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Dedicated to Professor Dr. Cozar Onuc on His 70th Anniversary

IDENTIFICATION OF ALKALOIDS IN THE *CLAVICEPS PURPUREA* FUNGUS

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ABSTRACT. Modern nutritionists worldwide require quality foods, based on crude vegetables and breads. Important nutritional benefits are provided by bakery products based on rye (*Secale cereale L*.), still contaminated rye flour with the fungus *Claviceps purpurea* may occur. Still a lot of dangerous substances can be found accidentally in natural products, causing accidental intoxication or even death. The present case study is meant to find quick analysis on unprocessed rye flour, *Secale cereale,* in order to define the presence of main compounds indicating contamination with *Claviceps purpurea.* Qualitative analysis using spectrofluorimetry confirmed literature data and revealed presence of gluten, ergometrine, growth factors, ergoalkaloids, lysergic acid and starch.

Keywords: *qualitative analysis,* Claviceps purpurea *fungus, alkaloids, rye flour, bread, spectrofluorimetry*

INTRODUCTION

Naturally produced alkaloids can be found in plants, synthesized for protection against herbivores and other enemies. For example common plant families which produce tropane alkaloids are the Brassicaceae (mustard family), Solanaceae (nightshade or potato family) Gramineae (rye) and Erythroxylaceae (coca family) [1].

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Claviceps purpurea is a fungus growth in food grain during wet seasons, particulary in rye. Ingested it disturbes activity of Central Nervous System, producing a disease named "ergotism" and, eventually, death [2]. Vasospasm related to ergot intoxication has been recognized since the Middle Ages, when it occurred due to ingestion of rye contaminated with the mold *Claviceps purpurea*. Today ergotism is a rare cause of peripheral ischaemia, most often associated with ergotamine tartrate therapy for migraine headaches [3]. Nevertheless, an awareness of this condition is important, as cases of ergotism may still occur. At present the dose of alkaloids in *Claviceps purpurea* in relation with the fungal species and, respectively, of geographical distribution of host plant it's not known. For example alkaloids from *Claviceps purpurea grown* up on rye (*Secale cereale L.)* are different than those grown up in/on other grasses. In order to identify and monitor all the factors responsible in speciation of alkaloids generated by *Claviceps purpurea* in every kind of grasses, targeted and performant research have to be organized [4].

Last decade specific literature contains some important references on qualitative and quantitative analysis of bioactive drugs of natural origin using fluorimetry principles. Techniques for the analysis of drug samples are classified into three categories (see Table 1) based on their maximum potential discriminating power. However, the classification of a technique may be lower, if the sample, analyte or mode of operation diminishes its discriminating power. Examples of combination of analytic techniques in efficient analysis of alkaloids may include:

- an infrared spectroscopy technique applied to a mixture which produces a combined spectrum;

- a mass spectrometry technique which only produces molecular weight information.

Categories of analytic techniques are determined mainly upon their complexity versus time and efficiency of results, meaning that most simple and rapid-giving useful information (confirming or denying the presence of a certain compound in the examined sample) are methods included in category C, and the most detailed information being obtained after careful examination using category A methods.

Table 1: Categories of Analytical Techniques [5].

Category A	*Category B*	*Category C*
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Raman Spectroscopy	Liquid Chromatography	Melting Point
X-ray Diffractometry	Microcrystalline Tests	Ultraviolet Spectroscopy

Rapid and efficient analytic techniques applied for quality control of common commercial products is widely reported, as modern bioprocess control of biomass, protein, and alkaloid concentrations during cultivation of *Claviceps purpurea,* using fluorescence spectroscopy, presenting the applicability of this instrumental analysis for bioproducts control and monitoring [5, 6].

FLUORESCENCE SPECTROSCOPY-METHOD

The fluorimetry gives enhanced sensitivity over other "Category C" analytic methods for those compounds which are naturally fluorescent. Drugs with good fluorophores include several antimalarials, natural alkaloids such as ergometrine, lysergic acid diethylamine (LSD), tetracycline derivatives, propanolol and derivatives, etc. Generally more rigid the substituted aromatic structures give increased fluorescence [10]. Excitation spectra are usually used to confirm the identity of components and to select an optimum excitation wavelength for quantitative analysis. The emission spectrum is then used for qualitative and quantitative analytic applications.

The fluorescence measurements were performed on rye flour and on *Claviceps purpurea sclerotia* powder respectively using the ABLE & JASCO FP-6500 spectrofluorimeter equipped with a xenon flash lamp for the excitation light. The measurements were carried out exciting the solid samples (disposed in a specific holder for solid samples) at excitation wavelengths corresponding to the main compounds possible to be found in the samples. For all the measurements an excitation band width of 3 *nm* and an emission band width of 5 *nm* were applied.

RESULTS AND DISCUSSION

In Fig. 1 the fluorescence spectra of flour of *Secale cereale* (SC) and *Claviceps purpurea* (CP) for gluten identification are represented.

Emission maxima at 318 *nm* and at 410 *nm* respectively found in both *Secale cereale* (SC) and of *Claviceps purpurea* (CP) samples are in good agreement with literature data [8]; the differences noticed are mainly due to the examination of analytically unprocessed samples in our present case study. Identification of ergot alkaloids in different products by HPLC with fluorescence detector is early reported in literature [9]. For excitation at 320 *nm* ergometrine and their derivatives fluoresce at an emission wavelength of 405 *nm*, as it is represented in Fig. 2, but most probably is a non-specific emission, or is covered by the gluten emission occurred at 410 *nm* [11, 12].

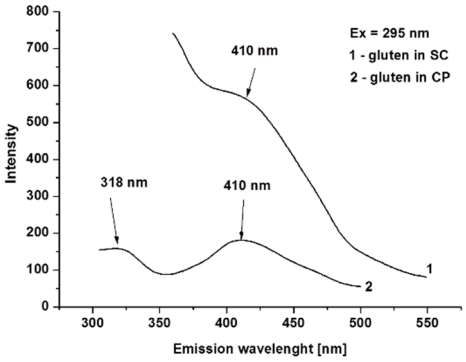

Fig. 1. Fluorescence spectra of flour of *Secale cereale* (SC) and of *Claviceps purpurea* (CP)

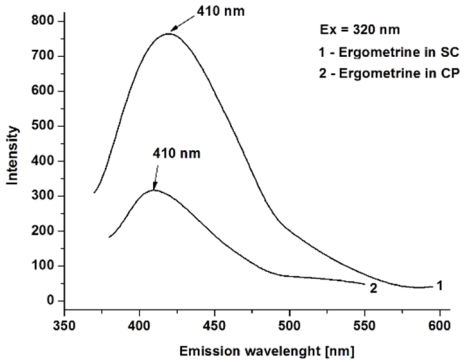

Fig. 2. *Fluorescence spectra of flour of* Secale cereale *(SC) and of* Claviceps purpurea *(CP) for the ergometrine identification*

In Fig 3 the fluorescence spectra obtained by excitation at 350 *nm* of the two samples - rye flour of *Secale cereale* (SC) and sclerota powder of *Claviceps purpurea* (CP), respectively are presented. As it can be seen rye flour (SC) exhibits an emission at 425 *nm* and sclerota powder (CP) has a broad emission band between 414 *nm* and 423 *nm*. Excitation at 360 *nm* reveals emissions at 431 *nm* for SC and at 423 *nm* – 426 *nm* for CP respectively, confirming presence of lysergic acid in both samples.

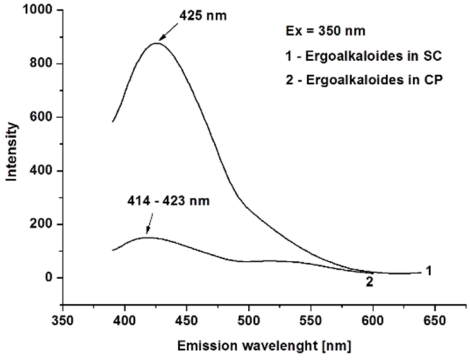

Fig. 3. Ergoalkaloides in integral rye flour, *Secalum Cereale*, and in sclerota powder of *Claviceps purpurea*

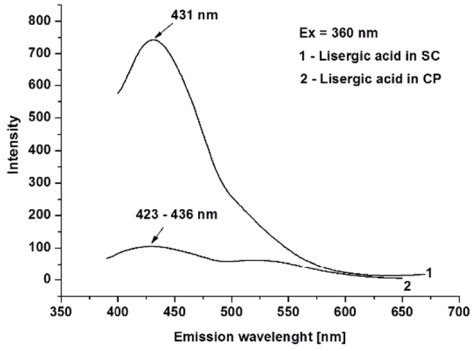

Fig. 4. Lisergic acid in integral rye flour, *Secalum Cereale*, and in sclerota powder of *Claviceps purpurea*

In the same time the growth factors, were put into evidence applying excitation wavelengths of 390 *nm* for NADPH, see Fig 5, or, respectively, of 450 *nm* for flavine, see Fig. 6. NADPH and flavine are biogenic fluorophors exhibiting emissions as follows: Em NADPH = 456 *nm* for both SC and CP, Em flavine = 507 *nm* for SC and Em flavine = 513 *nm*-516 *nm* for CP.

All these results confirmed the existence of ergoalkaloids in the two samples, as expected, and are in good agreement with literature data [7, 13].

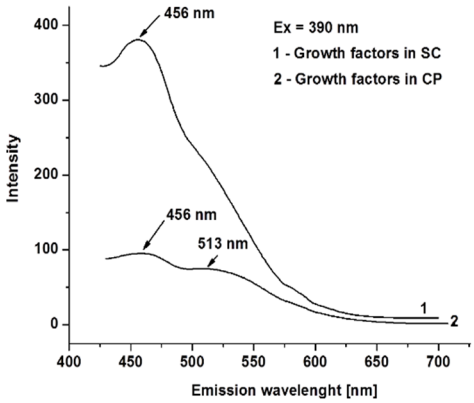

Fig. 5. Growth factors – NADPH – in integral rye flour, *Secalum Cereale*, and in sclerota powder of *Claviceps purpurea*

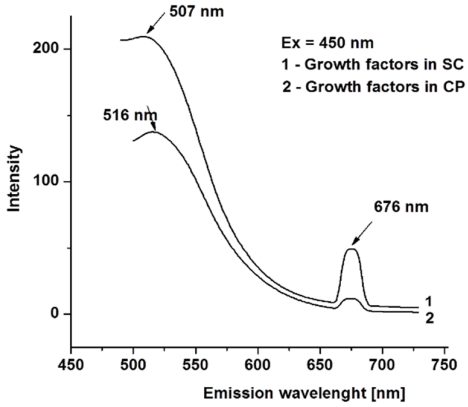

Fig. 6. Growth factors – flavine – in integral rye flour, *Secalum Cereale*, and in sclerota powder of *Claviceps purpurea*

Starches can be colorimetric identified in substances by using a solution of iodine and potassium iodide (I_2, KI). A color change from dark blue to black will occurs [14]. In Codex Alimentarius are not validated yet fluorimetric determinations [15], even if regional accepted protocols are applied. For quantitative determinations starch extracts incubated 30 min at room temperature exhibits fluorescence at 530 *nm* excitation and emission at 585 *nm*. [16]. Our experiment confirms the existence of starch, due to fluorescence emission signal at 795 *nm* excited at 530 *nm* (like in the protocol above specified). Shift of emission wavelenght from 585 *nm* to 795 *nm* can be caused by the fact that present fluorimetric identification of starch was done on solid unprocessed samples of integral rye flour, *Secalum Cereale* and of sclerota powder of *Claviceps purpurea*, respectively.

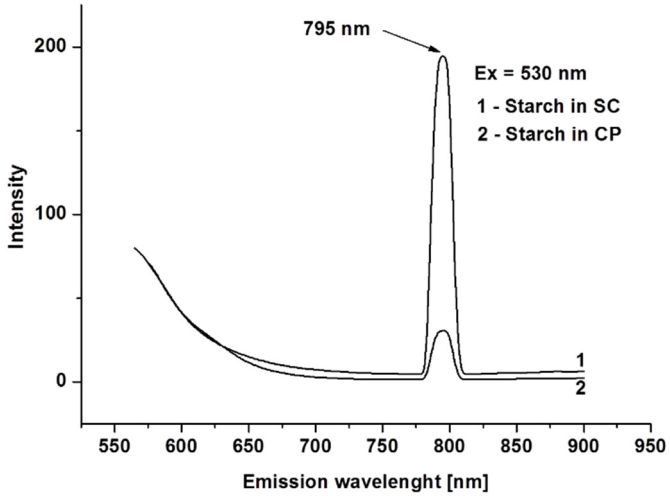

Fig. 7. Starch identification in *Secalum Cereale* (SC) integral rye flour and in sclerota powder of *Claviceps purpurea* (CP).

CONCLUSION

Qualitative identification of main constituents and alkaloids in integral rye flour, *Secalum Cereale*, and sclerota powder of *Claviceps purpurea* using spectrofluorimetry demonstrated the progress of application of above specified method in quick analysis of alkaloids. In further experiments extraction and purification of compounds of interest as well as quantitative analysis will follow, proving the efficacy and reliability of spectrofluorimetry for alkaloids quantification in food products. The fluorimetry has the advantage that the results could be read within 1–5 hours and the reproducibility was superior to other methods.

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