acid also proceeds in two steps, forming the ascorbyl radical $Asc^{\bullet-}$ as an intermediate, and is similarly catalyzed by trace amounts of transition metal, especially iron ions [47]. The hydrogen peroxide formed, in turn, can further oxidize phenolics or ascorbic acid, thus perpetuating the oxidation cycle [48].

An argument supporting this mechanism is the detection of the semiquinone radical by EPR in selected alcoholic beverages. The presence of a free radical EPR signal, attributed to the semiquinone radicals of polyphenols, was reported in red wines. Wine's exposure to

air leads to a transient increase in the intensity of this signal within minutes [49]. These data may serve as indirect evidence of the oxidation of wine polyphenols that should result in the production of hydrogen peroxide. Previously, the EPR signal of the semiquinone radical was identified in whisky [50].

Although detection of the characteristic signal of the ascorbyl radical could be expected, especially in the lemon liqueur, this radical was not observed. The absence of this signal can be explained by the interaction of the ascorbyl radical with flavonoids:

$$H_2Q + Asc^{\bullet} \rightarrow HQ^{\bullet} + Asc^{-} \quad (5)$$

Such reactions, originally postulated by Szent-Györgyi [51,52], has been demonstrated experimentally for some but not all flavonoids, depending on their redox potentials [53].

The formation of hydrogen peroxide in alcoholic beverages does occur, but the presence of hydrogen peroxide often cannot be measured since the hydrogen peroxide formed may react with components of the beverages, as evidenced by the scavenging of exogenously added hydrogen peroxide by polyphenols [21]. The presence and production of $H_2O_2$ could be detected in some but not all the wines studied due to the scavenging of hydrogen peroxide, i.a., by sulfites present in the wines [24]. A similar situation surely takes place in other alcohols, as the scavenging of exogenous hydrogen peroxide was demonstrated for beer [54] and strong alcoholic beverages [55].

The detection of superoxide generation in alcoholic beverages and the presence of semiquinone radicals in brandy, a liqueur and a beer is compatible with this mechanism.

The present data broaden the list of beverages that contain hydrogen peroxide. The whole set of studies devoted to this subject demonstrate that most, if not all, consumed beverages contain low concentrations of hydrogen peroxide. Low hydrogen peroxide concentrations may also be present in plant-derived food such as vegetables subjected to thermal treatment [19,20]. This hydrogen peroxide may have both deleterious and beneficial effects on human health. Hydrogen peroxide may be carcinogenic [56,57]. However, it may also act as a microbicidal agent.

The bactericidal action of such low concentrations of hydrogen peroxide as those found in the beverages was questioned, but it can be potentiated by the Fenton reaction and the action of peroxidases such as lactoperoxidase and myeloperoxidase [58,59]. In the oral cavity and esophagus, the ingested hydrogen peroxide serves as a substrate for salivary sialoperoxidase and myeloperoxidase, not only exerting antimicrobial action but also helping to inactivate toxic carcinogenic and genotoxic substances [60].

Some production of hydrogen peroxide from food components may also take place in the digestive tract. Holding green tea in the mouth, as well as chewing tea leaves, was found to generate hydrogen peroxide in the saliva. Holding 0.1–0.6% green tea in the mouth for 2 min generated 2.9–9.6 μM $H_2O_2$, while chewing 2 g of green tea leaves produced up to 31.2 μM $H_2O_2$ [61]. At the low pH of the stomach, no production of $H_2O_2$ can be expected, but it can be helpful in the protection against *Helicobacter pylori* [62]. In mice, hydrogen peroxide produced physiologically by DUOX2 was demonstrated to prevent *Helicobacter felis* infection and subsequent inflammation of the gastric mucosa [63]. This action can be succored by dietary hydrogen peroxide. A retrospective cross-sectional study revealed that consumption of honey, coffee and green tea had protective effects against infection by *H. pylori* [64]. All of them may contain or produce hydrogen peroxide.

The autoxidation of dietary components resulting in the production of $H_2O_2$ can be resumed in the intestine. The concentration of oxygen in the colonic mucosa is below 25% of that in air, but it still allows for autoxidation, albeit with a lower efficiency; then, the oxygen content falls further down the digestive tract [65]. Low concentrations of hydrogen

peroxide transmitted to or produced in the intestines from beverages and food may play a role in the functioning of this part of the digestive tract.

Hydrogen peroxide produced by DUOX controls the intestinal microbiota in *Drosophila*. This enzyme produces a unique epithelial oxidative burst, which limits the proliferation of microorganisms in the intestines [66]. Hydrogen peroxide is known to play a signaling and regulatory role in the digestive tract [65]. Some commensal bacteria, especially Lactobacilli, induce the generation of hydrogen peroxide by NOX1. This hydrogen peroxide stimulates the healing of mucosal wounds [67,68]. Low concentrations (1–5 μM) of lipid hydroperoxides were reported to promote the proliferation of colon-derived Caco-2 cells [69]. It can be expected that $H_2O_2$ may act similarly, as a similar pattern of cell growth stimulation was observed for low concentrations of various oxidants, including $H_2O_2$ [70]. Indeed, while high concentrations of $H_2O_2$ damage colon cells, low concentrations were found to stimulate colonic epithelial cells [71]. Hydrogen peroxide was also reported to promote gastric motility [72]. Exogenous hydrogen peroxide may contribute to all these effects.

Moreover, exogenous peroxide may react with ferrous ions in the intestines, producing hydroxyl and other radicals. Their reactions may facilitate digestion, as it was proven that proteins damaged by free radicals can be more susceptible to proteolytic enzymes [73].

It can be noted that, as far as analytics is concerned, the presence of hydrogen peroxide in alcoholic beverages could be a cause of artefacts in the methods of alcohol assay based on alcohol oxidase plus peroxidase [74–76], although the $H_2O_2$ concentrations are so low that a significant interference is not probable.

## 5. Conclusions

The presence of hydrogen peroxide was detected in six out of ten freshly opened beers studied, the brandy, the whisky and all nine fruit liqueurs studied. All the beverages generated $H_2O_2$ upon incubation in the atmosphere of air. These observations broaden the list of beverages in which the presence and/or generation of hydrogen peroxide was detected. The main source of hydrogen peroxide in alcoholic beverages is the autoxidation of polyphenols and other plant-derived antioxidants. The small amounts of hydrogen peroxide found in alcoholic beverages may have adverse health effects, contributing to the carcinogenicity of alcohol beverages and to the irritating effect of strong alcohols on the epithelium of the digestive tract. However. it may also have a beneficial, mainly microbicidal, action. This question, as well as the presence of hydrogen peroxide in a broader range of alcoholic and non-alcoholic beverages, requires further studies.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/pr13010277/s1, Table S1: Characteristics of the beers studied; Table S2: Characteristics of the strong alcoholic beverages studied; Table S3: Analysis of the dependence of the initial hydrogen peroxide concentration and hydrogen peroxide generation on the available parameters of the beverages determined by multiple regression.

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