Hydrogen peroxide is formed upon cooking of vegetables

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Hydrogen peroxide (H2O2) is generated under autoxidation of some components of beverages including flavonoids and ascorbate, especially in tea and coffee. As polyphenols are also present in solid food, especially in vegetables, we checked whether hydrogen peroxide is generated during cooking of several common vegetables. The formation of hydrogen peroxide was found in the decoctions of all cooked vegetables studied except for potato and in the homogenates of cooked vegetables except for garlic and purple potato. The highest concentration of hydrogen peroxide in 1:2 (w/v) homogenates was found for the broad bean (73.4±19.0 µM) followed by broccoli (18.6±0.3 µM), onion (10.4±1.6 µM) and leek (10.0±0.3 µM), while the H2O2 concentration in the decoctions was the highest for broccoli (24.4±0.8 µM), then for broad bean (21.4±1.1 µM), carrot (13.2±0.2 µM) and cauliflower (12.6±1.2 µM).

Keywords: hydrogen peroxide, vegetables, broad bean, broccoli, onion

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INTRODUCTION

Endogenous hydrogen peroxide is a reactive oxygen species, generated by activated phagocytes to kill ingested microorganisms (Clifford & Repine, 1982; Thomas, 2017) and by other cells for local signaling. Its elevated concentrations cause apoptosis and cell death (Sies, 2017; Lin et al., 2019). Hydrogen peroxide solution (3%) is used externally as disinfectant (Neely & Maley, 1999); virucidal efficiency of H2O2 vapor has also been previously demonstrated (Goyal et al., 2014). Concentrated H2O2 is used as a bleach, i.e., for hair discoloration (Watt et al., 2004; Smith et al., 2017). H2O2 is used for tooth whitening (Alkahtani, et al. 2020) and as a component of some mouthwashes (Ortega et al., 2020; Vergara-Buenaventura & Castro-Ruiz, 2020). Cases of poisoning with H2O2 have been reported (Watt et al., 2004), but low doses of H2O2 when ingested as solutions of low concentration, may not be toxic at all or may even have a beneficial action, mainly due to the antimicrobial activity. Interestingly, H2O2 ingestion has been used for treatment of dementia and enhancement of the immune system function and has been claimed to have anti-cancer effects and to promote well-being (Yutsis, 2003). Oral exposures to 3% H2O2 were reported to be mostly asymptomatic, with only one child among 670 patients developing multiple gastric ulcers and duodenal erosions (Henry et al., 1996). However, there are concerns that H2O2 contained in the mouthwash may be carcinogenic (Consolaro, 2013).

In the context of controversy concerning the health effects of low doses of ingested hydrogen peroxide, it is of interest to have a full picture of natural ingestion of H2O2 in food and beverages. Humans ingest H2O2 already as newborns since fresh human milk contains about 30 µM H2O2; in addition, baby saliva contains ca 10 µM H2O2 so the milk entering the digestive tract contains about 40 µM H2O2 sufficient to inhibit growth of the opportunistic pathogens like Staphylococcus aureus and Salmonella spp. (Al-Shehri et al., 2015). Later on, we ingest H2O2 in commonly consumed beverages, such as tea, coffee and cocoa. These beverages contain flavonoids and phenolic acids (Bubols et al., 2013; Galleano et al., 2010) which have many beneficial health effects, possibly related to their antioxidant activities (Chu et al., 2017; Ullah et al., 2020).

However, flavonoids and phenolic acids autoxidize in aerated solutions reacting with oxygen. H2O2 is generated as one of products of autoxidation of these compounds; therefore, tea and coffee (Akagawa et al., 2003; Grzesik et al., 2019, Long et al., 1999) and to a lesser extent cocoa and wine (Chai et al., Long et al., 1999; Tama et al., 2022) contain some amounts of H2O2, which usually increase with time after their preparation. H2O2 is also generated in formulated beverages due to oxidation of ascorbic acid and some other redox-active components (Bopitiya et al., 2021).

Flavonoids and other autoxidizing compounds are present not only in beverages, but also in solid food of plant origin, especially in vegetables, and can be expected to generate H2O2 in these products as well. The aim of the present study was to examine whether H2O2 is generated in cooked vegetables.

MATERIALS AND METHODS

Xylenol Orange was obtained from POCh (Gliwice, Poland). Other reagents were purchased from Merck (Poznań, Poland). Absorptiometric measurements were done in a Stark multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland), using Greiner 96-well flat-bottom clear polystyrene plates.

Vegetables (broad bean Vicia faba L., onion Allium cepa L., garlic Allium sativum L., leek Allium ampeloprasum L., cabbage Brassica oleracea L., broccoli Brassica oleracea var. italica Plenck, cauliflower Brassica oleracea var. botrydis L., carrot Daucus carota subsp. sativus (Hoffm.) Schübl. & G. Martens, carrot Daucus carota ssp. sativus and potato Solanum tuberosum L.) were purchased in a
local supermarket in Rzeszów. Ten grams of a vegetable were added with 20 mL of 50 mM phosphate buffer, pH 7.4, deionized water or tap water and heated in an open falcon tube at 95°C for 10 minutes. Distilled water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). A sample of decoction was withdrawn, and the remaining fraction was homogenized with the cooked vegetables in a blender. A sample of the homogenate was withdrawn, centrifuged, and the supernatant was collected. The remaining part was incubated at room temperature (21±1°C) and samples were collected and centrifuged after 3 h and 24 h.

The H$_2$O$_2$ concentrations in the decoctions and supernatants of fresh and incubated homogenates was essayed with Xylenol Orange (Gay & Gebicki, 2003; Grzesik et al., 2019) in duplicate aliquots, one of them being incubated with catalase (10 µg/mL) for 15 min prior to the assay. The difference between the results obtained for both aliquots was assumed to represent the H$_2$O$_2$ concentration in the sample. This method may underestimate the H$_2$O$_2$ concentration but ensures full specificity for this compound.

The experiments were run in triplicate and were repeated at least three times. Statistical significance of differences was estimated using one-tailed Student’s t-test.

**RESULTS**

The presence of H$_2$O$_2$ was detected in the decoctions of all studied cooked vegetables with the exception of potato and in the homogenates of all studied cooked vegetables except for garlic. The highest concentration of H$_2$O$_2$ in 1:2 (w/v) homogenates was found for the broad bean (73±9.0 µM) followed by broccoli (18.6±0.3 µM), onion (10.4±1.6 µM) and leek (10.0±0.3 µM). In the decoctions, the highest H$_2$O$_2$ concentration was recorded for broccoli (24.4±0.8 µM) and then for broad bean (21.4±1.1 µM), carrot (13.2±0.2 µM) and cauliflower (12.6±1.2 µM).

Incubation of the homogenates of cooked vegetables for 3 and 24 h generally decreased the concentration of H$_2$O$_2$ sometimes (carrot, garlic, cabbage) to zero values (Fig. 1).

When the vegetables were cooked in deionized or tap water, the formation of H$_2$O$_2$ was lower than upon cooking the phosphate buffer, pH 7.4, but still occurred, as reported for the broad bean (Table 1). This decreased generation of H$_2$O$_2$ in water is due to the lower pH of the homogenate and decoction of vegetables (Table 2).

**DISCUSSION**

The presence of H$_2$O$_2$ in cooked vegetables has not been reported to our best knowledge. There is no reason to suspect the presence of significant amounts of H$_2$O$_2$ in fresh vegetables since the catalase and peroxidase activities remove H$_2$O$_2$ inevitably produced in the living cells. However, when these activities are inactivated by cooking and redox-active compounds become exposed to ambient oxygen, this equilibrium is disrupted and H$_2$O$_2$ a by-product of autoxidation of polyphenols, can accumulate. The formation of H$_2$O$_2$ occurred at the highest rate during cooking at high temperature; upon cooling and subsequent incubation, the H$_2$O$_2$ concentrations in the vegetable homogenates decreased rather than increased, apparently due to reactions with components of the homogenates such as released heme and flavonoids themselves, which apart from generating H$_2$O$_2$, are also able to react with this compound (Bi et al., 2014; He et al., 2018).

Especially high H$_2$O$_2$ generation was found in the fava beans (Fig. 1), known to contain considerable amounts of pyridine derivatives, vicine and convicine, constitut-

**Table 1. Generation of hydrogen peroxide in the decoction and homogenate of broad bean cooked in phosphate buffer, pH 7.4, tap water and deionized water.**

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Phosphate buffer</th>
<th>Tap water</th>
<th>Deionized water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate 0 time</td>
<td>76.0±3.8</td>
<td>28.1±4.8</td>
<td>22.8±2.2</td>
</tr>
<tr>
<td>Homogenate, 3 h</td>
<td>61.7±3.5</td>
<td>29.5±7.0</td>
<td>8.0±4.3</td>
</tr>
<tr>
<td>Homogenate, 24 h</td>
<td>9.0±1.1</td>
<td>10.0±1.6</td>
<td>8.5±1.8</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001 with respect to zero (Student’s t-test)

**Table 2. pH of decoction and homogenate of broad been cooked in the phosphate buffer, tap water and deionized water.**

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Prepared in buffer</th>
<th>Prepared in tap water</th>
<th>Prepared in deionized water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoction</td>
<td>7.05±0.02</td>
<td>6.85±0.03</td>
<td>6.41±0.04</td>
</tr>
<tr>
<td>Homogenate 0 time</td>
<td>7.15±0.03</td>
<td>6.87±0.04</td>
<td>6.60±0.02</td>
</tr>
</tbody>
</table>

pH was measured after cooling to the room temperature.
is less than 0.85% of the total weight of the bean. Their aglycones autoxidize readily and, upon ingestion, undergo redox cycles, producing large amounts of \( \text{H}_2\text{O}_2 \) and hemolysis in sensitive individuals (famish) (Halliwell & Gutteridge, 1999). The present results demonstrate that significant generation of \( \text{H}_2\text{O}_2 \) by fava beans takes place also during cooking, under the conditions of limited redox cycling. Moreover, fava beans contain many flavonoids, especially catechin and rutin, and phenolic acids (Johnson et al., 2021); all these compounds can generate \( \text{H}_2\text{O}_2 \) upon autoxidation (Grzesik et al., 2019).

It should be pointed out that the concentrations of \( \text{H}_2\text{O}_2 \) formed in vegetables may be underestimated by our method since some flavonoids inhibit catalase (Krych & Gebicka, 2013) used to warrant specificity of the assay for \( \text{H}_2\text{O}_2 \).

Oxidation of flavonoids and many other compounds is pH-dependent and decreases as the pH is lowered (Akagawa et al., 2002; Arakawa et al., 2004; Grzesik et al., 2019). Elevated pH facilitates oxidation of phenolic compounds including flavonoids since the phenol groups dissociate at higher pH. The electron on the deprotonated phenol group reduces oxygen forming superoxide radical anion \( \text{O}_2^- \) and a phenoxyl radical. Superoxide can be further reduced by the phenoxyl radicals (oxidizing a phenoxyl radical to a quinone) or dismutate; both reactions generate \( \text{H}_2\text{O}_2 \) (Arakawa et al., 2004; Grzesik et al., 2019). The presence of transition metals, especially iron, ions facilitate autoxidation of phenolic compounds (Grzesik et al., 2019), which explains higher \( \text{H}_2\text{O}_2 \) concentrations in the homogenates of fava beans cooked in tap water rather than in deionized water (Table 1).

We compared \( \text{H}_2\text{O}_2 \) generation when cooking various vegetables in phosphate buffer, pH 7.4, to provide identical conditions for comparison. Cooking vegetables in deionized or tap water, resulting in decoctions and homogenates of lower pH (Table 2), decreased the rate of \( \text{H}_2\text{O}_2 \) generation but still resulted in the production of significant amounts of \( \text{H}_2\text{O}_2 \) (Table 1). Thus, an obvious means to reduce its generation during cooking is to keep low pH and avoid transition metal ion contamination during cooking.

The generation of \( \text{H}_2\text{O}_2 \) at low concentrations in some cooked vegetables does not appear to raise health problems and may even have beneficial effects. \( \text{H}_2\text{O}_2 \) can exert bactericidal and virucidal action, and thus contribute to the mouth hygiene and health. High \( \text{H}_2\text{O}_2 \) concentrations may damage colon cells (Wijeratne et al., 1996). In the digestive tract, \( \text{H}_2\text{O}_2 \) can react with available iron, form the hydroxyl radical and other free radicals, and facilitate digestion as proteins subjected to free radical action may show enhanced susceptibility to proteolytic enzymes (Wolff & Dean, 1986).

CONCLUSION

Cooking vegetables generates hydrogen peroxide both in the decoction and inside the solid food. These small amounts of hydrogen peroxide formed do not seem to pose a health danger and may even exert beneficial effects.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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REFERENCES


