PINK PEPPER (*Schinus terebinthifolius* Raddi) FRUIT: A PROMISING NATURAL ANTIOXIDANT AGAINST CHOLESTEROL OXIDATION IN FISH BURGERS DURING FROZEN STORAGE

ABTRACT

Plant materials from the Brazilian flora have gained attention in the scientific community due to their antioxidant potential and possible use as natural food additives. Thus, this study investigated the protective potential of pink pepper (*Schinus terebinthifolius* Raddi) fruit against cholesterol oxidation during frozen storage. Firstly, pink pepper extract was characterized by its total phenolic content (5.05 ± 0.19 mg GAE/g). Besides, it also showed antioxidant capacity by the DPPH (84.45 ± 1.61 % inhibition) and FRAP (121.54 ± 1.31 µmol Fe^{2+}/g) assays. The addition of pink pepper to fish burgers stored at -18°C proved to be effective limiting cholesterol oxides formation. Results showed that pink pepper was capable of reducing cholesterol degradation from 22.7 (control) to 9.77% and cholesterol oxides formation by 19.11% after 60 days of storage. Therefore, pink pepper may be highlighted as a suitable natural anti-cholesterol oxidation agent in fish burgers during frozen storage, contributing to the safety and quality of these products.

INTRODUCTION

Plants are sources of bioactive compounds that contribute to the maintenance and enhancement of human health mostly due to their antioxidant properties. Moreover, the concept of using food to manage health has become a trend over the last decade, highlighting the advantages of incorporating dietary sources of bioactive compounds in the global market as components of processed food (1,2).

Schinus terebinthifolius Raddi (Anacardiaceae family) is a native South American tree that produces fruits popularly known as pink pepper, Brazilian pepper, or aroeira. It is considered an ornamental and medicinal plant traditionally used in folk medicine. Besides, its fruit is appreciated as a condiment worldwide (2-4).

The great interest of the scientific community in pink pepper fruit is mainly due to its composition in antioxidants (e.g. phenolic acids, flavonoids, tannins, and terpenes) (2-5). This point supports the potential of pink pepper not only to improve food palatability but also to enhance food quality and shelf life by minimizing oxidative processes of lipids in food. Therefore, the application of pink pepper by the food industry may represent a promising alternative to meet the growing demand for functional foods and to reduce the use of synthetic antioxidants (2,4-6), which have been the subject of numerous debates due to their possible deleterious effects (7).

Lipid oxidation is a crucial deteriorative process of fish and fish products, which are extremely prone to oxidation due to their high levels of unsaturated lipids such as polyunsaturated fatty acids and cholesterol (2,8). Although frozen storage is one of the most applied methods of fish preservation, lipid oxidation still occurs under low temperatures, resulting in loss of sensorial and nutritional quality (8-10).

Moreover, cholesterol oxidation in fish may form oxidized compounds known as cholesterol oxides or cholesterol oxidation products (COPs), which are absorbed from food and contribute to the development of inflammations, cardiovascular and

neurodegenerative diseases, carcinogenesis, and other pathologies (11). Therefore, since the ingestion of COPs and synthetic antioxidants represent a public health concern, natural sources of antioxidants, like pink pepper, must be considered.

OBJECTIVE

This study aimed to investigate the effectiveness of pink pepper as an anti-cholesterol oxidation agent in fish burgers stored at -18°C for 60 days. Moreover, pink pepper extract was characterized by its total content of phenolic compounds and *in vitro* antioxidant capacity.

RESULTS AND DISCUSSION

Fresh pink pepper fruits were donated by local producers of Seropédica, Rio de Janeiro, Brazil. The samples were dried in an oven with air circulation at 30ºC for 24 hours and ground in a domestic processor. An ethanolic extract (70:30, %v/v) was prepared (12) to determine the total content of phenolic compounds (4) and the *in vitro* antioxidant capacity by the DPPH and FRAP assays (2).

For the fish burgers, fresh fillets of tilapia (*Oreochromis niloticus*) were processed in a grinder until a homogeneous mass was obtained. It was divided into two fractions: one fraction without addition of antioxidants (control) and other fraction with addition of ground pink pepper (0.05%). Then, fish burgers (40 ± 1 g) were shaped, packed, and stored in a domestic freezer at −18°C for 60 days, being analyzed on the day they were prepared (day 0) and after 15, 30, 45, and 60 days of storage.

Cholesterol and cholesterol oxides were obtained by direct saponification (8). Then, after derivatization (13), the samples were analyzed with a gas chromatographer (Shimadzu, GC-2010, Tokyo, Japan) with Rtx-5MS capillary column (60 m x 0.25 mm x 0.25 µm, Restek, Bellefonte, USA). The identification was done by evaluating the retention times of standards and quantification was performed by external standardization. To confirm their identity, a gas chromatographer (VARIAN 43-GC, Varian, Walnut Creek, CA) coupled to a Varian 210-MS /IT ion trap mass spectrometer (Varian, Walnut Creek, CA) with electron ionization was used (14).

Results of total content of phenolic compounds and antioxidant capacities of pink pepper extract are presented in Table 1. The total phenolic content was 5.05 ± 0.19 mg GAE/g, which was lower than the one reported by Menegali et al. (6) (12.17 mg GAE/g). Andrade, Poncelet, and Ferreira et al. (15), when evaluating ethanolic extracts, obtained by ultrasound-assisted extraction and Soxhlet, determined values ranging from 14.2 to 60 mg GAE/g extract.

Table 1: Total phenolic content and antioxidant capacities of pink peeper extract.

Methods	Values
Total phenolics mg GAE/g	5.05 ± 0.19
DPPH % Inhibition percentage	84.45 ± 1.61
FRAP µmol Fe^{2+}/g	121.54 ± 1.31

Results presented as mean ± standard deviation, n=3.

An inhibition percentage of 84.45 ± 1.61% was determined by the DPPH assay and 1.31 µmol Fe^{2+}/g was found applying FRAP (Table 1). Variable values can be observed in the literature. Lower inhibition percentages, from 42.68 to 95.03 %, were reported for methanolic extracts by the DPPH method (2,4). FRAP assay was previously performed in pink pepper by Silva et al. (16), who found 46.70 µmol Fe^{2+}/g.

The antioxidant capacity of pink pepper is well-documented and has been mainly attributed to the presence of phenolic compounds, which showed varied concentrations due to the influence of factors such as plant origin, harvest time, ripeness, climatic conditions, and genetic (3,4). Besides that, aspects related to the extraction procedures may lead to varied results (15).

Fish burgers presented a cholesterol level of 235.5 ± 9.5 mg/100 g, in dry basis, at day 0 (Table 2). Previous studies found values in the same range for raw fish meat from different species, 237.2 mg/100 g in *Sardinella brasiliensis* (17) and 249.30 mg/100 g in *Merluccius hubbsi* (10), while a higher content was assessed in *Sardina pilchardus* (342.20 mg/100 g) (8).

Table 2: Cholesterol (mg/100 g, dry basis) and cholesterol oxides levels (µg/g, dry basis) of fish burger samples.

		Days of storage				
		0	**15**	**30**	**45**	**60**
Cholesterol (mg/100 g)	**Control**	235.5 ± 1.5aA	225.7 ± 1.1bAB	223.2 ± 1.1bBC	215.1 ± 2.8bC	181.9 ± 1.7bC
	Pink pepper	---	229.1 ± 1.9aB	225.9 ± 2.8aB	225.6 ± 1.8aC	212.5 ± 4.2aD
Total COPs (µg/g)	**Control**	3.10 ± 0.4aA	5.9 ± 0.3aA	8.63 ± 0.4aB	17.30 ± 0.8aC	21.2 ± 0.9aD
	Pink pepper	---	5.14 ± 0.2bB	6.39 ± 0.3bB	11.98 ± 0.5bC	17.15 ± 0.8bD

Results presented as mean ± standard deviation, n=3. Values that are followed by different capital letters in the same row indicate difference among days of storage ($p < 0.05$) by the Tukey test. Values that are followed by different lowercase letters in the same column indicate differences among the treatments for each parameter ($p < 0.05$) by the Tukey test.

A reduction of cholesterol levels was observed during storage. The amount of cholesterol decreased from 235.5 ± 1.5 (day 0) to 181.9 ± 1.7 mg/100 g (day 60) in control samples ($p < 0.05$), which corresponds to a loss of 22.7%. Similar findings were determined in fish balls prepared with *Sardina pilchardus* during storage, where the cholesterol level reduced from 342.20 to 277.31 mg/100 g after 60 days (8).

However, a protective effect against cholesterol degradation was demonstrated by the addition of the natural antioxidant. For fish burgers containing pink pepper, the cholesterol content reduced from 235.5 ± 1.5 (day 0) to 212.5 ± 4.2 mg/100 g (day 60) ($p < 0.05$), which corresponds to a lower loss of 9.77%. Reductions of cholesterol levels may be attributed to oxidative processes; however, cholesterol may decompose by other routes leading to the formation of compounds such as hydrocarbons, ketones, aldehydes, and alcohols, in addition to cholesterol oxides (18).

The cholesterol oxides found in fish burger samples were 7α-hydroxycholesterol, 7-ketocholesterol, and 25-hydroxycholesterol. At day 0, 7α-hydroxycholesterol (3.10 ± 0.4 µg/g) and 25-hydroxycholesterol (1.20 ± 0.0 µg/g) were detected in samples. Storage led to an increase in these oxides amount during the 60

days: 7α-hydroxycholesterol (3.10 ± 0.4 - 14.5 ± 2.7 µg/g) and 25-hydroxycholesterol (1.20 ± 0.0 - 3.50 ± 0.0 µg/g), as well as the formation of 7-ketocholesterol (0 - 3.20 ± 0.1 µg/g). Thus, after 60 days the most relevant COP was 7α-hydroxycholesterol, followed by 7-ketocholesterol and 25-hydroxycholesterol, respectively.

Since cholesterol oxidation initiates with the abstraction of the allylic C7 hydrogen, COPs derived from C7 are commonly predominant (25). 25-hydroxycholesterol was formed in lower amounts, in agreement with the literature that describes as minorities oxides derived from the cholesterol side chain (8,10,18).

Pink peeper protected cholesterol from oxidation. The total COPs varied from 3.10 ± 0.4 (day 0) to 21.2 ± 0.9 1 µg/g (day 60) in control samples, while levels ranging from 3.10 ± 0.4 (day 0) to 17.15 ± 0.8 µg/g (day 60) were found in fish burgers with pink pepper. The addition of pink pepper was capable of reducing COPs formation by 19.11%, when comparing with COPs concentration in control samples ($p < 0.05$).

Cholesterol oxidation can be influenced by factors such as temperature, oxidation time, oxygen, light, medium composition, initial cholesterol concentration, among others (18,19). Studies have demonstrated that storage under low temperatures may increase COPs level in frozen fish (8-10), highlighting the importance of applying new strategies to control cholesterol oxidation. Pink pepper extracts have improved the oxidative stability of sardines (5), chicken burgers (6), and pork sausages (20) during refrigerated storage. However, there are no studies available regarding the direct addition of pink pepper fruit as anti-cholesterol oxidation agent in fish products during frozen storage.

CONCLUSION

The overall results highlight the remarkable potential of pink pepper fruit to minimize cholesterol oxidation in fish burgers during frozen storage. Due to the lack of time, more convenient and practical foods have fitted consumers' preferences. As a result, the consumption of ready-to-cook frozen food has increased significantly, poiting out the importance of limiting the formation of such deleterious compounds like cholesterol oxides. Thus, this study may contribute to the public health field in terms of COPs-reductions consumption. However, although promising results were found, further studies should be conducted to evaluate different levels of pink pepper addition and sensorial aspects, so that this condiment may be better explored by the food industry.

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