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Mutagenic and antimutagenic properties of aqueous and ethanolic extracts from fresh and irradiated *Tuber aestivum* black truffle: A preliminary study

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Abstract

A preliminary study was performed on fresh and irradiated *Tuber aestivum* black truffles to investigate the presence of mutagenic and antimutagenic activities in the fresh product and to examine the possible effects of treatment with gamma rays. The study was performed on aqueous and ethanolic extracts from truffles, untreated or irradiated with a final dose of 1.5 kGy. Two *Salmonella typhimurium* His$^-$ strains, TA 98 and TA100, were used. The preliminary results indicate that black truffles may contain compounds having an inhibitory effect against direct and indirect acting mutagenic compounds. The irradiation did not lead to the formation of mutagenic compounds, but the level of antimutagenic activity was slightly decreased after the treatment.

Keywords: Truffle; Ames test; Food irradiation; Food preservation

1. Introduction

A number of different "short-term tests" have been developed to investigate the genotoxic properties of chemicals in food and the environment. One of the best validated test is the "Ames test", which explores the capability of a certain compound or mixture to induce mutations in specific *Salmonella typhimurium* His$^-$ strains (Maron & Ames, 1983). For several years, it has been known that a large number of compounds that are carcinogenic in vivo also produce mutagenicity in the Ames test in vitro (about or over 70%). In order to detect also the so-called "indirect" or "pro-carcinogenic" agents, which by themselves are unable to produce a direct mutagenic effect but require a metabolic activation taking place in the host, the Ames test is supplemented with rat liver microsomal enzymes (S9 mix). Due to the generally accepted involvement of mutagenicity in the carcinogenic process, this test is not only widely used to screen for mutagenicity of chemicals but has also been found useful to examine for potential antimutagenicity of synthetic or natural molecules. In fact, compounds with antimutagenic and anti-carcinogenic potentials are found in many traditional herbal remedies and dietary therapies (Aruoma & Okezie, 2003; Ferguson, 2001; Surh & Ferguson, 2003) being antioxidants and thus capable of counterbalancing free radical activities that may cause cell injuries (Bravo, 1998; Tedesco, Russo, Nazzaro, Russo, & Palumbo, 2001). Many researches have reported a beneficial effect of different fungi, which have been used by some to combat emotional and physical stress, to improve the life quality of diabetics, for decreasing the blood level of cholesterol, and to be effective as antioxidant and anti carcinogen agents (Delmanto et al., 2001; Menoli, Mantovani, Ribeiro, Speit, & Jordao, 2001). Truffles of the genus *Tuber* are ectomycorrhizal Ascomycotina fungi, living in symbiosis with roots of different trees (Trappe, 1979). Italy is one of the

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most important countries in the world, both for the number of superior *Tuber* species present on its territory and for the global value of truffle production. The best-known species are *Tuber magnatum* var. Pico, or white truffle, and *Tuber aestivum* var. Vittadini, or black truffle. The typical taste of truffles is the result of an inimitable combination of several volatile organic compounds, such as aldehydes, alcohols, ketones, organic acids and some sulphur-compounds. However, one of the major problems is to store truffles with a long shelf life and good preservation of taste and aroma, essential parameters for its quality and the main reason why truffle is one of the most famous products in the world. As a consequence, several methodologies, such as use of gamma rays, are in searching and experimentation to improve its shelf life and safeguard its sensory and structural characteristics. Thus, studies comparing untreated and 1.5 kGy-irradiated truffles, demonstrated a good safeguarding of some biochemical characteristics after irradiation, with a related, noteworthy decrease in the microbial flora and an enhancement of the shelf life (Nazzaro et al., 2004; Nazzaro et al., 2005). However, if applied in an improper mode, irradiation could introduce unwanted sensory and chemical changes, i.e., resulting in free radicals, whose reaction with proteins, lipids, and polyphenols, could give rise to detrimental effects. Although several studies are present in the literature as regards the genus *Tuber*, mainly biochemical and biomolecular investigations (Percudani, Trevisi, Zambonelli, & Ottonello, 1999; Pierleoni et al., 2004), no studies have been performed about its antimutagenic activities. In this work, aqueous or ethanolic extracts of fresh or irradiated *T. aestivum* truffle were assessed to investigate the presence of mutagenic or antimutagenic activities in the fresh product and to evaluate the possible modifications in these aspects followed by irradiation.

2. Materials and methods

2.1. Preparation of extracts

Fresh samples of black truffle (*Tuber aestivum* var. Vittadini) were purchased in Molise (Italy). After packaging in N_2 atmosphere, truffles were irradiated using a ^{60}Co gamma ray source to achieve a final dose of 1.5 kGy. All samples were kept at −22 °C until examined.

Fresh or irradiated *T. aestivum* truffles were cut into small pieces, and divided into two portions, homogenised in cold sterile distilled water or in ethanol, respectively, and incubated for 2 h in the dark at 4 °C. After centrifugation at 15,700*g* for 30 min at 4 °C, the supernatants were evaporated under air to dryness and dissolved in dimethylsulphoxide to have a 10× concentration.

2.2. Ames test

For mutagenicity and antimutagenicity assays on extracts from *T. aestivum*, the Ames assay was performed (Maron & Ames, 1983) by using the *S. typhimurium* histidine-requiring strains TA98 and TA100, purchased from Molecular Toxicology Inc. (Moltox™, Annapolis, MD, USA). Bacteria were aerobically grown at 37 °C in Nutrient Broth n° 2 (OXOID) supplemented with ampicillin. As positive controls, the direct acting mutagens nitrofluorene was used for strain TA98 and sodium azide for strain TA100; the indirect acting mutagen was, for both strains, 2-aminoantracene. As negative control, 0.5 ml phosphate buffer (0.1 M, pH 7.4) was used both for the two strains and for all tests performed.

2.3. Mutagenicity test

The test was carried out by adding to 1.8 ml of molten agar 0.1 ml of the overnight bacterial culture, truffle extracts (20 μl of ethanol or 50 μl of aqueous extracts/plate), and 0.5 ml phosphate buffer (0.1 M, pH 7.4); the mixture was immediately plated onto minimal medium agar plates previously added with 0.2 ml of sterile 0.5 mM Bio-His. For the indirect test, the post-mitochondrial S9 fraction, prepared from livers of Sprague–Dawley rats treated with the polychlorinated biphenyl mixture Aroclor 1254, was purchased from Molecular Toxicology Inc. (Moltox™, Annapolis, MD, USA). The S9 metabolic activation mixture was prepared according to Maron and Ames (1983), and Mortelmans and Zeiger (2000). In the indirect test, 0.5 ml of S9 mix was supplemented instead of phosphate buffer.

2.4. Antimutagenicity test

For the antimutagenicity test, the mutagen standard was added to the mixture of truffle extract, bacterial culture, with S9 mix (indirect test) or phosphate buffer (direct test). After incubation for 30 min at 37 °C, the mixture was added to 2.0 ml of molten agar and plated as above. Positive and negative controls were also included in each assay. His^+ revertants were counted after 48 h of incubation at 37 °C. The antimutagenic activity was expressed as percentage of inhibition:

$[1-(T-S/M-S)]\times 100$, where T is the number of revertants/plate in the presence of mutagen and the test sample, M is the number of revertants/plate in the positive control and S is the number of spontaneous revertants/plate (Ong, Wong, Stewart, & Brockman, 1986).

3. Results and discussion

Table 1 shows the results obtained from the Ames test for mutagenicity of fresh or irradiated truffle extracts. The numbers of His^+ revertants produced by both extracts are low, being very similar to the negative control values, both for TA98 and for TA100. In some cases (ethanol extract from fresh truffle: 10 colony forming units (CFU)/plate for TA98, direct test; aqueous extracts from fresh truffle: 38 CFU/plate for TA100, direct test), the number of revertants was less than the spontaneous background

Table 1
Enumeration of His + revertants in strain TA98 (1a) and strain TA100 (1b) in the Ames test

	CFU/plate − S9 mean (SD)	CFU/plate + S9 mean (SD)
(1a): TA98 His + spontaneous revertants (normal values: 20–50)		
TA98 negative control (with phosphate buffer)	49 (2.08)	60 (6.24)
TA98 positive control (with mutagen standard)	322 (26.57)	361 (18.9)
TA98 + aqueous extract from fresh truffle	44 (3.51)	34 (4.35)
TA98 + aqueous extract from irradiated truffle	52 (4.5)	59 (6.55)
TA98 + ethanolic extract from fresh truffle	10 (1.52)	62 (7.81)
TA98 + ethanolic extract from irradiated truffle	65 (4.72)	69 (6.65)
(1b): TA100 His + spontaneous revertants (normal values: 80–200)		
TA100 negative control (with phosphate buffer)	98 (7.02)	140 (10.78)
TA100 positive control (with mutagen standard)	366 (15.52)	321 (22.34)
TA100 + aqueous extract from fresh truffle	38 (7)	90 (9.01)
TA100 + aqueous extract from irradiated truffle	54 (5.57)	169 (17.08)
TA100 + ethanolic extract from fresh truffle	72 (14.04)	122 (18.41)
TA100 + ethanolic extract from irradiated truffle	95 (9.29)	154 (16.52)

The data represent the mean of three independent experiments. Each experiment was performed in triplicate. SD values are shown in parenthesis. CFU: Colony Forming Units. For details: see Section 2.

revertants (49 and 98 CFU/plate, for TA98 and TA100, respectively). So, in strains TA98 and TA100, the truffle extracts did not induce gene mutations under the conditions applied.

Fig. 1 represents the antimutagenic effects exhibited by extracts of fresh and irradiated black truffles against nitrofluorene (direct acting) and 2-aminoanthracene (indirect acting) mutagenesis (for *S. typhimurium* TA98) and sodium azide (direct acting) and 2-aminoanthracene (indirect acting) mutagenesis (for *S. typhimurium* TA100). All extracts showed an inhibitory effect against these standard mutagens, ranging from 97% to 40%. For *S. typhimurium* TA98, the fresh truffle showed a stronger inhibitory effect than the irradiated truffle, both in the test for direct mutagenesis (plus 40% for water extracts and plus 12% for the ethanol extracts) and in the test for indirect mutagenesis. For the ethanol extracts, a decrease in antimutagenicity from fresh to irradiated samples of about 43% was observed. Concerning the TA100 strain, the trend was similar, except that the aqueous extracts of both fresh and irradiated truffles, tested without S9, had comparable inhibitory effects.

Our preliminary results did not reveal the presence of components in black truffles that are mutagenic in the

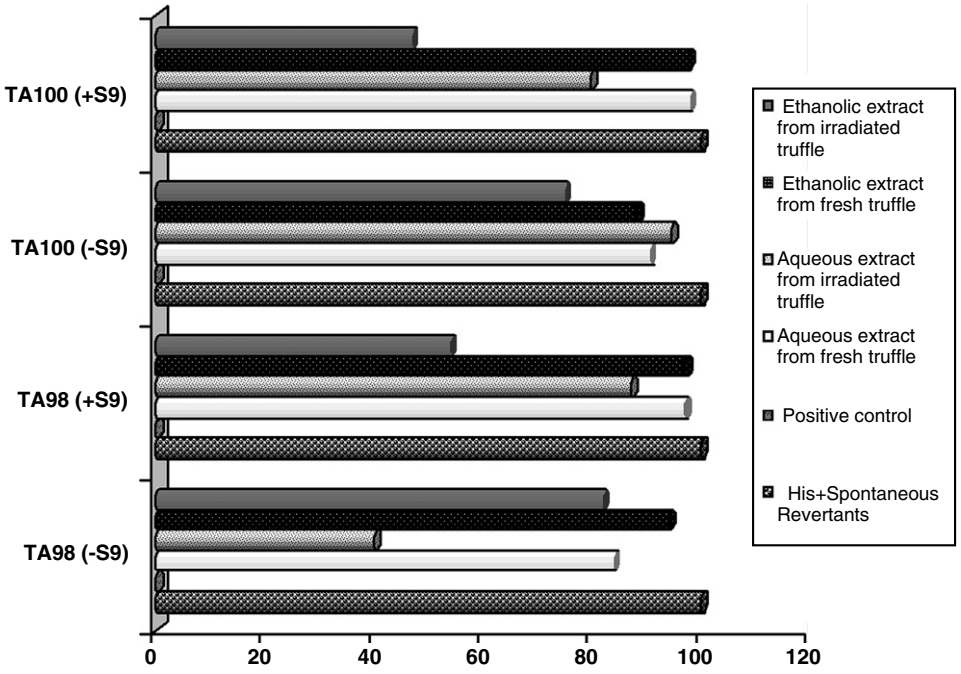

Fig. 1. Antimutagenic activity of extracts of fresh and irradiated black truffles against nitrofluorene (-S9) and 2-aminoanthracene (+S9) – induced mutagenesis in *S. typhimurium* TA98 and sodium azide (−S9) and 2-aminoanthracene (+S9) – induced mutagenesis in *S. typhimurium* TA100. The results are the mean of three independent experiments and are presented as percentage of inhibition of mutagenesis. Each experiment was performed in triplicate. For details: see Section 2.

Ames test. On the contrary, there were indications that black truffles may contain compounds that could possess an inhibitory effect against direct and indirect acting mutagenic compounds. Our experiments also indicated that irradiation (at 1.5 kGy) of truffles did not lead to the formation of mutagenic compounds. However, the results recorded with the extracts of the 1.5 kGy-treated truffles indicated that this treatment could result in a relative loss or modification of some antimutagenic constituents.

Further studies are in progress to better characterise the antimutagenic activities of *T. aestivum* extracts, by using other tester strains, and to specifically identify their active compounds and their mode of action.

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