# EFFECT OF VIRGIN OLIVE OIL PHENOLS ON THE FORMATION OF HETEROCYCLIC AMINES DURING FRYING OF BEEF BURGERS

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## Background

Several epidemiological studies have shown a correlation between the intake of fried, broiled or roasted meat and the development of cancer. It has been suggested that heterocyclic amines (HAs) play a role in the aetiology of human cancer. HAs are formed during cooking of muscle food and about twenty HAs have been identified so far. The International Agency on Cancer Research (IARC) [1] has recommended reduced human exposure to these compounds. The reaction mechanisms that lie behind the formation of HAs are very complex, but the Maillard reaction has been suggested to play an important role. Since the Maillard reaction involve free radical reactions, the effects of antioxidants have been examined in model systems [2]. It was shown that the addition of virgin olive oil or its phenols reduced the formation of HAs.

The phenols in virgin olive oil are secoiridoid derivates, formed by p-hydroxyphenylethanol or dihydroxyphenylethanol linked to elenolic acid, figure 1. These compounds and especially the dihydroxy derivates have strong antioxidative capacity and prevent the fatty acids in virgin olive oil from oxidative damage during processing and storage. The absolute concentration of these phenols depends of several factors for example cultivar, ripening age, climate and extraction process. Also the storage affects the phenolic compounds by oxidative and hydrolytic modifications.

# Objectives

The aim of the present study was to investigate the role of olive oil phenols in the formation of HAs in meat cooking experiments.

#### Methods

Freshly prepared virgin olive oil were used for the experiments. The phenols from the virgin olive oil were extracted by liquid-liquid extraction [3] and identified and quantified by LCMS. The antioxidant capacity of the phenols from virgin olive oil was measured by the ABTS-method [4]. Triplicate determinations were made for the samples and the blank. The percentage inhibition was calculated and the antioxidative capacity expressed as Trolox equivalents (trolox). For the frying experiments, virgin olive oil, refined olive oil, virgin olive oil depleted on phenols and refined olive oil with addition of phenols were tested.

Beef burgers were fried in the different oils at 200°C for 5 minutes per side. The crusts of the beef burgers were screened for HAs using solid phase extraction and HPLC analysis [5].

### **Results and discussion**

The phenol content in the virgin olive oil can be seen in Table 1. The total amount of phenols was 400ppm, which indicates an oil of good quality. The antioxidant capacity was 2,9 mmol trolox/100g oil. The refined olive oil contained no phenols.

The beef burgers were ordinary brown, looked appetizing and no difference in surface color between the beef burgers fried in different oils could be seen. The following HAs were detected in all samples: MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline), PhIP (2-amino-1-methyl-6-phenyl-imidazo[4,5-b]-pyridine), Norharman (9H-pyrido[3,4-b]-indole) and Harman (1-methyl-9H-pyrido[3,4-b]-indole). The amounts are shown in Figure 2. Virgin olive oil reduced the amount of HAs, compared with the other oils. When the phenols were discarded, the reducing effect disappeared. Addition of phenols to the refined olive oil did not reduce the amount of HAs, probably due to damage to the phenols during extraction.

The formation of HAs is promoted by lipid oxidation of the frying fat. Free radicals are formed that favour the condensation reactions leading to HAs. If the lipid oxidation is counteracted by the virgin olive oil phenols, less free radicals will be formed and thus also less HAs. Virgin olive oil phenols may also interact with the free radical mechanism of HA formation and make that less amount of HAs is formed.

#### Conclusions

Virgin olive oil reduced the formation of PhIP, Harman, and Norharman during frying of beef burgers, compared with refined olive oil. This is probably due to the presence of phenols.

The inhibitory effect disappeared if the phenols were discarded from the virgin olive oil.

The addition of phenol to refined olive oil did not show any effect, which indicates that the extraction of phenols may be a critical step.

## **Pertinent literature**

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# Acknowledgment

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Figure 1. Structure of phenols in vigin olive oil

Table 1. Phenol content in virgin olive oil

PHENOL	AMOUNT (PPM)
3,4-DHPEA-EA	84,4
3,4- DHPEA-EDA	79,2
p-HPEA-EA	97,6
p-HPEA-EDA	148
Totally	400



Figure 2. Heterocyclic amines formed during frying of beef burgers.