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*Original Research*

# Formaldehyde in Human Saliva as an Indication of Environmental Tobacco Smoke Exposure

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## Abstract

The aim of this study was to present the effectiveness of the proposed sample preparation procedure coupled with the spectrophotometric technique for the determination of formaldehyde in saliva samples collected from heavy, moderate, and passive smokers. Formaldehyde levels were determined in saliva samples collected from smoking and non-smoking individuals. Formaldehyde was found in about 60% of the samples analyzed, with concentrations ranging from 0.31 to 94 μg/g. The results confirmed the significant effect of tobacco smoking on formaldehyde levels in saliva samples.

**Keywords:** biological monitoring, biological fluids, human saliva, environmental tobacco smoke (ETS), formaldehyde (HCHO)

## Introduction

Humans may be exposed to a variety of chemical hazards. Tobacco smoking constitutes a significant source of indoor air pollution and carbonyl compounds, including formaldehyde, have been shown to be major air pollutants.

During tobacco smoking two streams of smoke are emitted:

- the mainstream smoke (MS), which is inhaled directly from a cigarette by the smoker, and
- the sidestream smoke (SS), which is emitted into the air from the smoldering cigarette tip. A large number of chemical compounds present in the mainstream and sidestream smokes are carcinogenic and also cause long-term toxic effects. A mix of the sidestream tobacco smoke and exhaled mainstream tobacco smoke has been defined as environmental tobacco smoke (ETS). The composition of environmental tobacco smoke depends on the content of compounds present in the tobacco plant and the various ingredients added to tobacco during the manufacturing process.

One of the ingredients frequently added to tobacco during the manufacturing process are sugars. Sugars are not only tobacco additives, but also natural tobacco components (Fig. 1). The tobacco companies claim that sugars are added to tobacco products in order to aid the production process and to realize brand specifications and give the individual brands their characteristic flavour. Sugars in some way promote tobacco smoking because they mask the harsh taste and the irritability of tobacco smoke [1-3].

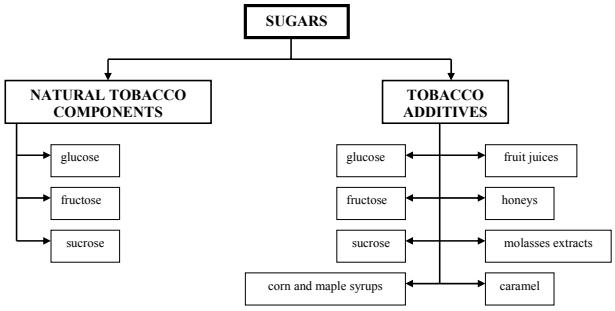


Fig. 1. General classification of sugars [1-3].

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Table 1. Parameters of calibration curves, limits of detection and quantification, and precision of the analytical results obtained by application of high-performance liquid chromatographic technique and spectrophotometric technique.

| Technique | Equation of the calibration curve* | Correlation coefficient (r) | Number of series | LOD | LOQ | Precision [%] | Expanded uncertainty [%] |
|---|---|---|---|---|---|---|---|
| Spectrophotometric | y=1.23x+0.010 | 0.996 | 7 | 0.050 mg/dm$^3$ | 0.150 mg/dm$^3$ | 5 | 10 |
| HPLC | y=151x+90.7 | 0.992 | 3 | 0.40 µg/dm$^3$ | 1.20 µg/dm$^3$ | 4 | 10 |
| HPLC/photometric (standards) | y=0.0070x-0.41 | 0.996 | 3 | | | | |
| HPLC/photometric (real samples) | y=0.0020x-0.0020 | 0.974 | 3 | | | | |

* measuring range 0.02-8.00 mg/dm$^3$.

Generally, sugars are recognized as being safe when used in food products, but this recognition does not imply their safety as tobacco additives. While sugars are approved as additives for foods, they were not tested by burning them. And it is the burning process that changes their properties, often for the worse. In burning tobacco, sugars undergo complex changes that result in a large number of highly toxic products such as formaldehyde (Fig. 2) [1, 4-7]. Due to the significant content of sugars added to tobacco products, their fate during tobacco smoking is an object of interest for many scientists.

Some methods have been reported for determining formaldehyde in indoor air, such as: liquid chromatography [8], high-performance liquid chromatography [9], and capillary electrophoresis [10-12]. However, only a few research studies have included the direct measurement of this compound by means of high-performance liquid chromatography [13-14] and capillary electrophoresis [14] in the saliva collected from smoking and non-smoking individuals.

The aim of this work was to:

- present the effectiveness of the proposed sample preparation procedure coupled with the spectrophotometric technique for the determination of formaldehyde in human saliva samples collected from heavy, moderate, and passive smokers
- determine to what extent formaldehyde is adsorbed orally by heavy, moderate, and passive smokers.

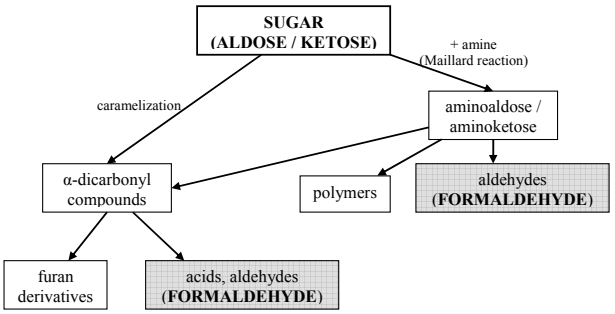


Fig. 2. Transformation of sugars into the toxic constituents of environmental tobacco smoke [1].

## Experimental

### Chemicals and Apparatus

The formaldehyde solution and sulphuric acid were purchased from Sigma-Aldrich (Schnelldorf, Germany). Chromotropic acid was obtained from Merck (Darmstadt, Germany). Deionized water was obtained from a Millipore Gradient A10 (resistivity: 18.2 MΩcm at 25°C) water purification system (Millipore, Bedford, MA, USA). A Spectroquant® Pharo 100 spectrophotometer was purchased from Merck (Darmstadt, Germany).

### Analytical Procedure

Human saliva samples were collected in the morning (before breakfast) in sterile glass bottles and transported immediately to the laboratory for analysis. A glass vial was weighed twice (without and with saliva sample), diluted with deionized water obtained from a Milli-Q water purification system and centrifuged (2,000 rpm, 20 minutes). After centrifuging, a clear supernatant fluid was collected, diluted to a final volume of 25 ml with deionized water (Milli-Q quality) and transferred into the ultrasound bath (15 minutes) in order for the sample to be homogenized. Formaldehyde was determined spectrophotometrically based on the reaction with chromotropic acid. In a solution acidified with sulphuric acid, formaldehyde reacted with chromotropic acid to form a violet dye that was measured (absorbance measured at 585 nm).

In order to apply the spectrophotometric technique for the determination of formaldehyde in human saliva samples, a comparison with the high-performance liquid chromatographic technique is required. Parameters of calibration curves, limits of detection and quantification, as well as precision of the obtained analytical results (estimated during comparative studies) are presented in Table 1.

## Results and Discussion

Human saliva samples were collected from heavy, moderate, and passive smokers, as well as from non-smoking

Table 2. Summary of data relative to individuals considered in this study.

| | Total number of individuals | Sex | Cigarettes/day |
|---|---|---|---|
| Heavy smokers | 48 | 19 ♀, 29 ♂ | > 10 |
| Moderate smokers | 43 | 22 ♀, 21 ♂ | < 10 |
| Passive smokers | 24 | 12 ♀, 12 ♂ | exposed to ETS (2-3 hours per day) |
| Non-smoking individuals | 21 | 11 ♀, 10 ♂ | not exposed to ETS |
| All individuals | 136 | 64 ♀, 72 ♂ | |

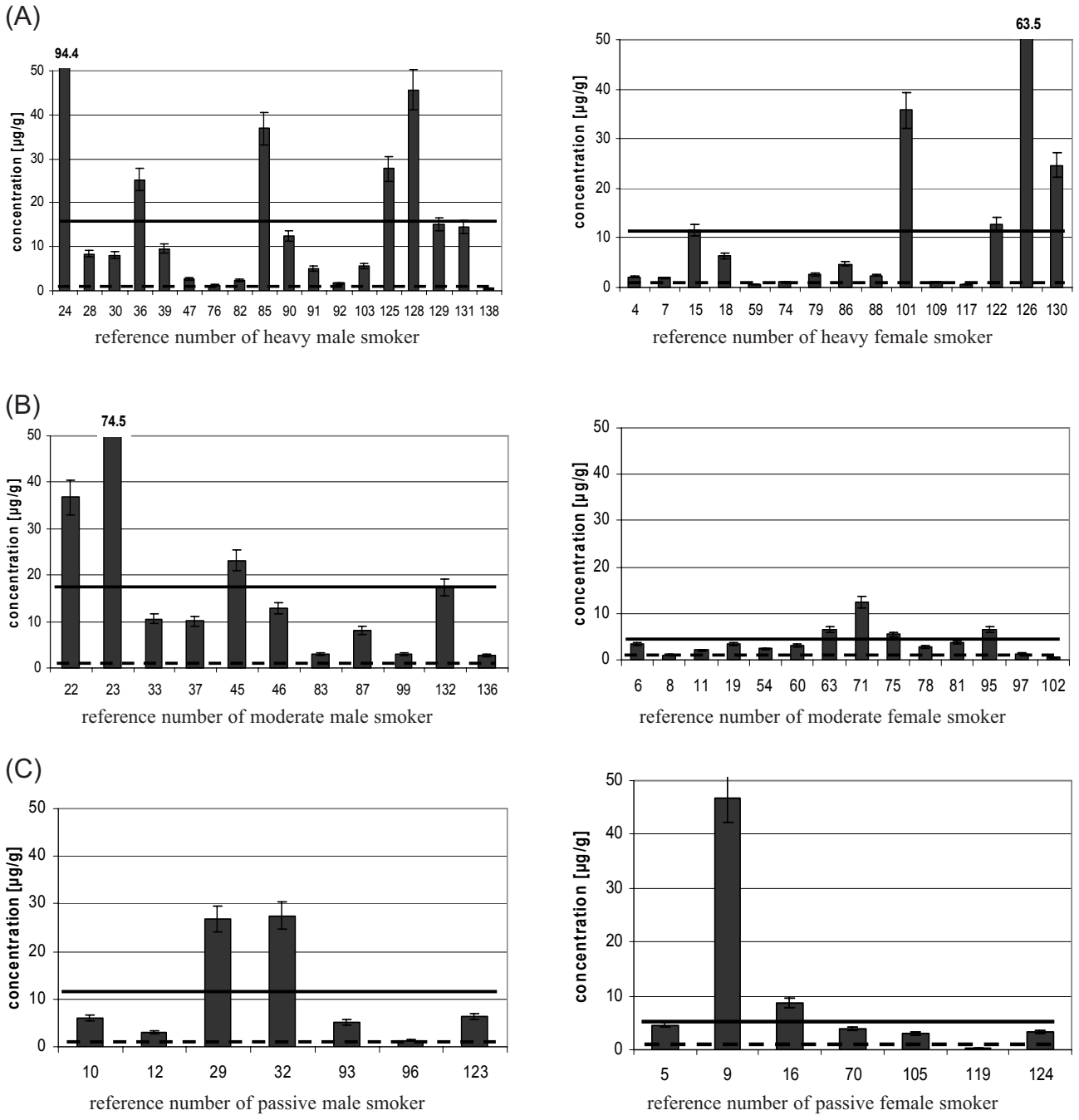


Fig. 3. Average formaldehyde concentration values determined in saliva samples collected from: (A) heavy male and female smokers, (B) moderate male and female smokers, and (C) passive male and female smokers. Each sample was analyzed in triplicate.
The average concentration level of formaldehyde determined in saliva samples collected from non-smoking individuals was indicated in the figures by a discontinuous horizontal line. The average concentration level of formaldehyde determined in saliva samples collected from heavy, moderate, and passive smokers is indicated by a horizontal black line.

individuals. The individuals were classified into the following groups by using their responses from the smoking habits questionnaire:

- Heavy smokers, who smoked more than 10 cigarettes per day.
- Moderate smokers, who smoked 1-10 cigarettes per day.
- Passive smokers, who were exposed to the influence of environmental tobacco smoke at home or in the workplace (exposure time: approximately 2-3 hours per day).
- Non-smoking individuals, who were not exposed to the influence of environmental tobacco smoke at home or in the workplace.

Selected data relative to these individuals are summarized in Table 2.

Average concentration levels of formaldehyde determined in saliva samples corresponding to the different tobacco consumption categories established from the self-reports are presented in Fig. 3 (only detected and determined formaldehyde concentration values were indicated in the figures). The percentage contribution of human saliva samples, in which formaldehyde was detected and determined is shown in Fig. 4.

The rising tendency between average formaldehyde concentration values and the number of cigarettes smoked was observed (this statement was made on a basis of a large number of individuals (136), from whom saliva samples were collected). The highest concentration levels of formaldehyde in saliva samples were observed for male heavy smokers, when compared with female ones (smoking the same number of cigarettes per day). The same situation was noticed in the case of male and female moderate smokers. However, in the group of passive smokers, the highest formaldehyde concentration value (46.7 μg/g) was determined in the sample collected from female individuals.

Generally, formaldehyde was detected and determined in 69%, 58%, 58%, and 15%, of the saliva samples collected from heavy, moderate, passive smokers, and non-smoking individuals, respectively. In the majority of cases, the exposure of passive individuals to the toxic constituents of environmental tobacco smoke in different public and workplaces can be compared to smoking a few cigarettes by them. Taking the obtained results into consideration, it can be concluded that the concentration level of HCHO determined in human saliva samples can be treated as an indication of environmental tobacco smoke exposure.

## Conclusions

The proposed sample preparation procedure coupled with the spectrophotometric technique for the analysis of formaldehyde in human saliva has been proved to be a simple, rapid, and cheap method. Moreover, the developed method can be useful for the determination of formaldehyde as a compound characterized by high toxicity in biological fluid samples [15]. The concentration level of HCHO determined in saliva samples can be treated as an indication of environmental tobacco smoke exposure. In the majority of cases, the highest concentration levels of HCHO were detected and determined in a group of heavy smokers. On the basis of the obtained results, the exposure of passive individuals to the toxic constituents of environmental tobacco smoke in different public and workplaces can be compared to their smoking a few cigarettes.

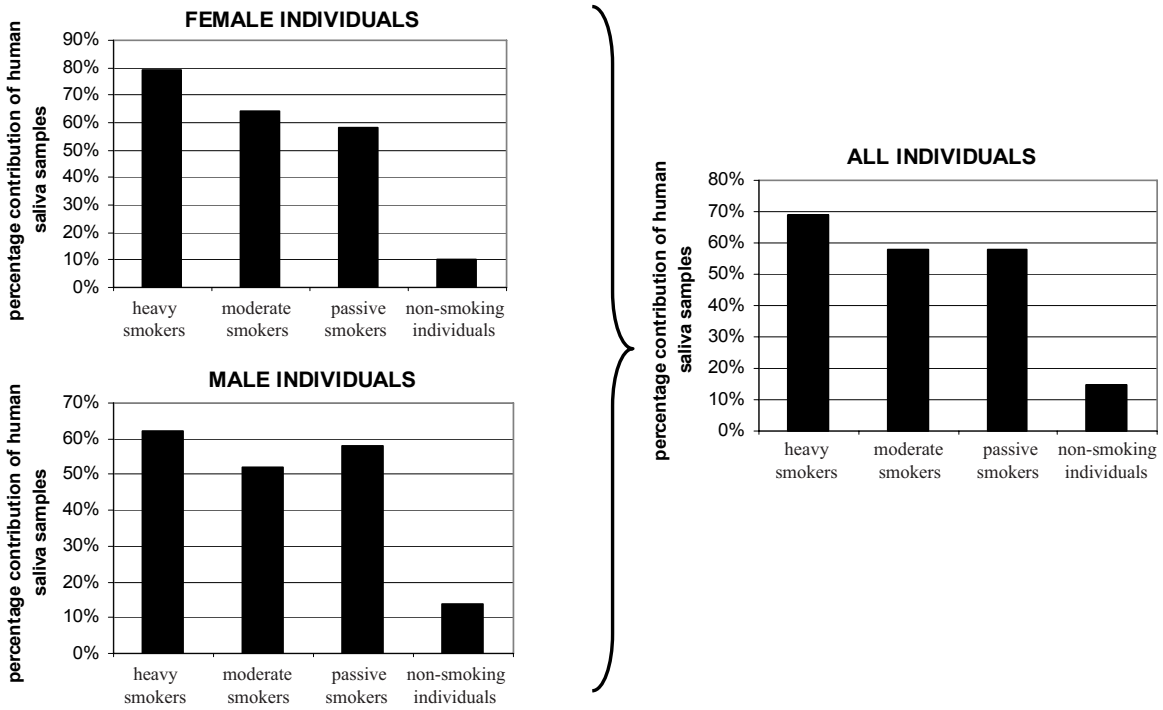


Fig. 4. Percentage contribution of human saliva samples in which formaldehyde was detected and determined.

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