SUMMARY OF Ph.D. THESIS

BIOMARKERS INVOLVED IN ESTABLISHING
THE QUALITY AND AUTHENTICITY OF
MEAT AND MEAT PRODUCTS

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INTRODUCTION

There are increasing events at global level which represent the illegal labeling and food adulteration. The trade of food is frequently disturbed by anomalies of food safety and quality of the products. Recent controversies on horse meat issues have brought a lot of control and supervises more frequently in connection with the authenticity of the products which are on the unique European market. The expression: “authentic product” refers to the ”intact product without additives or inferior ingredients, improper for the real quality of the product”. In the case of meat, the partial or total replacement/ substitute of raw meat with another one, cheaper than it, in order to have financial benefit is considered economical fraud. Even though these practices don’t affect the public health, in the big majority of cases, they are capable to cheat the consumer and they are breaking religious believes.

Therefore it is very important that the information of the producer is reflects the real content on the label of the product, so that the consumer has the chance to choose which ones contain the needed qualities. Nowadays, the consumers have became conscious of the dangers to which they are exposed because of the quality and the safety of the products. These aspects have to be specified on the label of the products. At a global level, lots of analytical techniques were put in practice, lately, in order to test the authenticity of different food products . The most important techniques have been proved to be those which are based on the protein analyses using immunological techniques and those of DNA, using electrophoresis methods. The polymerase chain reaction- PCR has became one of the most applied techniques in identifying the quality and authenticity of the products because of the high specificity and limits of detection. The technique is using particular sequences of DNA, which are called molecular markers into relevant genes for production or characteristic genes for the species.
Establishing the quality and the authenticity of meat products can be performed by analyzing some biochemical markers and molecular markers which are particular for each item. It is very important to know the particularities of the product. These particularities decide the selection of the biomarkers of the product. Because of them it is easier to choose the methods for analyzing.

This thesis represents the first step in order to certify some biochemical and molecular markers in the production pathway of processing meat and meat products. Furthermore, these biochemical and molecular markers could be selected for control units for testing authenticity and quality of items. Also, investigations and tested methods of analyzing help us know much more in this area. These types of investigations can be applied in any laboratories which are attested in molecular technique of analyzing.

In order to achieve this goal have been proposed to achieve the following objectives:

- The establish effective DNA extraction methods for optimal success of simplex and multiplex PCR for species identification in meat products by testing specific molecular markers;

- The establish a functional and fast PCR protocol for evaluating the authenticity of meat products obtained in the studied area and checking the appropriate labeling of products;

- The evaluation of infrared spectroscopy technique of the compositional biochemical markers of meat of bovine, swine, poultry and meat products;

- The identification of sex in meat obtained from cattle by PCR molecular technique and evaluation of compositional features by gender producing species.

The structure of the research

This study is divided in 6 chapters and 2 main parts. The first part has chapter 1 and 2 describing the current state of the art. The following part two represents “personal research” during 4 chapters. The thesis contains 198 pages, 53 graphics and images and
33 tables. All are structured and printed in conformity with the standards and requests of USAMV doctoral School, Cluj-Napoca. The biographical list includes 255 titles.

The first part is presenting the state of the art in the field of food control. **Chapter one** gives us information about biomarkers which are involved in the quality and the authenticity of meat and meat products and the methods used to put all of this in evidence. **Chapter II** is making reference to testing methods of meat compound parameters and meat products recorded in Romanian market.

The second part of the thesis is structured to reveal the results of this research. It debuts with the motivation of choosing this research theme and the purpose of it and the general objectives to point in this thesis. The description of the experimental part was performed in chapter 3, 4 and 5 which are followed by general conclusions and recommendations in chapter 6.

**Chapter III** of this research has pointed out the testing of some extracting protocols of DNA from meat but also from meat products under thermic treatment with the validation of the most efficient one concerning the aspects of quantity, purity as result after the applications of PCR technique. For the successful use of PCR technique, the extracted DNA has to have some essential characteristics so, in these directions we have proposed the following objectives:

- Evaluation of purity and quantity of DNA extracted from various samples of meat and meat products using three commercial kits with different specificity;
- Comparative evaluation of extraction methods tested based on the quantity, purity, result from the PCR application technique;
- The validation of the most efficient methods of DNA extraction for meat products obtained by processing high temperatures.

Evaluation of DNA purity and quantities extracted from meat and meat products, we didn’t identify important differences from a statistical point of view (p> 0.05) for none of the 3 kits. From a statistical point of view, for the values of DNA purity and quantities registered at meat products, under extracting with tree testing kits, we have identified significant differences (p< 0.05) between Bioline kit and FastID kit.
As a conclusion, we can state that from the point of efficiency in realizing the PCR technique, that the extracted DNA with kit I- Bioline from the meat products that were thermally treated, was proved to be more superior, the strips which were obtained were clear and there were no secondary or non-specific products in the reaction, and using of 3 commercial kits is trustful in case of meat.

![Figure 1](image)

Fig. 1: The results obtained following the amplification with specific primers for cow of the DNA samples extracted with kit I

Chapter IV evaluated and characterized biochemical markers used for compositional specificity with some of the meat products that came from several species (bovines, pork, sheep, chicken) studying the alteration of the quality parameters (protein, fat and moisture) depending on anatomical areas and sex. To establish our purpose we have had the following specific objectives:

- The testing and validation of PCR protocols in order to identify the sex in case of beef;
- The appreciation of biochemical markers in case of beef in relation with the sex of the animal which supplies raw meat;
- The application of biochemical markers in case of pork and chicken, in analyzing different anatomical areas;
- The evaluation of the main compound parameters in different sorts of meat products registered in the Romanian market.

For the PCR reaction we used two sets of primers: specific primers for bovines, which amplified both the female DNA (216bp) and the male DNA (301 bp) and the set of specific primers for y bovine chromosome. These primers have only amplified fragments
which came from male samples. For female samples there was just one specific light strip and for the male samples there were two characteristical strips.

**Fig.2: The electrophoretic profile of DNA fragments from female and male**

Using FoodScan Lab offers the advantage of a complete and fast analysis. Therefore, in case of values obtained for compositional parameters from beef split on sexes, insignificant differences (p>0.05) from a statistical point of view were obtained between the values of proteins, fats, moisture and collagen obtained from female meat and male meat. Concerning the significant difference from a statistical point of view (p<0.05) for pork, those were signaled in all these areas put under the analyses. The chicken meat presents a nutritional stability concerning the values of the main analyzed parameters (protein, fat, moisture). There weren’t significant differences between the percents obtained analyzing chest meat and pulp meat samples.

In analyzing the main compositional parameters from products of the fresh category, there were significant differences (p<0.05) revealed for percentages of protein and collagen and the average values of moisture for smoked products which were analyzed (sausages and salami) high values (64%) were registered, over the maximum admitted limit (50%). After the analysis were made, we may draw the conclusion that the wide variations of compositional parameters of the samples belonging to the same sort represent the deficitary aspect of processing and the unconformity of the labeling. Some products are registering more increased values than those which are mentioned.

**Chapter V** has had the purpose of testing and validating fast molecular methods (and very sure) (multiplex PCR) of identifying species in meat products and the
performance of an investigation about the authenticity of specific Romanian meat products found in the studied regional market. Our objectives were:

- The selection of specific primers of each meat supplying species in order to be analyzed;
- The testing and the validation of simplex PCR techniques for identifying of the species in meat products which were examined;
- The testing and validation of multiplex PCR technique for the meat products which were under processing with high temperatures;
- The identification of a possible mixed-meat in case of the products which might be brought into regional market through putting in practice the multiplex PCR validated protocol.

From the amount of 138 samples of analyzed meat products, for 58 of them we registered a negative amplification with species specific primers (fig. 3). A part of those were partially substituted, especially for chicken meat. This kind of meat, from an economical point of view, is bought to a much lower price than beef or pork meat.

![Fig.3: The amplification results with cattle, horse, pig and chicken specific primers from meat products. The lines are: MK- 50bp DNA ladder, Sample 1- Banat Salami, Sample 2- Beef Baloney, Sample 3- Italian Salami, Sample 4- Chicken Polish Sausage, Sample 5- Hunter Salami, Sample 6- Summer Salami, Sample 7- Rose Sausage, Sample 8- Rustic Susages, Sample 9- Chiken Baloney, Sample 10- Pig Baloney, Sample 11- Beef Baloney, Sample 12- Baloney from pig, beef and chicken, Sample 13- Chicken Baloney, Sample 14- Polish Sausage from pig and](image-url)
chicken, Sample 15- Meda Polish Sausage from pig and chicken, Sample 16- Beef Frankfurters, Sample 17- Pig Frankfurters, Sample 18- Chiken Frankfurters

On the first place of fraud acts, there is the category of fresh products (baloney-58.8%), followed by frankfurters 50% and Polish sausages 46.6%. Among the products registered negative for amplifying, the biggest part is represented by the products aquisited as “bulk” (72.2% from amount of negative samples). These products don’t have genuine label of the producer which is usually to be found for the majority products finding on the counter. These products have label which mark the ingredients of the product. This is in fact the label of the shop.

After putting in practice the PCR technique we may say that simplex and multiplex PCR can be applied with success in confirming the origin of raw meat as so of meat which is thermally treated.

**General conclusions and recommendations**

- The method of extraction described and validated in this study in order to isolate genomic DNA from meat and meat products could be adopted with full success in other labs of food control.
- The technique in infrared spectroscopy might be applied with success in order to determine the compositional parameters in meat and meat products.
- The PCR technique validated into this study has proved to be accurate and efficient in terms of applicability and specificity for identifying the meat species from meat and meat products. PCR could be easily adopted in food control labs.
- The statistical analysis of the food authentication for meat products reveals a value of 33.3 % for adulteration samples from all samples which were analyzed.
- Using of multiplex PCR in food control labs can be done in order to supervise more carefully the eventual meat mixes.
The implementation of a supervising system of food adulteration and practicing of drastic penalties in case of missed labeling of the products.