Doctoral Dissertation
Doctoral Program in Material Science (29th Cycle)

Biomacromolecules: A sustainable approach for the design of fire retardants for textiles

By

Annalisa Casale
******

Supervisor:
Prof. Giulio Malucelli

Doctoral Examination Committee:

Prof. Marta Giamberini, Referee, Universitat Rovira i Virgili
Prof. Baljinder Kandola, Referee, University of Bolton
Prof. Roberta Bongiovanni, Politecnico di Torino
Prof. Alberto Frache, Politecnico di Torino
Dott. Marco Zanetti, Università di Torino

Politecnico di Torino
2017

Declaration

I hereby declare that, the contents and organization of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.

Annalisa Casale

2017

* This dissertation is presented in partial fulfillment of the requirements for **Ph.D. degree** in the Graduate School of Politecnico di Torino (ScuDo).

Nella vita non c’è nulla da temere,
solo da capire.

Margherita Hack

Acknowledgments

I would like to acknowledge all the people that helped me in preparing and writing this thesis. First of all, I want to thank prof. Giulio Malucelli for the opportunity to follow this Ph.D. course, for his enthusiasm and his patience, and for the interesting talks, either about work or not. Thanks to prof. Francesca Bosco for having instilled in me the passion and the strictness required in scientific research. A great thanks to dr. Chiara Mollea, a friend more than a colleague, who helped me orienting in this world, and was always present with suggestions and support.

I would like to thank all the people from Politecnico of Alessandria, especially Daniele, Samuele and Silvia, for their hospitality, Giusi, for the technical support and the SEM analyses and Fabio, for the cone calorimetry tests.

A sincere acknowledgement is due to prof. Richard Hull from the UCLan University in Preston, who kindly hosted me in his labs for almost three months, and, besides teaching me new and different techniques, contributed to this work with fruitful talks.

A heart-felt thanks to Fiona and Antony, who welcomed me with open arms and made me feel at home, even in a different country. Thank you for your help and your friendship.

Thanks to Elena, Daniele, Erika and Gustavo, my office mates, for the moments of fun between a test and the other. A great thanks for all the coffees, the chats and the laughs.

Finally, I would like to thank my parents and my brother for their everlasting love and support, there's always need for it.

Last but not least, a special thanks to Alessio, who is always able to make me laugh and to make me see the silver lining. Thanks for your presence, your help and your hugs.

Abstract

Textiles are very hazardous materials when related to fires, because of the high surface to mass ratio and the open structure, which simplify the contact with heat and oxygen. Fabrics have a great impact on the development of fatal fires because of their easy ignition and high burning rate. For the flame retardancy of fabrics several strategies have been developed throughout the years, and nowadays additives and reagents are widely used for the different kinds of textiles. The most common commercial durable finishes for cotton fabrics contain phosphorous and nitrogen compounds. These products have been predominant in the field of flame retardants for cotton for 50 years, but recently the request for a reduction of environmental impact and of formaldehyde release during manufacturing and utilisation have pushed researchers towards new kinds of finishing.

This Ph.D. work aimed at investigating the effectiveness and the possibilities of the use of biomacromolecules as fire retardants for cellulosic textiles (i.e. cotton). To this aim, three biomacromolecules were taken in consideration: whey proteins, caseins and nucleic acids.

Whey proteins and caseins, derived from milk, were applied on cotton fabrics and their thermal and thermo-oxidative stability and fire behaviour were assessed through thermogravimetric analysis, cone calorimetry and horizontal flame spread tests. These biomacromolecules were effective in improving the fire retardancy of the treated fabrics, slowing down the combustion rate in flame spread tests and favouring the formation of a coherent carbonaceous residue (char).

Then, the fire retardant behaviour of DNA was thoroughly investigated. First, commercially-available DNA from herring sperm and testes, having different molecular weights, were applied on cotton fabrics: the experimental parameters (i.e., molecular weight of the biomacromolecules, pH of the aqueous solution, number of impregnations needed for achieving the final dry add-on on the treated fabrics) were optimized in order to achieve the highest flame retardant effectiveness. The distribution of the nucleic acids on the underlying fabric was studied through SEM analyses. Thermogravimetric analyses, cone calorimetry and horizontal flame spread tests were carried out for assessing the thermal and thermo-oxidative stability and the fire behaviour of the treated fabrics. In detail, the low-molecular-weight DNA solution prepared either at pH 4 or 8 and applied on cotton with multiple impregnation steps was the most effective flame retardant treatment.

Pursuing the research, the problem related to the high cost of commercially available DNA was considered: to overcome this drawback, a novel extraction method, starting from exhausted biomasses or agro-food crops, was developed. This method focused on the extraction of high quantities of nucleic acids, exploiting a low environmental impact approach. Overall, the

recovered nucleic acids showed a fire behaviour similar to that of commercially-available counterparts.

Finally, the washing fastness of the cotton fabrics treated with the biomacromolecules was considered: in fact, all the selected biomacromolecules are waterborne systems, which easily come off the fabrics when subjected to washing cycles, even in hot water only. This issue was taken on by treating cotton fabrics with nucleic acids and chitosan in mixture or as separate layers and also exploiting the layer by layer technique. The washing fastness of the treated fabrics was significantly improved by subjecting them to UV-curing, thus achieving the grafting of chitosan on cotton and, at the same time, entrapping the nucleic acid in the grafted chitosan coating. Notwithstanding the achieved fire retardancy, the fabrics treated with chitosan and nucleic acids also showed an antibacterial activity, due to the presence of chitosan. Furthermore, 30 bilayers of nucleic acids and chitosan provided the fabrics with self-extinction, either before and after a water washing cycle at 55°C.

As far as the effectiveness of the treatments is concerned, all the selected biomacromolecules conferred fire retardant features to cotton fabrics. In particular, low-molecular-weight nucleic acids and caseins were the most performing biomacromolecules either in forced combustion or in flame spread tests. Cotton fabrics treated with nucleic acids or caseins were able to achieve self-extinction in horizontal flame spread tests, with a reduction of the burning rate and an increase of the residue left. Similar reductions in the HRR were also observed in cone calorimetry tests.

The suggested approach is quite simple and does not involve the use of particular chemicals or expensive equipment; furthermore, the selected biomacromolecules are soluble/dispersible in water.

In conclusion, the proposed flame retardants for cotton may represent a new sustainable approach to face the challenges related to the increasing awareness of the health and environmental impact of traditional products and processes.

CONTENTS

1. INTRODUCTION 1

1.1 TEXTILE FIRE BEHAVIOUR AND FIRE HAZARD 2
1.1.1 Fibre chemical structure and thermal behaviour 4
1.1.1.1 Natural fibres 4
1.1.1.2 Regenerated fibres 6
1.1.1.3 Synthetic fibres 6
1.1.1.4 Inorganic fibres 8
1.1.2 Fabric construction 8
1.1.3 Fabric use, arrangement and combination with other flammable materials 9
1.1.4 Environmental conditions and fire stage 9
1.1.5 Smoke production and toxicity 10
1.2 FIRE RETARDANTS FOR TEXTILES 11
1.2.1 General mechanism 12
1.2.2 Textile flame retardants and recent developments 12
1.2.2.1 Inorganic flame retardants 12
1.2.2.2 Halogenated flame retardants 13
1.2.2.3 Phosphorous-containing flame retardants 15
1.2.2.4 Nitrogen-containing flame retardants 18
1.2.2.5 Intumescents 18
1.2.2.6 Inherently flame retardant fibres 19
1.2.2.7 Recent developments in flame retardancy 20
1.2.3 Health and environmental issues 23
1.3 BIOMACROMOLECULES 26
1.3.1 Caseins 26
1.3.2 Milk whey proteins 27
1.3.3 Hydrophobins 28
1.3.4 Deoxyribonucleic acid (DNA) 30
1.3.5 Extraction of biomacromolecules from wastes and by-products 31
1.4 FIRE CHARACTERIZATION 34
1.4.1 Thermogravimetric analysis (TGA) 34
1.4.2 Limiting oxygen index (LOI) and other flammability tests 35
1.4.3 Forced combustion tests - Cone calorimetry 36
1.4.4 Microscale Combustion Calorimetry (MCC) 37
1.4.5 NBS Smoke density chamber 39
1.4.6 Purser furnace (steady state tube furnace) 39

2. MATERIALS AND METHODS 41

2.1 MATERIALS 41
2.1.1 Whey proteins 41
2.1.2 Caseins 42
2.1.3 DNA 42
2.1.4 Chitosan 43
2.1.5 Agro-food wastes 43
2.1.5.1 Vegetable wastes 43
2.1.5.2 Spent brewer's yeast 44
2.2 METHODS 44
2.2.1 Fabric impregnation 44
2.2.2 Layer by layer (LbL) impregnation 45
2.2.3 Glass slide coating 45
2.2.4 UV-curing procedure 45
2.2.5 DNA extraction procedures 45
2.2.5.1 DNA extraction from the mixed vegetables 45
2.2.5.2 DNA extraction from the spent brewer's yeast 46
2.2.5.3 Evaluation of DNA yield and purity 47
2.2.6 Sample characterisation 47
2.2.6.1 Thermogravimetric analysis 47
2.2.6.2 Flame spread tests 48
2.2.6.3 Cone calorimetry tests 48
2.2.6.4 Microscale combustion calorimetry (MCC) 49
2.2.6.5 Washing fastness assessment 49
2.2.6.6 Antibacterial tests 49
2.2.6.7 Surface morphology analysis 50
2.2.6.8 Infrared spectroscopy 50
2.2.6.9 Elemental analysis 50
2.2.6.10 Agarose gel electrophoresis 50

3. RESULTS AND DISCUSSION 51

3.1 FLAMMABILITY, THERMAL AND COMBUSTION BEHAVIOUR OF COTTON FABRICS 51
3.1.1 Cotton 51
3.1.2 Whey protein isolates (WPI) 52
3.1.3 Caseins 56
3.1.4 DNA 61
3.1.4.1 Effect of pH of DNA solution 62
3.1.4.2 Effect of number of impregnations 69
3.1.4.3 Effect of molecular size 71
3.2 COMPARISON OF THE RESULTS OBTAINED FROM THE BIOMACROMOLECULE TREATMENTS 77
3.3 EXTRACTION AND APPLICATION OF DNA FROM VEGETABLE OR BREWERY WASTES 82
3.3.1 Vegetable wastes 82
3.3.2 Spent brewer's yeast 86
3.3.3 Further characterization of the extracted DNA 93

3.4 MIXTURES OF BIOMACROMOLECULES 95
3.4.1 HMW-DNA and LMW-DNA 95
3.4.2 DNA and chitosan 98
3.4.2.1 Washing resistance 99
3.4.2.2 Fire behaviour 104
3.4.2.3 Antimicrobial activity 108
3.4.3 Caseins and DNA 108
3.5 BIOMACROMOLECULES VS. PROBAN® 111
4. CONCLUSIONS AND FUTURE PERSPECTIVES 113
5. REFERENCES 117
APPENDIX A - Publications 131

List of Figures

Figure 1.1 – Combustion cycle of polymers 3
Figure 1.2 – Fire growth and fire stages 10
Figure 1.3 – Chemical structure of tris(tribromoneopentyl) phosphate 15
Figure 1.4 – Chemical structure of: a) 2-carboxyethyl(methyl)phosphinic acid; b) 9,10-dihydro-9-oxa-10-phosphaphenanthrenyl-10-oxide 16
Figure 1.5 – Chemical structure of tetrakis(hydroxymethyl) phosphonium salts 17
Figure 1.6 – Chemical structure of N-methylol dimethyl phosphonopropionamide 17
Figure 1.7 – Schematic structure of: a) Bovine β-lactoglobulin; b) Bovine α-lactalbumin; c) Bovine serum albumin; d) Bovine lactoferrin 27
Figure 1.8 – Hydrophobin structures 29
Figure 1.9 – DNA structure 30
Figure 1.10 – Cone calorimeter apparatus 37
Figure 1.11 – Schematic diagram of a microscale combustion calorimeter 38
Figure 1.12 – Schematic diagram of a Purser furnace. 40
Figure 2.1 – Agarose gels of the different DNAs 42
Figure 3.1 – TG and dTG curves of untreated cotton in nitrogen and air 51
Figure 3.2 – Residue after horizontal flame spread test of untreated cotton 52
Figure 3.3 – Heat release rate vs. time of pristine cotton, tested at 35 kW/m^2 52
Figure 3.4 – Residue after cone calorimetry test of untreated cotton 52
Figure 3.5 – TG and dTG curves of untreated cotton and WPI-treated cotton in nitrogen and air 53
Figure 3.6 – Residues after flame spread tests (horizontal configuration) of WPI-cotton samples 54
Figure 3.7 – Heat release rate vs. time of untreated cotton and cotton fabrics coated with WPI in static and dynamic conditions, tested at 35 kW/m^2 55
Figure 3.8 – Residue of WPI-cotton (dynamic conditions) after cone calorimetry test 55
Figure 3.9 – Heat release rate vs. temperature of cotton fabrics coated with WPI in dynamic conditions, obtained by MCC 56
Figure 3.10 – TG and dTG curves of untreated cotton and cotton treated with caseins in nitrogen and air 57
Figure 3.11 – Residues after flame spread tests (horizontal configuration) of CAS-cotton samples 58
Figure 3.12 – Heat release rate versus time of untreated cotton and cotton fabrics coated with caseins, tested at 35 kW/m^2 59
Figure 3.13 – Residue of CAS-cotton_A after cone calorimetry test 59

Figure 3.14 – CO and CO_2 yield in g/s from untreated cotton and casein-treated cotton, obtained by cone calorimetry 60
Figure 3.15 – Temperature measured under the fabric sample subjected to a heat flux of 35 kW/m^2 in the cone calorimeter 60
Figure 3.16 – Heat release rate vs. temperature curves of cotton fabrics coated with caseins, obtained by MCC 61
Figure 3.17 – Residues after flame spread tests (horizontal configuration) of LMW-DNA samples 63
Figure 3.18 – SEM micrographs of untreated cotton and cotton treated with low-molecular-weight DNA 63
Figure 3.19 – SEM micrographs of the residues after flame spread tests of LMW-DNA pH4, LMW-DNA pH6 and LMW-DNA pH8 64
Figure 3.20 – SEM micrographs of the residues after flame spread tests of LMW-DNA..... 64
Figure 3.21 – TG and dTG curves of untreated cotton, LMW-DNA pH4, LMW-DNA pH7 and LMW-DNA pH8 in air. 65
Figure 3.22 – Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions, tested at 35 kW/m^2 66
Figure 3.23 – Residue of LMW-DNA pH4 after cone calorimetry test 66
Figure 3.24 – CO and CO_2 yield in g/s from untreated cotton and LMW-DNA-treated cotton at different pHs, obtained by cone calorimetry 67
Figure 3.25 – Temperature measured under the fabric sample subjected to a heat flux of 35 kW/m^2 in the cone calorimeter 68
Figure 3.26 – Heat release rate versus temperature of cotton fabrics coated with LMW-DNA at pH 4 and pH 8, obtained by MCC 69
Figure 3.27 – Residues after flame spread tests (horizontal configuration) of LMW-DNA samples with single impregnation 70
Figure 3.28 – Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions, tested at 35 kW/m^2: A) Single impregnation at different pH values; B) Comparison between single and multiple impregnation for LMW-DNA pH4 71
Figure 3.29 – SEM micrographs of fabrics treated with DNA with different mlecular sizes. 72
Figure 3.30 – TG and dTG curves of LMW-DNA, HMW-DNA and MMW-DNA in air. 73
Figure 3.31 – Residues after flame spread tests (horizontal configuration) of HMW-DNA and MMW-DNA samples with multiple impregnation 74
Figure 3.32 – Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA pH8, MMW-DNA pH8 and HMW-DNA pH8, tested at 35 kW/m^2..... 75
Figure 3.33 – Heat release rate versus temperature of cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA at pH 8, obtained by MCC 76
Figure 3.34 – TG and dTG curves of untreated cotton and biomacromolecules-treated cotton in nitrogen and air 77
Figure 3.35 – Heat release rate versus time of untreated cotton and cotton treated with biomacromolecules, tested at 35 kW/m^2 79

Figure 3.36 – Heat release rate versus time of polyurethane foam plus untreated cotton and cotton treated with biomacromolecules, tested at 35 kW/m^2 80
Figure 3.37 – Reverse side of the residue after cone calorimeter test of cotton plus polyurethane foam 81
Figure 3.38 – Residues after cone calorimetry tests of cushion-like samples 81
Figure 3.39 – Extraction yields (EYDNA%) obtained with: (A) ethanol at different degrees of purity; (B) different precipitation times 84
Figure 3.40 – SEM micrographs of LV-DNA treated and untreated fabrics 85
Figure 3.41 – Residues after flame spread tests (horizontal configuration) of untreated cotton and LV-DNA treated cotton 86
Figure 3.42 – SEM micrographs of residues after horizontal flame spread tests of cotton treated with LV-DNA 86
Figure 3.43 – EY_{DNA}% obtained for different purification steps 88
Figure 3.44 – Extraction yields (%) and purity data obtained with different cell debris separation methods 89
Figure 3.45 – SEM micrographs of yeast-DNA treated and untreated fabrics..... 90
Figure 3.46 – Residues after flame spread tests (horizontal configuration) of AY-DNA treated fabrics 92
Figure 3.47 – Residues after flame spread tests (horizontal configuration) of FY-DNA treated fabrics 92
Figure 3.48 – SEM micrographs of residues after horizontal flame spread tests of yeast-DNA treated cotton 93
Figure 3.49 – Agarose gel electrophoresis analysis of extracted DNA..... 94
Figure 3.50 – FTIR-ATR spectroscopy of extracted DNA 95
Figure 3.51 – DNA concentration in water during the washing of DNA treated cotton fabrics ... 96
Figure 3.52 – Residues after flame spread tests in horizontal configuration of cotton treated with mixture of LMW-DNA and HMW-DNA in different proportions..... 97
Figure 3.53 – DNA concentration in water during the washing test of fabrics treated with LMW-DNA and HMW-DNA 98
Figure 3.54 – DNA concentration in water during the washing tests performed on glass slides coated with DNA and chitosan. 100
Figure 3.55 – FTIR spectra of chitosan and DNA samples 101
Figure 3.56 – DNA concentration in water during the washing test of fabric samples treated with DNA and chitosan. 102
Figure 3.57 – SEM micrographs of samples before and after washing at 55°C 103
Figure 3.58 – Flame spread tests in horizontal configuration of untreated and cotton treated with chitosan. 105
Figure 3.59 – Flame spread tests in horizontal configuration of cotton treated with DNA and chitosan before washing. 105
Figure 3.60 – Flame spread tests in horizontal configuration of cotton treated with DNA and chitosan after washing 106
Figure 3.61 – SEM micrographs of fabric samples treated with DNA and chitosan after flame spread tests. 107

Figure 3.62 – Flame spread tests in horizontal configuration of cotton treated with DNA and caseins alone 110
Figure 3.63 – Flame spread tests in horizontal configuration of cotton treated with DNA and caseins in different combinations 111
Figure 3.64 – Heat release rate vs. temperature of untreated cotton, cotton fabrics coated with biomacromolecules and cotton treated with Proban®, obtained by MCC 112

List of Tables

Table 1.1 – Home structure fires by item first ignited (only textile items reported). 2007-2011 annual averages [3] 2
Table 1.2 – LOI values of some fibres [181] 35
Table 1.3 – Classes of polymers tested according to UL 94 standard 36
Table 2.1 – WPI composition 41
Table 3.1 – Flammability data of WPI-treated and untreated cotton fabrics 53
Table 3.2 – Cone calorimetry data of untreated cotton and cotton fabrics coated with WPI 55
Table 3.3 – MCC results for untreated cotton and cotton fabrics coated with WPI in dynamic conditions 55
Table 3.4 – Flammability data of untreated and casein-treated cotton fabrics with different AO%. 58
Table 3.5 – Combustion data of untreated cotton and cotton fabrics coated with caseins, obtained by cone calorimetry 59
Table 3.6 – MCC results for untreated cotton and cotton fabrics coated with caseins 61
Table 3.7 – Flammability data of untreated and LMW-DNA-treated cotton fabrics at different pH conditions. 62
Table 3.8 – Cone calorimetry data of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions with multiple impregnations 66
Table 3.9 – MCC results for untreated cotton and cotton fabrics coated with LMW-DNA 68
Table 3.10 – Flammability data of untreated and LMW-DNA-treated cotton fabrics at different pH conditions with multiple impregnation (MI) or single impregnation (SI) 70
Table 3.11 – Combustion data of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions with single impregnation, obtained by cone calorimetry 71
Table 3.12 – Flammability data of untreated and DNA-treated cotton fabrics with different molecular sizes at different pH conditions. 74
Table 3.13 – Combustion data of untreated cotton and cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA, obtained by cone calorimetry 74

Table 3.14 – MCC results for untreated cotton and cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA at pH 8 75
Table 3.15 – Flammability data of untreated cotton fabrics, LMW-DNA pH4_MI, CAS-Cotton and WPI-Cotton samples 78
Table 3.16 – Combustion data of untreated cotton and cotton fabrics coated with WPI, LMW-DNA pH4 and caseins, obtained by cone calorimetry 78
Table 3.17 – Phosphorous and nitrogen content of the selected biomacromolecules 79
Table 3.18 – Combustion data of polyurethane foam samples covered with untreated cotton and cotton fabrics treated with WPI, LMW-DNA pH4 and caseins, obtained by cone calorimetry 80
Table 3.19 – Extraction yields (EY_{DNA}%) obtained with fresh or frozen vegetable material 82
Table 3.20 – Flammability data of the cotton fabrics coated with LV-DNA obtained from horizontal flame spread tests 85
Table 3.21 – EY_{DNA}% and purity data obtained for different extraction solutions of spent brewer's yeast 87
Table 3.22 – EY_{DNA}% and purity data obtained for the different ball grinding times 88
Table 3.23 – Combustion data of the cotton fabrics coated with different extracted DNA obtained from horizontal flame spread tests 91
Table 3.24 – Flammability data of untreated cotton fabrics and cotton treated with LMW-DNA and HMW-DNA in different combinations 97
Table 3.25 – Prepared samples. 99
Table 3.26 – Flammability data of untreated cotton fabrics and cotton treated with DNA and chitosan in different combinations, before the washing tests. 105
Table 3.27 – Flammability data of untreated cotton fabrics and cotton treated with DNA and chitosan in different combinations, after the washing test 106
Table 3.28 – Antibacterial activity against Staphylococcus aureus of treated cotton samples 108
Table 3.29 – Flammability data of untreated cotton fabrics, cotton treated with caseins alone and cotton treated with DNA and caseins in different combinations 110
Table 3.30 – MCC results for untreated cotton, cotton fabrics coated with biomacromolecules and cotton treated with Proban® 111
Table 4.1 – Tested biomacromolecule costs (2016) 114

List of abbreviations

ϕ – Porosity of a filter
AO% - Add-on (percentage)
ATR – Attenuated total reflectance
AY – Autolysed yeast
BFRs – Brominated flame retardants
CAS – Caseins
DNA – Deoxyribonucleic acid
EDTA – Ethylenediaminetetraacetic acid
EY_{DNA}% – Extraction yield for DNA
FTIR – Fourier Transform Infrared Spectroscopy
FY – Frozen yeast
HRR – Heat release rate
HMW-DNA – High molecular weight DNA
LbL – Layer by layer
LOI – Limiting oxygen index
LMW-DNA – Low molecular weight DNA
LV – Leafy vegetables
MCC – Microscale combustion calorimeter
MI – Multiple impregnation
MLV – Mixed leafy vegetables
MMW-DNA – Medium molecular weight DNA
MR – Microorganism reduction
$OD_{260/280}$ – Ratio between the absorbance of a sample at 260 and 280 nm. It represents the purity of DNA in a solution.
PB – Phosphate buffer
PBDEs – Polybrominated diphenyl ethers
PCBs – Polychlorinated biphenyls
PI – Isoelectric point
pkHRR – Peak of heat release rate
PU-foam – Polyurethane foam
RNA – Ribonucleic acid
SDS – Sodium dodecyl sulphate
SEM – Scanning electron microscopy
SI – Single impregnation
TGA – Thermogravimetric analysis
THR – Total heat released
$T_{onset10\%}$ - Temperature at which the sample has lost the 10% of its mass in a thermogravimetric test
Tris-HCl – Tris (hydroxymethyl) aminomethane Hydrochloride
TTI – Time to ignition
WPI – Whey protein isolate

1. INTRODUCTION

Textiles are ubiquitous materials in almost every aspect of human life. From clothes to furnishing, from transportation to industrial and technical textiles, the applications of textiles are different and numerous.

For these reasons, the easy flammability and the safety of textile materials have always been a great issue for humanity over the centuries.

Every year there are about 3 million fires in the world, which cause important life and economic losses. The fires related to residential buildings (that correspond to 30-50% of all fires) are responsible for the 90% of fire casualties. More than 25000 people die during home and building fires every year [1]. In USA, which have been the first country for number of fires in the last five years, in 2013 the percentage of residential fires, injuries and deaths was 31.7, 76.5, and 78%, respectively, of all fires, injuries and deaths [2].

Smoking, cooking and bad use of electrical and heating devices are the most frequent causes of accidental fires, and frequently the first item ignited is a textile product, which can easily catch fire.

Fabrics used indoor have a great impact on the development of fatal domestic fires because of their ease of ignition and high burning rate. As reported by the U.S. National Fire Protection Association for the period 2007-2011, the object first ignited in dwelling fires was a textile item in one tenth of the fires, without considering the unknown cases (Table 1.1). On the other hand, 42% of home fire deaths occurred in the case of first ignition of textiles, while it occurred only in 18% of fires, where structural framing, flammable liquids and gas or cooking materials considered altogether were the first item ignited [3]. This is a consequence of the burning rate, which is a fundamental factor concerning fire fatality: the faster a fire spread is, the lower is the time people have to react and extinguish it or escape.

In general, smoking is the most frequent cause of fire when textiles are involved, and most of the provoked fires are cause of fatalities [4]. Besides smoking, candles represent another cause of fires, which involves textiles. A great percentage of candle fires start in the bedroom where mattresses, bedding, or curtains are the first items ignited [5].

Smoke inhalation is the most frequent cause of death in fires. Smoke causes moving limitations, confusion, unconsciousness and suffocation. Only when the fire is related to clothes, death is mostly caused by burns.

In different renowned big fires (for example the Woolworth store fire in Manchester in 1979 or the Monarch Underwear Company fire in New York in 1958), textiles played a significant role in the ignition phase or in the fast spread of flames and fire growth. Frequently, after these high-

profile incidents, regulations have been introduced or revised and they are often a driving force for the development of products and technologies, with an improvement in people wellness. A well-known example of this process is the adaptation of the “Furniture and Furnishings Fire Safety Regulations” in 1988 in UK. Thanks to this legislation and the successive amendments, the level of fire retardancy of domestic upholstered furniture and other upholstery increased and thus at least fifty lives every year have been saved since then [6].

Statistics have demonstrated that, along with the utilisation of fire alarms and smoke detectors, the use of non-flammable or low-flammable items in homes is an effective way to improve fire safety.

Table 1.1 - Home fires by item first ignited (only textile items reported). 2007- 2011 annual averages [3].

Item first ignited	**Fires**	**Fire deaths**	**Fire injuries**
Mattress or bedding	9900 (3%)	330 (13%)	1360 (10%)
Clothing	7700 (2%)	130 (5%)	520 (4%)
Upholstered Furniture	6300 (2%)	450 (18%)	810 (6%)
Floor covering	- (<2%)	100 (4%)	-
Unclassified soft goods or wearing apparel	- (<2%)	50 (2%)	-
Total of textiles	23900 (≈10%)	1060 (42%)	2690 (20%)

1.1 Textile fire behaviour and fire hazard

When fire safety is related to textiles, it is important to be aware of the flammability and fire hazards associated with each kind of fabric.

The flammability of materials is correlated to the ease of ignition, which is, in turn, associated to the thermal and thermo-oxidative degradation of the polymer.

In the case of textiles, and more generally of polymers, thermal degradation begins when an external heat is provided to the fabric and the decomposition reactions start. Volatile combustible compounds are formed and released in the gas phase, where the presence of oxygen and heat can start the combustion. If the heat released from the combustion is enough to continue the degradation in the condensed phase and the production of combustible species, the process is self-sustaining (Figure 1.1).

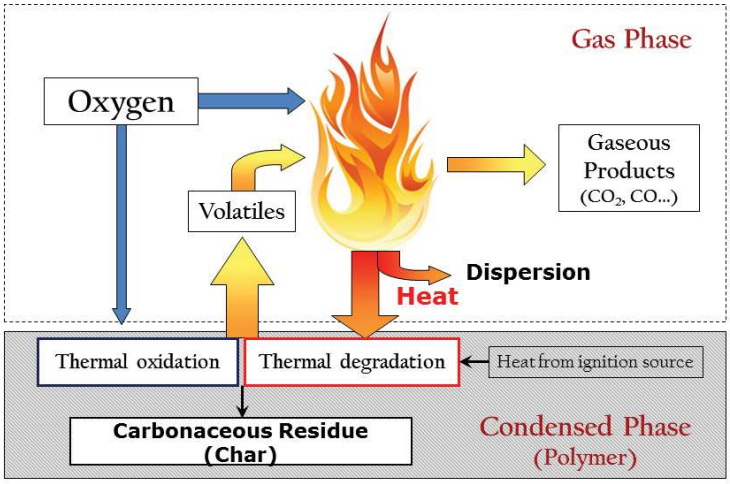


Figure 1.1 – Combustion cycle of polymers

The initial degradation may occur according to different chemical mechanisms depending on whether the involved atoms belong to the main chains, side chains or functional groups. The breaking of bonds in the main chain follows radical reaction rules and can occur at the chain end (end-chain scission) or at random positions in the chain (random chain scission), with the production of monomers and chain fragments. The main chain can undergo cross-linking reactions as well, where close polymer chains are linked after the cleavage of some lateral substituents. This process is opposite to the scission reactions as it increases the molecular weight of the polymer. Side chains and functional groups are usually implicated in elimination or cyclisation reactions. In the case of elimination, side groups are separated from the main chain, which maintains the initial molecular weight but has an altered chemical composition. Cyclisation reactions result in the creation of a cyclic structure due to the formation of bonds between adjacent functional groups or side chains. The cross-linking and the cyclisation mechanisms promote the formation of a stable carbonaceous residue that is called “char”, instead of releasing volatile species.

The different degradation reactions take place, singularly or in combinations, according to the chemical structure of the polymers, as well as the decomposition temperature. At low temperature, side chains and functional groups are more easily involved, while main chain reactions are favoured at high temperature.

When atmosphere includes oxygen, the radical decomposition reactions are accelerated by the formation of oxygen radicals. This implies that a lower amount of external heat is needed to start and carry on the degradation process. In addition, new degradation products are generated with fragmentation and combination reactions.

The fire hazard of every material is not an intrinsic property, but depends on different chemical or physical conditions, which, for textiles, can be summarized as follows:

- Fibre chemical structure and thermal behaviour;
- Fabric construction;
- Fabric use, arrangement and combination with other flammable materials;
- Environmental conditions and fire stage;
- Smoke production and toxicity.

1.1.1 Fibre chemical structure and thermal behaviour

The fire behaviour of fibres is very variable, depending on the origin of the fibre, hence its chemical composition. Fibres can be natural, regenerated, inorganic or synthetic.

1.1.1.1 Natural fibres

Cotton is one of the oldest and most versatile fibres. Nowadays it is the most used natural fibre, comprising about 25-30% of global fibre market [7]. It is produced from the seeds of cotton plant and cellulose is almost its only component (88-96%) [8].

The main applications of cotton and its blends are in the home furnishing field, for example for towels, sheets, curtains and upholstered furniture, and in the apparel field, for clothes, bags and shoes. The use of cotton is common in hospitals since it can be washed at very high temperatures.

The widely spread use of cotton is due to its very favourable properties, such as breathability, softness, good strength, absorption features, washability, easiness of dyeing and manufacturing [9]. On the other hand, cotton is a very flammable fibre, and this implies many problems and restrictions on its applications.

The natural cotton fibre ignites very easily and burns quickly, leaving a light and grey ash. In some cases, a glowing combustion consumes cellulose. This behaviour derives from the cellulose thermal and thermo-oxidative decomposition, which has been detailed in several works [10,11].

The decomposition starts within 300 and 400°C with two competitive processes: a depolymerisation of the chains, where the reaction of hydrogen transfer leads to the formation of monomeric fragments (levoglucosan) subsequently converted in volatile combustible species (e.g. furans and aldehydes), and a dehydration, which leads to the formation of aliphatic char. In the presence of oxygen, the temperature would be shifted downward of about 20°C [12]. A second decomposition step occurs in between 400 and 800°C. In this stage the aliphatic char is further oxidised and transformed in a graphitic carbon structure, releasing water, CO, CO_2 and methane. The aromatic char formation is largely influenced by the heating rate: at low heating rate, the char yield is maximum, due to the synergistic effect between low temperature dehydration reactions and the higher probability of re-polymerisation of levoglusan [13].

Flax fibre, usually called linen, is obtained from the stem of the flax plant and is composed mainly of cellulose (80 wt.%), although it also contains small percentages of hemicellulose and lignin (about 10 and 5 wt.%, respectively)[14]. This composition provides a high stiffness and a low resiliency to the fibres that easily crease. The use of linen fabrics is very limited nowadays, due to the higher production costs in comparison with cotton and other natural fibres. On the other hand, flax fibres have excellent strength, resistance to abrasion, good absorbency and chemical resistance. For these reasons, its main applications are in luxury apparel and furnishing (about 1% of world apparel fibre consumption [15]). Recently, the use of flax fibres in the field of eco-friendly polymer composites is growing, along with other natural fibres, as an alternative to polymer composites containing inorganic fillers.

Since flax is a cellulosic fibre, its burning behaviour is very similar to that of cotton fibres: the ignition is very easy and the combustion is fast and complete.

Unlike cotton and linen, wool and silk are natural fibres with animal origin and they are not based on cellulosic compounds.

Wool is a fibre derived from the fleece of different animals, such as sheep, goats, camelids, lamas and muskoxen. Wool represents less than 2% of the global fibres market [16]. Australia is the most important wool producer, owning 25% of the world market, followed by New Zealand and China [16].

Wool fabrics are mostly used in clothing production. Other applications are in home furnishing, for blankets, carpets and quilts, and in technical textiles, for thermal and acoustic insulation, for example.

Wool is a natural polyamide, since wool fibres are protein-based fibres. Their main component is keratin, a protein that contains sulphur besides carbon, oxygen, nitrogen and hydrogen. The high sulphur content is due to the presence of the amino acid cysteine (13 wt.% in keratin [17]). Keratin proteins are highly cross-linked through the disulfide bonds of cysteine and are, to a great extent, rolled up with hydrogen bonds to form α-helixes.

Thanks to this chemical composition (15-16 wt.% of nitrogen and 3-4 wt.% of sulphur [18]), wool has a low flammability and an inherent fire resistance. In addition, this protein fibre has 8-16 wt.% moisture content at ambient conditions [19], which favours the fire retardancy: in fact, water molecules are the first compounds that are emitted by wool upon heating, and their release decreases the material temperature. When the temperature approaches 230-240°C the helical structures start to undergo some breaks and the proteins start to melt. Above 250°C the disulphide bond of cysteine starts to break and H_2S is released in the gas phase, hence diluting the combustible species. This process is later followed by the dehydration of the α-substituents of cysteine and the formation of a stable char [20].

Because of this mechanism, usually wool does not favour the flame spread and is self-extinguishing.

Silk fabrics have always been considered a luxury by normal people, for their exotic origin, peculiarities (e.g. softness, shininess and strength) and their rareness. In fact, silk represents around 0.2% of global textile fibres production [21].

Although silk is mainly used for luxury apparel, clothing accessories and interior decoration, recently a new variety of technical textiles and biomedical applications has been investigated by industry and academic researchers [22].

The silk fibre is produced by different kinds of insects, especially butterflies, moths and spiders. *Bombyx mori* moth is the most used producer, from an industrial point of view. This insect has been bred through the centuries and is famous for living on mulberry trees. Silk is a natural polyamide mainly consisting of two proteins: fibroin and sericin, which comprise 18 different amino acids. The most abundant are glycine (50% mol.) alanine (30% mol.) and serine (10% mol.) [23].

Silk shows charring characteristics when exposed to a temperature increase. At around 250°C the thermal degradation starts with the serine α-substituents that are involved in dehydration and cross-linking reactions [24]. The formation of char and the high nitrogen content give rise to an intrinsic fire retardancy of silk.

1.1.1.2 Regenerated fibres

Regenerated fibres are obtained from the chemical modification of cellulose. The most known regenerated fibres are viscose, acetate, lyocell and modal. These fibres are made by dissolving cellulose fibres from trees or wastes in suitable solvents and then extruding the obtained viscous solution. The different types of fibres are obtained with some process modifications. Apparel and furnishing are the main applications of these fibres, thanks to their low cost and ease of manufacturing. Since these fibres derive from cellulose, their thermal behaviour is very similar to that of cotton or flax, but they can melt and drip during combustion, especially in the case of acetate fibre.

1.1.1.3 Synthetic fibres

Synthetic fibres started to replace natural fibres in the early ‘50s, when the industrial production of polymers begun. Their market went through a continuous growing and is expected to continue this trend. In 2015, synthetic fibres share of global production was around 62% [25]. Polyester production, in particular, marks the most significant growth.

Synthetic fibres are produced from the polymerization of hydrocarbons and other chemicals that are subsequently extruded. Polypropylene, polyamide, polyacrylonitrile and polyester represent the main synthetic fibres.

These fibres are used in various fields, from industrial and technical textiles (e.g. filters, nonwovens, transportation upholstery, ropes, medical applications, etc.), to apparel and home furnishing (e.g. curtains, draperies, upholstery, carpets, etc.). Furthermore, synthetic fibres, especially polyester fibres, are used in blends with other manmade fibres and with natural fibres to obtain the best comfort, aesthetic and mechanical properties.

The degradation of these synthetic polymers is strictly correlated to their chemical structure and composition.

In the case of polypropylene, which has an olefin origin, the thermal degradation occurs with a random chain scission mechanism. Polypropylene starts to decompose at around 250°C [26]. The macroradicals that are generated in chain scission are very reactive, because all the carbon radicals are tertiary carbons. In addition, there are mobile hydrogen atoms that can be extracted from the chain. For these reasons, intramolecular hydrogen transfer is the predominant propagation mechanism for the radical degradation reaction. The main degradation products are low molecular weight hydrocarbons. These compounds are very flammable species, thus justifying the high flammability of polypropylene. Furthermore, no cross-linking and cyclisation occur with consequently negligible char formation.

Aliphatic polyamide fibres, usually known as nylon fibres, are made from a class of nitrogen-containing polymers. Nylon started to be produced during the II World War to replace silk. Thanks to its high strength, it was used principally in parachute covers and military equipment. After the war it became famous for the production of women pantyhose.

In air, nylons begin to decompose from 340°C onward, while in nitrogen temperatures are about 80°C higher. [26] In nitrogen, the primary random chain scissions occur with the breaking

of the C-N bonds, the weakest bonds in the chain. Then, different reactions, i.e. homolytic scission, C-H intramolecular transfer and hydrolysis, occur according to the external conditions [27]. In some cases (e.g. nylon 6.6), the decomposition can lead to the formation of rings [28]. In the presence of oxygen, the methylene groups adjacent to the nitrogen are firstly oxidised. Then, the decomposition proceeds with the breaking of N-C or C-C (adjacent to nitrogen) bonds. The main volatile products are CO, CO_2, H_2O, NH_3 and low-molecular weight hydrocarbons [29].

Aliphatic polyamides tend to shrink, melt and drip in contact with a flame or a heat source. In this way the propagation of the fire is usually stopped and the material self-extinguishes. In case of ignition, the release of ammonia and carbon dioxide can dilute the fuel species and interfere with the combustion cycle. Due to this behaviour, nylon has an intermediate flammability.

On the other hand, aromatic polyamides have a high thermal stability and a low flammability. Kevlar® fibres are the main example of these materials. Aromatic polyamides are very expensive and are used when high mechanical and chemical performances are necessary. Their decomposition in air begins at temperatures higher than 450°C [26] with the breaking of some aromatic N-H bonds and the formation of aromatic nitriles [30]. The main oxidative degradation products are a solid highly cross-linked residue containing most of the nitrogen and some volatile species (i.e. CO, CO_2 and H_2O).

Polyacrylonitrile fibres are lightweight, soft and warm and are often used for replacing wool fibres. Polyacrylonitrile is a polymer with a carbon main chain with nitrile (-C≡N) side groups. This polymer starts to decompose at about 250°C and follows different pathways depending on the heating rate. At low heating rate, before any mass loss occurs, a cyclisation reaction links nitrogen atoms with adjacent carbon atoms. When the temperature increases, ammonia and hydrogen cyanide are produced from not-cyclised side or end groups [31]. When temperature reaches 350°C, hydrogen starts to be released from the rings, which become aromatic. A subsequent condensation process occurs, with the development of nitrogen and the formation of a carbon residue. At high heating rates, chain scission and volatile formation are prevalent [32]. This is the case in fire development and for this reason polyacrylonitrile fibres have a high flammability.

The most produced synthetic fibres are polyesters, among which the most famous is poly(ethylene terephthalate), PET. This fibre is used for the production of ropes, tire cords, filters, automotive textiles, sports equipment, sportswear, clothes (frequently in blends with cotton), carpets, blankets and other furnishing items.

PET is obtained from the polycondensation of terephthalic acid and ethylene glycol and contains aromatic rings, ketones and ester bonds in its structure.

PET is easily flammable, it can melt away from the ignition source and, when ignited, burns slowly, but it can drip and spread the flame on other surfaces and materials.

The thermal decomposition of PET starts above 300°C and follows a random chain scission mechanism. The initial step is an intramolecular β-hydrogen transfer that gives rise to a six-membered ring transition state and the breaking of an ester bond [33]. Then, an exchange reaction can release acetaldehyde. Besides, when the temperature grows, rearrangements of the chain favour the loss of CO, CO_2 and water with some cross-linking reactions, not relevant for

the char formation. Along with CO, CO_2 and H_2O, acetaldehyde, ethylene, anhydride groups, acid compounds and aromatic polymer fragments are released and can fuel the flame [34].

When polyester is blended with other fibres with different properties (e.g. cotton fibres), it is more difficult to predict the thermal and fire behaviour of the system, as it can be quite different from the behaviour of each component. For example, in the case of PET and cotton, PET tend to melt, at temperatures at which cotton is already burning and the melted material, instead of dripping and shrinking away from the ignition source, covers the charring cotton fibres. In addition, cotton fibres act as a candlewick. This phenomenon is called the "scaffolding effect" and is responsible for the intense and dangerous burning of these blends [35].

1.1.1.4 Inorganic fibres

Inorganic fibres are mainly composed by inorganic molecules. These fibres have particular features and usually are not applied in traditional textiles. They are mostly used in composite materials, thanks to their high mechanical, chemical and thermal resistance.

Examples of these fibres are glass, metal and ceramic fibres.

Inorganic fibres do not ignite and burn, they can melt but at very high temperatures (>1000°C). Glass fibres, in particular, are also used in textiles strictly related to fire protection, such as fire blankets, and are frequently combined with other advanced textiles (e.g. Nomex®).

1.1.2 Fabric construction

The high surface-to-mass ratio and the open structure represent the main issues of textiles in relation to flammability. These characteristics facilitate the contact with oxygen and heat, promoting the ignition and the combustion. A fabric with a low thickness or a loose yarn disposition is more easily ignited than a thick one with close fibres. This latter will ignite more difficulty and burn slower. Gauzes and tulles are the most flammable kinds of fabrics. Horrocks et al. [36] showed that, especially for cotton fabrics, the fire behaviour is dependent on the area density and the fabric thickness. The yarn structure and geometry can also represent an influent factor, but its effect is complex and not easy to investigate.

The surface is important, too: a napped or brushed surface (e.g. in carpets) is more flammable than a smooth surface. Finally, the presence of laces and fringes favours the ignition.

1.1.3 Fabric use, arrangement and combination with other flammable materials

The different application of fabrics obviously affects the fire hazard. The presence of heating or electric devices in the proximity, as well as of stoves or fireplaces with open flames near textile furnishing or clothes increase the risk of the fire spread.

In some of their applications, fabrics assume a three-dimensional arrangement, which can increase the ease of ignition and flame spread. For example, in the case of curtains, the vertical geometry is very favourable for the fire diffusion, since the heat above the flames continuously promotes the ignition of additional material.

When fabrics are joined with other materials, such as foams in upholstered furniture or mattresses, the fire hazard has to be described evaluating the combination of the materials. Stuffed items combine the negative aspects of the two materials: the high surface of textiles favours the ignition and the higher mass of the filling provides fuel for the flames and can produce a lot of smoke. As reported by statistical papers [3], when fires started from upholstered furniture and mattresses there were the higher percentages of fire deaths, both because most of these fires originate at night, when people are sleeping and cannot react in time, and because fires from these products are very hazardous.

1.1.4 Environmental conditions and fire stage

In general, the development of a fire depends on many environmental factors: fire location, ventilation, atmosphere temperature and humidity, volume and disposition of the space.

A fire passes through different stages (Figure 1.2). In most cases, fires begin from small points of origin, for example a burning cigarette, a kitchen appliance or an electric device. In the presence of a flame, the heat is transferred by convection with the air above the flame and by radiation all around and downwards. Since the fire propagates with subsequent ignition processes and the convective heat transfer is more effective, the flame spread is easier in the vertical direction (growth stage). The oxygen quantity is higher than the stoichiometric amount and the combustion is complete (well-ventilated conditions). Up to this stage, the thermal behaviour of materials and the presence of flame retardants are important and can have a big influence on the spread of the fire.

However, when the flame reaches significant dimensions (1-2 m height), the heat release becomes relevant also in the radiation directions (typical heat flux on the floor of 15-20 kW/m^2 [37]), the temperature of hot gases is very high, 500-600°C [38], and also the objects not directly in contact with the flame can self-ignite. This stage is called “flashover” and at this point the fire cannot be handled any more. In a fully-developed fire, the oxygen concentration is much lower than the stoichiometric concentration (under-ventilated conditions), but the fire is not limited by the lack of oxygen. In fact, at this stage a great part of the fuel pyrolises, instead of burning with the oxygen. Temperatures are much higher than the spontaneous ignition temperatures (>600°C [39]). Until all the fuel is consumed, the fire continues.

If, during the growth stage, oxygen or fuel lacks, the fire can decay and self-extinguish: this is one of the purposes of flame retardants.

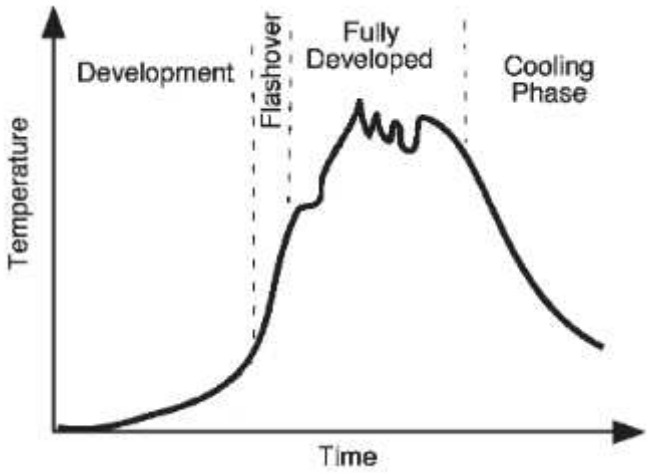


Figure 1.2 - Fire growth and fire stages.

1.1.5 Smoke production and toxicity

Smoke is an aerosol of solid and liquid particulates and gaseous products of combustion, whether complete or incomplete. The composition and the optical density of smoke are two important parameters in the safety possibilities of involved people. These depend on the stage of the fire, the pyrolysis or the flaming combustion, the ventilation and the quantity and the nature of burning material.

Usually textiles have a low mass ratio in fires, with respect to the other materials. This reduces the percentage of smoke produced by them.

Fire gases comprise a great variety of compounds. In the case of well-ventilated combustion, the most important gaseous compounds are oxides, especially carbon dioxide (CO_2), nitrogen and sulphur oxides (NO_x, SO_x). Carbon monoxide (CO) yields are very low at the beginning of a fire, because CO is almost completely oxidised to CO_2. A great increase in the CO formation occurs when the fire reaches the flashover stage. In fact, at this stage, the oxygen concentration is lower than the stoichiometric one. The CO_2 to CO ratio is only dependent on the oxygen concentration and is almost irrespective of the material composition [40].

Different acids are released from halogen- and nitrogen- containing materials. Textiles fibres usually do not contain halogenated compounds, notwithstanding that these latter can be introduced to improve some features (e.g. fire retardant properties). Hydrogen chloride (HCl), hydrogen bromide (HBr), hydrogen fluoride (HF) and hydrogen cyanide (HCN) are produced both from smouldering and flaming combustion. In the presence of wool, hydrogen sulphide (H_2S) can be released in the smoke. HCl, HF and HBr are not further oxidised, while H_2S and HCN are transformed in SO_x and NO_x in some cases.

When the fire is in under-ventilated conditions, different organic molecules, such as benzene, formaldehyde and other aldehydes (e.g. acrolein in cotton pyrolysis), can be formed due to the incomplete oxidation of more complex volatile species.

Water vapour can be also present in smoke.

The hazard from smoke and fire gases is related to the peoples' ability to escape from fire, as well as to the pathological effects in the long term.

Some fire gases are identified as asphyxiants, which means that they cause the depletion of oxygen to the body tissues. Brain is usually the first that suffers from the lack of oxygen and the consequence is the loss of consciousness and the subsequent death. The most important asphyxiant fire gases are CO, HCN and CO_2: CO effect is to replace oxygen in haemoglobin; HCN forms cyanide ions in the blood, which are distributed in the body and interfere with one of the main cell respiratory enzymes; CO_2 has a low toxicity itself, but increases the breathing depth and rate, causing the inhalation of a higher quantity of other toxicants. The oxygen consumption due to the combustion reaction is usually considered as an asphyxiant agent, too.

Other gases are defined as irritants. This is the case of halogen acids, nitrogen oxides and some organic compounds, such as formaldehyde and acrolein. These gases cause painful irritation of eyes, nose, mouth, respiratory track and lungs, with difficulties in breathing and sometimes fatal pulmonary inflammations.

Nowadays it is recognised that the toxicity of most burning materials can be attributed to the previously described toxic gases [40].

Smoke not only causes visibility obscuration, but also contains different size particulates. Quite recently, the presence, size distribution and the toxicity of micrometric and nanometric soot particles in the smoke has been investigated in different works [41,42]. Particulates can interfere with the lung efficiency, cause inflammation and transport other adsorbed toxicants deep into the organism.

It was demonstrated that the heat released by a fire is associated to the smoke toxicity and that the use of flame retardant treated products, unlike untreated counterparts, significantly reduces the heat release rate, thus decreasing the toxicity hazard [43].

1.2 Fire retardants for textiles

Textile fire retardant research was mainly developed starting from the 1950s. As usual, the legislation was a driving force for the refinement and the improvement of products and manufacturing techniques. Nonetheless, almost all the currently employed flame retardant commercial products for fibres and fabrics have their origin in chemicals designed before 1980 [44]. Nowadays, the major challenges are related to the development of low environmental impact and non-toxic products.

The production of a flame retardant textile is not simple. It requires an equilibrium among safety, aesthetic and comfort properties, performances and ease of manufacturing.

There are different approaches in the development of a flame retardant textile. One consists of using fibres that are intrinsically flame retarded (e.g. aromatic polyamides). A second

approach is to chemically adapt the composition of a synthetic fibre by copolymerisation or grafting, or to introduce suitable flame retardant additives in the manufacturing process. The last approach involves a post-treatment of fibres or fabrics after the manufacturing.

1.2.1 General mechanism

The goal of flame retardants is to delay or block the ignition and the combustion of materials, in order to allow the people to be safe and to save structures and properties.

Flame retardants can act with chemical or physical modes of action to interrupt the combustion cycle. The physical action may involve a reduction of the temperature of the material, by starting endothermic reactions or by water evaporation. In a second physical mode of action, the flame retardant can cause the production and release of inert gases that dilute the fuel species in the gas phase under the lower ignition limit. The last physical mechanism involves the formation of a solid layer over the condensed phase (e.g a ceramic layer) that prevents the heat and mass transport from the bulk material to the gas phase and vice versa. The chemical mode of action usually occurs in the gas phase, with an interference with the free radical reactions (radical quenching). In some cases, chemical reactions take place in the condensed phase, favouring the char formation, for example through the dehydration of the material.

The overall flame retardant mechanism is quite complex because it can involve a combination of the aforementioned modes of action.

The final desired effect of these treatments is the inhibition of combustion, by interfering with the ignition, the heating of the surface, the degradation of the material and the flame spread.

1.2.2 Textile flame retardants and recent developments

Many of the strategies usually adopted for conferring flame retardant properties to bulk polymers are not successful in the case of textiles. This finding is attributable to the interference with the spinning process, the deterioration of fibre and fabric properties and the washing fastness. Usually it is not possible to introduce the necessary amount of flame retardant because of the changes in the flexibility, softness, mechanical and aesthetic properties of the fabric.

1.2.2.1 Inorganic flame retardants

Some metal hydroxides are recognized for their flame retardant activity. The most important are aluminium and magnesium compounds (e.g. aluminium trihydrate (ATH), bauxite (AlOOH) and magnesium hydroxide (MH)). These additives degrade endothermically and release water, with a cooling effect of the material and a diluting effect in the gas phase. Furthermore, the hydroxide becomes an oxide through the degradation reactions and forms a protective ceramic layer on

the surface of the material [45]. This layer is a catalytic wide area that can promote the oxidation of carbon on its surface, reducing the obscuration effects of smoke [46,47].

The thermal degradation of metal hydroxides should take place near the beginning of the polymer decomposition for the maximum efficacy: polymers should be chosen rationally, according to their decomposition temperatures.

This kind of flame retardants has no toxic drawbacks for human beings and environment and is cheap. On the other hand, the use of metal hydroxides is limited by the high loading needed for their effectiveness (40-60 wt.%). In the case of textiles, the applications are confined to the backcoating of heavy fabrics, such as carpets [48].

Another inorganic flame retardant is antimony oxide. This is rarely used on its own and is preferably added to other flame retardants (e.g. halogenated compounds) to enhance their activity [49].

In textile fibres, inorganic particles could be introduced in nanometric sizes. In this way, the total amount to obtain flame retardant properties and their effect on fibre manufacturing and features can be reduced. Some investigations have been made in this direction, especially considering nanoclays, but the efficacy of nanoparticles was primarily related to the coupling with other flame retardant species in a synergistic system and in some cases there was a detrimental effect. An example is a study of nylon flame retardancy, where nanoclay were coupled with phosphorous and nitrogen-phosphorous flame retardants: there was almost always a synergistic effect, while in the case of nanoclay alone the flame retardant properties were deteriorated [50].

A special case is that of the commercial flame retardant for wool, Zirpro® by IWS, where inorganic salts, such as zirconium and titanium salts, form complexes with hexafluoride. This treatment promotes the formation of an intumescent char, which shows a particular porous structure that insulates the material [51]. The principal application of Zirpro treated wool is in aircraft upholstered seats.

Other inorganic flame retardants are boron-based. Their action mechanism is similar to metal oxides, i.e it involves endothermic decomposition, release of water and boric acid and formation of a boron oxide glassy layer. The most used boron flame retardants in polymeric materials are zinc borates that are effective on their own or can be combined in synergistic systems with halogenated flame retardants [52]. For cotton fabrics, widely used boron additives are boric acid and sodium borate, applied for example in mattresses and upholstered furniture; however these treatments do not resist to laundry cycles.

1.2.2.2 Halogenated flame retardants

Halogenated flame retardants were among the first developed flame retardants, but their use has lately decreased due to the high toxicity and environmental impact of some of these species. To be applied as flame retardants, halogens should have a high efficiency, which depends on the atomic weight, and a defined stability, which is related to the carbon-halogen bond strength. The most efficient halogen would be iodine, but it has a very low thermal stability and it cannot be compounded with polymers. On the contrary fluorine is the most stable halogen, but it is not

efficient enough. For these reasons halogenated flame retardant include only chlorine and bromine. Recently, chlorine compounds have been drastically reduced due to their toxicity and carcinogenicity, especially polychlorinated biphenyls (PCBs).

Although halogenated flame retardants have different modes of action, the main one involves a chemical mechanism and takes place in the gas phase. In this case, a halogen atom or a hydrogen halide is released and reacts with the radicals that carry on the combustion reaction (i.e. $H^{\bullet}$ and $OH^{\bullet}$). In this way the radicals are blocked and the combustion reaction is inhibited. This chemical action is called "radical trapping" or "flame poisoning". The inhibiting reactions are the following (1):

$$H^{\bullet} + HX \rightarrow H_2 + X^{\bullet} \quad (1)$$

$$OH^{\bullet} + HX \rightarrow H_2O + X^{\bullet}$$

where X is the halogen atom.

Then the halogen atom can react with hydrogen in the fuel gases (reaction (2)) and become active again:

$$RH + X^{\bullet} = HX + R^{\bullet} \quad (2)$$

A physical action also occurs in the gas phase, with the dilution of fuel species and the absorption of part of the heat released by the combustion.

When considering textile materials, bromine is usually found as decabromodiphenyl ether and hexabromocyclododecane. These species are applied to synthetic fabrics in combination with a binder resin with the backcoating method, and find applications in public building furnishing and automotive upholstery [48]. Other strategies were developed for polyester and its blends. For example, one involves the application of an aqueous suspension of hexabromocyclododecane to the fibres and a thermal treatment (thermosol treatment, described later) to induce the migration of the additive into the fibres (CD-75PM® by Chemtura). A second one consists in the coating of the fabrics with a brominated acrylate copolymer containing 35-45 wt.% of bromine (TexFRon® by ICL-IP). These methods produce durable finishes (resistant up to 50 launderings) where the bromine species are fixed and cannot be released in the environment and fabrics can be used in apparel, work wear and bedding.

Typical loadings are in 10-25 wt.% range, depending on the chemical species and the fabric nature and application. These compounds are more effective than other kinds of flame retardants, but their use is restricted because of their potential environmental toxicity and the release of corrosive acid gases during combustion.

In some cases, when halogen compounds are mixed with synergistic species, the flame retardant action can involve the condensed phase, as well. The main synergist is antimony oxide. It enhances the activity of radical trapping and fuel dilution of halogens, impeding the penetration of oxygen into the polymer [49]. In addition, the presence of antimony oxide favours the char formation in the condensed phase [53]. Unfortunately, the solid oxide particulates should be avoided in synthetic fibres, because they can interfere with the spinning process and reduce fibre mechanical properties.

Another common combination is that of bromine and phosphorous containing flame retardants, which act in the condensed phase, in addition to the enhancement of the halogen activity. For example, a commercial melt additive for polypropylene is tris(tribromoneopentyl) phosphate (FR-370® by ICL-IP)(Figure 1.3), and another one, designed for polypropylene carpet fibres, contains 63 wt.% bromine and 2.6 wt.% phosphorus (SaFRon® 5371, by ICL-IP) . In the particular case of polyacrylonitrile, the use of hexabromocyclododecane and ammonium polyphosphate produces a fully intumescent action, where the halogen does not act as flame quencher, but as blowing agent [54].

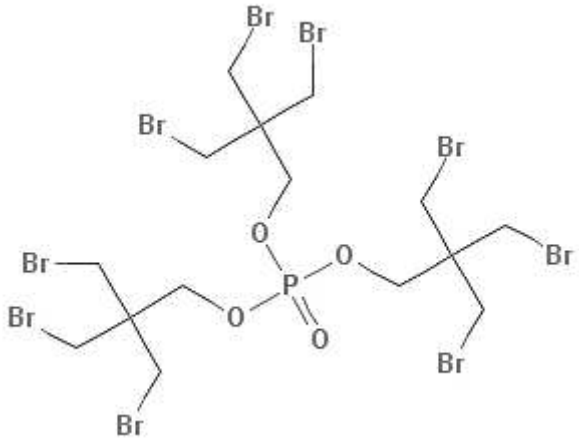


Figure 1.3 – Chemical structure of tris(tribromoneopentyl) phosphate.

1.2.2.3 Phosphorous-containing flame retardants

Phosphorous-based flame retardants are the most used flame retardants for textiles. They are especially applied on natural fibres and fabrics, but find application also for synthetic textiles. These compounds comprise phosphine, phosphine oxide, phosphates, phosphonates, phosphites, phosphonium species, phosphinates and red phosphorous.

The main mechanism of action of phosphorous in oxygen-containing polymers takes place in the condensed phase. The phosphorous species are transformed in phosphoric acids as a consequence of the temperature increase. These species interfere with the polymer degradation reactions and promote the dehydration of the polymer end chains towards the char formation. Char can act as a protective layer on the material and reduce the release of fuel species. Besides promoting the char formation, phosphorous improves the stability of char at temperatures in the range 300-800°C, by inhibition of its oxidation and therefore reduction of smouldering [55,56]. In addition, in the presence of phosphorous compounds, the barrier effect of char is enhanced by a reduction of char permeability [57]. Phosphorous species have also the ability to promote the melt flow and the related heat dispersion.

In nylons, phosphorous flame retardants were found to be not much effective in char formation. Conversely, there were evidences of vapour phase action (i.e. flame inhibition by radical quenching) in some cases. This mechanism was also found in the case of PET treated with the commercial flame retardant Trevira® CS [58]. These flame retardants are also not

significantly effective in char formation in polyolefins, which do not contain oxygen in their chains, and they have to be combined with other charring agents.

In PET, two main approaches are used for the treatment with phosphorous flame retardants: incorporation of comonomeric units into the polymer chains or addition of a melt additive. Trevira® CS polyester, for example, contains comonomeric units of phosphinic acid (Figure 1.4 a), while HEIM® from Toyobo contains a phosphaphenanthrenyl oxide comonomer (Figure 1.4 b). Common melt additives are based on phosphonate, usually cyclic phosphonate (Antiblaze® by Albemarle, Aflammit® PE by Thor). These additives are applied by means of the thermosol process, an adaptation of the fixation method for dispersed dyes [59]: the fabric is impregnated with a water solution of the flame retardant and, after drying, a thermal treatment (at about 200°C for a few seconds) causes the migration of the active compounds into the more permeable fibres. Some phosphinate salts are also used as melt additives and can be applied on nylons, too (Pyrapex® by Apexical, Exolit® OP, by Clariant).

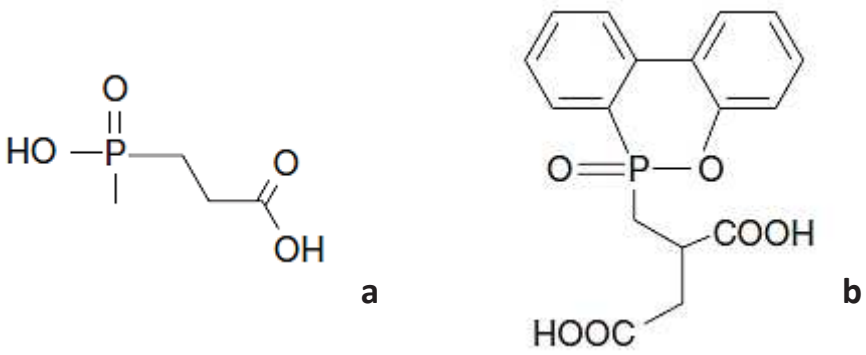


Figure 1.4 – Chemical structures of: a) 2-carboxyethyl(methyl)phosphinic acid; b) 9,10-dihydro-9-oxa-10-phosphaphenanthrenyl-10-oxide.

A synergism between phosphorous and nitrogen compounds is well documented in literature, although it greatly depends on the nature of the polymer and of the nitrogen species and the actual mechanism is still in doubt [53].

The most common commercial durable finishes for cotton fabrics contain phosphorous and nitrogen compounds. One group is based on tetrakis(hydroxymethyl) phosphonium salts (Figure 1.5). These salts react with urea and with gaseous ammonia to form a highly cross-linked polymer network on the fibres, which is then oxidised to obtain a very stable structure. This finishes are resistant to 100 hot alkaline launderings [60]. Such products as Proban®, by Rhodia, and Aflammit® P, by Thor, are obtained with this process. These finishes can be also used for cotton-polyester and cotton-nylon blends, where cotton is the main component. Besides the durability, there are several positive features of these products: i) cotton is not treated in acid environment and therefore cellulose is not damaged; ii) the fibres remain soft and flexible; iii) the finishing treatment is very effective and can be used for military textiles, hotels, hospitals and public buildings. The major drawbacks refer to the need of a special equipment for the “ammoniation” step and the use of formaldehyde during the process, with subsequent issues of its possible release in the environment [61].

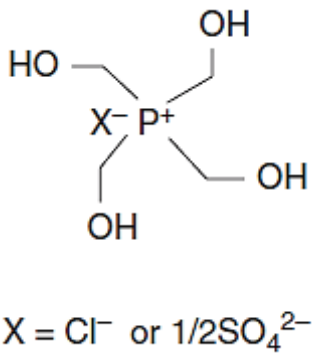


Figure 1.5 – Chemical structure of tetrakis(hydroxymethyl) phosphonium salts.

Another group of flame retardants for cotton is based on N-methylol dimethyl phosphonopropionamide, which can be linked to cellulose in acid conditions. Usually, this treatment is more firmly fixed with the addition of an amino resin (e.g. melamine resin). Two example are Pyrovatex® CP, by Ciba, and Aflammit® KWB, by Thor. This finishes are applied by means of pad-dry-cure methods and can withstand 50 launderings. Pyrovatex is also the most used flame retardant for regenerated fibres such as viscose and lyocell and provides optimal flame retardant features to silk [62]. Although different improvements were made for the reduction of formaldehyde release [63] and the conservation of fibre mechanical properties during storage, the necessity to fasten the finish make it impossible to eliminate formaldehyde-related issues.

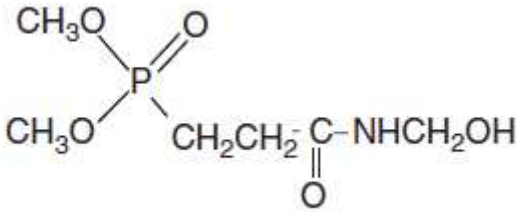


Figure 1.6 – Chemical structure of N-methylol dimethyl phosphonopropionamide.

Proban and Pyrovatex have been predominant in the field of flame retardants for cotton from the '50s, but recently the request for a reduction of environmental impact and of formaldehyde release during process and utilisation have pushed researchers towards new formaldehyde-free and halogen-free finishes. Unfortunately, there are still some concerns on the durability of the treatments and the conservation of fabric properties.

Several non-durable and semi-durable treatments for cotton are based on ammonium polyphosphate (applied usually as a backcoating with a binder) or on guanidine phosphates (e.g. Flammetin® FMB, by Thor Specialties). These treatments are quite inexpensive and find application on items that do not need to be washed, that can be dry cleaned or that are disposable, such as curtains, upholstery, disposable medical gowns and party costumes.

A complex of alkylphosphonic acid ammonium salt and ammonium chloride has been recently proposed as flame retardant for wool by Firestop Chems under the trade name of Noflan®.

Phosphorous and nitrogen species are also important in the effectiveness of intumescent flame retardants.

1.2.2.4 Nitrogen-containing flame retardants

Besides being used as synergist with phosphorous compounds, nitrogen can be effectively exploited on its own or coupled with other active species (e.g. sulphur or halogens). The mechanism of action is usually physical in the gas phase and chemical in the condensed phase.

An example is a commercial flame retardant for polyamides. Melt additives and reactive units are very difficult to be introduced in polyamides for fibre applications, due to the effects on melt spinning, fibre features and flame retardant mode of action. For these reasons, the most common treatment for aliphatic polyamide fibres is a finishing treatment. The commercial product was developed by Bayer under the name Flamegard® and consists of a thiourea-formaldehyde resin. The mode of action probably involves the decrease of the melting temperature and the melt-flow improvement [64].

In polypropylene, the melt flow enhancement for flame retardant purposes was obtained with a hindered amine (Flamestab® NOR 116, by Ciba). It also improves the UV resistance. Furthermore, it was observed that the use of bromine as ammonium bromide was more effective than in the usual form.

1.2.2.5 Intumescents

In comparison to halogenated and phosphorous compounds, these products are relatively new in the flame retardant market, as they were introduced in this field in the middle of the ’90s [65].

Intumescent materials are characterised by a particular behaviour as a consequence of the exposure to heat or fire: they tend to swell, increase their volume and form a porous structure, similar to that of a foam or sponge. This structure is usually composed by char. In the case of flame retardants, this char foam can act as a protective barrier for the underlying material: in fact, it can prevent the heat transfer and the oxygen and volatile fuel species exchange with the gas phase.

Intumescent flame retardants are composed by three agents that act together:

- A source of acidic species. This compound should decompose at a lower temperature than the polymer and the carbonizing agent, it should release acid species that esterify the carbon and promote its degradation through dehydration and formation of cross-linked char, thus reducing the gaseous fuel. These active compounds are usually based on phosphorous, which can be sometimes replaced by sulphur.

- A carbonizing agent. This is an organic compound rich in carbon and oxygen that has the tendency to form char. The carbonizing agent acts in synergism with the acid species to increase

the yield of char formed. Polyols, for example, can be used for this purpose and in some cases the substrate itself can behave as a char forming agent (e.g. cellulose).

- A blowing agent. This species decomposes during the char formation and releases gases that swell the char to obtain the foamed structure. Usually, foaming agents are based on nitrogen compounds and release ammonia or nitrogen.

There have to be a strong compatibility among the three agents, to obtain a concerted formation of the char and its expansion in an intermediate semi-liquid state without subsequent structural failures.

The most common flame retardants with intumescent features consist of ammonium polyphosphate or melamine phosphate plus pentaerythritol or similar polyols and can be applied to textiles (especially cotton and its blends) as coatings or backcoatings. For polyacrylonitrile and some fibre-forming copolymers, ammonium polyphosphate was found to be more effective than other common flame retardants, both phosphorous- and halogen-based [66].

In some cases, a synergism was achieved exploiting the combination of inorganic compounds. For example, the addition of small quantities of metal ions could catalyse the degradation of ammonium polyphosphate at lower temperatures, thus enhancing its efficacy in cellulose and polypropylene [67,68].

1.2.2.6 Inherently flame retardant fibres

Inherently flame retardant fibres can be of different origin. They can be synthetic or regenerated fibres where a flame retardant is added to the chemical formulation; in other cases they can be fibres that are intrinsically flame retardant on their own.

The most common models of the first group are modified polyesters and acrylics (also called modacrylics).

The active species are introduced in these polymers as comonomers or as functionalizing groups. In the case of PET, this approach allows achieving the flame retardant features with a low concentration of phosphorous (1 wt.%). Some examples are the aforementioned Trevira® CS polyester and the HEIM® polyester, as well as Avora® from Invista. Modacrylics are copolymers of acrylonitrile and vinyl chloride or vinylidene dichloride and, unlike modified PET, can contain a high percentage of non-acrylic comonomers (from 15 to 65 wt.% [69]); sometimes, antimony oxide can be added as synergist. Some commercial products are Velicren FR®, by Montefibre, and Kanecaron®, by Kaneca Corporation.

The intrinsically flame retardant fibres can be either organic or inorganic.

Inorganic fibres are based on glass, silica, alumina and basalt. These fibres are resistant to very high temperatures and to chemicals, therefore their applications are mostly in harsh or special environments. They are also used in blends with different polymers.

The most famous inherently organic flame retardant fibres are aramids, i.e. aromatic polyamides. Nomex® and Kevlar® by DuPont and Twaron® by Teijin are the most popular. Aramids are naturally flame retardant, thanks to their aromatic structure with high C/H ratios and high quantity of aromatic rings. They can be *meta*-aramids, like poly(m-phenylene isophtalamide) (Nomex®) and poly(aramid-arimid) (Kermel® by Rhodia), which have acceptable

aesthetics and physical properties similar to nylons and therefore are utilised for protective clothes, such as fire-fighter uniforms. There are also some *para*-aramids, like poly(p-phenylene terephtalamide) (Kevlar® and Twaron®), which have highly symmetric polymer chains that improve their mechanical properties and their thermal stability, but, due to the high cost, are almost always used in blends. Finally, copolymeric fibres can be also exploited (for example copoly(p-phenylene-3,4-oxidiphenylene-terephtal-amide), Technora® by Teijin), which have higher chemical and abrasion resistance, with respect to the aforementioned aramids.

Other inherently flame retardant organic fibres are based on heterocyclic polymers derived from rigid-rod polymers. As regards the thermal and burning behaviour, these fibres have better properties than aramids: higher thermal degradation temperature, higher limiting oxygen index (LOI) and lower rate of heat released [70]. Two examples are poly(p-phenylene-2,6-benzobisoxazole), commercially available as Zylon® by Toyobo, and poly(2,6-diimidazo(4,5-b:4',5'-e)pyridinylene-1,4(2,5-dihydroxy)phenylene (M5 by Magellan).

Some thermosets have been developed for application as high performance fibres with high thermal stability. One of them is a melamine-based fibre called Basofil® and produced by BASF. The thermal degradation of this material occurs with an endothermic cross-linking mechanism that releases ammonia and ends with the formation of a highly heat resistant char. The same behaviour for char formation is observed with a phenol-formaldehyde fibre, Kynol® by Nippon Kynol Inc. These fibres are applied in mattresses, aircraft upholstery, protective clothing, automotive insulation, friction items and gaskets.

The last kind of inherently flame retardant organic fibres are oxidised polyacrylonitrile fibres (OPF). They are obtained by a controlled oxidation of polyacrylonitrile with a high molecular orientation. They have low compressibility and tenacity, but they are highly flame resistant. Some commercial products are Pyromex®, by Toho Rayon, and Lastan®, by Asahi.

1.2.2.7 Recent developments in flame retardancy

Nowadays, scientific research is focused on different challenges, from the environmental and safety concerns about traditional chemicals and processes, to the reduction of costs and raw materials. Both the reconsideration and the improvement of established technologies and the development of new products and techniques are being investigated. The most important efforts have been made in developing new strategies for the deposition, absorption and fixation of active additives on fabric surfaces. Also the reduction of flame retardant loading, necessary to obtain the desired features, has been investigated through the construction of different architectures, an efficient dispersion of particles and the coating homogenization. The utilisation of inexpensive inorganics in synergistic systems is growing with the development of these techniques: in fact these latter do not significantly affect the aesthetics, flexibility and softness of the resulting fabrics. Another interesting field that has very recently arisen is that of bio-based flame retardants, such as compounds obtained from biomasses, milk or vegetables.

A quite innovative technique for flame retardant additive deposition is the layer by layer method (LbL). It was already established in 1966 [71], but the first application in the area of textile treatments was implemented just in 2009 by Li et al. [72]. This technique consists in the

alternate impregnation of the fabric in two (or more) oppositely charged polyelectrolyte solutions/nanoparticles suspensions, possibly followed by a water rinsing. The procedure is repeated until the desired number of bilayers (or trilayers, or quadlayers) is formed on the substrate, which is then dried. These layers are very thin and held together mainly by electrostatic forces.

The first attempts to impart flame retardant features to fabrics were achieved by means of inorganic LbL coatings. Silica, alumina and montmorillonite were successfully applied on cotton and PET fabrics to enhance their thermal and fire stability [73,74,75]. Inorganic species were also applied on fabrics by spray, instead of dipping: in particular the horizontal spray deposition was found to be more homogeneous and effective than the vertical one [76]. Afterwards, organic compounds have been considered, as well, for example phosphorous and nitrogen compounds for the construction of intumescent coatings. More specifically, ammonium polyphosphate was coupled with silica and chitosan solutions both in bilayers and quadlayers for the efficient treatment of cotton/polyester blends [77,78]. In the same period poly(sodium phosphate) was coupled with poly(allylamine) in a layer by layer assembly to create an intumescent system for cotton fabrics [79]. In all these cases, the desired mechanism of action was the enhancement of the formation of a protective ceramic or char layer and the blocking of melt dripping in polyester fibres.

In the last years, this technique has been developed with different approaches and combinations of polyelectrolytes: bio-based coatings have been built with chitin, chitosan, DNA and starch [80,81,82]; furthermore, the technique has been applied on other kinds of fabrics (e.g. polyamides) [83] and the application at large scale has been investigated [84].

Another recently re-discovered technique for fabric treatment is cold plasma. Although cold plasma is a surface treatment already well known since the ‘80s, the low-pressure equipment was complex and expensive for an industrial development. In the last few years, atmospheric-pressure plasma has become a reality and new possibilities are open.

Some investigations were made with the application of plasma as pre-treatment of cotton fabrics before the flame retardant deposition, to improve the absorption and the grafting of the active species [85]. Other researchers focused on the use of plasma-induced graft-polymerisation, which consists in the simultaneous activation of the surface and the grafting and polymerisation of the active monomer units. This method was applied successfully on cotton, polyacrylonitrile and silk [86,87,88].

Different kinds of coatings, either inorganic or hybrid organic-inorganic, have also been deposited by means of the sol-gel process. With this technique, very thin and homogeneous films can be obtained starting from a colloidal water solution, which, through hydrolysis and condensation reactions, becomes a gel phase, finally dried.

In the case of flame retardancy, the generally used inorganic precursors are metal alkoxides, based on silicon, titanium, aluminium or zirconium. The precursor nature and reactivity, the molar ratio with water and the time and temperature of the treatments are all process parameters that may influence the resulting finishing treatment and should be optimised when required. For example, the flame retardant efficacy on cotton fabrics of different silica precursors was compared and the results indicated that the best fire retardant performances were obtained using silica precursors bearing a high number of hydrolysable groups [89].

With the aim of improving the effectiveness of the coating, inorganic materials have been successfully combined with phosphorous- and nitrogen-based flame retardants. This way, in presence of a heat source, the formation of a protective ceramic layer is accompanied with the formation of a stable char, instead of volatile fuel species. In addition, the presence of metal ions can catalyse the dehydration reactions of the substrate (e.g. cellulose) [90] and the early degradation of phosphorous compounds (e.g. ammonium polyphosphate, as already mentioned) [91,92]. Some examples of these systems involve silica, aluminium and zinc compounds with phosphorous species [93-96]. The sol-gel process can be an effective deposition method for the *in situ* formation of nanoparticles, which have recently generated great interest in the flame retardant field. For example, alumina, silica and bohemite nanoparticles have been introduced in sol-gel coating systems [97,98].

The deposition of very thin films on fabrics can be achieved via admicellar polymerisation, a recent technique in fabric finishing. Similarly to LbL and sol-gel processes, admicellar polymerisation can be used to confer different properties to fabrics, such as hydrophobicity, barrier properties, flame retardancy or ultraviolet protection and for providing fabrics with multifunctional features. This technique consists of four steps: 1) formation of admicelles on the fabric surface with a specific surfactant, 2) solubilisation of monomeric units into the admicelles, 3) thermal polymerisation, 4) washing of the excess of surfactant. This way, nanometric films can be obtained [99]. A few works are based on admicellar polymerisation of phosphorous-containing monomers on cotton fabrics. Both Siriviriyanun et al. [100,101] and Nehra et al. [102] found self-extinguishing features for fabrics having a low phosphorous content.

Due to the growing consciousness of environment need for protection, sustainable products and processes are more and more desired. The reduction of energy and raw material consumption leads to recycling, exlpoitation of renewable materials and reuse of waste products. Nowadays, several biopolymers and bio-based polymers are under investigation or already on the market; the scientific research started to be inspired by this success towards the development of bio-based additives also including flame retardants.

Recently, some efforts in this direction have been made investigating the use of biomacromolecule coatings for flame retardation of cotton and polyester. Some milk-derived proteins, such as whey proteins and caseins, were deposited on cotton fabrics and their thermal and fire behaviour were investigated. In horizontal flame spread tests, a reduction of the burning time and an increase in the final residue were obtained [103,104]. The mode of action of these biomacromolecules probably occurs in the condensed phase with an enhancement of char formation. A similar behaviour was obtained with hydrophobins, proteins produced by filamentous fungi that contain high quantity of sulphur [104]. Another investigated biomacromolecule was DNA, which represents a potential intumescent-like flame retardant because of its structure and composition. In fact, DNA treated cotton fabrics were self-extinguishing in horizontal flame spread tests and the formation of a char stable at temperatures below 600°C was confirmed in thermogravimetric tests [105]. As already mentioned, DNA was later coupled with chitosan in layer by layer assemblies. All these biomacromolecules contain nitrogen with phosphorous or sulphur, which are the active species in traditional flame retardants. A more detailed description of these biomacromolecules in general and their application as flame retardants will be carried out in section 1.3.

There are other few examples of bio-based flame retardants for fabrics. Cheng and co-workers considered the application of phytic acid, which can be extracted from plant seeds and contains a high concentration of phosphorous, to poly(lactic acid) nonwoven fabrics and to wool fabrics with good results in terms of increase of the limiting oxygen index, extinguishment of treated fabrics in vertical flame spread tests and reduction of heat release rate in microscale combustion calorimetry tests [106,107]. An Indian group applied another plant derivative, i.e. sap from banana plant pseudostem, to cotton fabrics, proposing a char enhancing mode of action [108].

1.2.3 Health and environmental issues

The general use and abuse of chemicals in the second half of the 20th century raised the need for a critical investigation of their possible health and environmental effects. In the case of flame retardants, this operation started at the beginning of the '70s, with the first claims related to halogenated flame retardant toxicity and environmental impact. Afterwards, the inquiry was extended to the other flame retardants and to the manufacturing processes, too.

It is fundamental to know that the use of flame retardants, in general, does improve public and environment safety, by reducing the probability of fires and, in case, the emission of heat, smoke and hazardous combustion products. A balance between the benefits and the risks should always be taken into account.

In developed and developing countries, people are in contact with flame retarded materials during the whole day and night, in their houses, vehicles, workplaces and public buildings. The animal and human exposure may occur by inhalation, dermal absorption or ingestion. In addition, these chemicals can be released in the environment during the different stages of their existence, from manufacturing, to consumer use, to disposal (i.e. landfill confinement, incineration) or recycling.

Initially, the chemicals under investigation were polychlorobyphenyls (PCBs), a class of compounds consisting of a biphenyl unit, which can carry up to 10 chlorines in the two aromatic rings. 209 congeners can be obtained by changing the number and the position of chlorine atoms in the structure. These compounds, usually produced as mixtures, have physical and chemical properties attractive for several industrial applications (e.g. thermal and chemical stability, electrical insulation), therefore their usage is largely spread. The first indications of PCB toxicity were already published in 1937 [109], but they did not receive the appropriate attention. It was only in 1966 that PCBs were defined as possible environment contaminants by Jensen, who was working on dichlorodiphenyltrichloroethane (DDT) pollution [110]. An increased consciousness of PCB hazard resulted in their ban during the '70s (Germany in 1972, Sweden in 1973, U.S. in 1977) while the worldwide production ended in 1989. Nowadays, PCBs are only produced under strict regulations for scientific purposes. Besides flame retardancy, PCBs were commercialized for many applications, from insulation to lubrication, as cement and insecticides additives, for the sealing of instrumentations: they were widespread worldwide. Due to their diffusion, their inadequate disposal and their high stability, PCBs can be found in every kind of

environment, in air, water and soil, in wild and domestic animals, fishes, plants, human adipose tissue, blood and milk. They are persistent and bioaccumulative in fat tissues and can be carcinogenic and toxic for plants, animals and humans. In addition, their improper combustion can give rise to the formation of dioxins and furans, well-known toxic and carcinogenic compounds [111].

Nowadays chlorine is still used in the flame retardancy of textiles in chlorinated paraffins for the backcoating of fabrics and in combination with some phosphorous species, but all these compounds are under examination to define the related level of hazard.

Brominated flame retardant compounds (BFRs) are also of great concern, since they can form dioxins and furans following an incomplete combustion and they can be toxic for humans and the environment.

The most debated BFRs are polybrominated diphenyl ethers (PBDEs), which consist of two aromatic rings linked by an ether bond. The aromatic hydrogens can be replaced by different numbers of bromine atoms and the compounds of this family can be classified in 209 congeners as PCBs. PBDEs application was restricted in Europe and in USA, especially the utilisation of pentaBDE and octaBDE, respectively in 2004 and in 2006. Some USA states banned all PBDEs in 2007 and Sweden banned the use of decaBDE in 2007. In Europe, decaBDE is under examination since then, but so far it is not identified as a significant risk for humans or the environment [112].

PBDEs were found in air and soil, fishes, birds and animals and in some human tissues; pentaBDE was detected in the highest quantities. Several studies have been conducted on PBDEs toxicity and health effects, most of them with laboratory tests and some on human records. Studies on mice indicate that the acute effect is low, while the chronic exposure can damage liver, kidney and thyroid systems, with lower-brominated BDEs having the heaviest effect [113]. The major examined effect is the endocrine system damaging. In fact, some thyroid hormones, like triidothyronine and thyroxin, have a similar structure to PBDEs and therefore there can be interferences. Some studies on mice report irregular levels of these hormones after the exposure to PBDEs, and data on humans, even with some lacks, generally indicate a correlation with some PBDEs and altered amounts of thyroid hormones [114]. Some studies also report an interference of PBDEs with the thyroid-stimulating hormone [115]. Another health issue related to PBDEs is developmental neurotoxicity, especially for pentaBDE. Behavioural and cognitive alterations, such as hyperactivity and dysfunctions in learning and memory, were observed in mice, which have been exposed to PBDEs during neonatal period [116]. The two areas where data and studies mainly lack are the carcinogenicity of these compounds and the degradation and modification of PBDEs to lower brominated PBDE and to other bromo-containing compounds that can be more mobile and toxic.

Other BFRs are polybrominated biphenyls (PBBs), the brominated counterpart of PCBs. Also these compounds were banned in USA and Europe at the end of the ‘70s. The trigger was an accidental contamination of animal feed in Michigan in 1973, which resulted in the block of 500 farms, and the destruction of thousands of farm animals and products. The production of PBBs ended definitely in 2000.

Two other BFR compounds are under examination, due to their persistent presence in the environment: namely, tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCDD). TBBPA toxicity can be related to the disruption of thyroid homeostasis, it can interfere with

triidothyronine, thyroxin, thyroid-stimulating hormone and transthyretin [117,118]. Also HBCDD seems to target the thyroid system, along with the liver [119,120].

Unfortunately, halogenated flame retardant market is still lively because of the difficulties in replacing these compounds, which are cheap, show high effectiveness and work well on a wide range of polymers and textiles.

The substitution is slowly taking place with phosphorous-based flame retardants, which are generally recognized as more environmentally friendly and less toxic for humans. Some studies have started to investigate the impact of these compounds, especially the ones directly concerned in the replacement of halogens [121]. Up to now, the compounds where phosphorous is combined with halogens are considered as the most toxic and possibly carcinogenic among phosphorous flame retardants.

Antimony oxide, an inorganic synergist, is another substance that created some concerns about its health effects. This product is often combined with halogenated flame retardants, especially in backcoatings, and its presence could promote the formation of dioxins [122]. In addition, antimony trioxide is a suspect carcinogenic compound and irritant and the chronic exposure can damage lungs, liver, kidney and heart [123]. Actually, in flame retardancy, antimony oxide is well attached to the material and should not be a cause of exposure, nonetheless the US National Research Council recommended additional studies on its exposure pathways and its toxicity to evaluate the related risk. [124].

Environmental concerns increased the seeking for more sustainable manufacturing processes: this includes a more strict control of effluent disposal and a reduction in water consumption. Some European directives pushed the industry towards the control and neutralization of gaseous and liquid effluents, as well as the optimization of production to minimize the formation of wastes and by-products and the recycling of water and materials. Among these measures, the reduction of formaldehyde utilization and emission mainly affects the field of cotton flame retardants (e.g. Proban® and Pyrovatex®). Formaldehyde is used in different steps of their production and is essential for the reaction of nitrogen and phosphorous compounds with cellulose anhydroglucopyranose-OH groups.

Formaldehyde is a gaseous species, which can affect human and animal health and is persistent in water environments. The exposure routes of formaldehyde are mainly inhalation and ingestion and it can irritate nose and throat and damage lungs and stomach. The International Agency for Research on Cancer (IARC) defined formaldehyde as a carcinogen for humans after some studies on human inhalation, where throat and nose cancer were detected in significant amounts [125].

Especially in the case of N-methylol dimethyl phosphonopropionamide, where an equilibrium reaction is involved and free formaldehyde remains in the treated fabrics, the release of this species cannot be avoided, but only minimised. In addition, during storage, an auto-hydrolysis may occur, with an additional release of formaldehyde.

The replacement of these finishes is a key issue, even because they are market leaders and innovative processes and products must have comparable or superior characteristics regarding ease of application, effects on textile flame retardancy, cost and environmental impact.

1.3 Biomacromolecules

The recent interest in environment protection, the reduction of energy and raw material consumption and the regulation of toxic chemicals have pushed the academic world towards the seeking for more sustainable products and processes. The same trend is observed in flame retardancy, as previously reported.

The application of biomacromolecules derived from renewable biologic sources started from the recognition that some of them contain the same active elements as traditional flame retardants, comprising the simultaneous presence of nitrogen and phosphorous or of nitrogen and sulphur atoms. The possibility to recover these compounds from agro-food industry by-products and wastes increases their relevance in the development of environment-friendly flame retardants.

Examples of biomacromolecules with the desired composition are nucleic acids and some proteins. In fact, proteins bear nitrogen atoms and in some cases they can contain amino acid residues with sulphur atoms or they can be phosphorylated. This latter means that they have one or more phosphate groups linked to their chain. DNA and RNA are composed by long chains that contain both phosphorous and nitrogen atoms.

An extensive review about the first results from the use of commercially available biomacromolecules in fire retardancy for textiles was published a couple of years ago [126].

1.3.1 Caseins

Caseins are a group of phosphoproteins and are the main component (80%) of milk proteins. There are four types of caseins, which differ for the number and position of phosphorylations and for their molecular structure, namely: α_{s1}, α_{s2}, β, and κ-caseins. κ-caseins contain also some sulphur atoms. In milk, caseins are disposed in micelles with the κ-caseins on the outside. Caseins have an isoelectric point of 4.6 and near this pH value they are insoluble in water [127]. This circumstance is very important for the production of fermented milks, cheese, and other dairy products. Besides the cheese production, caseins have several applications in food industry, especially in manufacturing of yoghurt, ice-cream, low-fat dairy products, sauces, bread and pastry, and meat products. In fact, they have appealing properties for food processing: they are used as emulsifying, foaming, swelling and thickening agents, as binders in meat processing and for improving nutritional features [128]. Other uses of caseins are related to medical, catalytic and coating applications. For example, caseins have been combined with metal nanoparticles (e.g. gold and iron) to improve their biocompatibility and confer particular surface properties for biomedical applications [129,130].

Caseins contain an average of 0.8 wt.% of phosphorous [131]. This high phosphorous content suggested the application of caseins as flame retardants on cotton [104]. The thermal behaviour of caseins was found very similar to that of ammonium polyphosphate, a well-known flame retardant additive, although caseins showed a lower stability. More specifically, the casein coating (dry add-on of 20 wt.%), shifted cotton decomposition to lower temperatures and, in

horizontal flame spread tests, caused the reduction of the burning rate of about 35%, releasing a consistent residue. Some tests were also made in a cone calorimeter under 35 kW/m^2 heat flux: the casein coating caused a 30% decrease in the peak of heat release rate.

1.3.2 Milk whey proteins

Whey proteins constitute 20% of milk proteins. They are globular proteins that remain in the liquid fraction (i.e. whey) after the precipitation of caseins. This protein group comprises β-lactoglobulin (58 wt.%), α-lactalbumin (20 wt.%), bovine serum albumin (5 wt.%), immunoglobulins (10 wt.%), lactoferrin (5%) and lactoperoxidase (1 wt.%) (Figure 1.7). Whey proteins can be separated from whey with different techniques (i.e. precipitation, adsorption and membrane filtration) and subjected to several treatments to obtain products with different properties and applications [132]. The most used whey protein products are whey protein concentrates (WPC), with a protein concentration that ranges in between 35 and 80 wt.%, whey protein isolates (WPI), with a protein content beyond 90 wt.%, and whey protein hydrolysates (WPH), where proteins have been enzymatically hydrolysed to peptides.

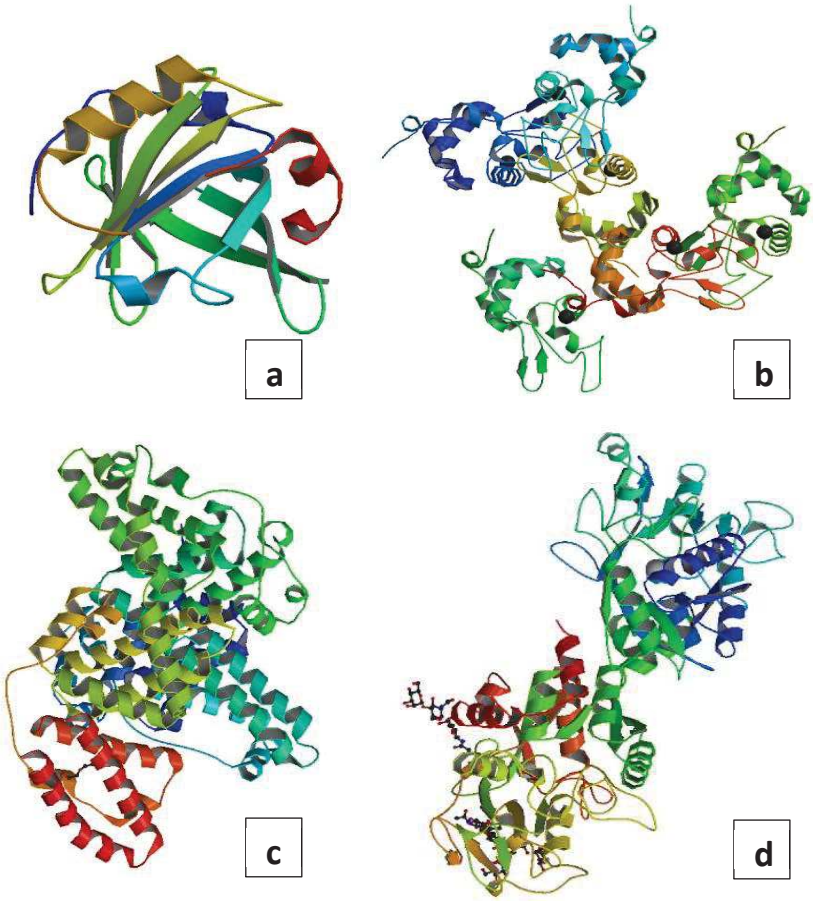


Figure 1.7 – Schematic structure of: a) Bovine β-lactoglobulin; b) Bovine α-lactalbumin; c) Bovine serum albumin; d) Bovine lactoferrin.

Whey proteins show several interesting physical, chemical and biological properties: they are largely soluble in water, have emulsifying tendency, can form gels, show antimicrobial properties and probable anticarcinogenic activity [133,134].

The most widespread applications of whey proteins are related to the food industry. For example, they are used in bakery products, ice-creams and in some meat-based products [135]. They are also used as nutritional supplement because of their high content of essential amino acids and sulphur amino acids [136]. Recently, the application of whey proteins in fields rather far from food industry has been investigated. Their major applications refer to the production of coatings, edible films and membranes and the encapsulation of compounds for drug delivery [136,137].

Whey proteins have a lower thermal stability than other milk proteins, such as caseins. Their denaturation starts approximately at 70°C [138], but this depends on the heating rate and the protein solution pH [139].

The fire retardant behaviour of coatings of whey proteins on cotton fabrics was investigated by Bosco et al. [103]. The high sulphur and nitrogen content and the capability to form films with high oxygen barrier features were considered of interest for the application in fire retardancy. Both folded and unfolded (denatured) whey proteins were tested with a dry add-on of 20-25 wt.%. By means of thermogravimetric analyses (TGA) it was assessed that the thermal decomposition of whey proteins starts at about 100°C and when they were applied on cotton, they cause a sensitization of cotton degradation, but at the same time promote the formation of a stable carbon residue. In horizontal flame spread tests, the presence of whey proteins reduced the burning rate and, especially in the case of folded proteins, significantly increases the final residue.

1.3.3 Hydrophobins

Hydrophobins are proteins with particular amphipathic features. They are produced by filamentous fungi for their growth and development. Hydrophobins have the capability to place themselves at the interfaces and reverse the hydrophilicity or hydrophobicity of a surface. Traditionally, two classes of hydrophobins can be distinguished, depending on the stability of their assemblies: class I hydrophobins form very stable assemblies, while class II are less stable.

Hydrophobins are small and globular proteins, with a molecular mass of 7-10 kDa and 70-350 amino acids [140] (Figure 1.8). All hydrophobins have the same cysteine number and distribution and form 4 disulphide bridges between cysteines. These bridges stabilise the protein and are essential for the proper self-assembly of hydrophobins at interfaces [141].

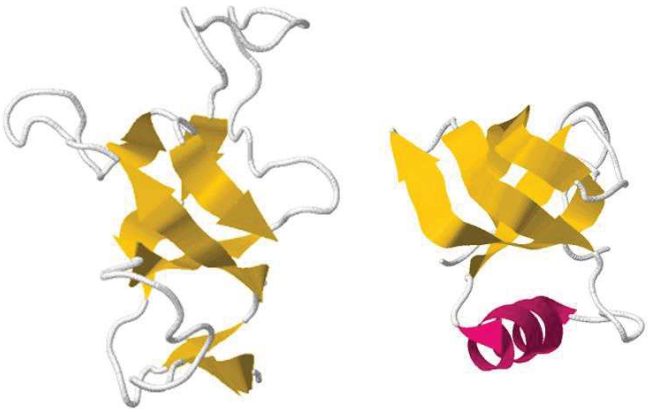


Figure 1.8 – Hydrophobin structures [142].

The applications of hydrophobins are various and cover different fields. Their ability to control and change surface properties is a very important feature. Furthermore, hydrophobins are not toxic, immunogenic or cytotoxic, therefore can be used in medical and food applications. They can be utilised as coating of liquid droplets or solid particles and nanoparticles to enhance their dispersion and stability in water, for pharmaceutical, cosmetic and food applications [143]. Foams can be stabilised by hydrophobins, in a more effective way than with other proteins [144,145]. In medical and biomedical research, hydrophobins have been used for the immobilisation on a substrate (e.g. sensors, scaffolds, implants etc.) of other proteins, enzymes, biological compounds and even living cells and microorganisms [146-148]. With the combination of hydrophobins with antibiotics and with their modification, it has been possible to obtain also the opposite effect, i.e. to prevent the adsorption of undesired materials [149] and, especially, of bacteria [148]. For biomedical applications, hydrophobins can also be used as lubricants for catheters and other surfaces to reduce their friction [150]. The great interest generated in the last few years by the production and applications of carbon nanotubes, nanoparticles and graphene affected also the biological field. The exploitation of hydrophobin interface features for the enhancement of the stability and processability of these materials is under investigation and may have interesting outcomes [151].

The industrial production of hydrophobins at large scales is still not developed enough to face a growing market, and this is the major obstacle to their diffusion.

The presence of sulphur-containing amino acids, in a similar way to wool keratin, can be exploited in the application of hydrophobins as flame retardants. Cotton fabrics treated with a mixture of class I and class II hydrophobins, with 20 wt.% dry add-on, were tested by means of TGA, cone calorimetry and horizontal flame spread tests [104]. The results showed a sensitization of cotton degradation. At the same time, the peak of heat release rate was reduced by 45% and the burning rate in the horizontal flame spread tests was lowered by 35%.

1.3.4 Deoxyribonucleic acid (DNA)

DNA is the genetic material in living organisms and is the basic element for genes conservation, replication and expression. DNA can be considered a biopolymer, as it is composed by two chains (i.e. *strands*) that are winded up in the characteristic double helix (Figure 1.9). These strands are formed by monomeric units, nucleotides, each containing a phosphate group, a deoxyribose sugar and a nitrogen base. While the phosphate group and the sugar are the same for every unit and form the backbone of the chains, there are four different nitrogen bases that are linked to the backbone and can form hydrogen bonds with each other. These hydrogen bonds are responsible for the coupling of the two strands in the double helix.

Since the discovery of its structure and function, DNA has been thoroughly studied for its role in genetic information. Only in the last three decades, the investigation of the use of DNA for its chemical and physical features, independently from the genetic information, has begun. One example refers to the modification of metal nanoparticle surface for biosensor applications: the DNA sequence can be designed to bind desired analytes and give the response [152]. Another example is the artificial sequencing of DNA to obtain self-assembling nanostructures (also called tile-DNA and origami-DNA), which can be both static and dynamic. These nanostructures can have multiple applications as scaffolds for the assembling of other materials, as sensors and nano-robots [153]. DNA can be also used in drug delivery, both as part of the physical transport system or as drug itself in gene therapy [154,155]. In tissue engineering, DNA can have applications as coating in order to increase the surface bioactivity of scaffolds and implants [156,157]. For example, it was observed that the presence of negatively charged groups, especially phosphates, is very effective in inducing the formation of chemical bonds with bone tissues [158].

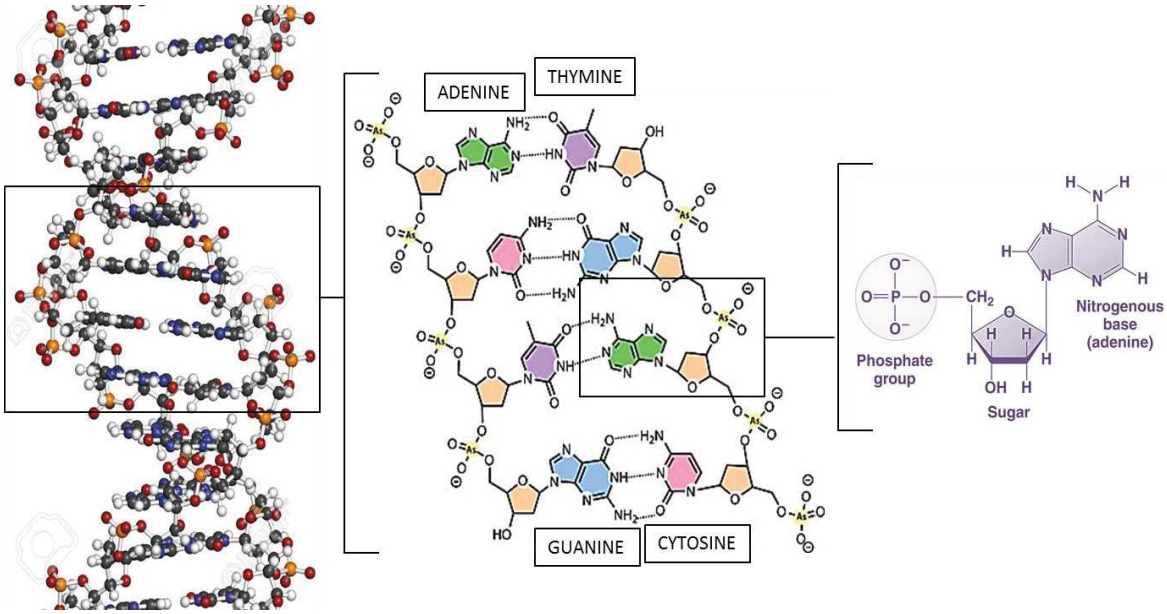


Figure 1.9 – DNA structure.

The presence of phosphates groups was also at the base of the investigation of DNA flame retardant features. Alongi et al. [159] were the first to recognize the potentiality of DNA as flame retardant for cotton. They applied DNA from herring sperm on cotton by an impregnation-exhaustion method with a 19 wt.% dry add-on: in these conditions the treated fabrics achieved self-extinguishment in horizontal flame spread tests. From these results, the intumescent features of DNA were explained according to its composition: upon heating, the phosphate groups release phosphoric acid that promotes cotton dehydration and char formation, the deoxyribose sugars act as carbonizing agent and the nitrogen bases can behave as blowing agents with the release of ammonia and nitrogen. This behaviour was confirmed by scanning electron microscopy (SEM) performed on the residues after flammability tests: the fibre surface showed multiple bubbles and swelling phenomena; meanwhile hollow fibres were formed.

The investigation of DNA fire behaviour was pursued in a successive work [105], aimed at finding the lowest biomacromolecule loading (10 wt.%) to achieve the self-extinguishment of treated fabric in horizontal flame spread tests. Furthermore, cone calorimetry tests performed under a heat flux of 35 kW/m^2, showed a 50% decrease of the peak of heat release rate for the fabrics treated with DNA. In all the above mentioned studies, the pH and molecular size of DNA were not considered.

By changing the pH of DNA solution, it was also possible to apply the biomacromolecule in a layer by layer assembly, in combination with chitosan, to confer flame retardant features to both cotton fabrics and polyurethane foams [81,160].

The DNA treatment can be a potential alternative to traditional flame retardants, as it can be recovered from renewable sources, dissolved in water baths and applied exploiting the impregnation-exhaustion method. Nevertheless, there are still some key issues, particularly referring to the biomacromolecule washing resistance, cost and toxicity assessment.

Since DNA and RNA (ribonucleic acid) have an almost identical chemical structure, it could be presumed that also RNA would be able to confer flame retardant features to cotton fabrics, with performances similar to DNA.

1.3.5 Extraction of biomacromolecules from wastes and by-products

The necessity for a global environment protection and the development of a circular economy, leads to the minimisation and valorisation of wastes and by-products. The agro-food industry is widespread and represents a significant portion of economy, especially in Europe. A recent European study reports that in Europe, every year, food waste during manufacturing corresponds to an average of 76 kg per capita. In particular, the processes that generate the higher quantities of wastes and by-products are the cheese manufacturing (85-90% of raw material becomes waste or by-product), sugar production from beetroot (85%), fish and crustacean processing (50-75%), fruit and vegetable processing (30-50%) and vegetable oil production (40-70%) [161]. The main wastes are usually wastewater, biomass and food residues and they are traditionally dispersed in agricultural soils, added in animal feed or disposed in landfills. In some cases, wastes are exploited as carbon sources in microbial fermentations, for

the extraction of added-value compounds, or for the production of energy [162,163]. In fact, organic wastes, if not handled thoughtfully, can have a high environmental impact, but with the proper treatments they can become a resource.

The dairy industry produces great amounts of liquid wastes. These fluids contain residual proteins, lactose and fats. For example, the cheese whey or the liquid from the concentration of milk contain high quantities of valuable components that can be recovered. As regards the recovery of caseins and whey proteins, several methods have been developed in the last 50 years [127]. For casein extraction, the most traditional ones involve the increase of the temperature and/or the decrease of the pH of skim milk to cause their precipitation. Then, the obtained whey is delactosed and desalted. The resulting whey proteins can be purified by means of ultrafiltration and diafiltration, in order to obtain products with different whey protein contents. When highly purified products are required, filtration techniques are preferably used. Microfiltration is exploited to separate from skim milk casein micelles that are retained and can be further purified with diafiltration. Ultrafiltration and repeated diafiltration is applied to obtain whey protein isolates (WPI). Other, more recent, industrially applied techniques for the extraction and purification of whey proteins imply the use of ion exchange resins and gel filtration [132].

Other methods have been established for the extraction of proteins from wastes. For example, wastes from the fish and crustacean processing can be treated with alkaline chemicals and extracted proteins precipitated with acid addition [164,165], while meat proteins can be extracted with detergents [166].

Extraction and recovery methods have been developed also for other wastes with interesting components such as carbohydrates, fibres, phytochemicals, flavours and fats. Still, a lot of research is in progress for the optimisation of extraction conditions and the use of new techniques [163].

On the other hand, the utilisation of DNA, independently from the study of genetics, is a quite recent outcome and all the current applications, e.g. in biosensors, drug delivery and tissue engineering, require very small amount of DNA (in the order of μg) with high purity degrees and sometimes with specific nucleotide sequences. Therefore, the so far developed extraction and recovery methods, have the purpose of obtaining a highly pure and intact product, suitable for the application in medicine and molecular biology. This implicates the necessity of controlled sources, expensive and highly pure reagents and complex and time-consuming processes.

For the application as flame retardant, a considerable quantity of DNA (in the order of g and kg) is required, while high integrity and high purity grades are not mandatory. In fact, it is not important the sequence of nucleotides or the completeness of the genetic code, but only the overall elemental composition. In addition, the presence of impurities such as proteins, which contain nitrogen, may have an additional and/or synergistic effect on flame retardancy. For these reasons, the utilisation of low-cost and easily available starting materials and the investigation of new, sustainable and cost-effective methods for the extraction of high quantities of DNA should be taken in consideration.

Traditional DNA extraction methods vary depending on the starting substrate (vegetable or animal tissue, microorganism, human cell, soil, etc.) and the final application of extracted nucleic acids. The operation method is similar in all the cases and involves an initial disruption of tissues

and cells with physical, chemical or biological methods, followed by the recovery of extracted nucleic acids and their purification. Usually, the disruption and the purification steps are the most expensive, as they require high-priced chemicals or enzymes, complex equipment and sometimes several repetitions of the same step.

Tissues and cell lysis is a critical phase of nucleic acids extraction, as they are intracellular components. Usually the starting material, especially if it is a tissue, is firstly frozen with liquid nitrogen, and then ground to powder, to improve the tissue disruption with minimum DNA damaging. Then, cell walls and membranes have to be broken in order to obtain the release of DNA. In the case of human cells and tissues, most of protocols involve the use of enzymes, such as lysozyme or proteinase K, for the cell lysis [167-169]. Such enzymes are sometimes used also for plant or animal tissues [170], but most commonly chemical reagents are exploited, especially for soil samples [171]. Such detergents as sodium dodecyl sulphate (SDS), 2-mercaptoethanol or sarkosyl are generally used. Other chemicals are also comprised in extraction solutions, namely: a pH buffer (e.g. NaH_2PO_4, Tris-HCl); a chelating agent (e.g. EDTA), which prevents the DNA damaging and a salt (e.g. NaCl), which facilitates DNA precipitation. In some cases, for example with plant tissues and soil, hexadecyltrimethylammonium bromide (CTAB) or polyvinylpolypyrrolidone (PVPP) or both are added to improve DNA purity [172]. The denaturing and lysing effect is usually improved with an incubation at medium-high temperatures (60-100°C) for some hours (from 1 to 12 h). Chemical and enzymatic cell lysis are milder than mechanical lysis, and thus prevent the shearing of DNA, but they can lose in efficacy with some kinds of samples (as for soil samples), because of incomplete penetration in solid particles [172]. Mechanical cell lysis methods can be of four types: mill grinding, bead-milling, sonication and freeze-thaw cycles. Bead-milling can be considered the most effective cell disruption method, achieving the highest DNA yields, but at the same time is also the most aggressive technique and can cause a significant shearing of DNA and the extraction of non-desired contaminants [173]. For this reason, mechanical cell disruption is rarely applied on animal or human tissues, while it is sometimes applied on plant tissues and soil samples, which need a more intense disruption [172]. Extraction of DNA from microorganisms, such as bacteria and yeasts, usually employs both enzymatic or chemical methods and mechanical methods, since the cell walls can be highly resistant to disruption [174,175].

Chemical, enzymatic and mechanical methods can be used in different combinations to obtain the most suitable procedure for the target kind of sample.

After the incubation in the extraction solution, the addition of organic solvents (phenol, chloroform and isoamyl alcohol, usually in proportion 25:24:1) promotes the deproteinisation and purification of samples [168,176]. Since these compounds are toxic and carcinogenic, some researchers prefer to use salts (e.g. sodium chloride, potassium chloride, ammonium acetate, potassium acetate and sodium acetate) to improve protein precipitation [177,178]. Then, a centrifugation, performed at low speed, helps in separating cell fragments and precipitates from the extracted-DNA solution.

In order to concentrate and separate DNA from extraction chemicals and other contaminants, a precipitation is usually carried out with the addition of ethanol or isopropanol. It is generally reported that the use of one or the other mainly depends on the size of the sample. In fact, ethanol needs to be added at least in a 2:1 vol/vol proportion, while isopropanol can be

added also in a 0.5:1 vol/vol proportion. On the other hand, isopropanol causes the precipitation of some contaminants, especially salts, and the DNA needs consequently to be washed with ethanol after precipitation.

The precipitated biomacromolecule frequently needs further purification treatements, especially in the case of DNA from soil. For this purpose different techniques can be used. The most popular are chromatography (using size exclusion or ion-exchange columns)[179,180] and gel electrophoresis (with agarose, polyacrylamide or dextran gels) [179]. These techniques are labour- and time-consuming and can cause DNA losses, but are necessary for some applications, such as polymerase chain reaction.

Starting from these thoroughly experimented methods, it is possible to develop new sustainable procedures for the extraction of nucleic acids, specifically tailored on agro-food wastes and by-products as starting materials. This implicates the necessity of high DNA yields, a less relevant DNA purity and large-scale processes without the use of toxic and expensive chemicals.

1.4 Fire characterization

As already mentioned, the fire hazard of materials is not an intrinsic property, but depends on several factors. Therefore, to investigate the different aspects of the fire behaviour of a material, different tests are needed. Some tests assess the ease of ignition and the flame spread, some others the thermal behaviour or the factors related to the heat released, even the production of smoke and toxic gases. Except for some large-scale tests, single tests cannot mimic a real fire, but the information provided by every test can figure out the fire behaviour of a material. Some of the test methods and related instrumentations that can be applied for textiles will be described as follows.

1.4.1 Thermogravimetric analysis (TGA)

The thermogravimetric analysis is not a specific fire characterizing test, as it is a standard technique used in very different fields of materials science. It measures the weight changes in a sample subjected to defined heating up and in specific atmosphere conditions.

Very briefly, the equipment consists of a furnace, in which a balance supports the sample in a pan and a gas flow controls the atmospheric conditions. Temperature can be modified with defined heating rates in a temperature range or can be maintained at a specific value (isothermal tests). Atmosphere can be inert (e.g. N_2, Ar), oxidative (e.g. air, O_2) or reducing (e.g. H_2). The instrument can evaluate all the weight changing transitions, such as loss of water or solvents, degradation, pyrolysis and oxidation. From these results, the thermal and thermo-oxidative behaviour of a material can be detailed and indications on its composition can be obtained. The sample mass, usually between 5 and 20 mg, can be a drawback regarding the

reliability of the test, especially with non-homogenous materials. Therefore, repetition of the test is mandatory.

TGA can be very useful to identify the temperatures, at which the different polymeric materials start to degrade, the rate of degradation and the fraction of the final residue.

1.4.2 Limiting oxygen index (LOI) and other flammability tests

The limiting oxygen index test is a standard fire test widely used for assessing polymer flammability. The instrument consists of a glass tube, where a particular air composition is maintained by a gas flow from the bottom to the top. In this test, the burning is carried out in steady conditions and it aims at determining the minimum concentration of oxygen in volume percentage that can support a flaming candle-like combustion of the polymer. ASTM D2863 and BS EN ISO 4589 are the standards that describe the procedure for this test. The sample is placed in a vertical position in the chimney with the desired nitrogen:oxygen atmospheric ratio. The top of the specimen is ignited and its behaviour is observed. If the specimen burns for 3 minutes or the flame covers at least 75 mm of sample, the oxygen concentration will be decreased; vice versa, if the specimen does not burn the oxygen concentration will have to be increased. The test is repeated with different samples of the same material until the minimum oxygen concentration is determined. Generally, materials with an LOI of 21% or less are highly flammable and burn quickly, materials with 21-25% LOI burn more slowly and materials with LOI beyond 26% show flame retardant features. Typical LOI values for some fibres are presented in Table 1.2.

Table 1.2 – LOI values of some fibres [181].

Fibre	**LOI (%)**
Cotton	18.4
Viscose	18.9
Wool	25
Acrylic	18.2
Polypropylene	18.6
Nylon 6	20-21.5
Polyester	20-21.5
Proban-treated cotton	31-33 [36]
Pyrovatex-treated cotton	29-30 [36]
Modacrylic	29-30
Nomex	28.5-30
Kevlar	29
Oxidised polyacrylonitrile	55

Limiting oxygen index can give an idea of the flammability of a material, but is not representative for the burning behaviour in a real fire. In fact, materials with a limiting oxygen

index much higher than 21% can burn completely in a fully developed fire, where the oxygen concentration is lower than 10%. In addition, this test can be highly influenced by some structural features of the fabric.

Flammability tests comprise different procedures with a similar purpose. The major differences among the procedures are related to the positioning of the sample: ASTM D3801 characterises solid plastics in vertical position, ASTM D635 considers solid plastics in horizontal position, ASTM D4804 is related to non-rigid plastics in vertical position, while ASTM D1230 is specific for textiles in a 45° tilted position. Procedures for flammability tests in vertical and horizontal positions are reported also in the UL 94 standard from the American Underwriters Laboratories. These are just pass/fail tests, where the material can be classified in different classes depending on the flame spread and the burning behaviour (Table 1.3 reports UL 94 standard classes, as an example). In all the cases, a flame is applied to the short side of the specimen (the bottom side in the vertical and the 45° tilted positions) for 1 to 30 seconds and if the sample is ignited, the burning time and the rate of flame spread is measured. Usually, more than one flame application is required. In case of samples, which may undergo dripping, a layer of absorbent cotton should be placed 300 mm under the sample to verify its possible ignition.

Table 1.3 – Classes of polymers tested according to UL 94 standard.

Class	**V0**	**V1**	**V2**	**HB**
Sample position	Vertical	Vertical	Vertical	Horizontal
Burning time after flame application	≤ 10 sec	≤ 30 sec	≤ 30 sec	Burning rate of less than 75 mm/min OR Burning stops before 100 mm
Total burning time	≤ 50 sec	≤ 250 sec	≤ 250 sec	
Burning time plus glowing time after second flame application	≤ 30 sec	≤ 60 sec	≤ 60 sec	
Complete burning	NO	NO	NO	
Ignition of underneath cotton	NO	NO	YES	

1.4.3 Forced combustion tests - Cone calorimetry

Cone calorimetry is a bench-scale test that measures different parameters related to the combustion behaviour of a material. The most important parameter is the heat release rate (HRR), which is calculated from the oxygen consumption during combustion [182].

The equipment consists of a conical electric heater with a balance underneath and an exhaust hood and tube system above (Figure 1.10). The sample, which is usually a square of 100 mm x100 mm x10 mm, is placed on the balance in a sample holder where it is separated from the metal by a glass fibre layer. The sample holder can have closed or open edges. When the test begins, the sample is irradiated by a specific heat flux, which raises its temperature. Usually, the heat flux is set in a range between 15 and 75 kW/m^2. A spark ignites the volatile fuel species

released from the sample and data are recorded until combustion ends. Smoke and gases are collected in the hood, analysed and discharged.

Besides the heat release rate, several other data can be collected and calculated during the experiment, namely: time to ignition (TTI), total heat release (THR), peak of heat release rate (pkHRR), time to pkHRR, sample mass variation, smoke density, CO and CO_2 concentration in the smoke, specific extinction area (SEA), total smoke release (TSR) and effective heat of combustion. Charts of these combustion parameters versus time are usually obtained. HRR, mass loss rate and CO_2 production rate usually have a similar evolution, since they are all correlated to the burning rate. From the combination of the pkHRR and the time to peak the fire growth rate (FIGRA) can be calculated.

ASTM E1354 and ISO 5660 standards describe the use of cone calorimetry for building materials and for polymers; new strategies for sampling disposition have allowed its application with textiles [183]. For example, the use of a metal grid over the sample can limit the bending, the shrinking and the moving of the fabric sample.

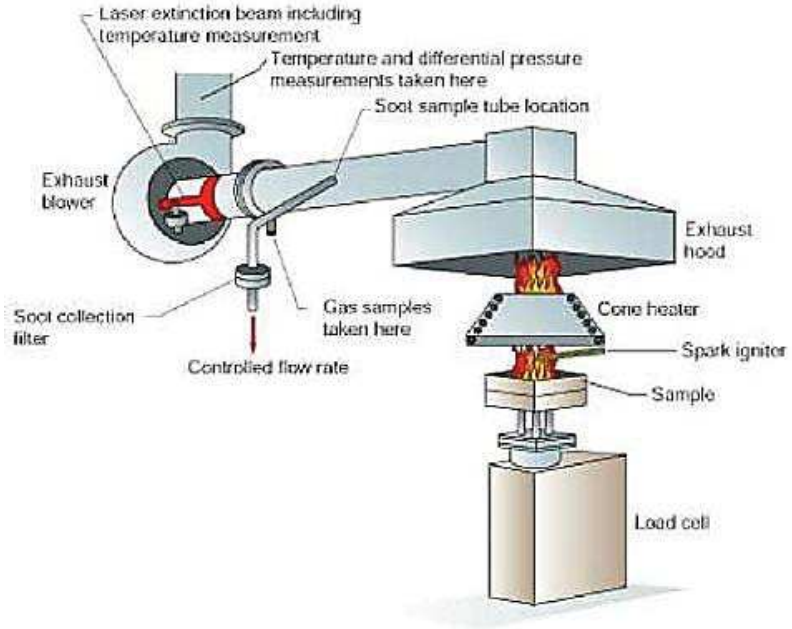


Figure 1.10 – Cone calorimeter apparatus. Adapted from [184].

1.4.4 Microscale Combustion Calorimetry (MCC)

The heat released can be measured by means of microscale combustion calorimetry, too. As reported in the name, this apparatus analyses very small samples in comparison to the cone calorimeter (order of milligrams). The apparatus consists of two overlapping chambers (Figure 1.11):

- in the lower chamber (i.e. the pyrolyzer), the sample can be pyrolyzed at increasing temperatures, up to 750-800°C, with a high heating rate (in the range of 0.2-2 K/s); the released volatiles move to the second chamber in plug-flow, which means without axial mixing.

- in the upper chamber (i.e. the combustion chamber), the gaseous products are completely burnt at 900°C in presence of oxygen in sufficient quantity for the complete combustion; the atmosphere is usually air or oxygen-enriched air.

MCC can be used with two methods, depending on the kind of decomposition in the lower chamber. In the case of thermal anaerobic decomposition (ASTM D7309, Method A), which simulates the fire conditions, nitrogen is introduced into the pyrolyzer. This way, only the heat released by the volatile species that reach the combustion chamber is measured. On the contrary, in the case of thermal aerobic decomposition (ASTM D7309, Method B), air is introduced into the pyrolyzer and the entire sample is oxidized (if the maximum temperature is sufficiently high), thus measuring the total calorific value of the sample, including that of a possible solid char residue.

Heat release rate can be plotted against time or temperature and the total heat release, the peak of heat release rate and the time/temperature of pkHRR can be measured.

The size of the specimen is both an advantage and a limitation of this technique. It is an advantage, especially in the early phases of a fire retardant development because a small amount of material is used and it is not necessary to produce, synthesize, or treat big quantities of material. It is a limitation in an in-depth study of fire behaviour, since a milligram sample is not representative of an entire object. Furthermore, it cannot reproduce the physical effects of melting, dripping, swelling, shrinking or barrier formation, and neither can take in consideration physical parameters of the object, such as thickness, edges or orientation.

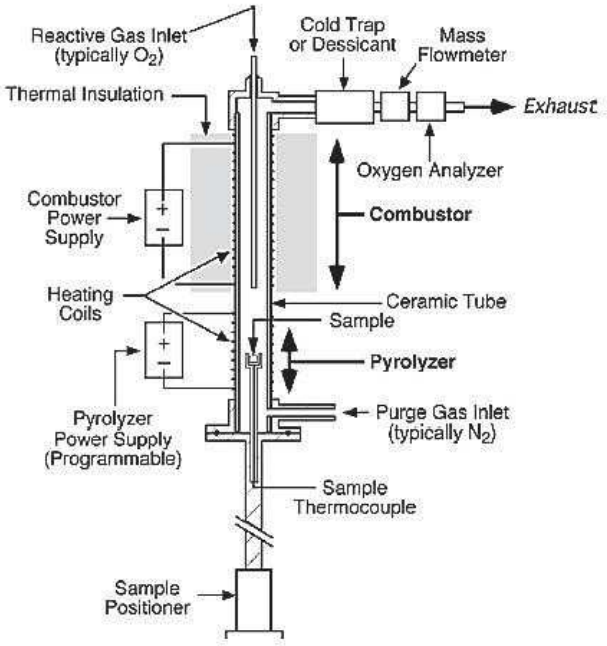


Figure 1.11 – Schematic diagram of a microscale combustion calorimeter.

1.4.5 NBS Smoke density chamber

The generation of smoke and some of its characteristics can be investigated with the smoke chamber (ASTM E662). The tendency to release smoke and the related optical density of a sample exposed to a heat source are measured with a light beam. In some cases, it is possible to extract smoke samples with a probe for the assessment of the presence of toxic gases.

The sample is placed in a closed chamber, where it is subjected to a heat flux of 25 kW/m^2. The use of a pilot flame is optional. The sample can be horizontally placed with a cone heater (e.g. in maritime and railway tests) or vertically with a cylindrical heater (e.g. in aircraft tests). Aside, a light beam passes through the chamber and is collected by a photo sensor that evaluates the difference in intensity due to the obscuration of smoke.

In the smoke chamber, burning does not undergo steady state conditions and the development of the system can give raise to issues for the collection of samples at the right time or the data interpretation. In a smoke chamber only well-ventilated conditions, which are less dangerous for the production of smoke and toxic gases, can be tested. Furthermore, the obscuration measurement depends on the orientation of the specimen, the ventilation, and the incident heat flux.

1.4.6 Purser furnace (steady state tube furnace)

Steady state tube furnace is a bench-scale apparatus that allows evaluating the emission of gases and smoke during the combustion of a material (according to the ISO 19700 standard). A schematic diagram is presented in Figure 1.12. It consists of a quartz tube in a cylindrical furnace linked to a small chamber, where smoke and gases are gathered. This chamber is linked to an exhaust hood and to a second smaller furnace at 900°C where the complete oxidation of combustible materials to CO_2 occurs. Two controlled air fluxes are directed to the first and the second furnace. O_2, CO and CO_2 can be evaluated in real time with sensors, while samples of smoke and gases can be extracted from the middle chamber for the estimation and quantification of HCN, HCl, HBr and other toxic gases and with particular instruments (e.g. a cascade impactor) of micro- and nano- particles.

This can be exploited for long samples, usually in form of pellets, which are pushed slowly into the tube furnace to obtain a combustion in steady conditions. This way it is possible to study complex reactions more easily and deeply than in an unstable system. It is possible to investigate different stages of a fire development by varying the temperature of the main furnace and the air flows.

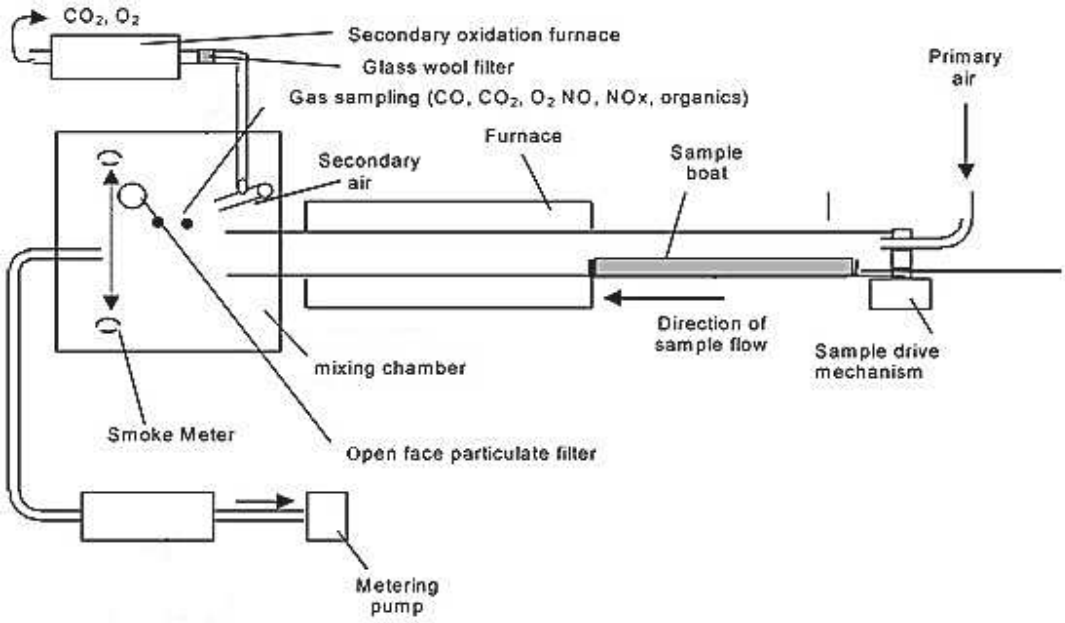


Figure 1.12 – Schematic diagram of a Purser furnace. Adapted from [185].

2. MATERIALS AND METHODS

2.1 Materials

Cotton fabrics (200 g/m^2) were purchased from Fratelli Ballesio S.r.l. (Torino, Italy). The textile samples were washed with a non-ionic detergent at 40°C for 20 min. Then, fabrics were thoroughly rinsed with water and dried. They were cut into 50x50 mm^2 and 100x100 mm^2 squares for performing the treatments and the characterisation tests.

Chitosan (deacetylation degree of 75–85%) was purchased from Sigma Aldrich S.r.l., Milano, Italy.

Irgacure 1173 (BASF Corporation, Florham Park, NJ, USA) was used as radical photoinitiator.

NaOH, glacial acetic acid, NaCl, ethanol (at 99.8% purity), tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) (pH 8) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich S.r.l. (Milano, Italy) and used as received.

2.1.1 Whey proteins

Whey protein isolate (WPI) powder was purchased from Anderson Research (Cervaro (FR)), Italy. Table 2.1 reports the composition of the powder.

Table 2.1 – WPI composition.

	Wt.%
Proteins **of which cysteine**	93.5 3.2
Carbohydrates	1
Lipid	0.5
Moisture	2.8
Ash	2.2

WPI solution was prepared by dissolving the powder in distilled water with a concentration of 10 wt.%. The dissolution was carried out under magnetic stirring (300 rpm) and at a temperature of 35°C for 30 min. The measured pH value of the obtained solution was 6.

2.1.2 Caseins

Casein powder was purchased from Sigma Aldrich S.r.l., Milano, Italy. It has a composition of 12-15 g/l of α-s_1-casein, 3-4 g/l of α-s_2-casein, 9-11 g/l of β-casein and 2-4 g/l of κ-casein.

Casein solutions at three different concentrations (namely 0.5, 2.5 and 5 wt.%), were prepared by dissolving the powder in 0.5 M NaOH under magnetic stirring (300 rpm) for 24 h. The final pH value of the obtained solutions was 13.

2.1.3 DNA

DNA from herring sperm (#D3159) and DNA from herring testes (Type XIV sodium salt, #D6898) were purchased from Sigma-Aldrich S.r.l., Milano, Italy, and stored at 4 °C before use. A reduced-molecular-weight DNA was obtained by sonicating herring testes DNA solution (2.5 wt.%) in an ice bath with an XL-2020 sonicator (Misonix) equipped with a Misonix microtip probe 419 (3.2-mm tip diameter; 16.5 cm length) at 550W.

The different deoxyribonucleic acids were characterised by means of gel electrophoresis on 0.8% w/v agarose gels (Sigma-Aldrich S.r.l. Milano, Italy) at 5 V/cm for 2 h in 1 × Tris–acetate–ethylenediaminetetraacetic acid buffer containing 0.5 µg/mL of ethidium bromide. Gel pictures were taken using a Gel Doc XR+ Imaging System (BioRad). 100 bp (BioRad) and 1 kb (Thermo Scientific) molecular rulers were run with the samples to estimate DNA lengths. Figure 2.1 reports the resulted picture. It was possible to determine that DNA from herring sperm had size range of 100–200 bp, DNA from herring testes had a size range of 3000–10,000 bp and the DNA with reduced molecular weight had a size range of 300–800 bp [186].

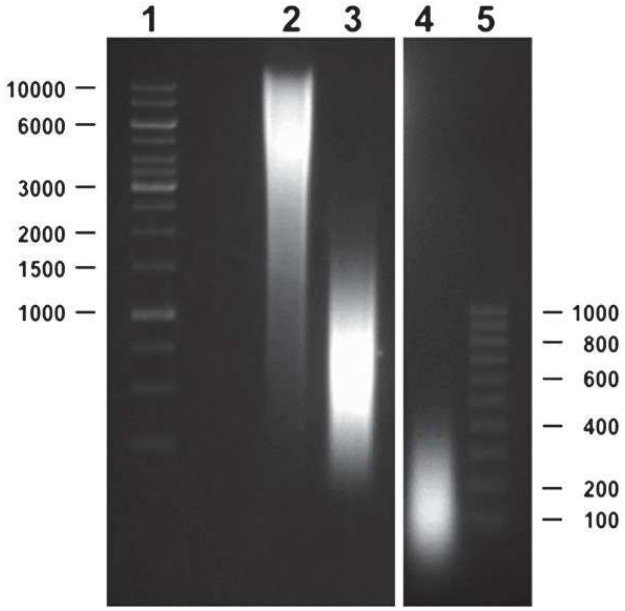


Figure 2.1 – Agarose gels of the different DNAs. Lanes: 1) molecular mass markers, 1 kb ladder; 2) herring testes DNA; 3) reduced-molecular-size DNA; 4) herring sperm DNA; 5) molecular mass markers, 100 bp ladder. Adapted from [186].

Herring sperm DNA solutions at 2.5, 6 and 8 wt.% were prepared by dissolving the powder in distilled water under magnetic stirring at room temperature for 2 hours. The pH was modified with 0.1 M NaOH to improve the dissolution. Solutions at pH 4, 6, 7, 8 and 9 were prepared.

Herring testes DNA solutions at 0.5 wt.% were obtained by slowly dissolving the DNA powder in distilled water under low magnetic stirring (50 rpm) for 12 hours. Two solutions were prepared, namely: a solution without pH correction (pH=8) and a solution with pH corrected at 4 with 0.1 M HCl.

Two solutions with a mixture of DNA and caseins were prepared by dissolving herring sperm DNA powder in a solution of caseins (at 0.5 and 2.5 wt.%), with concentrations of 2.5 and 0.5 wt.%, respectively. The resulting solutions had a pH of about 12.

2.1.4 Chitosan

Chitosan solutions at concentration of 0.5, 2 and 5 wt.% were prepared by dissolving the powder in 2 vol.% acetic acid water solution, under magnetic stirring (300 rpm) for 24 hours. The pH of the prepared solutions was 4.

A solution with a mixture of DNA and chitosan was obtained through the mixing of a 8 wt.% herring sperm DNA solution at pH 4 with an equal volume of a chitosan solution at pH 4 and 2 wt.% concentration.

The photoinitiator (Irgacure 1173) was added to all the containing-chitosan solutions in proportion of 4% with respect to chitosan weight, just before use.

2.1.5 Agro-food wastes

2.1.5.1 Vegetable wastes

Vegetable wastes were kindly supplied by a food processing plant (Stroppiana Ortofrutticola S.p.A. Chieri, TO, Italy). Vegetables of different kinds arrived altogether in different batches, and the composition of these mixtures changed on the basis of the seasonal variance of the cultivations. Almost all of the batches consisted of a portion of leafy vegetables (lettuce, chard, celery, cabbage leaves, red chicory, rocket salad leaves) and another portion made up of small pieces of turnip, onions, peppers, courgettes, potatoes, carrots and pumpkin. Batches will be distinguished in those containing leafy vegetables and a small portion (about 10 %) of other vegetables (herein after coded as MLV matrices) and in those containing only leafy vegetables (LV matrices). The matrices were separated into small portions (500 g) and stored at +4°C (for a maximum of 48 hours) or at -20°C before use.

Nucleic acid content in vegetable tissues is not precisely identifiable, as it depends on several factors, from the species to the part of the plant (leaves, roots, seeds, flowers, fruits, etc.), to the growth stage. A range of extraction yields can be defined, on the basis of results

reported in published papers: yields usually fall in the range of 0.005-0.1% on plant tissue dry weight [187-189].

2.1.5.2 Spent brewer's yeast

Spent brewer's yeast was kindly supplied by Heineken Italia S.p.A. (Aosta, Italy) and Birrificio Della Granda (Cuneo, Italy) and stored at +4°C before use.

The content of nucleic acids in yeast depends on the species and growing conditions (e.g. the culture medium and the growth rate and stage) and can vary in an average range of 5-15% of dry cell weight, where DNA usually corresponds to less than 0.5% [190].

2.2 Methods

2.2.1 Fabric impregnation

Cotton fabric pieces were dried at 105°C for 6 h, cooled down at room temperature and then weighted with an analytical balance (accuracy:±10−4 g) (W_i). After the impregnation and the subsequent drying steps, fabrics were weighted again (W_f) to evaluate the final dry add-on (AO%), using the following equation (Eq.1):

$$AO\% = \frac{W_f - W_i}{W_i} \cdot 100 \qquad \text{(Eq.1)}$$

The impregnation of cotton with the WPI solution was obtained by dipping the fabrics in the solution for 1 min at room temperature and then drying them at 105°C for 30 min. The impregnation process was repeated three times to achieve an AO% ≥ 20. In one case the solution was stationary and a final AO% of 24 was obtained; in a second case, it was kept moving with a rocker shaker and an AO% of 22 was reached.

The impregnation with caseins was carried out by dipping the fabric in the solution on the rocker shaker for 1 min at room temperature. After impregnation, fabrics were dried at 30°C for 12 h and then at 105°C for 2 h. This double-step drying was adopted in order to prevent the rolling up of fabrics and to ensure an homogeneous distribution of the coating on the fabric substrate. An AO% of 8, 10, 13 and 20% was obtained with the different solutions, according to the number of impregnations.

The impregnation with DNA was made by dipping the fabric in the DNA solutions for 1 or 5 min at room temperature with the rocker shaker. Then, the fabrics were dried at 105°C for 30 min. Single or multiple impregnations were carried out in order to achieve the desired AO% with the different DNA solutions.

Impregnations with chitosan were obtained by spreading an appropriate amount of the chitosan solution over the fabrics. These latter were successively dried at 105°C for 30 min. The same technique was applied for the impregnation with the DNA and chitosan mixture.

2.2.2 Layer by layer (LbL) impregnation

Layer by layer assemblies were constructed on cotton fabrics by alternate immersion of the samples into a positively charged solution (chitosan solution, 0.5 wt.%, pH 4) and in a negatively charged solution (low-molecular-weight DNA solution, 0.5 wt.%, pH 7). The fabrics were washed in distilled water and squeezed in between different applications. The first layer of chitosan was obtained with a 5-min immersion, all the subsequent layers were obtained with a 1-min immersion. After the repetition of the process up to 30 bilayers the samples were dried at 105°C for 30 min. Half of the samples had DNA as the last layer, chitosan was the last layer for the other specimens.

2.2.3 Glass slide coating

The coating of glass slides with chitosan or DNA or with the mixture of DNA and chitosan was performed by spreading a proper amount of solution over the surface using a glass rod to obtain a micrometric layer. The glass slides were successively dried at 105°C for 30 min and used for the washing fastness assessment.

2.2.4 UV-curing procedure

The grafting reaction of chitosan and the cross-linking of high molecular weight DNA was induced by means of UV radiation, when required. The treatment was carried out using a medium pressure mercury lamp, with a light intensity of about 60 mW/cm^2, in a small box equipped with a quartz window, under nitrogen atmosphere. The distance between the sample and the lamp and the exposure time were set to 20 cm and 1 min, respectively. The treated cotton samples were irradiated on both sides to ensure the complete curing.

2.2.5 DNA extraction procedures

2.2.5.1 DNA extraction from the mixed vegetables

The extraction was carried out with the following procedure:

- Grinding of the thawed vegetable tissue (30g) with mortar and pestle or with a hand blender.

- Addition of the aqueous extraction buffer (liquid detergent 10% v/v, NaCl, 10% w/v). The extraction buffer weight:vegetable dry weight ratio was set to 1:7.
- Incubation at 65°C for 30 min.
- Filtration with nylon gauze and qualitative paper to separate and recover the aqueous fraction.
- Addition of cold (-20°C) ethanol to the filtrate (1:1 vol ratio).
- Precipitation at -20°C overnight.
- Recovery of the upper ethanol phase, containing the precipitated nucleic acids.
- Centrifugation of ethanol (4100 rpm, 10 min) and re-suspension of the pellet in distilled water.

Different parameters were modified to optimise the extraction:

1) the starting material was either stored at -4°C or at -20°C before extraction;
2) 99.8% ethanol was further diluted with bi-distilled water to 96 and 90% purity grade and used for the precipitation step;
3) the precipitation was carried out at two different temperatures (+4 and -20°C);
4) the precipitation was performed at -20°C for different incubation times, namely 1, 2, 3, 4, 8 hours and overnight.

2.2.5.2 DNA extraction from the spent brewer's yeast

Two alternative pre-treatments were adopted for the yeast cells:

A. Conservation of the beer slurry at 4°C for 8 months. Then, the yeast cells were centrifuged and washed three times with distilled water (batch labelled AY).
B. Immediate centrifugation and repeated washing of yeast cells for the separation from beer residues. Then pelleted yeast cells were stored at -20°C until utilisation (batch labelled FY).

The extraction procedure that was applied to both the batches was the following:

- Addition of aqueous extraction buffer (Tris-HCl 50 mM, EDTA 10 mM, NaCl 100 mM) to yeast pellet.
  Extraction buffer volume: yeast dry weight ratio equal to 1 ml:8 mg.
- Freeze-thawing for three times (Freeze: -20°C, 24 h; Thawing: 65°C, 15 min).
- Ball milling with glass beads (1 and 2 mm diameter) with 1mg:5g yeast:glass beads ratio for 10 min (150 rpm).
- Recovery of glass beads and centrifugation (4100 rpm, 10 min) for the removal of cell debris. Discard of the pellet.
- Microfiltration on glass fibre filter (porosity 0.7 µm).
- Ultrafiltration (cut-off 10 kDa).

An optimisation of the procedure was obtained by changing some parameters, one at a time, as follows:

1) different extraction buffers were tested, namely:
   - TrisHCl50 mM + NaCl 50 mM + EDTA10 mM , pH 8 (labelled TNE 50/50/10)

- TrisHCl 50 mM + NaCl 100 mM + EDTA 10 mM, pH 8 (labelled TNE 50/100/10)
- TrisHCl 50 mM + NaCl 50 mM, pH 8 (labelled TN 50/50)
- TrisHCl 50 mM + NaCl 100 mM, pH 8 (labelled TN 50/100)
- Sodium phosphate, pH 8 (labelled PB);

2) different times of ball milling (from 10 to 90 min) were compared;
3) to separate cell debris after the extraction of nucleic acid, mixtures were centrifuged, or microfiltrated (ϕ=1.2 μm), or filtrated through Celite as reported by Hatti-Kaul and Mattiasson [191].

2.2.5.3 Evaluation of DNA yield and purity

The measurement of nucleic acids (DNA and RNA) content in the extraction solutions was performed by evaluating the absorbance of solution samples at the wavelength of 260 nm, after a proper dilution, with a HP 8452A diode array spectrophotometer. The NA concentration in the extract was determined according to the following equation (Eq.2) [192]:

$$C\ (\mu g/ml) = OD_{260} \cdot DF \cdot 40 \qquad \text{(Eq. 2)}$$

where OD_{260} is the absorbance value at 260 nm, DF is the dilution factor and 40 is a constant (40 μg/ml of RNA have an absorbance value of 1 with an optical path of 1 cm). This concentration is slightly underestimated because DNA, which has a constant of 50, is also present in the extract; however, RNA should be predominant in quantity [193].

The extraction yield (EY_{DNA}%) was calculated by means of the following equation (Eq.3):

$$EY_{DNA}\% = \frac{DNA\ quantity\ (mg)}{Starting\ dry\ weigth\ material\ (mg)} \cdot 100 \qquad \text{(Eq. 3)}$$

The purity of the nucleic acids was assessed from the absorbance ratio at 260 and 280 nm [194].

2.2.6 Sample characterisation

2.2.6.1 Thermogravimetric analysis

Thermogravimetric analyses (TGA) were carried out using a TA Q500 thermo balance (TA Instruments) (experimental error: ±0.5 wt%, ±1 °C). The samples (about 10 mg) were placed in open alumina pans and fluxed with nitrogen or air (gas flow: 60 mL min^{-1}) and the temperature was raised from 50 to 800°C with a heating rate of 10°C/min. $T_{onset10\%}$ (temperature, at which 10 % weight loss occurs), T_{max} (temperature, at which maximum weight loss rate is achieved) and the mass of the final residues at 600, 700 and 800°C were evaluated.

2.2.6.2 Flame spread tests

The fire behaviour of all the treated and untreated cotton fabrics was assessed by flame spread tests performed in horizontal configuration. A methane flame (25 mm long) was applied for 5 s to the short side of the samples (50x100 mm^2), which were clamped to a U-shaped metallic frame tilted 45° with respect to the plane. At least three tests were performed for each kind of sample. The total burning time (s), char length (mm), total burning rate after flame application (mm/s, ratio between the char length and total burning time) and the final residue (%) were measured. The flammability tests were conducted with the aim of mimicking the procedure described in the ASTM D4804 standard, which is usually employed for thin films, although the specimen size is different (50x200 mm^2 in the ASTM D4804 standard). Prior to the tests, all the specimens were conditioned at 23 ± 1°C for 48 h at 50 % R.H. in a climatic chamber.

2.2.6.3 Cone calorimetry tests

The forced combustion of square fabric samples (100x100x0.5 mm^3) was investigated by cone calorimetry (Fire Testing Technology, FTT). The measurements were carried out under 35 kW/m^2 irradiative heat flow, placing the samples in horizontal configuration. Samples were placed on an aluminium foil and blocked with a metal grid (size of the wires of 1 mm of diameter). This grid is necessary with low weight samples, such as fabric, to avoid movements of the sample during the test and to maintain a uniform irradiance over the whole area, obtaining similar results to tests without grid but with a reduced experimental error [195,196].

Time to ignition (TTI, s), total heat release (THR, MJ/m^2g), peak of heat release rate (PHRR, kW/m^2g), CO and CO_2 release (CO, CO_2 yield, g/s) were measured. The last four parameters were normalized with respect to the initial sample mass due to the significant difference of weight between untreated and treated fabrics. The experiments were repeated three times for each kind of sample to ensure reproducible and significant data. Before combustion tests, all the specimens were conditioned at 23 ± 1 °C for 48 h at 50 % R.H. in a climatic chamber.

The cone calorimeter was also exploited for the measurement, by means of a thermocouple, of the temperature on the unexposed side of specimens (100x100x0.5 mm^3), while it was subjected to a heat flux of 35 kW/m^2, to evaluate the thermal protection of treated fabrics.

Furthermore, a cone calorimeter test was carried out on cushion-mimicking samples. In this case samples consisted of flexible polyurethane foam (100x100x20 mm^3) and by untreated or treated cotton fabrics (140x140x0.5 mm^3), which covered the upper and the lateral surfaces of the foam. These samples were tested under a heat flux of 35 kW/m^2; time to ignition (TTI, s), total heat release (THR, MJ/m^2) and peak of heat release rate (PHRR, kW/m^2) were measured. In this case the weight of the samples was not influenced by the fabric treatment and the AO% and the normalisation with respect to the initial mass was not necessary.

2.2.6.4 Microscale combustion calorimetry (MCC)

MCC tests were carried out with a Govmak MCC-2. To this aim, 3 mg of the fabric samples were heated to 750°C at a heating rate of 1 K/s in a nitrogen gas stream (80 ml/min). The pyrolysis products were then mixed with oxygen (20 ml/min) before entering a second chamber at 900°C, where the combustion of volatile species took place. The heats of combustion of the pyrolysis products were measured by the oxygen consumption principle. The test was repeated three times for each kind of sample.

2.2.6.5 Washing fastness assessment

Some samples treated with mixtures of biomacromolecules were subjected to a water soaking test, to investigate the resistance to a prolonged contact with hot water.

Chitosan and DNA treated glass slides were immersed in water at 30°C under low stirring for 1 h, while all the treated fabric samples (50x50 mm^2) were immersed in water at 55°C for the same time. The temperature of 55°C was chosen as an average temperature in textile colour fastness tests. The release of DNA in water was assessed by collecting samples of the water at defined intervals and analysing them by means of a spectrophotometric method, using a HP 8452A diode array spectrophotometer. The absorbance of the samples after proper dilution at 260 nm was measured and the corresponding concentrations were calculated according to the following equation [192] (Eq.4):

$$\text{Concentration } (\mu g/ml) = OD_{260} \cdot DF \cdot 50 \qquad \text{(Eq.4)}$$

where OD_{260} is the absorbance value at 260 nm, DF is the dilution factor and 50 is a constant (50 µg/ml of DNA have an absorbance value of 1 with an optical path of 1 cm).
Tests were repeated at least twice.

2.2.6.6 Antibacterial tests

The antibacterial activity of the cotton treated with chitosan and DNA was determined according to ASTM E 2149-01 using a Gram-positive bacterium: (*Staphylococcus aureus* ATCC 6538). Cultures of the microorganism in a nutrient medium were incubated for a sufficient growth and then diluted to give a concentration of 1.5–3.0×10^5CFU/ml. Each fabric sample (of about 1 g) was transferred to a flask containing 50 ml of the diluted culture. All flasks were shaken for 1 h at 190 rpm. 1 ml of the obtained solution, with proper dilution, was plated in nutrient agar. After an incubation at 37°C for 24 h, surviving cells were counted. The antimicrobial activity was expressed as the percentage reduction of the microorganisms after contact with the specimen, compared to the number of bacterial cells surviving after contact with the inert control.

2.2.6.7 Surface morphology analysis

The surface morphology of the treated fabric samples and of the residues after flame spread tests was studied using a LEO-1450VP Scanning Electron Microscope (beam voltage: 5 kV). Fabric pieces of 5x5 mm^2 were cut and pinned up to conductive adhesive tapes and gold-metallized for the analysis.

2.2.6.8 Infrared spectroscopy

Pellets of KBr, covered with films of herring sperm DNA, chitosan and a DNA-chitosan mixture, following the same procedure as the glass slide coating, were analysed by means of Fourier Transformed Infrared (FTIR) spectroscopy to investigate any interaction between chitosan and DNA. For this purpose, a Nicolet FTIR 5700 spectrophotometer was used: each spectrum was collected by cumulating 128 scans, at a 4 cm^{-1} resolution and gain 8, in the 4000–600 cm^{-1} wavenumber range.

Attenuated Total Reflectance (ATR) spectroscopy of commercial herring testes DNA and of the DNA extracted from FY yeast and leafy vegetables was performed using a Frontier FT-IR/FIR spectrophotometer (16 scans and 4 cm^{-1} resolution), equipped with a Universal ATR Sampling Accessory (diamond crystal).

2.2.6.9 Elemental analysis

The concentrations of the P element on the fabrics treated with commercial DNA and the yeast NA extracts was determined by performing inductively coupled plasma mass spectrometry (ICP-MS) tests. These measurements were carried out with a ICAP-Q apparatus (Thermo Fisher, USA). The DNA-treated fabrics (100 mg) were dissolved in 80 mL of an $HNO_3/HClO_4$ aqueous solution (4:1 molar ratio) at 50°C, then cooled to room temperature; bi-distilled water was added to the obtained solution until a final volume of 100 mL was achieved and then diluted in order to reach a suitable concentration for the tests (between 100 and 1000 ppb). The apparatus had previously been calibrated considering a multi-element standard for phosphorus.

2.2.6.10 Agarose gel electrophoresis

Aliquots of the different DNA samples were digested with a DNAase-free RNase (20 µg/ml), and then analyzed by means of gel electrophoresis on 0.8% w/v agarose gels (Sigma-Aldrich S.r.l. Milano, Italy) at 5 V/cm for 2 h in a 1 × Tris–acetate–ethylenediaminetetraacetic acid buffer containing 0.5 µg/mL of ethidium bromide (Sigma-Aldrich S.r.l. Milano, Italy). A 1 kb molecular ruler (Thermo Scientific) was run with DNA samples in order to estimate the lengths. Gel pictures were taken using a Gel Doc XR+ Imaging System (BioRad).

3. RESULTS AND DISCUSSION

3.1 Flammability, thermal and combustion behaviour of cotton fabrics

3.1.1 Cotton

Cotton is a very flammable material, it ignites easily and burns quickly.

The thermal and thermo-oxidative degradation of cotton has been widely studied [10]. In nitrogen, the degradation follows two competitive pathways: in fact, cellulose can be dehydrated and decomposed to form carbonaceous residue (char) or can depolymerise, giving rise to volatile flammable species. As shown in Figure 3.1 (left), the thermal degradation of the selected cotton occurs in one step that starts within 310 and 330°C and ends between 360 and 380°C, leaving a residue of about 8%.

In air cotton fabrics show a two steps degradation (Figure 3.1 (right)): the first main mass loss is attributed to dehydration and depolymerisation reactions, similarly to what has already been mentioned in inert atmosphere. The second stage can be ascribed to the further oxidation of the aliphatic and aromatic char previously formed.

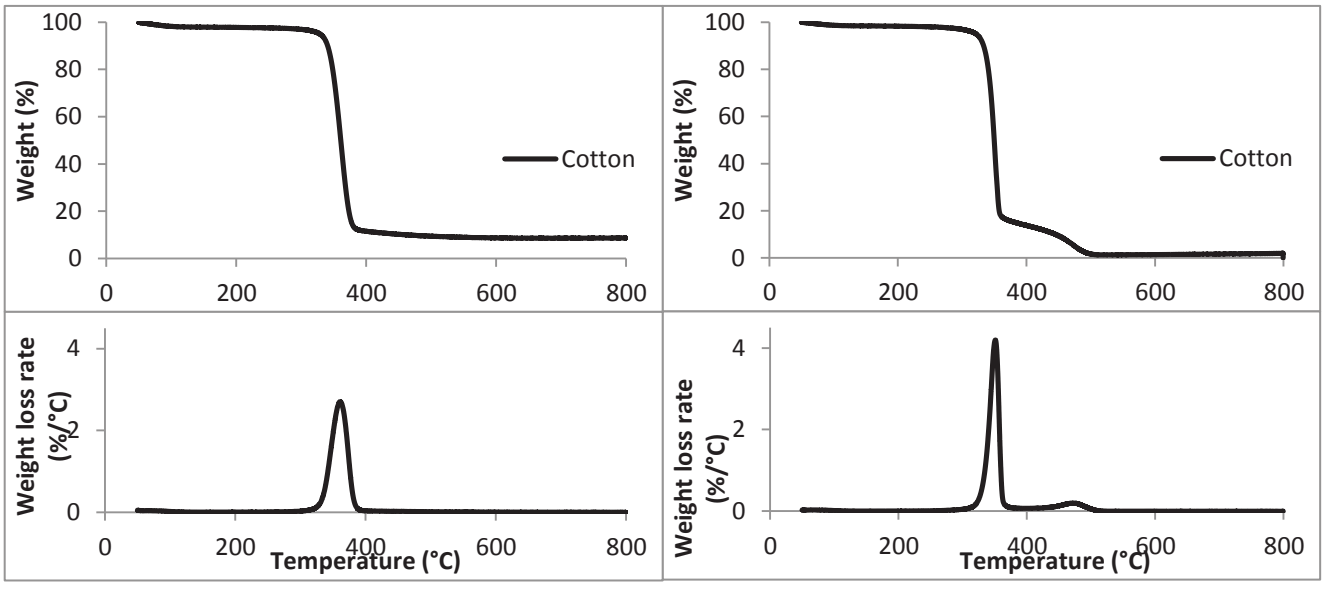


Figure 3.1 - TG and dTG curves of untreated cotton in nitrogen (left) and air (right).

When subjected to horizontal flame spread tests, cotton burns completely and leaves a negligible residue (Figure 3.2).

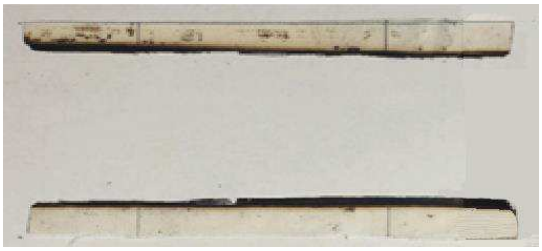


Figure 3.2 – Residue after horizontal flame spread tests of untreated cotton.

In cone calorimeter tests, under a heat flux of 35 kW/m^2, cotton ignites within 25-35 s and burns usually for 20-40 s, depending on the fabric texture and density. The HRR vs. time curve shows one sharp peak (Figure 3.3); a grey and very light residue is found at the end of tests (Figure 3.4).

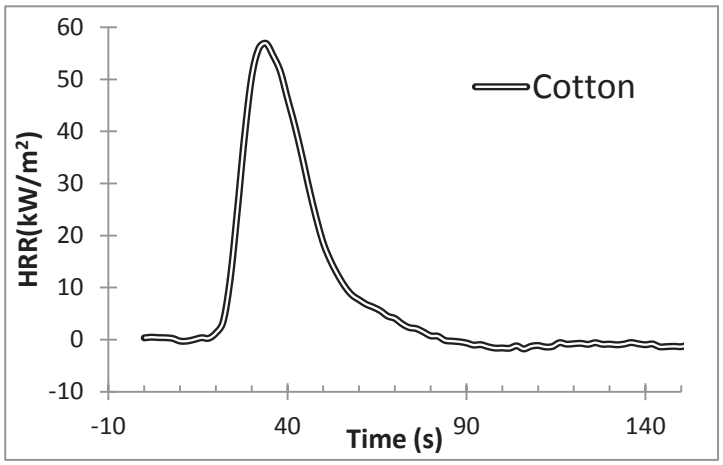




Figure 3.3 - Heat release rate vs. time of pristine cotton, tested at 35 kW/m^2.

Figure 3.4 – Residue after cone calorimetry test of untreated cotton.

3.1.2 Whey protein isolates (WPI)

It has been already shown that whey proteins decrease the onset of the degradation temperature of cotton fabrics, while, at the same time, reduce the burning rate of the fabrics subjected to flame spread tests. Specifically referring to folded whey proteins, these biomacromolecules were found to increase the final residue in flammability tests by 30% [103].

TG analyses showed a reduction of the onset of degradation temperature of cotton both in inert and oxidative atmosphere, due to the presence of the WPI coating (Figure 3.5). The $T_{onset10\%}$ of pure cotton was lowered by exactly 20°C in the presence of WPI. The peak of the rate of mass loss in the first degradation step was halved in both the cases. In nitrogen, the degradation

occurred in one step with a final residue of 15% at 800°C for the treated fabric, as compared with untreated cotton (8%). The thermo-oxidative degradation of WPI-treated cotton occurred in two main steps: the protection exerted by the protein coating is clearly shown by the higher residues found both at the end of the first degradation step (40% at 355°C for WPI-cotton vs. 20% for untreated cotton) and at the end of the analysis (2.7% for WPI-cotton vs. 0% for untreated cotton). These results indicate that, despite the sensitisation on the thermo oxidative degradation of the fabric, exerted by the biomacromolecule coating, this latter favours the formation of a char residue, presumably aromatic [197].

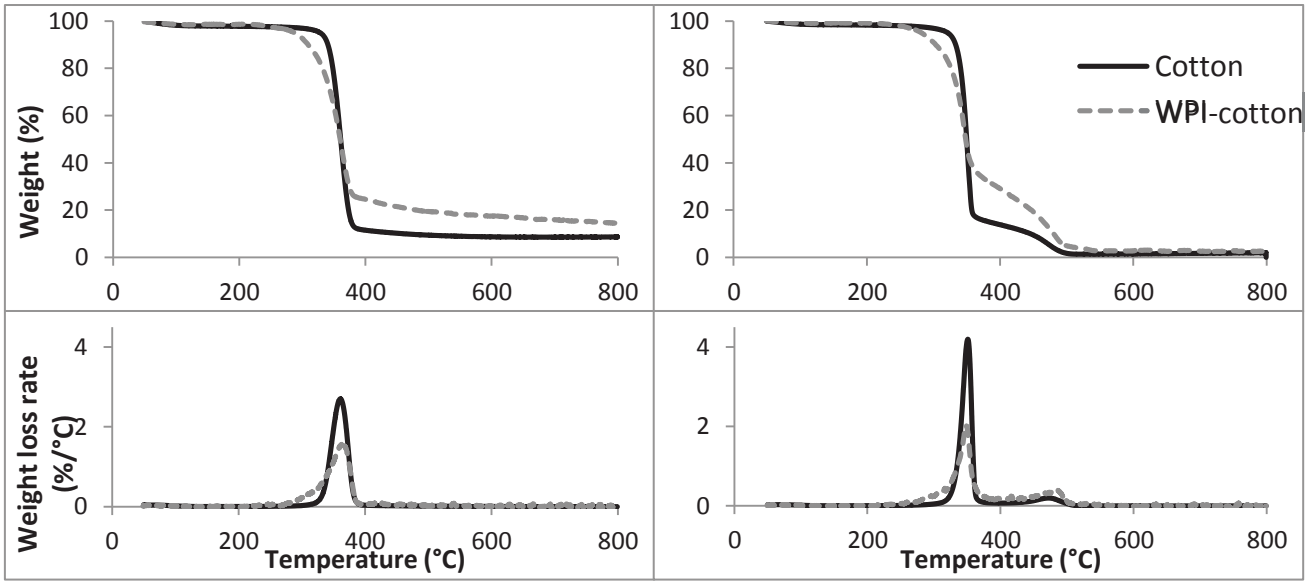


Figure 3.5 - TG and dTG curves of untreated cotton and WPI-treated cotton in nitrogen (left) and air (right).

In order to investigate the possible effect of the impregnation technique on flame retardancy, fabrics were impregnated either in static and in dynamic conditions (i.e. a rocker shaker was used to keep the solution in motion during the fabric finishing). In doing so, a slight difference in the final AO% values was observed: more specifically, static conditions allowed achieving a slightly higher AO% with respect to dynamic ones (24 vs. 22 wt.%, respectively).

Three samples of each kind of fabrics were subjected to horizontal flame spread tests and the average results are collected in Table 3.1 and Figure 3.6.

Table 3.1 – Flammability data of WPI-treated and untreated cotton fabrics. (Accuracy ±5%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction	AO(%)
Untreated cotton	66	-	1.50	-	NO	-
WPI-cotton (static)	66	58	0.88	79	YES	24
WPI-cotton (dynamic)	67	54	0.81	80	YES	22

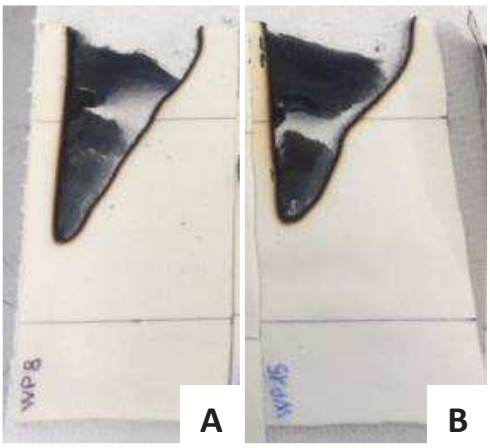


Figure 3.6 - Residues after flame spread tests (horizontal configuration) of WPI-cotton samples: A) static impregnation; B) dynamic impregnation.

In both the cases the samples achieved self-extinction, with the flame arriving at about the half of the specimen. The total burning rate was significantly decreased in comparison with the untreated fabric (from 1.50 to 0.8-0.9 mm/s) and a high final residue of about 80% was achieved. Despite the slightly different AO%, the results from the two kinds of impregnation were very similar.

Whey proteins do not contain phosphorous and their mechanism of action does not involve the dehydration of cellulose, but may be related to a dilution of combustible gases and to a charring tendency of the protein itself, as confirmed by horizontal flame spread test.

Three samples of each kind of fabric were tested with the cone calorimeter to assess the resistance to a heat flux and the combustion behaviour. Table 3.2 collects the obtained data. In this case, a more relevant difference between the two treatments (static vs. dynamic impregnation) is observed, both for TTI and pkHRR. The fabrics treated in dynamic conditions behave more similarly to untreated cotton: TTI is slightly decreased (24 instead of 29 s, for WPI- and untreated cotton, respectively) and pkHRR is almost the same (52 kW/m^2 for WPI-cotton and 57 kW/m^2 for untreated cotton)(Figure 3.7). Using the static impregnation, both TTI and pkHRR were found to decrease in comparison with cotton (-17 and -19%, respectively) and were lower than those obtained from samples subjected to dynamic impregnation. TTI decrease is probably due to the effect of sensitization of the whey proteins on cellulose decomposition, which promotes the initial formation of a stable char that slightly helps in reducing the heat release rate.

The final residues were negligible in all the cases (Figure 3.8).

The slightly better results obtained with the static impregnation are probably due to the AO% difference between the two treated fabrics.

Table 3.2 - Cone calorimetry data of untreated cotton and cotton fabrics coated with WPI.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^2g)	THR (MJ/m^2g)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	1.0 ± 0.5
WPI-cotton (static)	18 ± 3	47 ± 3	29 ± 6	45 ± 2	1.0 ± 0.1	1.0 ± 0.5
WPI-cotton (dynamic)	24 ± 1	50 ± 3	26 ± 4	52 ± 1	1.2 ± 0.2	1.0 ± 0.5

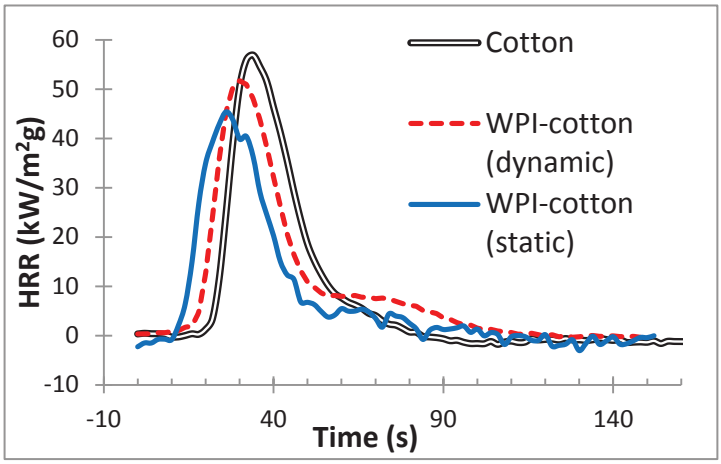




Figure 3.7 – Heat release rate vs. time of untreated cotton and cotton fabrics coated with WPI in static and dynamic conditions, tested at 35 kW/m^2.

Figure 3.8 – Residue of WPI-cotton (dynamic conditions) after cone calorimetry test.

It is worth noticing that the self-extinction in the flammability tests was not accompanied by the reduction of the heat release rate in cone calorimetry tests. This is probably due to the different heating conditions adopted for these tests, which affect the biomacromolecule reactivity in a different way.

Finally, cotton fabrics treated with WPI in dynamic conditions were tested with the microscale combustion calorimeter (MCC). Table 3.3 collects the results concerning the peak of heat release rate (peak HRR) and its temperature (T peak HRR) and the total heat release (Total HR). In comparison with untreated cotton, WPI-cotton showed a 4°C slightly lower temperature to peak HRR, while the decomposition was anticipated of about 25°C with respect to cotton (Figure 3.9). The peak HRR was reduced by 20% and the residue % was increased from 10.3 (untreated cotton) to 15.7% (treated counterpart).

Table 3.3 - MCC results for untreated cotton and cotton fabrics coated with WPI in dynamic conditions.

	peak HRR (W/g)	T peak HRR (°C)	Total HR (kJ/g)	Residue (%)
Cotton	257 ± 10	396 ± 2	10.1 ± 0.6	10.3 ± 0.5
WPI-cotton (dynamic)	206 ± 12	392 ± 2	10.2 ± 0.3	15.7 ± 2.1

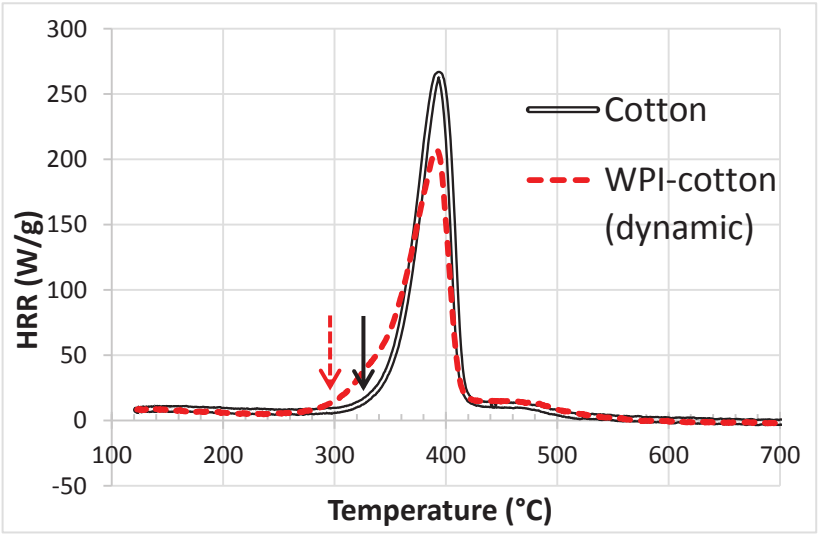


Figure 3.9 - Heat release rate vs. temperature of cotton fabrics coated with WPI in dynamic conditions, obtained by MCC.

3.1.3 Caseins

Casein efficacy as flame retardant for cotton has been demonstrated in a quite recent work [104]. In this Ph.D. thesis, the solution preparation step was simplified and the efficacy of the resulting biomacromolecule coating was investigated.

First, cotton fabrics were impregnated with a casein solution at 5 wt.% to achieve 20 wt.% AO (samples coded as CAS-cotton_A) and then characterised.

Then, with the aim of assessing the lowest AO% necessary to provide the fabrics with self-extinction, cotton fabrics were treated with 2.5 wt.% caseins solutions and the impregnation step was repeated until different AO%, namely 13, 10 and 8 wt.% (samples coded CAS-cotton_B,C,D)) were obtained.

The thermal stability of CAS-cotton_A was assessed with TGA (Figure 3.10). The first degradation step occurs at almost the same temperature range both in inert and oxidative atmosphere. In nitrogen, a stable char is formed and a significant 24% final residue is observed at 800°C. In air, there are two more degradation steps that oxidise the char, leaving a final residue of 8% at 800°C. The mass loss rate in the first degradation step was reduced significantly in both the atmospheres: in nitrogen the reduction of the peak was of 70%, while in air it was of about 80%.

The probable mechanism of action of caseins is based on the presence of phosphorous-containing groups, which upon heating release acidic species, promoting the dehydration of cellulose and the formation of an aliphatic char, which is subsequently transformed into an aromatic one. At the same time, the production of volatile combustible species is inhibited and the release of nitrogen species from the protein itself improves the dilution of fuel gases.

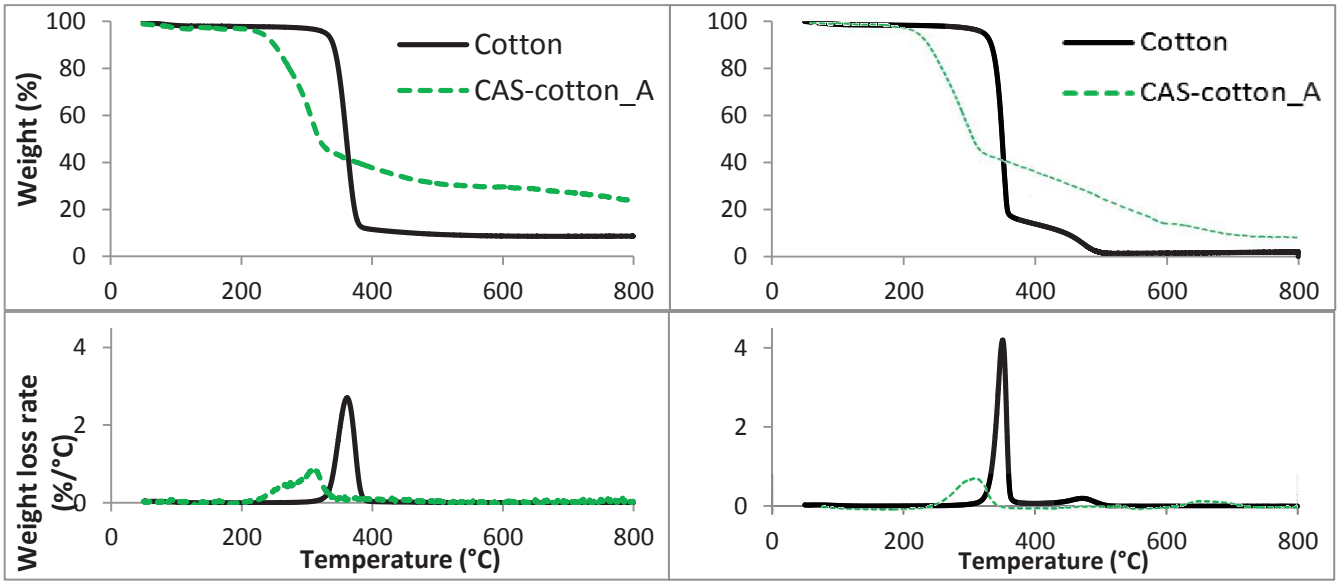


Figure 3.10 - TG and dTG curves of untreated cotton and cotton treated with caseins in nitrogen (left) and air (right).

Four samples of each kind of fabrics were subjected to horizontal flame spread tests. The average results are shown in Table 3.4 and Figure 3.11. In all the cases, after the flame out, the samples continue burning with a smouldering combustion for a long time. The smouldering combustion differs from the flaming combustion as the oxidation reaction occurs in the solid phase instead of the gas phase. Cotton is a material that can sustain smouldering and probably the casein coating promotes the formation of a char with a porous structure, which is permeable to oxygen and favours the smouldering process. This latter consumes all the char residue previously formed in the flaming combustion, leaving only a light and brittle residue (Figure 3.11 (C)). Values in brackets in Table 3.4 correspond to the smouldering combustion time. It can be seen that, with the exception of the CAS-cotton_A samples, the flaming combustion has a similar duration (of about 20 s), irrespective of the AO%. Besides, the rates of combustion are comparable for all the samples and much lower than that of untreated cotton (0.21, 0.28, 0.21, 0.22 mm/s for A, B, C and D respectively, in comparison with 1.50 mm/s for cotton). The amount of caseins in the coating affects the duration of the smouldering combustion: with decreasing AO%, an increase in the smouldering time is observed. The final residue decreases as the amount of caseins decreases. This can be due to the fact that from 8 to 13% the add-on is enough to stop the flaming combustion, though the smouldering goes further on.

Finally, 8 wt.% AO of caseins was sufficient to provide self-extinction to cotton fabrics.

Table 3.4 - Flammability data of untreated and casein-treated cotton fabrics with different AO%. (Accuracy: ±3%).

	Total flaming time (s) (Total flaming + smouldering time (s))	**Char length (mm)**	**Total flaming + smouldering rate (mm/s)**	**Residue (%)**	**Self-extinction**	**AO(%)**
Untreated cotton	66	-	1.50 (NO smouldering)	-	NO	-
CAS-cotton_A	12 (61)	13	0.21	94	YES	20
CAS-cotton_B	20 (126)	36	0.28	85	YES	13
CAS-cotton_C	18 (353)	74	0.21	58	YES	10
CAS-cotton_D	21 (418)	90	0.22	50	YES	8

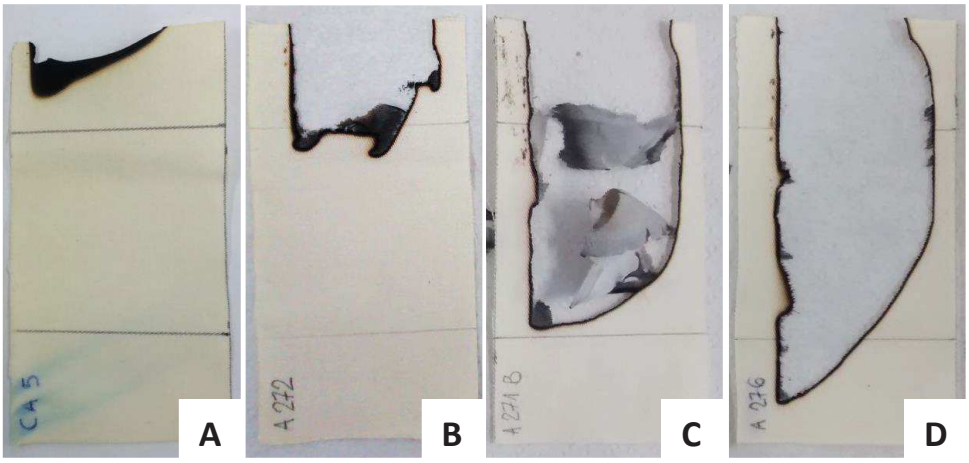


Figure 3.11 - Residues after flame spread tests (horizontal configuration) of CAS-cotton samples: A) 20 wt.% AO; B) 13 wt.% AO; C) 10 wt.% AO; D) 8 wt.% AO.

The results from cone calorimetry tests were very interesting as far as the heat release is concerned (Table 3.5 and Figure 3.12). In the presence of the casein coating both THR and pkHRR decreased by 50 and 43%, respectively and an almost halved TTI value (from 29 s to 16 s) was observed. The combustion time decreased, too. CAS-cotton_A samples left a significant carbonaceous residue after the test (17% of initial mass) (Figure 3.13), which differed a lot from the weightless white residue of untreated cotton (Figure 3.4).

Table 3.5 - Combustion data of untreated cotton and cotton fabrics coated with caseins, obtained by cone calorimetry.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^2g)	THR (MJ/m^2g)	pkCO yield (g/s)	pkCO_2 yield (g/s)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	0.00116 ± 0.00021	0.039 ± 0.003	1.0 ± 0.5
CAS-cotton_A	16 ± 3	34 ± 4	18 ± 7	32 ± 6	0.6 ± 0.2	0.00190± 0.00019	0.030 ± 0.002	17 ± 5

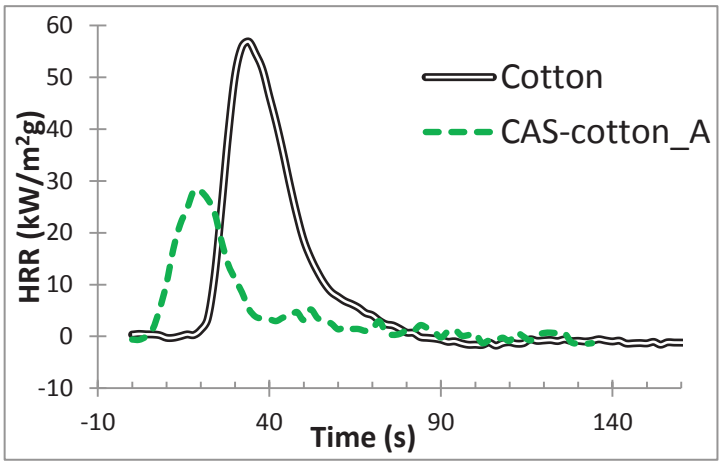




Figure 3.12 - Heat release rate versus time of untreated cotton and cotton fabrics coated with caseins, tested at 35 kW/m^2.

Figure 3.13 – Residue of CAS-cotton_A after cone calorimetry test.

The production and release of CO and CO_2 were also evaluated in cone calorimeter tests (Table 3.5 and Figure 3.14). As regards CO_2, the release trend from CAS-cotton is similar to that of untreated cotton, with a shift towards lower times due to sensitisation and a lower peak. In the case of CO yield, the fabric treated with caseins displays a single high and sharp peak after the ignition of the sample, which is almost the double of the highest peak of untreated cotton. The CO production is usually higher in the early stages of a fire when fire retarded materials are compared to untreated materials, without implicating a higher fire hazard [198]. Conversely, it is almost independent from the chemical composition in fully developed fires (which cannot be simulated by the cone calorimetry tests).

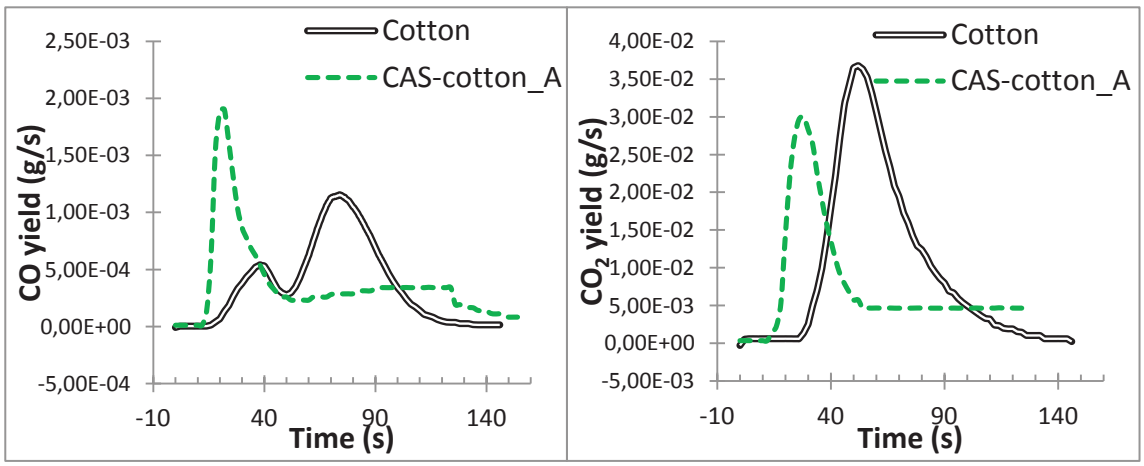


Figure 3.14 – CO and CO_2 yield in g/s for untreated cotton and casein-treated cotton, obtained by cone calorimetry.

In a second test, the temperature of the lower side of the samples was measured during irradiation. The test was repeated three times and the results averaged. Temperature trends are shown in Figure 3.15, in which the combustion periods are delimited by vertical lines. The casein treatment creates a protective layer on the fabric surface, thus reducing the heat transmission to the lower side of the fabric. The temperature of untreated cotton exceeds 600°C with a peak near the end of combustion, while with the casein treatment the temperature remaines below 500°C during the whole combustion step and even after. The cotton combustion follows the typical evolution of a fire, with the temperature that reaches a maximum when the fire is fully-developed and then decreases when the fire extinguishes because of the lack of fuel. The casein coating slows down the emission of volatile species and the burning rate and the combustion does not reach a complete development.

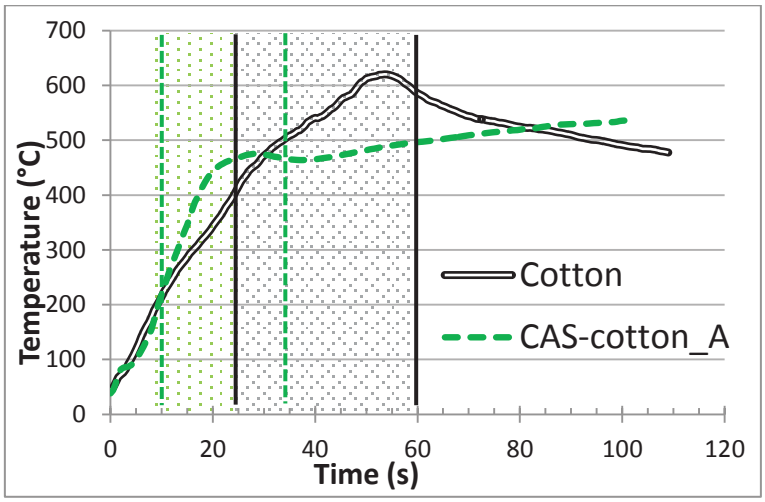


Figure 3.15 – Temperature measured under the fabric sample subjected to a heat flux of 35 kW/m^2 in the cone calorimeter.

MCC tests were carried out on casein-treated cotton fabrics with 20 wt.% AO. Results and HRR trend are shown in Table 3.6 and Figure 3.16. The presence of caseins has a significant effect on the decomposition behaviour of cotton. A strong sensitisation shifts the beginning of the degradation onset and the HRR peak by 65°C (Figure 3.16): this allows the formation of a very stable char, which does not undergo oxidation in inert atmosphere. This way, the combustible volatiles are reduced and 28.5% of residue remains. As a consequence, the total HR is reduced by 50% and the peak HRR by 69%, with a significant decrease of the fire hazard.

Table 3.6 - MCC results for untreated cotton and cotton fabrics coated with caseins.

	peak HRR (W/g)	T peak HRR (°C)	Total HR (kJ/g)	Residue %
Cotton	257 ± 10	396 ± 2	10.1 ± 0.6	10.3 ± 0.5
CAS-cotton_A	80 ± 3	331 ± 2	5.1 ± 0.3	28.5 ± 1.2

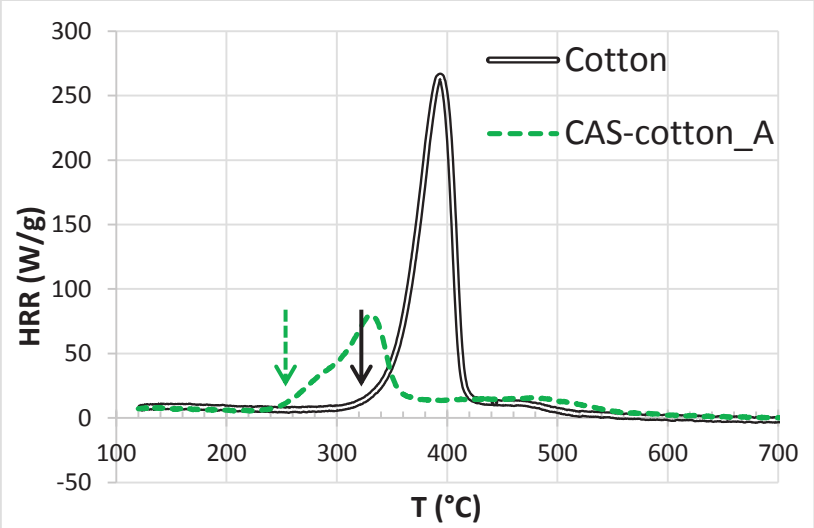


Figure 3.16 - Heat release rate vs. temperature curves of cotton fabrics coated with caseins, obtained by MCC.

3.1.4 DNA

DNA was the most promising flame retardant additive among the investigated biomacromolecules. It was shown that a DNA coating with 10 wt.% AO was sufficient for providing cotton fabrics with self-extinction in horizontal flame spread tests [105].

In order to obtain the best flame retardant performances, in this Ph.D. thesis different process parameters were considered, namely: number of impregnations to reach the desired add-on, DNA molecular weight and pH of the impregnation solution that influences the electrical charge of DNA. In all the cases, a 8 wt.% AO was achieved on the fabrics, as this amount allowed discriminating the effects of the different selected parameters on the fire behaviour. This AO% provided the self-extinguishment in horizontal flame spread tests only in some cases: this way, it

was possible to identify the most effective conditions. The investigation was carried out by means of horizontal flame spread tests, cone calorimetry, MCC, TG analyses, scanning electron microscopy, and ICP-MS tests.

The results reported in this section have been partially published in a paper in 2015 [186].

3.1.4.1 Effect of pH of DNA solution

DNA powder from herring sperm was used in this study. It has a low molecular weight (100–200 bp), as described in the "Materials and methods" section (for this reason the fabric samples will be coded as LMW-DNA), and is not completely soluble in water, unless a desired pH value is obtained. This correction was obtained with the addition of NaOH, which modified DNA charge in the solution.

Solutions with 2.5 wt.% DNA concentration were prepared with different pH values, namely 4, 6, 7, 8 and 9 and applied on cotton with multiple impregnations to reach the desired AO% (i.e. 8 wt.%).

The results from flame spread tests in horizontal configuration are reported in Table 3.7. The flame out of the samples was obtained using the biomacromolecule solutions at pH 4, 8 and 9. At pH 4 and 8, comparable results in terms of burning time and char length were observed. The percentage of self-extinction was quite high (74% and 86% of samples were self-extinguishing for LMW-DNA at pH 8 and LMW-DNA at pH 4, respectively) and the final residue was consistent for both the kinds of samples (Figure 3.17). LMW-DNA samples at pH 9 showed a very low burning rate (0.97 mm/s vs. 1.50 mm/s for untreated cotton). On the other hand, the flame out, in the 50% of cases where occurred, was near the end of the samples, as can be observed from the char length (96 out of 100 mm of sample), leaving a slightly lower residue than LMW-DNA at pH 4 and LMW-DNA at pH 8 (59, 60 and 76%, respectively). Self-extinction was not achieved for the samples treated with the solutions at pH 6 and 7, but the burning rate was reduced (1.25 and 1.24 mm/s, respectively) and a significant char residue remained after the test, especially in the case of LMW-DNA at pH 7 (68%), comparable with that of the self-extinguishing samples.

Table 3.7 - Flammability data of untreated and LMW-DNA-treated cotton fabrics at different pH conditions. Adapted from [186]. (Accuracy ±3%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction (%)	pH
Untreated cotton	66	-	1.50	-	NO	-
LMW-DNA pH4	57	68	1.20	76	YES (86)	4
LMW-DNA pH6	80	100	1.25	56	NO	6
LMW-DNA pH7	81	100	1.24	68	NO	7
LMW-DNA pH8	52	68	1.30	60	YES (74)	8
LMW-DNA pH9	101	96	0.97	59	YES (50)	9

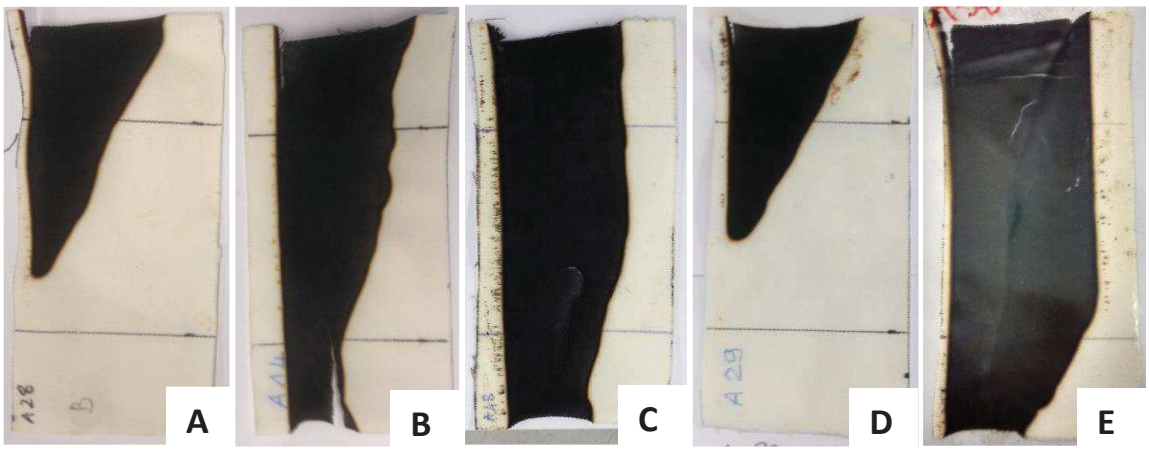


Figure 3.17 - Residues after flame spread tests (horizontal configuration) of LMW-DNA samples: A) LMW-DNA pH4; B) LMW-DNA pH6; C) LMW-DNA pH7; D) LMW-DNA pH8; E) LMW-DNA pH9.

The morphology of fabrics treated with DNA before and after flammability tests was assessed by means of SEM microscopy (Figures 3.18, 3.19 and 3.20). In Figure 3.18, on the left, it is possible to see the typical morphology of cotton fibres, with a non-homogenous and rough surface. The treatment with DNA creates a smooth and uniform coating on the fibres. The typical morphology of the residues of LMW-DNA pH4, LMW-DNA pH6 and LMW-DNA pH8 is shown in Figure 3.19. The warp and weft of the burnt residues are still visible and with a higher magnification it is possible to observe a slight difference in the intumescent behaviour of DNA at different pHs. With LMW-DNA at pH 4 very small similar bubbles (diameter of about 0.1 µm) are formed, with a uniform spread all over the fibres, while the fabrics treated with LMW-DNA at pH 6 show few bubbles having different sizes (from 0.1 to 2 µm of diameter) non-homogenously distributed on the fibre surface. In the case of LMW-DNA at pH 8, bubbles are bigger than those of LMW-DNA pH4 and show different sizes; furthermore, they can be observed in higher quantity and with a better distribution than those of LMW-DNA pH6. This different behaviour in the bubble formation could be the reason for the different effectiveness in flame spread test. Finally, as already described in recent papers [105], the residue consisted of hollow fibres (Figure 3.20), irrespective of the pH values of DNA solutions.

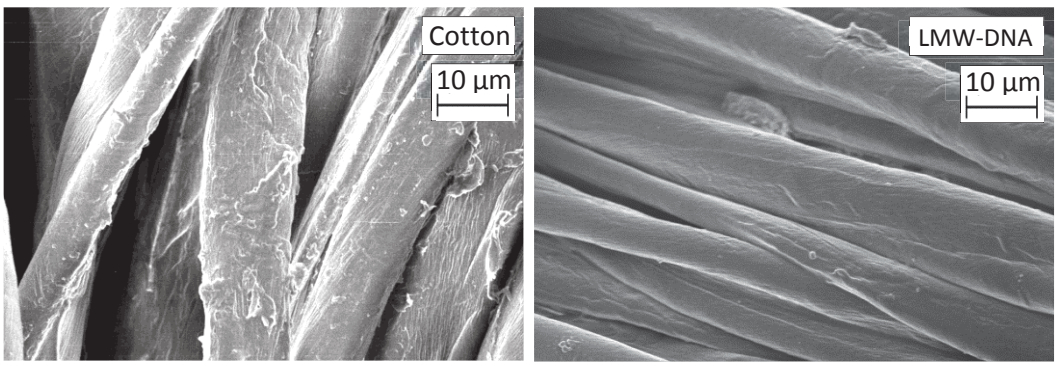


Figure 3.18 – SEM micrographs of untreated cotton and cotton treated with low-molecular-weight DNA.

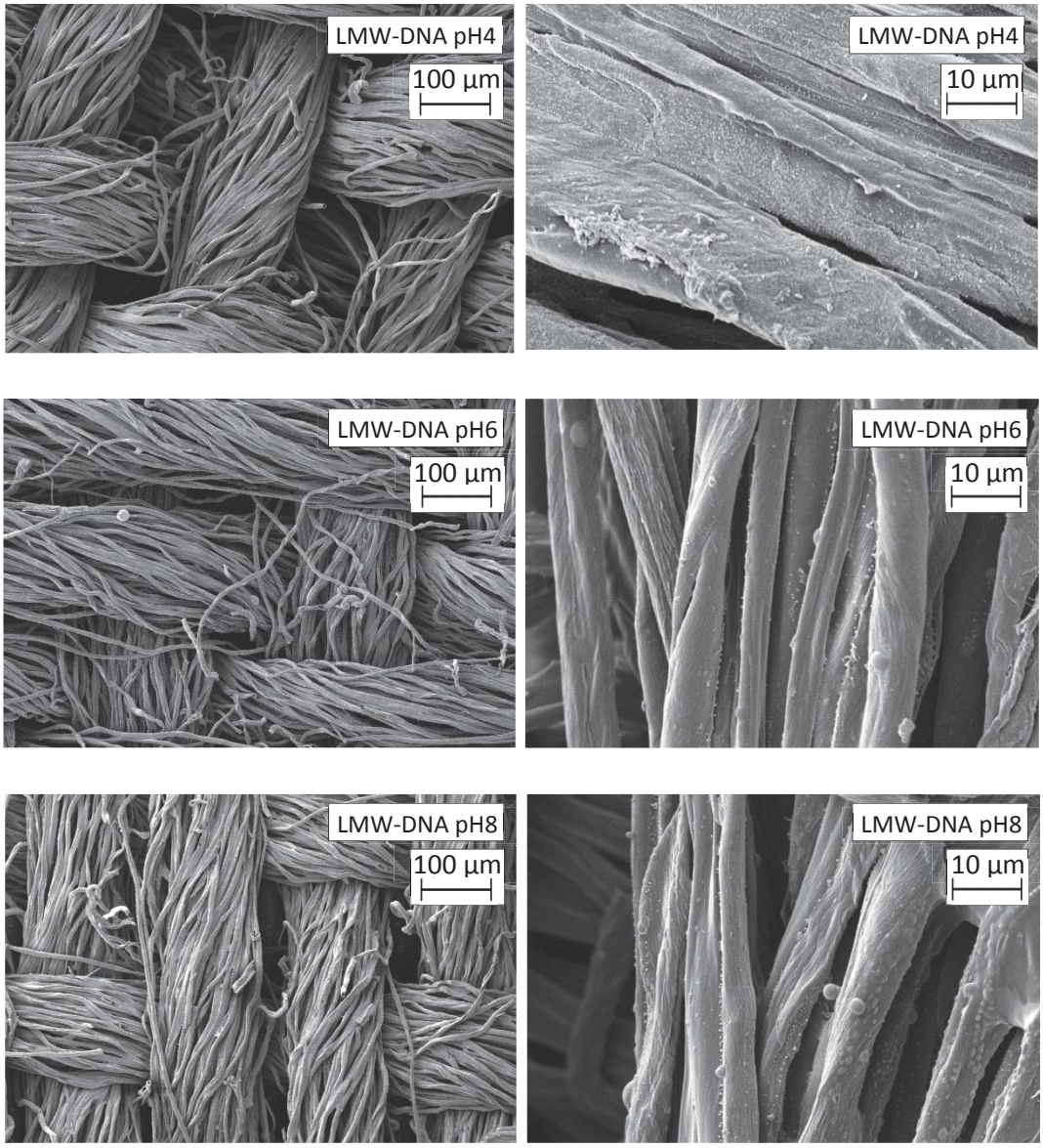


Figure 3.19 – SEM micrographs of the residues after flame spread tests of LMW-DNA pH4, LMW-DNA pH6 and LMW-DNA pH8.

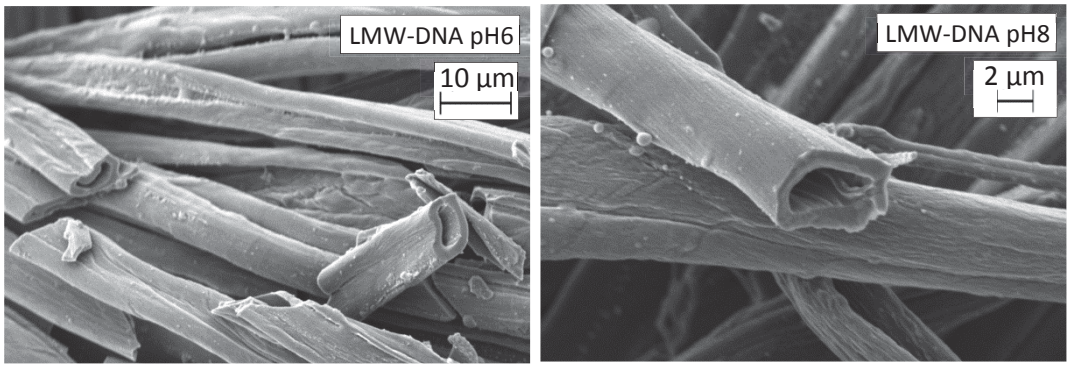


Figure 3.20 - SEM micrographs of the residues after flame spread tests of LMW-DNA.

A possible explanation of the different behaviour of the DNA coating at different pH conditions, can be related to the isoelectric point (PI) of DNA. Despite conflicting values reported in the literature, a PI around 6.5 is accepted [199]. Samples treated with DNA at pH 4 and 8 are quite far from the PI and DNA is charged uniformly (positively or negatively). As a consequence the fabric surface could be expected to be covered homogenously. Conversely, when the fabrics are treated with DNA at pH 6 or 7 the charge is not well defined; as a consequence, phosphate groups could be randomly oriented (i.e. towards the inside or the outside of the coating) and this could promote a less homogeneous covering, although not visible in SEM pictures.

The samples treated with LMW-DNA at pH 4, LMW-DNA at pH 7 and LMW-DNA at pH 8 were tested with the TGA and the cone calorimeter, to highlight potential differences in thermo-oxidative degradation and in the combustion behaviour.

Figure 3.21 shows the TG and dTG curves in air for untreated cotton, LMW-DNA at pH 4, LMW-DNA at pH 7 and LMW-DNA at pH 8. In all the LMW-DNA samples, the degradation begins at lower temperatures in comparison with untreated cotton ($T_{onset10\%}$: 283°C for LMW-DNA pH4, 292°C for LMW-DNA pH7 and LMW-DNA pH8, 334°C for cotton). This early degradation allows the release of phosphoric acid that promotes the dehydration of cellulose and the formation of char, instead of volatile compounds. In fact, at the end of the first decomposition step it is possible to observe a higher residue in the case of DNA-treated cotton (50% for LMW-DNA at pH 4 and 45% for LMW-DNA at pH 7 and LMW-DNA at pH 8, instead of 20% of untreated cotton) (Figure 3.21). This residue is then further oxidised at high temperatures, leaving a residue of 5% for LMW-DNA pH4 and 8% for LMW-DNA pH7 and LMW-DNA pH8 at 600°C.

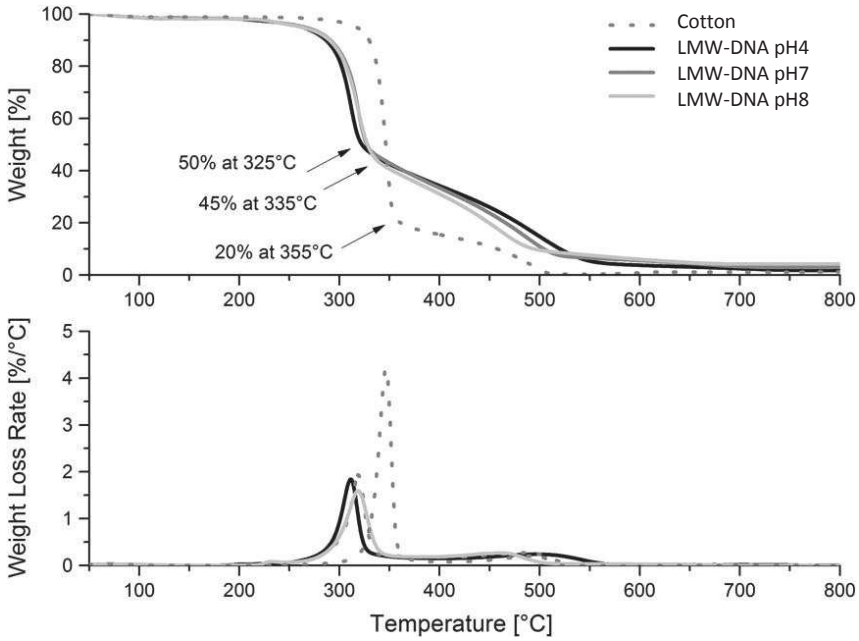


Figure 3.21 - TG and dTG curves of untreated cotton, LMW-DNA pH4, LMW-DNA pH7 and LMW-DNA pH8 in air. Adapted from [186].

Cone calorimetry tests show a similar behaviour of the treated cotton fabrics, especially in the increased formation of char and reduced development of volatile combustible gases (Table 3.8 and Figure 3.22). THR is lowered by about 35% and the combustion duration by 36%, irrespectively of the pH of the impregnation solution. The reduction of pkHRR is remarkable: -42, -46 and -47% for LMW-DNA pH4, LMW-DNA pH7 and LMW-DNA pH8, respectively. During the test, fabrics shrink and the final char residue is thick and with an almost halved surface area (Figure 3.23).

The differences that could be observed in the flame spread tests for the three pH values, are not noticeable in cone calorimetry tests, probably because of the different heating conditions.

Table 3.8 – Cone calorimetry data of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions with multiple impregnations.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^2g)	THR (MJ/m^2g)	pkCO yield (g/s)	pkCO_2 yield (g/s)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	0.00116 ± 0.00021	0.039 ± 0.003	1.0 ± 0.5
LMW-DNA pH4	27 ± 3	45 ± 2	18 ± 5	33 ± 4	0.75 ± 0.05	0.00291 ± 0.00011	0.017 ± 0.002	6.0 ± 0.5
LMW-DNA pH7	31 ± 3	49 ± 1	18 ± 4	31 ± 4	0.8 ± 0.1	0.00249 ± 0.00001	0.026 ± 0.005	7 ± 1
LMW-DNA pH8	32 ± 3	50 ± 1	18 ± 4	30 ± 4	0.8 ± 0.1	0.00264 ± 0.00005	0.018 ± 0.0025	6 ± 1

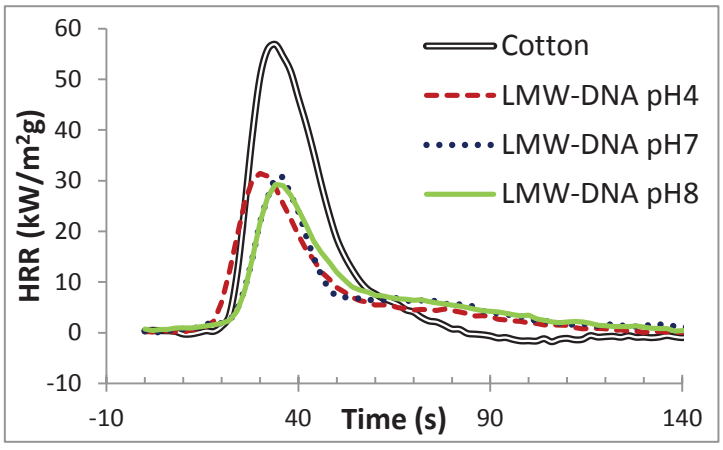




Figure 3.22 - Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions, tested at 35 kW/m^2.

Figure 3.23 - Residue of LMW-DNA pH4 after cone calorimetry tests.

Cone calorimetry results obtained in terms of CO and CO_2 yields are shown in Table 3.7 and Figure 3.24. As far as CO yield is concerned, the behaviour of the treated fabrics is similar, irrespective of the solution pH: in particular, a wide CO release occurs after combustion, mimicking the trend of untreated cotton but with higher values. As reported earlier, this is not usually considered as a significant increase of the fire hazard, taking into account the enhancement in fire retardancy (e.g. referring to HRR, pkHRR and THR) provided by the designed coatings [198]. The CO_2 release is comparable for the LMW-DNA at pH 4 and 8, while it has a slightly higher peak for LMW-DNA at pH 7. However, in all the cases, the curves remain below that of untreated cotton.

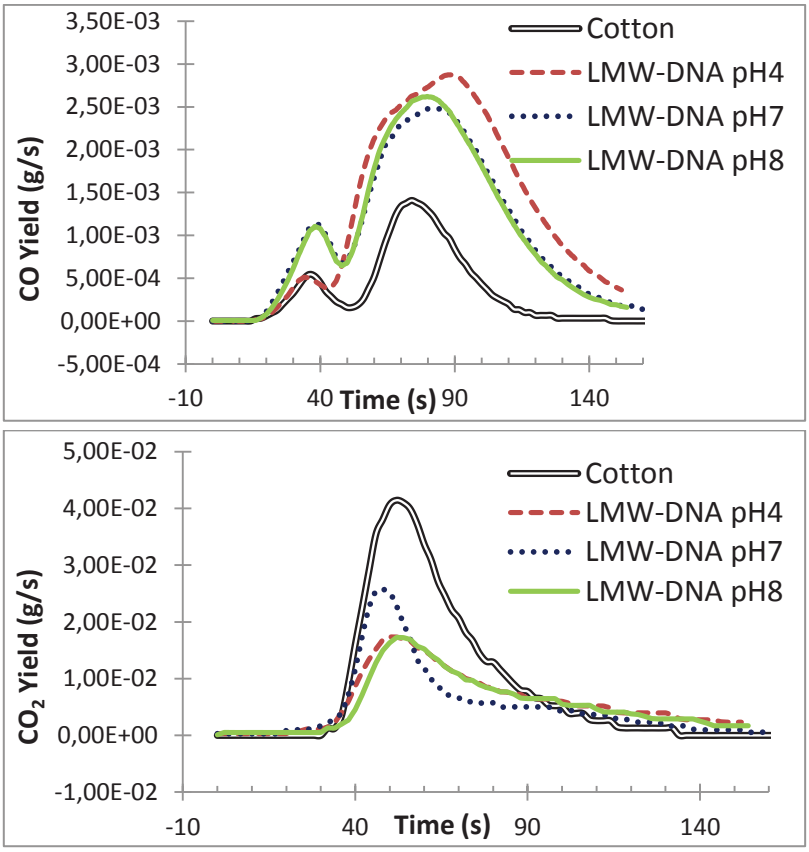


Figure 3.24 - CO and CO_2 yield in g/s from untreated cotton and LMW-DNA-treated cotton at different pHs, obtained by cone calorimetry.

When temperature is measured on the lower side of the treated fabric samples subjected to 35 kW/m^2 heat flux, a protective effect can be observed (Figure 3.25), as indicated by the smooth temperature profile, according to which the temperature always remains below 575°C. LMW-DNA at pH 4 and at pH 8 show a similar behaviour. More specifically, the char formation limits the heat and oxygen transfers, as well as the volatile diffusion, preventing the complete development of the combustion.

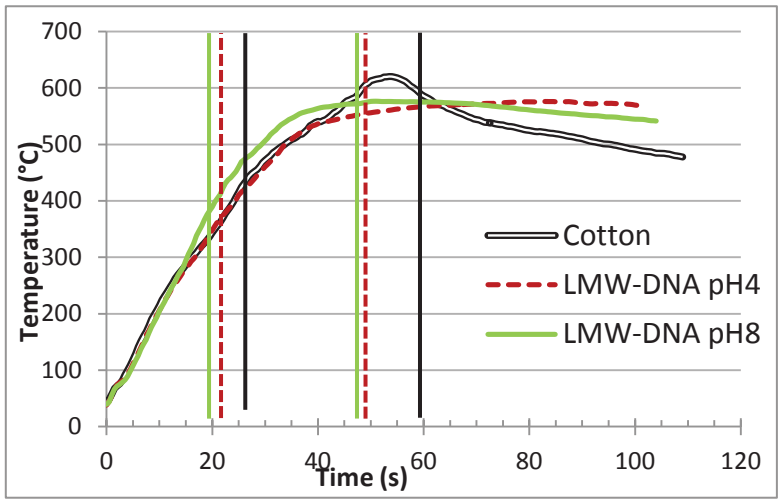


Figure 3.25 – Temperature measured under the fabric sample subjected to a heat flux of 35 kW/m^2 in the cone calorimeter.

Furthermore, MCC tests were performed on LMW-DNA pH4 and LMW-DNA pH8 to investigate whether one treatment could be more effective than another. As shown in Table 3.9 and Figure 3.26, the differences are very small, with a slightly better behaviour of LMW-DNA pH4. In fact, the reduction of peak HRR and Total HR are higher with the DNA solution at pH 4 in comparison to the solution at pH 8 (45% vs. 30% in Total HR and 41% vs. 36% in peak HRR, respectively). The residue is also higher (25 wt.% instead of 21.9 wt.%, for the solution at pH 4 and 8, respectively). Another difference refers to the presence of a small peak at about 250°C for LMW-DNA at pH 8. This peak could be attributed to the release of phosphorous species in the gas phase and a consequent slight reduction in the char formation.

Table 3.9 - MCC results for untreated cotton and cotton fabrics coated with LMW-DNA.

	peak HRR (W/g)	**T peak HRR (°C)**	**Total HR (kJ/g)**	**Residue %**
Cotton	257 ± 10	396 ± 2	10.1 ± 0.6	10.3 ± 0.5
LMW-DNA pH4	151 ± 2	350 ± 2	5.5 ± 0.3	25 ± 0.3
LMW-DNA pH8	165 ± 8	369 ± 1	7.1 ± 0.1	21.9 ± 0.5

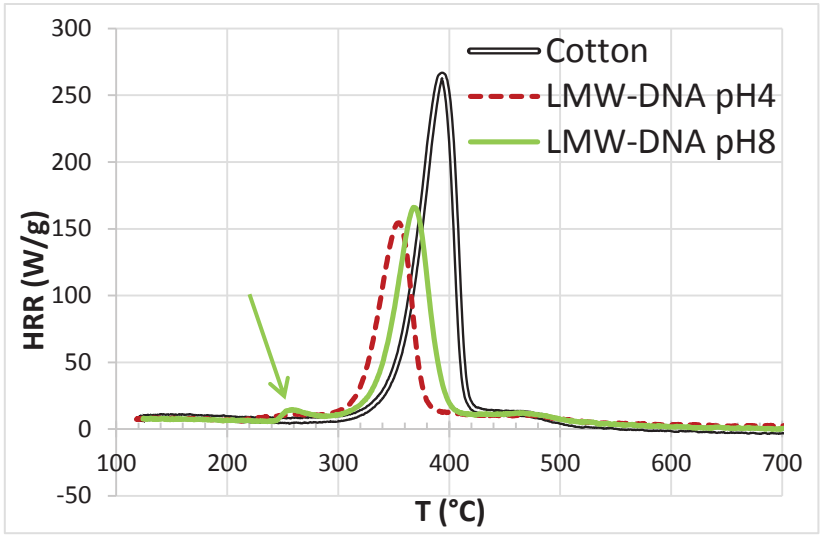


Figure 3.26 - Heat release rate versus temperature of cotton fabrics coated with LMW-DNA at pH 4 and pH 8, obtained by MCC.

3.1.4.2 Effect of number of impregnations

The number of impregnation and drying steps can be an important parameter in the coating process, as far as the consumption of time and energy is considered. For this reason, the reduction of the number of impregnations was taken into consideration. The reduction to a single impregnation step to achieve the desired AO% (8 wt.%), was achieved by using a 6 wt.% DNA solution. Solutions at pH 4, 7 and 8 were utilised for the fabric treatment.

Flame spread tests in horizontal configuration showed that, despite the same DNA quantity on the cotton fabric, the effectiveness of the coating is significantly lower with a single impregnation (SI) than with multiple impregnations (MI). In fact, as shown in Table 3.10 and Figure 3.27, the percentage of self-extinguishing samples is much lower with the solution at pH 4 (33% instead of 86% with multiple impregnations) and self-extinction is not achieved with the solution at pH 8 (0% instead of 74% with multiple impregnations). In addition, for LMW-DNA pH7_SI and LMW-DNA pH8_SI, where the flame out did not occur in any case, the burning rate is higher than with multiple impregnations and the final residue lower (46 vs. 68% and 39 vs. 60%, with LMW-DNA pH7 and LMW-DNA pH8, respectively).

Table 3.10 – Flame spread data of untreated and LMW-DNA-treated cotton fabrics at different pH conditions with multiple impregnation (MI) or single impregnation (SI). Adapted from [186]. (Accuracy ±3%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction (%)	pH
Untreated cotton	66	-	1.50	-	NO	-
LMW-DNA pH4_MI	57	68	1.20	76	YES (86)	4
LMW-DNA pH7_MI	81	100	1.24	68	NO	7
LMW-DNA pH8_MI	52	68	1.30	60	YES (74)	8
LMW-DNA pH4_SI	76	89	1.17	65	YES (33)	4
LMW-DNA pH7_SI	79	100	1.27	46	NO	7
LMW-DNA pH8_SI	74	100	1.36	39	NO	8

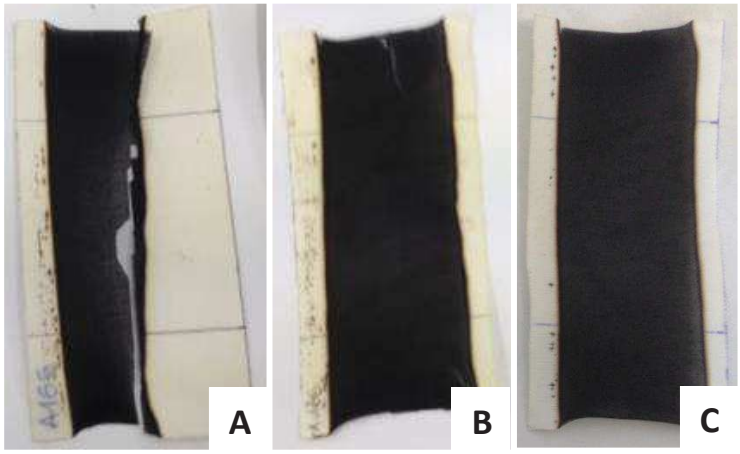


Figure 3.27 - Residues after horizontal flame spread tests of LMW-DNA samples with single impregnation: A) LMW-DNA pH4_SI; B) LMW-DNA pH7_SI; C) LMW-DNA pH8_SI.

The combustion behaviour of the samples was assessed by means of cone calorimetry and the obtained results are presented in Table 3.11 and Figure 3.28A. In the case of single impregnation, the effect of pH is more evident, as revealed by higher differences in TTI, Flame out and pkHRR. The best results are obtained with LMW_DNA pH7_SI, showing a lower pkHRR and THR in comparison with untreated cotton (-32% and -25%, respectively). LMW_DNA pH4_SI and LMW_DNA pH8_SI show a more pronounced decrease of TTI (-10 and -6 s, respectively), while the decrease of pkHRR is about 21% for both.

Significant differences can be noted when the behaviour of samples treated with multiple impregnation and single impregnation is compared. The higher effectiveness of MI samples in the flame spread tests was further confirmed with the cone calorimeter. In fact, for all the tested pH values, the multiple impregnation caused a more significant reduction in pkHRR and THR values. As an example, in the case of LMW-DNA pH4 (Figure 3.28B) for MI and SI samples the pkHRR was 33 and 46 kW/m^{2}g and THR was 0.75 and 0.9 MJ/m^{2}g, respectively.

Table 3.11 - Combustion data of untreated cotton and cotton fabrics coated with LMW-DNA at different pH conditions with single impregnation, obtained by cone calorimetry.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^{2}g)	THR (MJ/m^{2}g)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	1.0 ± 0.5
LMW-DNA pH4_SI	19 ± 4	42 ± 0	23 ± 4	46 ± 4	0.9 ± 0.1	5.0 ± 0.5
LMW-DNA pH7_SI	28 ± 1	49 ± 1	21 ±2	39 ± 3	0.8 ± 0.1	6.0 ± 1.0
LMW-DNA pH8_SI	23 ± 3	43 ± 3	20 ± 7	45 ± 3	0.95 ± 0.04	6.0 ± 1.0

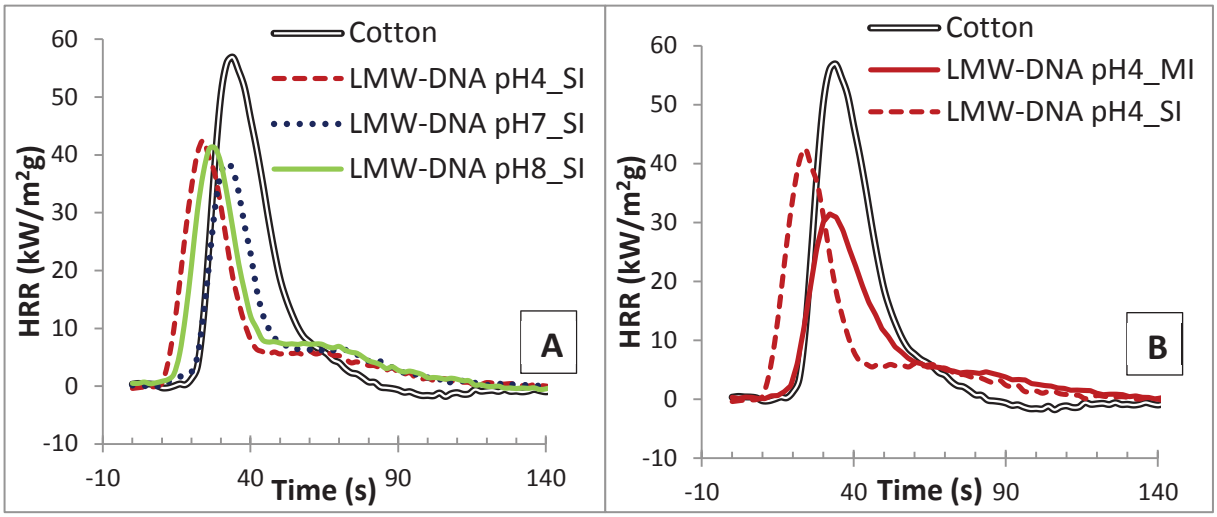


Figure 3.28 - Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA at different pHs, tested at 35 kW/m^2: A) Single impregnation at different pH values; B) Comparison between single and multiple impregnation for LMW-DNA pH4.

The application of consecutive layers of DNA, with a drying step in between, is more effective in providing fire retardancy than single layers, being constant the final dry add-on. Probably, a too concentrated solution could create a non-homogeneous coating of the fibres in a single layer and the DNA coating seems to fill the fabric interstices. Conversely, a low concentration solution is used with multilayers, hence favouring the formation of an homogenous biomacromolecule coating.

3.1.4.3 Effect of molecular size

DNA can be purchased with different molecular weights, hence different prices. High molecular weight DNA, in fact, is very expensive, since it usually requires more complex extraction and purification processes.

The effect of molecular size on the flammability and thermal behaviour of cotton fabrics treated with DNA was investigated. To this aim DNA from herring testes having a size range in between 3000–10,000 bp was selected. This product is a sodium salt and is soluble in water with

no need of pH correction. The obtained solution has a pH of 8 and a high viscosity at a concentration of 2.5 wt.%. Therefore, a solution at 0.5 wt.% was used for multiple impregnations of cotton fabrics. In addition, a solution at pH 4 was prepared using HCl for the pH correction and applied on cotton. The samples treated with this DNA will be coded as HMW-DNA.

In parallel, a solution of herring testes DNA was also subjected to sonication, as reported in the "Materials and methods" chapter, to obtain a DNA with a lower molecular size (300–800 bp), aiming at further investigating the effectiveness of DNA with different size ranges. Again, a 0.5 wt.% solution at pH 8 was applied on cotton with multiple impregnations. The samples treated with this DNA will be coded as MMW-DNA.

SEM micrographs of the treated fabrics are shown in Figure 3.29. In all the cases, the DNA coatings cover the fabric homogenously. In particular, the LMW-DNA forms a very smooth cover on the fibres and penetrates into the fabric texture. MMW-DNA and even more HMW-DNA, cover the fibres but fill also the fabric interstices.

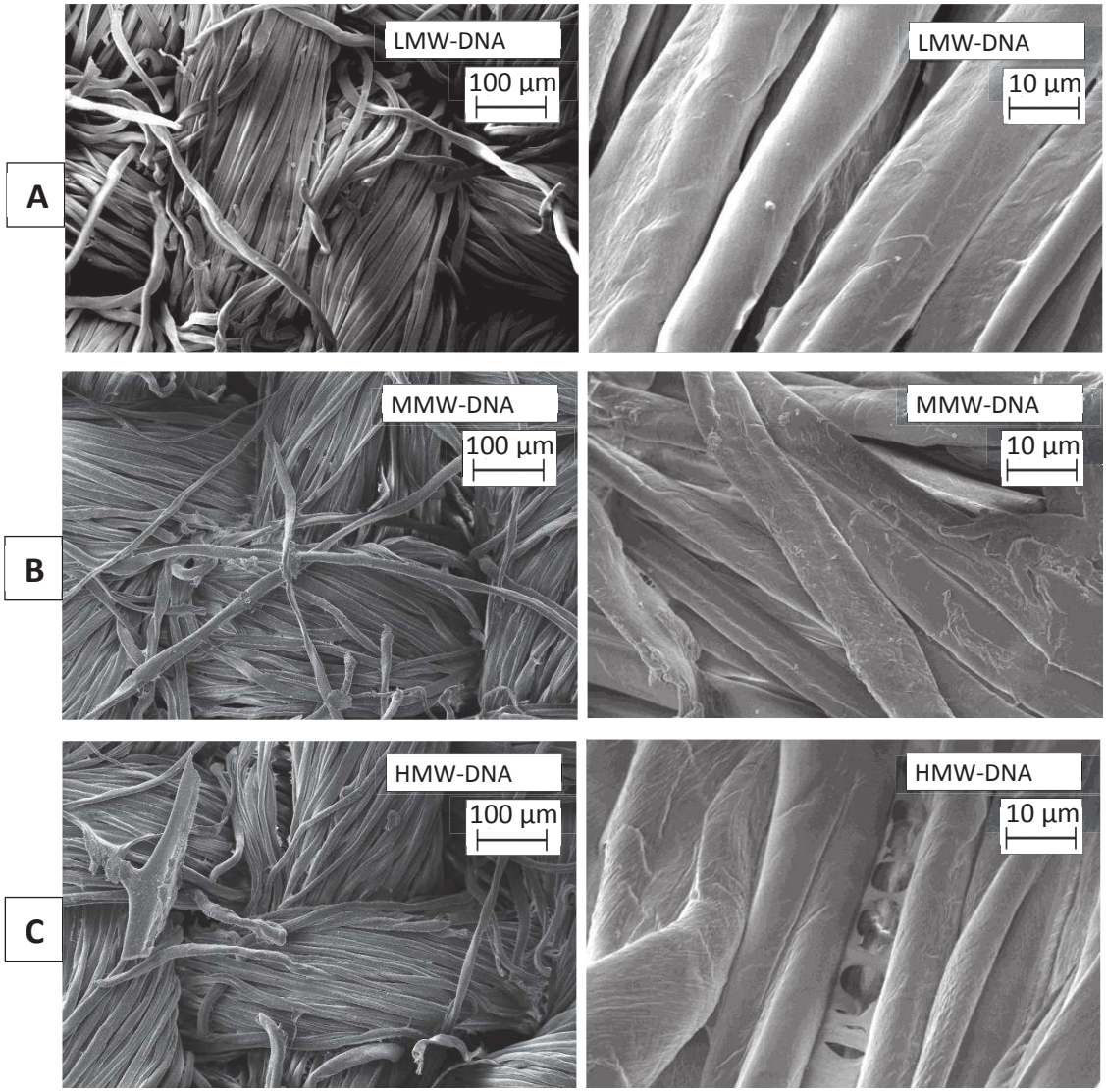


Figure 3.29 - SEM micrographs of fabrics treated with DNA with different molecular sizes: A) LMW-DNA; B) MMW-DNA; C) HMW-DNA. Adapted from [186].

The thermo-oxidative degradation of cotton treated with DNA of different size ranges is shown in Figure 3.30. The behaviour seems to be almost independent from the DNA molecular size, especially in the first decomposition step ($T_{onset10\%}$ of 295, 305 and 305°C for LMW-DNA, HMW-DNA and MMW-DNA, respectively). From the dTG curves, it is possible to observe that with the two DNAs at higher molecular weight, the peak of the weight loss rate is almost the double of that for low-molecular-weight DNA. In other words, higher-molecular-weight DNAs show higher rate of mass loss. Besides, the degradation starts 10°C above, but is faster and the onset of the second step occurs almost at the same temperature for all the samples. In the cases of HMW-DNA and MMW-DNA, the end of the second degradation step is shifted to higher temperatures with respect to LMW-DNA, notwithstanding that their final residue at 800°C is comparable to that of LMW-DNA (3 and 5%, respectively).

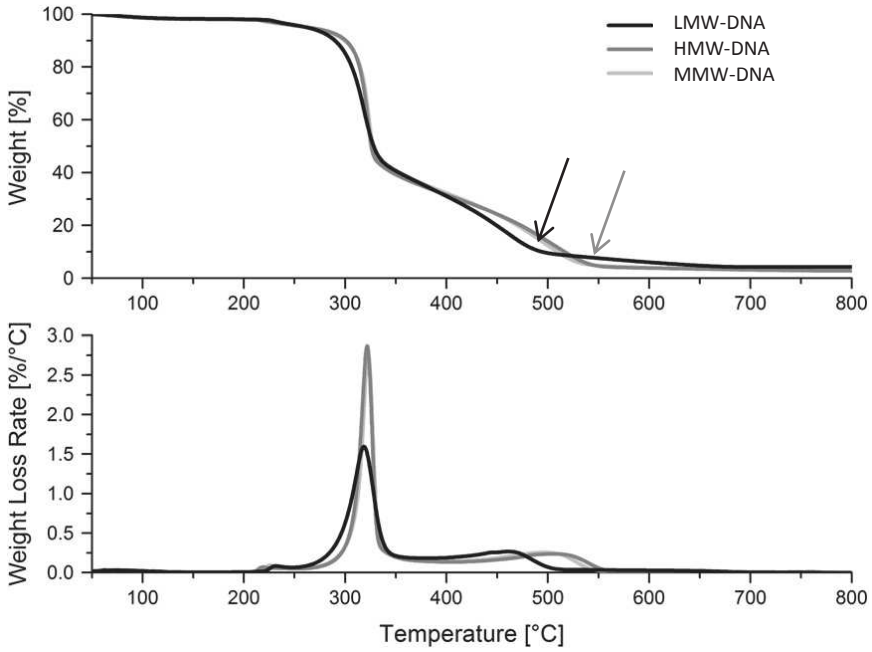


Figure 3.30 - TG and dTG curves of LMW-DNA, HMW-DNA and MMW-DNA in air. Adapted from [186].

The results obtained from flame spread tests are reported in Table 3.12 and Figure 3.31. HMW-DNA samples at both pHs do not reach self-extinction, notwithstanding a significant reduction of the burning rate with respect to untreated cotton (1.04 and 1.15 mm/s for HMW-DNA pH4 and HMW-DNA pH8, respectively, vs. 1.50 mm/s for untreated cotton). The differences between the effectiveness of the solution at pH 4 and the solution at pH 8 are negligible. It is noteworthy that MMW-DNA samples burn completely and show an intermediate behaviour between the high-molecular-weight and the low-molecular-weight DNA. In fact, the burning rate of MMW-DNA was lower than that of LMW-DNA, while the final residue was higher than that of HMW-DNA.

Table 3.12 - Flammability data of untreated and DNA-treated cotton fabrics with different molecular sizes at different pH conditions. Adapted from [186]. (Accuracy ±3%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction (%)	pH
Untreated cotton	66	-	1.50	-	NO	-
HMW-DNA pH4	87	100	1.15	38	NO	4
HMW-DNA pH8	98	100	1.04	35	NO	8
MMW-DNA pH8	88	100	1.14	46	NO	8
LMW-DNA pH4	57	68	1.20	76	YES (86)	4
LMW-DNA pH8	52	68	1.30	60	YES (74)	8

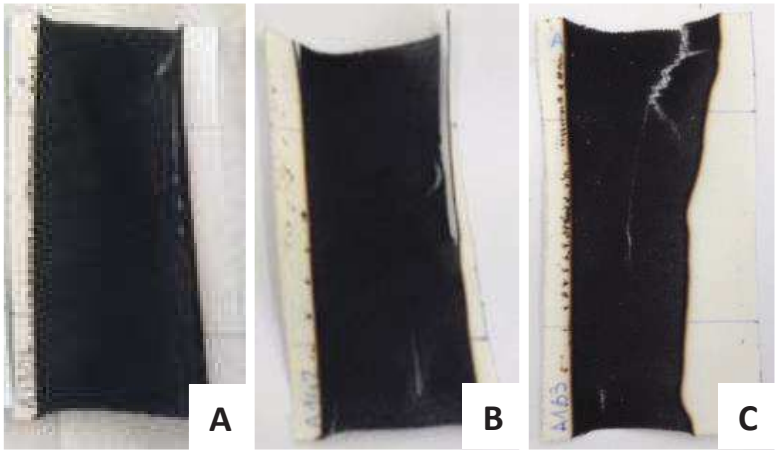


Figure 3.31 - Residues after flame spread tests (horizontal configuration) of HMW-DNA and MMW-DNA samples with multiple impregnation: A) HMW-DNA pH4; B) HMW-DNA pH8; C) MMW-DNA pH8.

Cone calorimetry tests showed that the combustion behaviour of cotton treated with DNA with different molecular weights is comparable. All the treatments are effective in decreasing pkHRR and THR (Table 3.13 and Figure 3.32). Besides, TTI, combustion duration and THR do not seem to be affected by the molecular size of the biomacromolecules, notwithstanding a slight difference in pkHRR values, for which LMW-DNA is responsible for 47% decrease, MMW-DNA for 41% and HMW-DNA for 37%.

Table 3.13 - Combustion data of untreated cotton and cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA, obtained by cone calorimetry.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^2g)	THR (MJ/m^2g)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	1.0 ± 0.5
LMW-DNA pH8	32 ± 3	50 ± 1	18 ± 4	30 ± 4	0.80 ± 0.05	6.0 ± 1.0
MMW-DNA pH8	30 ± 3	50 ± 1	20 ± 4	34 ± 4	0.8 ± 0.1	6.0 ± 1.0
HMW-DNA pH8	28 ± 4	49 ± 1	21 ± 5	36 ± 5	0.8 ± 0.1	6.0 ± 1.0

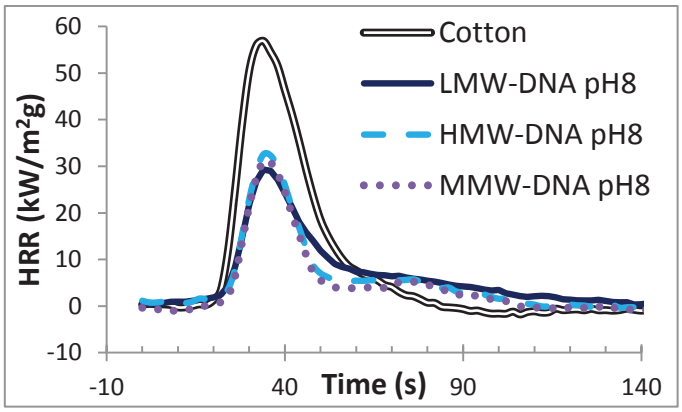


Figure 3.32 - Heat release rate versus time of untreated cotton and cotton fabrics coated with LMW-DNA pH8, MMW-DNA pH8 and HMW-DNA pH8, tested at 35 kW/m^2.

MCC tests confirmed the observed thermal behaviour of DNA treated cotton. The reduction of the peak HRR and of Total HR is significant with all the biomacromolecules (Table 3.14 and Figure 3.33). As already observed in the first degradation step in air in TG analyses (Figure 3.31), the decrease in the degradation kinetics is more pronounced with the LMW-DNA (peak HRR reduction of 18, 22 and 36% for HMW-DNA, MMW-DNA and LMW-DNA, respectively). A similar trend is observed for total HR (-10 for HMW-DNA and MMW-DNA and -30% for LMW-DNA). The increased residue at the end of the tests confirmed the formation of a more stable char in LMW-DNA pH8. The small peak that was already present in LMW-DNA at pH 8 is slightly more pronounced in the other two samples (Figure 3.33).

Table 3.14 - MCC results for untreated cotton and cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA at pH 8.

	peak HRR (W/g)	T peak HRR (°C)	Total HR (kJ/g)	Residue %
Cotton	257 ± 10	396 ± 2	10.1 ± 0.6	10.3 ± 0.5
LMW-DNA pH8	165 ± 8	369 ± 1	7.1 ± 0.1	21.9 ± 0.5
MMW-DNA pH8	201 ± 9	379 ± 1	8.9 ± 0.2	16.7 ± 1.1
HMW-DNA pH8	210 ± 10	383 ± 0	9.1 ± 0.3	15.6 ± 1.5

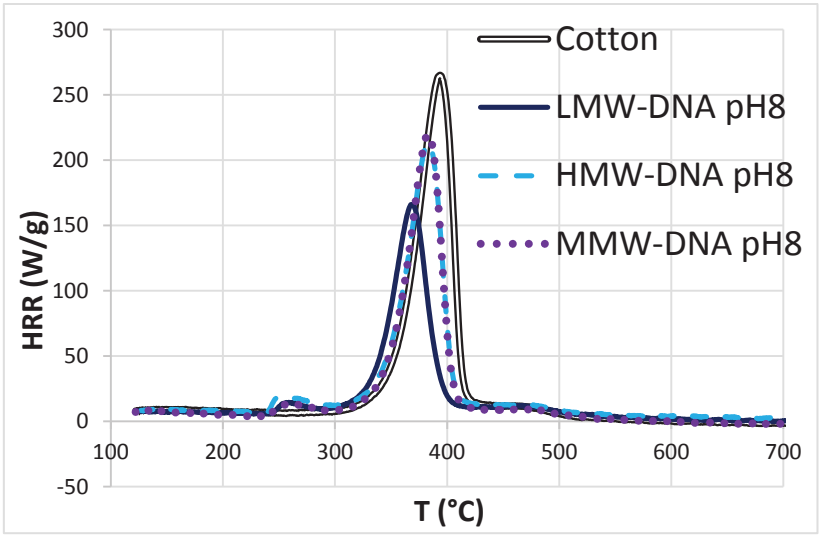


Figure 3.33 - Heat release rate versus temperature of cotton fabrics coated with LMW-DNA, MMW-DNA and HMW-DNA at pH 8, obtained by MCC

With the purpose of investigating the reason of the different effectiveness with different DNA sizes, the concentration of phosphorous in the DNA products and in the treated fabrics was assessed by means of ICP-MS analysis. In particular, this test was carried out for investigating the possible contribution of such impurities as proteins to the overall phosphorous content. The results showed that there are no significant differences in DNA or coating phosphorous content. The percentage of phosphorous is 7.9% in both low- and medium-molecular-weight DNA and 8% in high-molecular-weight DNA. On the fabrics, the phosphorous concentration is approximately 0.5% in all the cases.

For this reason, the different fire behaviour of cotton treated with DNA at different size ranges could be ascribed to the slightly different thermal behaviour and to the different coating morphology, as evaluated by SEM. In fact, this analysis clearly shows that a portion of high-molecular-weight DNA is blocked within the fabric interstices; as a consequence, the remaining part gives rise to a too thin coating around the fibres and therefore could be less effective in the formation of a stable and protective char.

From the obtained results, it was possible to prove that the treatment that confers the most effective flame retardant features involves the use of low-molecular-weight DNA, with a solution pH of 4 or 8, applied with multiple impregnation steps.

3.2 Comparison of the results obtained from the biomacromolecule treatments

In summary, the different treatments described in the previous chapters can be compared on the basis of the behaviour in fire tests.

Therefore, the following treatments were considered:

- Multiple impregnations with low molecular weight DNA (final AO%: 8).
- Multiple impregnations with caseins (final AO%: 20).
- Multiple impregnation in dynamic conditions with whey proteins (final AO%: 22).

First of all, the thermal and thermo-oxidative behaviour of treated fabrics was assessed by TG analyses (Figure 3.34). In nitrogen atmosphere, the degradation of all the treated samples starts at lower temperatures, as already mentioned, with respect to untreated cotton. The degradation of both LMW-DNA and WPI treated cotton fabrics starts almost at the same temperature ($T_{onset10\%}$=310 °C for LMW-DNA and 309°C for WPI), but proceeds with a higher weight loss rate for WPI leaving a very different residue at 700°C (40% vs. 15% for LMW-DNA and WPI, respectively). The casein treatment causes a more pronounced decrease of the degradation onset, with a $T_{onset10\%}$ of 250°C only, but the degradation step proceeds more slowly than with the other coatings and the residue formed is gradually degraded approaching 25% at 700°C.

In oxidative atmosphere, the degradation follows two main steps. In this case, the behaviour of LMW-DNA- and WPI-treated fabrics is similar, with slightly lower weight loss rate and a higher residue at 700°C for LMW-DNA (4% vs. 2.7%). The CAS-cotton degradation occurs according to three steps and with very low weight loss rates in comparison to the other treatments (for the first step: 0.7, 1.85, 2 %/°C for CAS-cotton, LMW-DNA-cotton and WPI cotton, respectively). The residue at 700°C is also higher and equal to 8%.

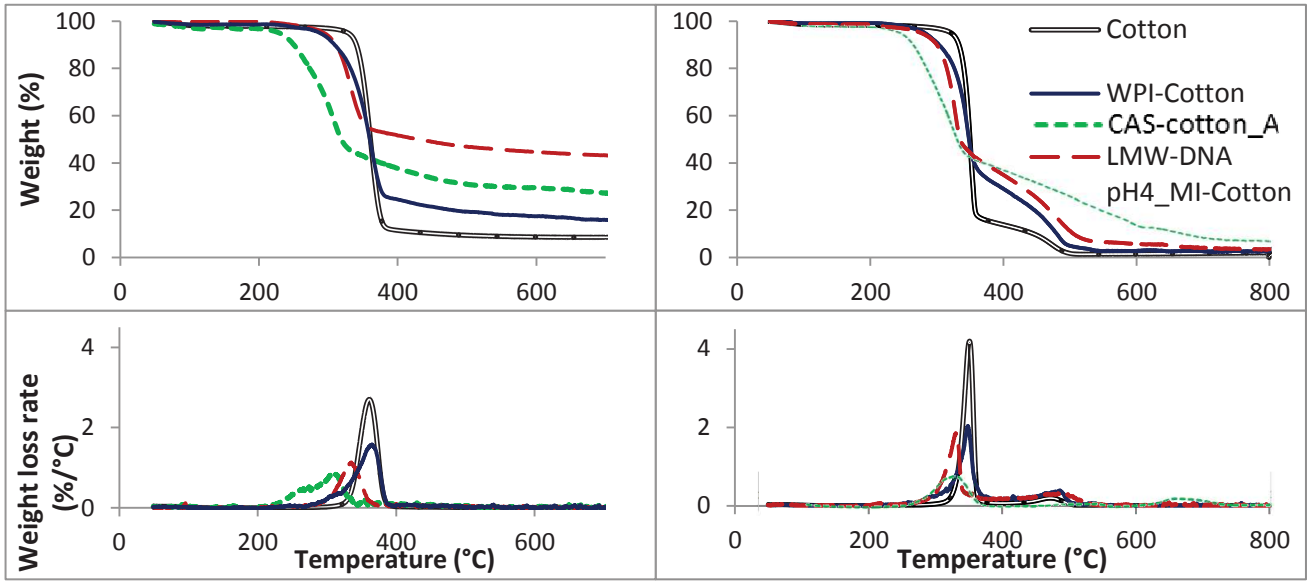


Figure 3.34 - TG and dTG curves of untreated cotton and biomacromolecules-treated cotton in nitrogen (left) and air (right).

In horizontal flame spread tests, all the tested treatments provide the treated fabrics with self-extinction (Table 3.15). Although the reduction of burning rate is observed in all the cases, it is more pronounced in the case of WPI treatment, notwithstanding the smouldering rate of caseins. The casein treatment shows the lowest char length and the highest final residue (13 mm and 94%, respectively). LMW-DNA is also very effective on cotton, bearing in mind the lower AO%.

Table 3.15 – Flammability data of untreated cotton fabrics, LMW-DNA pH4_MI, CAS-Cotton and WPI-Cotton samples. In brackets: smouldering time and smouldering rate of CAS-cotton samples (Accuracy ±3-5%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction (%)	AO%
Cotton	66	-	1.50	-	NO	-
LMW-DNA pH4_MI - Cotton	57	68	1.20	76	YES (86)	8
WPI-Cotton	67	54	0.81	80	YES	22
CAS-Cotton	12 (61)	13	1.08 (0.21)	94	YES	20

The combustion behaviour of biomacromolecules-treated fabrics was assessed by cone calorimetry; the results are shown in Table 3.16 and Figure 3.35. The effectiveness of LMW-DNA and casein in reducing the fire hazard of cotton is very similar, with a reduction of pkHRR (-41 and -44%, respectively) and of THR (-38 and -50%, respectively) is observed. On the other hand, the effect of the WPI treatment on cotton is negligible, as pkHRR and THR values are very similar.

Table 3.16 - Combustion data of untreated cotton and cotton fabrics coated with WPI, LMW-DNA pH4 and caseins, obtained by cone calorimetry.

	TTI (s)	Flame out (s)	Combustion duration (s)	pkHRR (kW/m^2g)	THR (MJ/m^2g)	Residue (%)
Cotton	29 ± 6	57 ± 6	28 ± 12	57 ± 3	1.2 ± 0.1	1.0 ± 0.5
CAS-Cotton_A	16 ± 3	34 ± 4	18 ± 7	32 ± 6	0.6 ± 0.2	17 ± 5
WPI-Cotton	24 ± 1	50 ± 3	26 ± 4	52 ± 1	1.2 ± 0.2	1.0 ± 0.5
LMW-DNA pH4_MI-Cotton	27 ± 3	45 ± 2	18 ± 5	33 ± 4	0.75 ± 0.05	6.0 ± 0.5

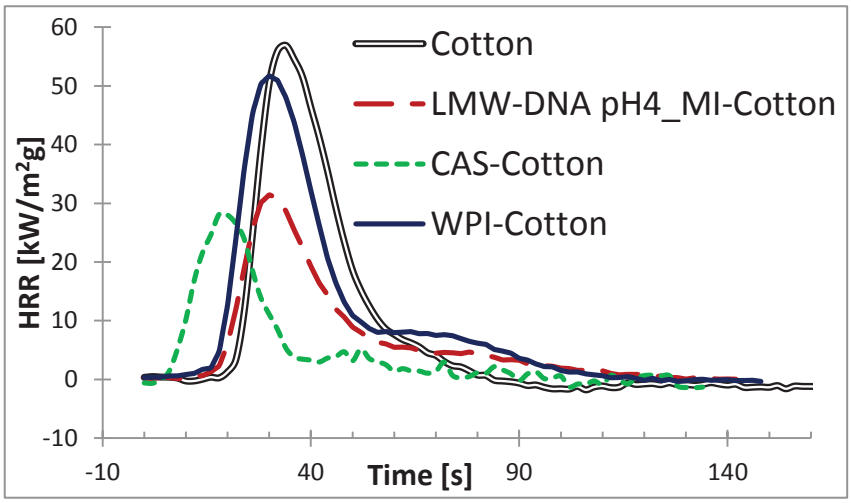


Figure 3.35 - Heat release rate versus time of untreated cotton and cotton treated with biomacromolecules, tested at 35 kW/m^2.

A possible explanation of the different behaviour of these biomacromolecules can be related to the phosphorous content. Whey proteins do not contain phosphorous, and their effectiveness is the lowest among the proposed treatments. Caseins contain a tenth of DNA phosphorous and a higher AO% is necessary to achieve performances similar to DNA, especially in cone calorimeter tests. Finally, it is worthy to note that all these biomacromolecules have comparable nitrogen content (Table 3.17).

Table 3.17 – Phosphorous and nitrogen content of the selected biomacromolecules

	Whey Proteins	Caseins	DNA
Phosphorous content (wt.%)	0	0.8 [131]	8 [200]
Nitrogen content (wt.%)	15.6 [201]	15.8 [201]	15.1 [200]

Fabrics treated with biomacromolecules were also tested in combination with a non-flame-retarded flexible polyurethane foam (PU-foam), aiming at mimicking the combustion behaviour of an upholstered cushion. The results of cone calorimetry tests are shown in Figure 3.36 and Table 3.17. Polyurethane foam squares of 10x10x2 cm^3 were covered with the fabrics and tested under a heat flux of 35 kW/m^2. In the heat release rate curves, two peaks are visible, due to the presence of the PU-foam. The most evident flame retardant effect of the biomacromolecules is the reduction of the first and main peak of heat release rate. TTI values are very short, with no differences among the all treated fabrics and cotton. Furthermore, in the case of WPI-Cotton, the combustion duration is also comparable to that of cotton. Conversely, for caseins and LMW-DNA, the combustion duration is about 50 s longer. Comparable results are also obtained for THR values, where the most influencing factor is the weight of the polyurethane foam. The most pronounced reduction in the first pkHRR is observed with caseins (-37%), then LMW-DNA (-24%) and, lastly, with WPI (-16%). Caseins and LMW-DNA are also responsible for a slight anticipation of the peak (of about 5 s), which can be associated to their degradation mechanism. The second

peak is mostly reduced by LMW-DNA (-21%) and WPI (-9%), while it is almost unchanged with caseins (150 vs. 153 kW/m^2 for untreated cotton). In all the cases, the residue at the end of the tests consists of the fabric shell, while the PU-foam is almost completely consumed (Figure 3.37). The highest residue is left by CAS-Cotton (15% vs. 11% of cotton): the residue is brittle and white, unlike its black colour in the other cases (Figure 3.38). Only with the WPI-cotton samples, the residue shell is broken in the middle. All these findings further support the results already discussed in the previous sections, which indicate a flame retardant action mainly in the condensed phase (i.e. dehydration of the cellulosic substrate and formation of a stable char).

Table 3.18 - Combustion data of polyurethane foam samples covered with untreated cotton and cotton fabrics treated with WPI, LMW-DNA pH4 and caseins, obtained by cone calorimetry.

	TTI (s)	Flame out (s)	Combustion duration (s)	1st pkHRR (kW/m^2)	THR (MJ/m^2)	Residue (%)
Cotton	9 ± 1	255 ± 27	246 ± 28	294 ± 22	21.8 ± 0.55	11 ± 2
CAS-Cotton_A	8 ± 0	300 ± 40	292 ± 40	186 ± 17	23.3 ± 2.7	15 ± 5
WPI-Cotton	10 ± 1	256 ± 26	246 ± 27	246 ± 23	22.1 ± 1.6	13 ± 5
LMW-DNA pH4_MI-Cotton	8 ± 2	299 ± 14	291 ± 16	224 ± 26	22.4 ± 0.75	11 ± 2

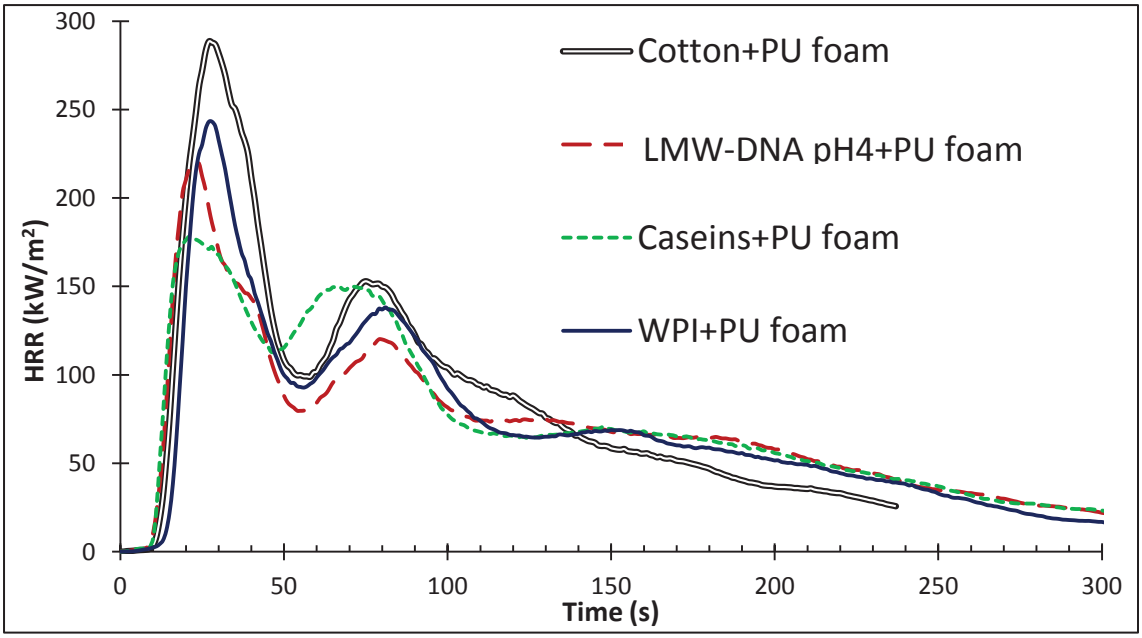


Figure 3.36 - Heat release rate versus time of polyurethane foam plus untreated cotton and cotton treated with biomacromolecules, tested at 35 kW/m^2.



Figure 3.37 – Reverse side of the residue after cone calorimeter tests performed on cotton covering the polyurethane foam.

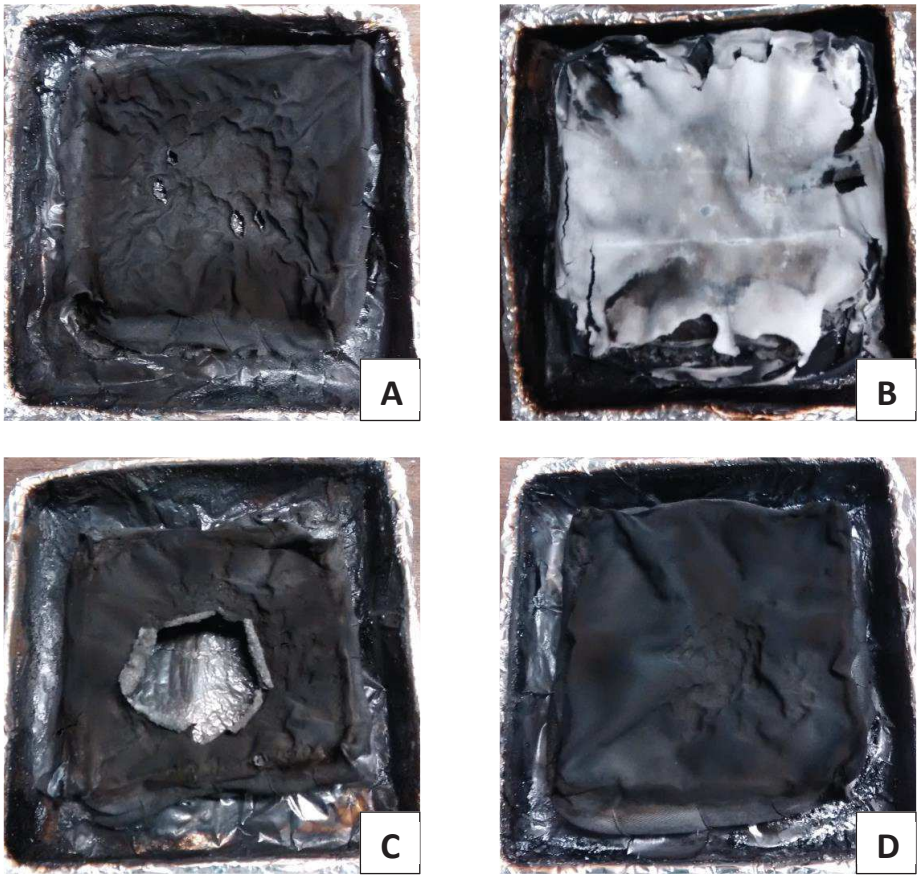


Figure 3.38 – Residues after cone calorimetry tests of cushion-like samples: A) Untreated cotton + PU foam; B) CAS-Cotton + PU foam; C) WPI-Cotton + PU foam; D) LMW-DNA-Cotton + PU foam.

3.3 Extraction and application of DNA from vegetable or brewery wastes

Wastes from the agro-food industry can be good sources of DNA for the application as flame retardant for textiles. This particular application needs high quantities of DNA, with no necessity of high purity or high molecular sizes. Actually, low molecular weight DNA is more effective in conferring flame retardancy, as shown in the previous section.

In the present work, two wastes of very different origins were exploited for the development of two methods for the extraction and recovery of DNA. Mixed vegetable scraps were supplied by a vegetable and fruit processing plant and spent yeasts were provided by two breweries. The extracted DNA was then applied on cotton fabrics, for which the flammability behaviour was assessed.

In the following sections only DNA will be referred to as extracted product, bearing in mind that the contamination from RNA could be significant but without negative drawbacks.

3.3.1 Vegetable wastes

DNA extraction from the vegetable wastes was carried out on a mixed scrap derived from a IV range vegetable production line. Owing to the high heterogeneity of the supplied wastes, the extraction results refer to two matrices, i.e. MLV (mixed leafy vegetables) and LV (leafy vegetables).

The two matrices were treated using a basic protocol that considers a detergent-mediated cell lysis at high temperature, a removal of solid particles and a precipitation of DNA in cold ethanol. Different parameters were taken into account in order to optimize the extraction procedure.

The influence of the storage conditions on the vegetable matrix was evaluated with a set of preliminary tests. For this purpose, the DNA extraction was carried out on either frozen or fresh MLV and LV samples. As reported in Table 3.18, the extraction yields obtained from the frozen material were almost doubled, compared to those obtained with the fresh substrate. The DNA purity values ($OD_{260/280}$) were almost the same, and ranged from 1.12 to 1.17.

Table 3.19 - Extraction yields (EY_{DNA}%) obtained with fresh or frozen vegetable material. The standard deviation values are indicated in brackets.

	MLV	**LV**
Fresh	0.070 (±0.008)	0.070 (±0.010)
Frozen	0.140 (±0.006)	0.120 (±0.008)

Freezing offers the advantage of preserving vegetables for long periods before use. At the same time, the freeze-thawing cycle had the effect of softening the vegetable material, which became easier to handle since vegetable tissues were broken down. This procedure allowed

higher quantities of DNA to be extracted with the same purity obtained by treating the fresh material.

The main parameters that influence the cost effectiveness of a recovery process, at large scale, are time and temperature, as well as quantities and purity grade of the employed reagents. The phase of ethanol precipitation can affect significantly the process economy, since it requires the use of ethanol and an incubation period at low temperature. For this purpose, the ethanol purity grade, the temperature, and the duration of the precipitation phase were modified one at a time to obtain the best extraction yields.

The ethanol precipitation step was performed at two different incubation temperatures, namely +4 and -20°C. The influence of this parameter on the EY_{DNA}% was evident. For MLV, higher DNA yield values (0.1%) were obtained at -20°C in comparison to the significant lower percentage (0.02%) extracted from the same source at +4°C. Again in this case, there were no differences in the DNA purity, which was close to 1.13. For these reasons the extractions were performed at -20°C.

The influence of the ethanol purity grades (90, 96 and 99.8%) on the DNA yields and purity was then evaluated on the two matrices. As shown in Figure 3.39A, no significant differences in the extraction yields were found for the MLV and LV matrices: the EY_{DNA}% values were within 0.25 and 0.32%. On the other hand, the ethanol with the lowest purity provided the highest DNA yields, but with a high standard deviation value. No differences were found for the DNA purity, which was within 1.11 and 1.17 for all the samples. On the basis of these results, it was proven that the ethanol with the lowest purity grade (90%) could be utilized for the precipitation step, hence achieving the same extraction yields at lower cost.

The results obtained for the investigation of the effect of the incubation time on LV matrix are compared in Figure 3.39B. It could be noted that the time of this step affects the extraction yields: in particular, EY_{DNA}% reached a plateau (at about 0.3%) after 4 hours of incubation. The protraction of the incubation time did not provide any further increase in the extraction yields. 4 hours were enough for the highest yield to be obtained. Purity was not affected by the time of the precipitation step: the purity values ($OD_{260/280}$) were equal to 1.1 for all of the samples.

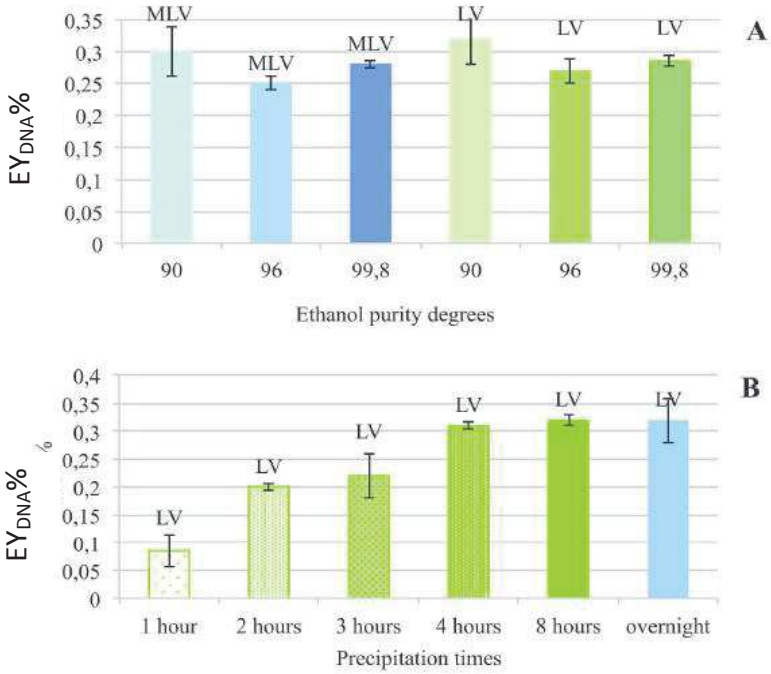


Figure 3.39 - Extraction yields (EY_{DNA}%) obtained with: (A) ethanol at different degrees of purity; (B) different precipitation times.

The results of the above trials, taken together, allowed defining the best conditions to extract DNA from mixed vegetable wastes. First, the MLV and LV sources gave comparable EY_{DNA}% and purity values for the same extraction conditions. Nevertheless, the LV source was preferable, as it was only composed of leaves that are characterized by a similar composition, irrespective of the origin of the vegetable tissues. On the other hand, the MLV source contained small pieces of vegetables (e.g. potatoes, carrots and pumpkin), which are rich in complex polysaccharides and which can interfere negatively with DNA extraction and recovery [202,203]. In fact, they contribute to the formation of macromolecular complexes, which are able to embed the nucleic acids in a sticky and gelatinous matrix. This makes the precipitation and separation of DNA difficult during the subsequent recovery steps.

Second, the best results were obtained by starting from a frozen material that was subsequently thawed, mashed up and mixed with the extraction buffer in a 1:7 ratio. This ratio was chosen on the basis of the results reported in the scientific literature [204]; it also corresponded to the minimum buffer quantity necessary to completely soak the selected matrices.

Third, the DNA precipitation could be carried out with 90% ethanol for 4 hours at -20°C.

These optimized conditions were adopted to process the LV matrix in a scaled up process (where quantities were fivefold multiplied), in order to obtain sufficient DNA to impregnate the cotton fabrics. It was necessary to adjust the extraction temperature, which was raised to 68°C, and time, which was doubled (60 minutes). However, the scale-up did not affect the recovery yields. In fact, the obtained EY_{DNA}% was equal to 0.33% and comparable with that gathered previously in small-scale experiments.

The LV-extract allowed reaching 5 wt.% dry add-on on cotton fabrics.

The morphology of the treated fabrics was assessed by means of SEM microscopy (Figure 3.40). LV-DNA coating showed a rough surface, due to the presence of impurities that aggregated, hence giving rise to an "island" growth of the coating.

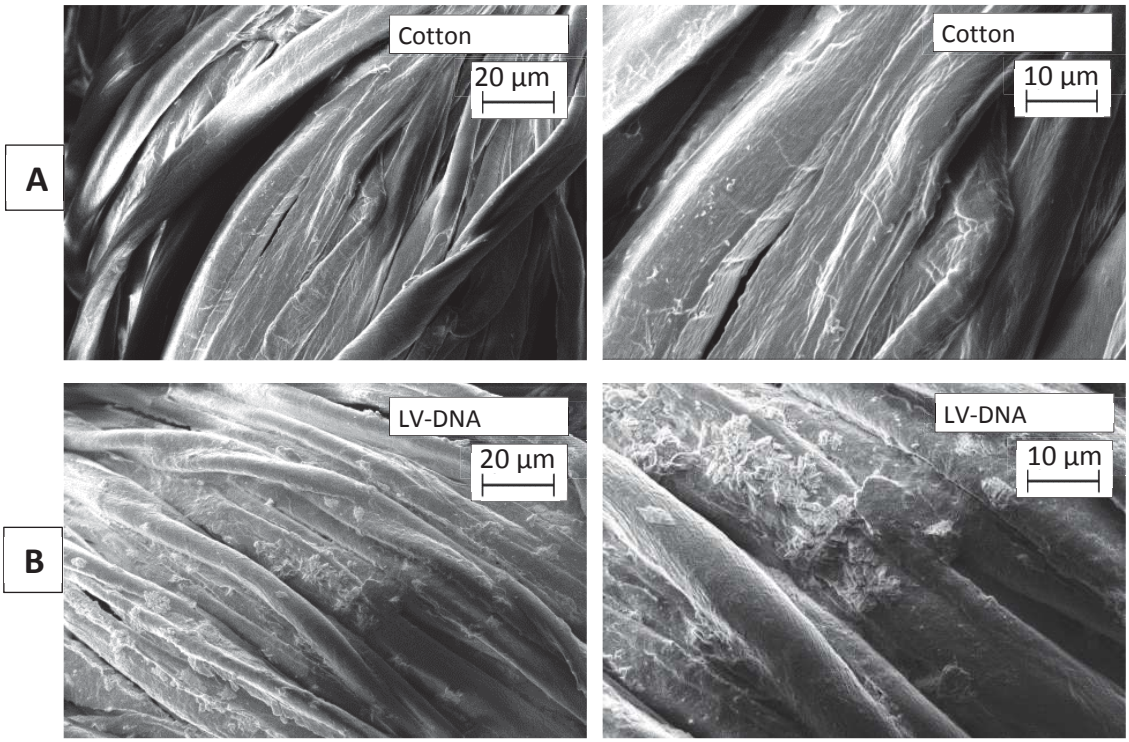


Figure 3.40 - SEM micrographs of treated and untreated fabrics: A) untreated cotton; B) cotton treated with LV-DNA.

The treated fabrics were subjected to flame spread tests in horizontal configuration (Table 3.19). The effect of the LV-derived extracts in flame retardancy was almost negligible: just a decrease in the burning rate was observed (1.3 mm/s vs. 1.5 mm/s for treated and untreated fabrics, respectively). Furthermore, an incoherent and very limited final residue remained (Figure 3.41). These findings could also be ascribed to the low dry add-on of the biomacromolecules on the fabrics (i.e. about 5 wt.%, which represents the maximum value achievable in the adopted experimental conditions), as compared to commercial DNA samples (8 wt.%, as reported in previous chapters). SEM micrographs of the residue after the tests showed that the fibres and the fabric texture were still present, but they were incoherent and brittle, with many little holes and a thin structure (Figure 3.42). Therefore, the extraction and recovery of nucleic acids from vegetable wastes does not seem to be a very promising strategy for the design of fire retardants for cotton, probably because of the very low extraction yields obtained.

Table 3.20 – Flammability data of the cotton fabrics coated with LV-DNA obtained from horizontal flame spread tests. Accuracy ±4%.

	Total burning time (s)	**Char length (mm)**	**Total burning rate (mm/s)**	**Residue (%)**	**Self-extinction**
Cotton	66	100	1.5	0	NO
LV-DNA	77	100	1.3	3	NO

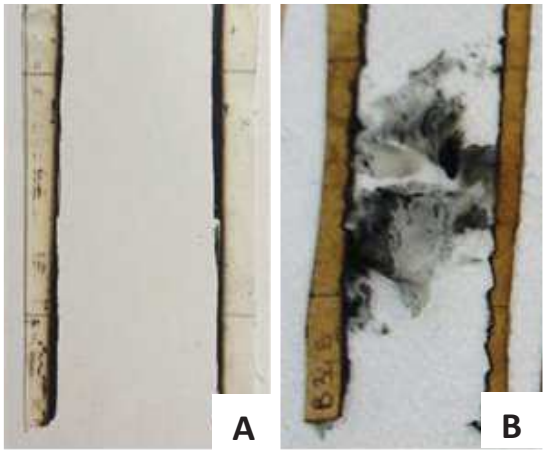


Figure 3.41 - Residues after flame spread tests (horizontal configuration) of A) untreated cotton; B) LV-DNA treated cotton.

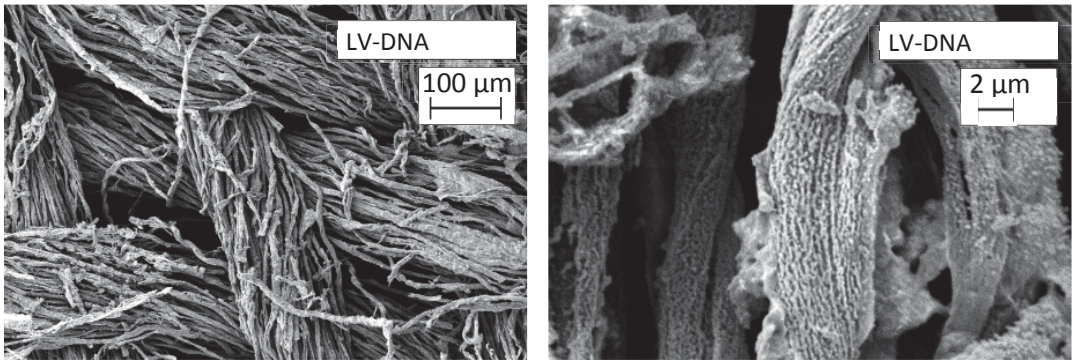


Figure 3.42 – SEM micrographs of residues after horizontal flame spread tests of cotton treated with LV-DNA.

3.3.2 Spent brewer's yeast

The second food industry waste that was selected as a potential source of DNA was spent brewer's yeast. The basic extraction procedure of this matrix required the addition of a defined volume of extraction buffer, followed by a series of freeze-thaw cycles and a ball milling step, which was performed by adding glass beads to the yeast/buffer slurry. Then, after the removal of the glass beads and a centrifugation for cell debris elimination, the extract was microfiltrated and ultrafiltrated to obtain an aqueous solution containing DNA.

As for vegetable wastes, different parameters of the basic procedure were changed in order to maximize yields and purity of the extracted DNA. The starting material was beer slurry that had been subjected to two different pre-treatments: one batch was maintained at +4°C for 8 months and, just before being used, yeast cells were centrifuged and washed three times with bi-distilled water (hereinafter these samples will be coded as AY); in a second batch, beer slurry cells were immediately centrifuged to separate yeast cells that were then washed three times

with bi-distilled water and stored at -20°C until their utilization (FY samples). The basic procedure was applied on these two starting materials and different extraction buffers, ball milling conditions, and filtration methods were taken in consideration.

To reduce the cost and environmental impact of extraction buffer, the use of detergents and enzymes for the cell lysis was avoided; they were replaced by a combination of physical (freeze-thaw) and mechanical (glass beads milling) methods. Therefore, the optimization of the extraction buffer was investigated through three different combinations of the remaining essential components. The first consisted of 50 mM Tris-HCl, 50 or 100 mM NaCl and 10 mM EDTA (coded as TNE 50/50/10 and TNE 50/100/10, respectively). EDTA was not included in the second formulation, which just contained Tris-HCl and NaCl, at the same concentrations as the first one (coded as TN 50/50 and TN 50/100). The third extraction solution was a phosphate buffer (PB), without any of the other components.

The different extraction buffer efficacy was evaluated for both the AY and the FY starting materials. The EY_{DNA}% and purity values, calculated after the centrifugation step, are shown in Table 3.20. For AY, high yields were obtained with TNE 50/50/10 (13.1%), TNE 50/100/10 (13.1%) and PB (13.5%). Conversely, lower yields were observed for TN 50/50 and TN 50/100 (11.1 and 11.6%, respectively). When the yields and purity values were combined, TNE 50/100/10 and PB resulted to be the best extraction solutions. In the case of FY, the results were similar for all of the utilized extraction solutions and both the yield and purity values were lower, compared to those of AY.

Table 3.21 - EY_{DNA}% and purity data obtained for different extraction solutions of spent brewer's yeast.

	AY		FY	
Extraction solutions	**EY_{DNA}%**	**Purity ($OD_{260/280}$)**	**EY_{DNA}%**	**Purity ($OD_{260/280}$)**
TNE 50/50/10	13.1 (±0.9)	1.85 (±0.01)	5.5 (±0.3)	1.57 (±0.01)
TNE 50/100/10	13.1 (±0.8)	1.95 (±0.01)	5.3 (±0.2)	1.60 (±0.01)
TN 50/50	11.1 (±0.5)	1.90 (±0.02)	5.6 (±0.2)	1.65 (±0.01)
TN 50/100	11.6 (±0.5)	1.95 (±0.01)	5.4 (±0.3)	1.60 (±0.02)
PB	13.5 (±1)	1.95 (±0.02)	5.7 (±0.3)	1.50 (±0.02)

The PB solution had to be discarded: in fact, the fabrics treated with PB alone achieved self-extinction with only 3 wt.% AO, as it will be discussed later. TNE 50/100/10 was therefore selected for all the subsequent extraction tests.

As previously reported, a mechanical cell disruption method was employed to improve the release of the intracellular components, especially DNA. For this purpose, glass beads were added to the samples after the last freeze-thaw cycle, and a ball milling apparatus, with a rotation speed of 150 rpm, was employed. The influence of two different grinding times (namely 10 and 90 minutes) on cell disruption was evaluated. The DNA EY_{DNA}% and purity values, obtained for AY and FY after the centrifugation, are reported in Table 3.21. The treatment time was found to affect the EY_{DNA}%: an increase from 13.1 to 15.5% for AY and from 5.3 to 9.4% for

FY was observed as the ball milling time was increased. On the other hand, the purity was not affected substantially for both the yeasts.

Table 3.22 - EY_{DNA}% and purity data obtained for the different ball grinding times

	AY		FY	
Grinding time	**EY_{DNA}%**	**Purity ($OD_{260/280}$)**	**EY_{DNA}%**	**Purity ($OD_{260/280}$)**
10 min	13.1 (±0.8)	1.95 (±0.01)	5.3 (±0.2)	1.60 (±0.01)
90 min	15.5 (±0.5)	2.00 (±0.01)	9.4 (±0.9)	1.50 (±0.01)

Two alternative filtration methods were applied to samples extracted with TNE 50/100/10 and then centrifuged. Microfiltration was carried out by using a glass fibre filter (ϕ=0.7 μm) to remove any solid particles that might still be present in the DNA solution. Ultrafiltration was performed with a membrane cut-off of 10 kDa in order to reduce the concentration of the molecules with a molecular weight below 10 kDa. The permeate was discarded, while the retentate, rich in DNA, was recovered.

The EY_{DNA}% obtained for AY and FY after the two purification steps is shown in Figure 3.43. A decrease of the yield after ultrafiltration was particularly evident for AY (about 11%), likely due to the presence of DNA with a lower molecular weight than the membrane cut off (Figure 3.49), able to pass through the membrane. However, purity of AY was further increased by the filtration steps, since it reached $OD_{260/280}$ values higher than 2. This was probably ascribed to the removal of the smaller cell debris by microfiltration and to the removal of low molecular size proteins by ultrafiltration.

The filtration steps did not significantly affect the purity of the FY extract: unpurified samples had an $OD_{260/280}$ value of 1.5, whereas 1.6 was obtained for both the microfiltrated and ultrafiltrated samples.

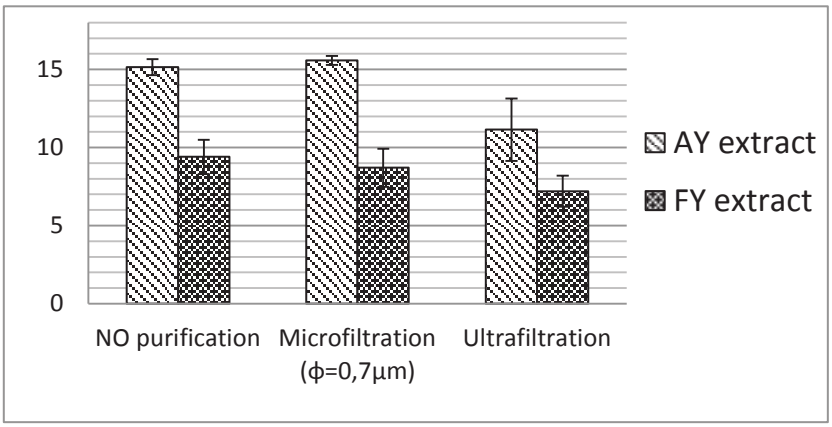


Figure 3.43 - EY_{DNA}% obtained for different purification steps.

Taking into account that the yields and purities of FY were lower than those of AY, further attempts were made to improve them. The centrifugation was replaced by a Celite filtration or a microfiltration (ϕ=1.2 μm) step. As shown in Figure 3.44, Celite filtration allowed obtaining a

DNA solution with yield and $OD_{260/280}$ values (9.1% and 1.45, respectively) very similar to those obtained from centrifugation (9.4% and 1.50). Microfiltration, on the other hand, led to the worst results, in terms of yield (6%) and a negligible increase in purity (1.56). In this case, a cake of cell debris obstructed the pass through of both nucleic acids and proteins. As a consequence, a significant decrease in the DNA yield was obtained together with a small increase of the purity value.

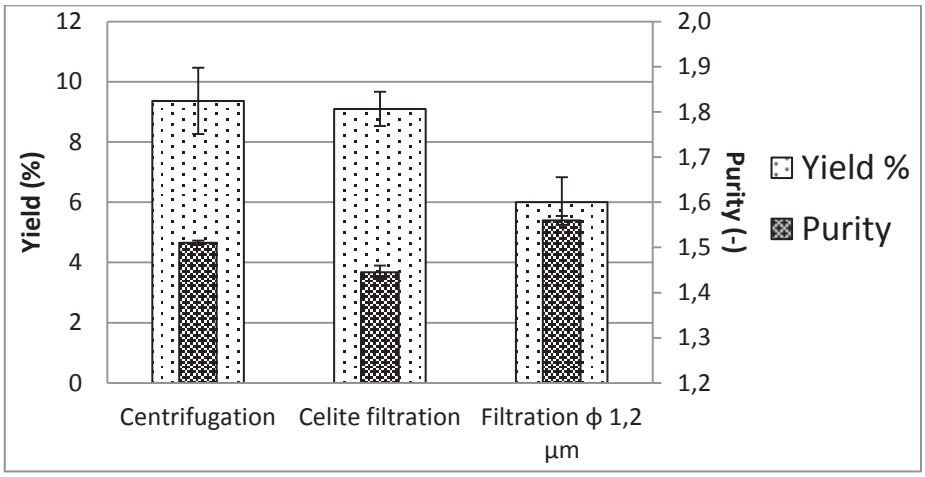


Figure 3.44 - Extraction yields (%) and purity data obtained with different cell debris separation methods.

From all of the obtained results with AY and FY, an optimized extraction procedure was defined. First, the most effective extraction buffer was composed by Tris-HCl 50 mM, EDTA 10 mM and NaCl 100 mM. Second, the cell disruption could be carried out with 3 alternate freeze-thaw cycles and ball milling with glass beads for 90 minutes. Finally, post-extraction purification steps were not strictly necessary, but the most performing method implied the use of microfiltration with glass fibre filter (ϕ=0.7 µm).

The extracts showing the highest DNA yields and purity were applied on cotton fabrics and subjected to horizontal flame spread tests.

Figure 3.45 shows some typical pictures of the fabrics, as assessed by SEM, before and after the treatment with the extracted biomacromolecules (namely, FY and AY). The roughness of pure cotton (Figure 3.45A) disappeared when the fabrics were treated with the extracted yeast DNA: these latter samples showed a smooth surface, because of the presence of the biomacromolecule coating (Figures 3.45B and C), which was also located in the fabric interstices, as clearly shown in Figure 3.45B.

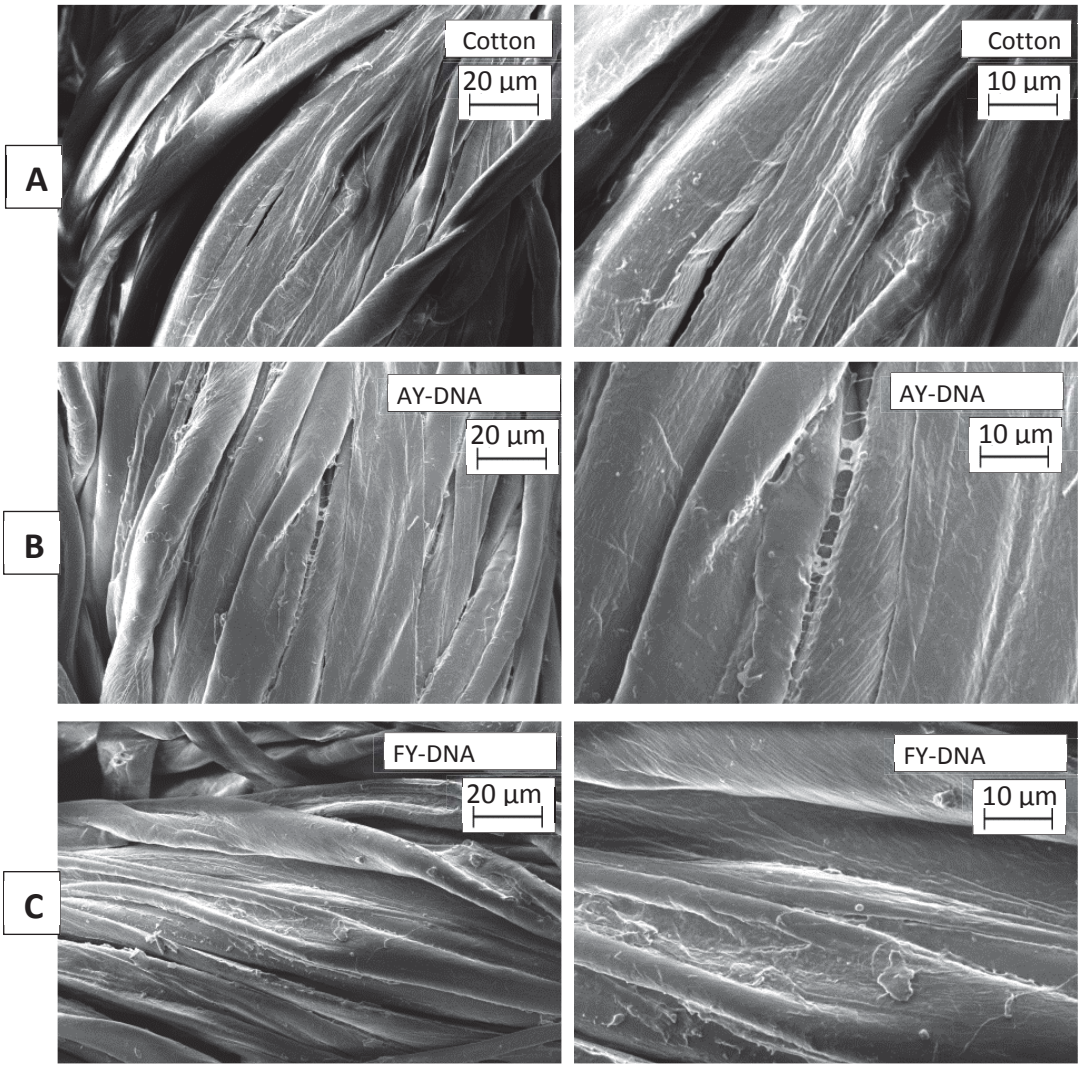


Figure 3.45 – SEM micrographs of treated and untreated fabrics: A) untreated cotton; B) cotton treated with AY DNA; C) cotton treated with FY DNA.

Table 3.22 collects the data obtained from flame spread tests in horizontal configuration and Figures 3.46 and 3.47 show some pictures of the treated fabrics at the end of the tests.

As already mentioned, the fabrics treated with the phosphate buffer alone were found to be self-extinguishing with only 3 AO% and therefore PB was not considered for the evaluation of the flame retardant effectiveness of the DNA extracts. Also the TNE 50/100/10 buffer alone was able to confer some flame retardant features to cotton (AO%=8): a few samples self-extinguished and quite thin residues were found at the end of the tests.

An add-on of 8% was also considered for yeast-derived DNA samples, as optimized in previous studies [186]. Interesting results were observed with the application of the AY-derived DNA-containing extract. In fact, all the tested samples achieved self-extinction, and the best performances were obtained for the AY extract even without purification (60% residue). The solutions after microfiltration and ultrafiltration provided flame retardant features to cotton with similar effectiveness, as shown by almost comparable residues (50% and 47%, respectively)

and burning rates (1.15 mm/s) (Table 3.22 and Figure 3.46). Furthermore, a long afterglow combustion was observed for all of the investigated samples. The good flame retardant properties of unpurified AY-derived extract are likely due to the high content of low molecular weight DNA (Figure 3.49), which makes the biomacromolecule more effective in protecting the underlying fabric [186].

Different results were obtained for fabrics treated with FY-derived DNA-containing solution. In particular, the extract showed a significant decrease in the burning rate, compared to the untreated cotton (1.05 vs. 1.5 mm/s), with no self-extinction of the samples (Figure 3.47 and Table 3.22). Better results, i.e. self-extinction and residues of almost 50%, were achieved with samples filtrated by Celite (Table 3.22).

As regards the post-extraction purification steps, the most effective treatment for fire retardancy for both AY- and FY-derived samples was microfiltration performed with a glass fibre filter (ϕ=0.7). In this case, self-extinction was achieved, together with a high final residue (72%) and a low burning rate (1.15 mm/s). A similar behaviour, but without the flame out being reached, was found for the cotton treated with the ultrafiltrated FY extract.

FY was less effective than AY, probably because of the higher molecular weight of extracted DNA [186]. Nevertheless, microfiltration probably caused a better homogenization of the extract and allowed the formation of a more protective coating, in comparison with unpurified FY-derived extract.

Since phosphorus is a key component of DNA, and is necessary to provide flame retardant features, ICP-MS was exploited for assessing the phosphorous content of the cotton fabrics treated with the DNA extracted from yeast. The ICP-MS measurements showed that the concentration of this element in the fabrics coated by AY- and FY-derived DNA samples was 0.47 wt.%, hence comparable to that obtained from the commercial DNA coatings (0.5%) [186].

Table 3.23 - Combustion data of the cotton fabrics coated with different extracted DNA obtained from horizontal flame spread tests. (Accuracy ±5%)

	Total burning time (s)	**Char length (mm)**	**Total burning rate (mm/s)**	**Residue (%)**	**Self-extinction (%)**
Cotton	66	100	1.50	0	NO
AY-DNA	51 (181)	60	1.17	60	YES
AY-DNA after microfiltration (φ=0.7μm)	65 (203)	75	1.15	50	YES
AY-DNA after ultrafiltration	64 (227)	75	1.15	47	YES
FY-DNA	95	100	1.05	38	NO
FY-DNA (Celite filtration)	80	87	1.08	49	YES (50%)
FY-DNA after microfiltration (φ=0.7μm)	61	70	1.15	72	YES
FY-DNA after ultrafiltration	83	100	1.20	34	NO
PB	71	69	1	69	YES
TNE 50/100/10	70	83	1.20	45	YES (50%)

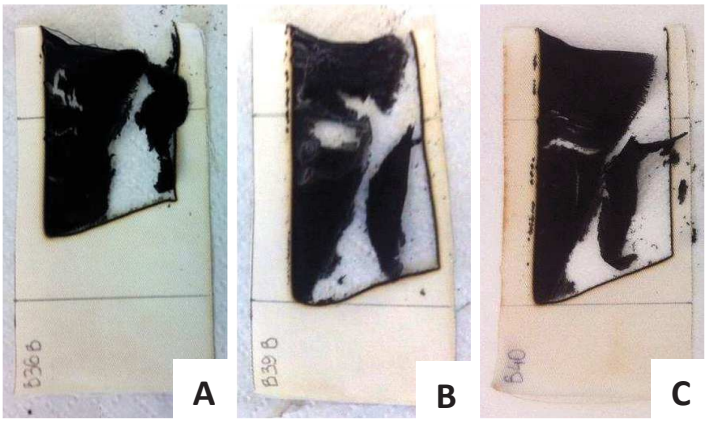


Figure 3.46 - Residues after flame spread tests (horizontal configuration) of AY-DNA treated fabrics: A) Extract; B) Extract after microfiltration (ϕ=0.7μm); C) Extract after ultrafiltration.

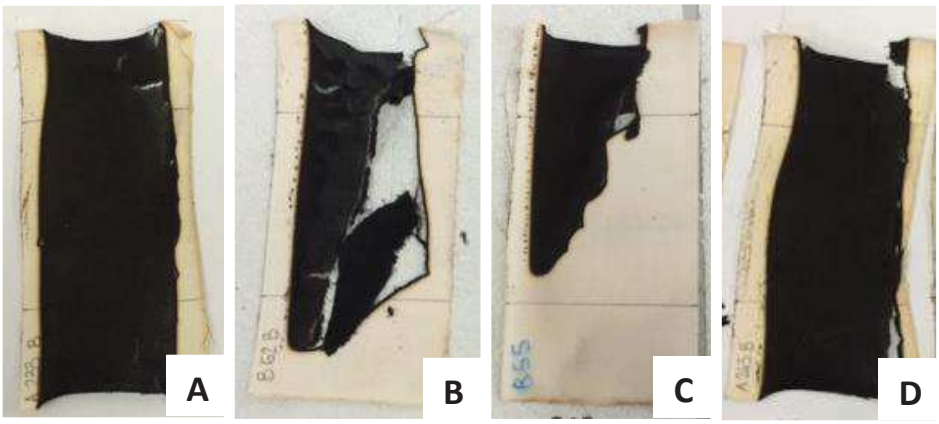


Figure 3.47 - Residues after flame spread tests (horizontal configuration) of FY-DNA treated fabrics: A) Extract, B) Extract with Celite filtration, C) Extract with microfiltration (ϕ=0.7μm), D) Extract with ultrafiltration.

SEM analyses were also exploited for assessing the morphology of the residues after the horizontal flame spread tests. As clearly shown in Figure 3.48, the texture of all the treated fabrics was maintained, thus indicating a significant protection exerted by the biomacromolecule coating on the underlying fibres. Furthermore, the fibres showed a hollow structure (Figure 3.48 B), derived from the dehydration reaction promoted by the DNA degradation, which favours the formation of a stable char. Finally, as compared to the fabrics treated with the commercial DNA coating, which showed the presence of several bubbles as a consequence of the intumescent process occurring after the flame application, the residues of the substrates treated with extracted DNAs displayed a different surface morphology, without a direct evidence of the intumescent process (i.e. no bubble formation).

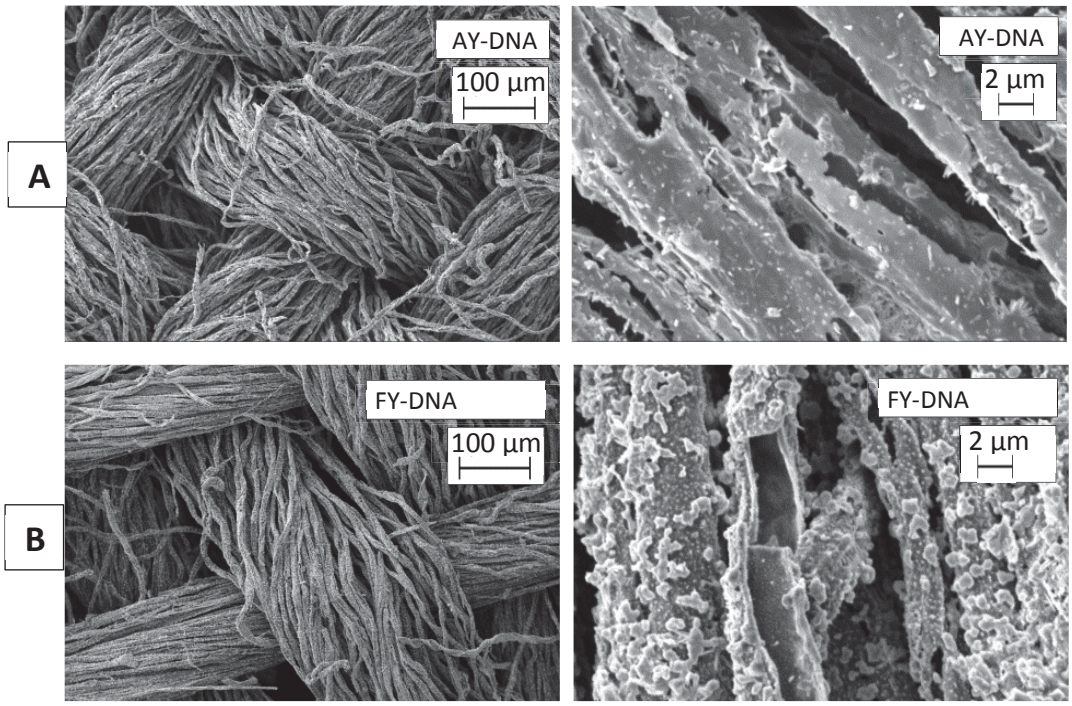


Figure 3.48 – SEM micrographs of residues after horizontal flame spread tests of yeast-DNA treated cotton: A) cotton treated with AY DNA; B) cotton treated with FY DNA.

3.3.3 Further characterization of the extracted DNA

DNA extracted from the three different matrices (FY, AY, and LV) were digested with DNase-free RNase and then characterized with agarose gel electrophoresis to establish their molecular sizes in comparison to those of commercially available herring DNA (Figure 3.49). The DNA extracted from FY showed a continuous smear between 3 and more than 15 kb comparable to that of commercial DNA from herring testes (HMW-DNA), thus indicating that FY-DNA did not degrade during the extraction process. In contrast, the DNA obtained from AY showed a partial degradation with a smear lower than 3 kb, comparable with that of commercial DNA from herring sperm (LMW-DNA). This degradation is probably due to the cells autolysis occurred during the prolonged storage of the yeast at +4°C.

Conversely, the DNA extracted from FY cells remained almost intact and enzyme degradation did not occur. For this reason, for FY, harder conditions for cell disruption were required to get good recovery of extracted DNA. Thus, FY was more sensitive to both the physical and the mechanical cell disruption processes. As shown in Table 3.21, the influence of the glass bead grinding time was more pronounced for FY than for AY with a 4% difference between 10 and 90 min grinding.

Finally, the LV-DNA sample did not show a detectable smear and only a very low quantity of oligonucleotides likely derived from the RNA degradation was visible.

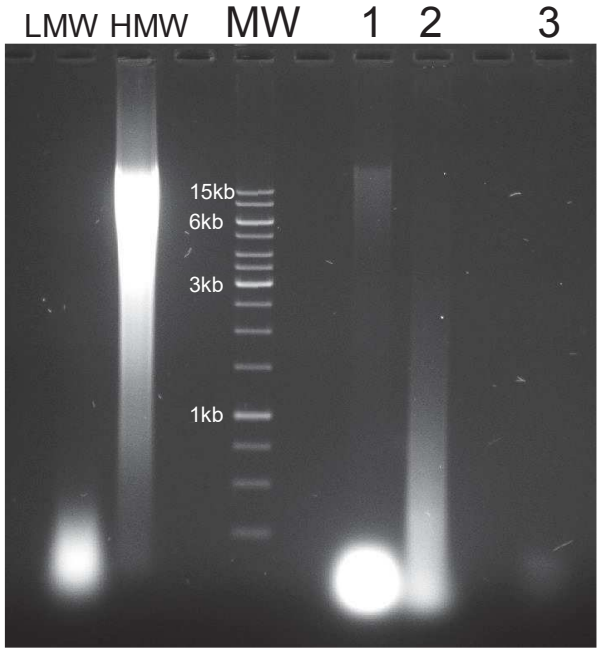


Figure 3.49 – Agarose gel electrophoresis analysis of extracted DNA: LMW) LMW-DNA from herring sperm (Sigma-Aldrich S.r.l.); HMW) HMW-DNA from herring testes (Sigma-Aldrich S.r.l.); MW) ladder; 1) DNA from FY; 2) DNA from AY; 3) DNA from LV.

FTIR-ATR spectroscopy was then used to evaluate the quality of the yeast DNA (extracted from FY) and vegetable DNA (extracted from LV). The corresponding spectra were compared with that of commercial herring testes DNA (Figure 3.50). First of all, it is noteworthy that, despite a difference in intensity, all the spectra showed signals in the 1250-1000 cm^{-1} region that corresponds to phosphate vibrations; furthermore, they also exhibited C=N vibrations at about 1600 cm^{-1}, attributable to the nitrogen bases. Some absorption peaks with different intensity at about 1400 cm^{-1}, at least for FY DNA, could be referred to uracil bases and thus to the presence of RNA in the extract [205]. The two FTIR-ATR spectra of the extracted DNA show similarities, notwithstanding a slight shift of the peaks for the LV extract toward higher wavelengths.

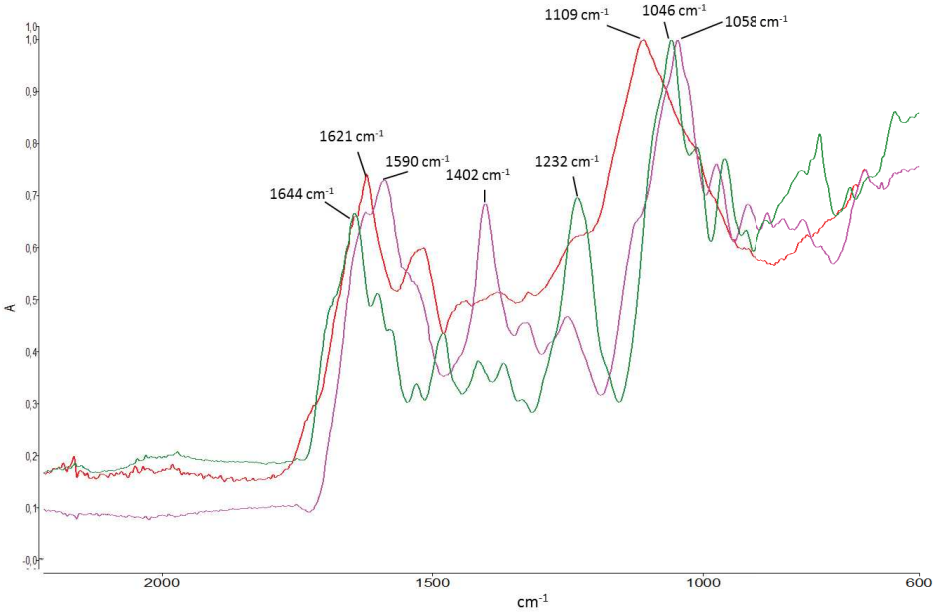


Figure 3.50 – FTIR-ATR spectroscopy of extracted DNA: DNA from FY (purple line); DNA from LV (red line); commercial DNA from herring testes (green line).

3.4 Mixtures of biomacromolecules

Combinations of different biomacromolecules were made with the purpose of going beyond some limits of the treatments with DNA, in particular its low washing fastness and high cost. Besides the impregnation process, a UV-curing step was added in some cases to favour the formation of bonds within the coating and between the coating and the substrate. The washing resistance was assessed by performing a washing cycle at 55°C for 1 hour. More details on the UV treatment and the washing procedure are reported in the “Materials and Methods” section.

3.4.1 HMW-DNA and LMW-DNA

In water, the DNA treated fabrics completely lose the coating and this is not a desirable feature for developing durable flame retardants.

DNA immobilisation has been widely studied for the application on sensors, nanoparticles and other supports [206,207] and has been usually improved with the combination with other active species [208,209]. Yamada et al. described the immobilisation of salmon milt DNA on non-woven cellulose fabrics through the formation of bonds among the chains due to UV irradiation [210]. In fact, it was demonstrated that, when exposed to UV irradiation, thymine bases in DNA chains react with each other to form dimers [211].

In this work, a similar approach was adopted for the immobilisation of DNA on cotton fabrics.

First of all, DNA from herring testes (3000–10,000 bp), DNA from herring sperm (100–200 bp), and DNA from herring testes previously sonicated (300–800 bp) were applied on cotton fabrics, reaching the same AO%; then, the treated fabrics were exposed to UV irradiation and finally washed. It was possible to observe that the UV treatment is effective in the immobilisation of the integer DNA from herring testes and only partially effective on the sonicated DNA. No modification of the UV irradiation time or intensity were effective in favouring the immobilization of the DNA from herring sperm. This is probably due to its low molecular weight. Figure 3.51 plots the concentration of DNA in the washing bath of LMW-DNA, MMW-DNA and HMW-DNA after UV exposure and of HMW-DNA (not UV irradiated), as a function of time. Both LMW-DNA UV-cured and HMW-DNA not UV-cured release the whole DNA uptake during the first 10 minutes. On the other hand, HMW-DNA UV-cured did not release DNA. MMW-DNA-treated fabrics release an intermediate quantity of DNA: the UV irradiation is more effective on the longest chains as compared to the shortest ones.

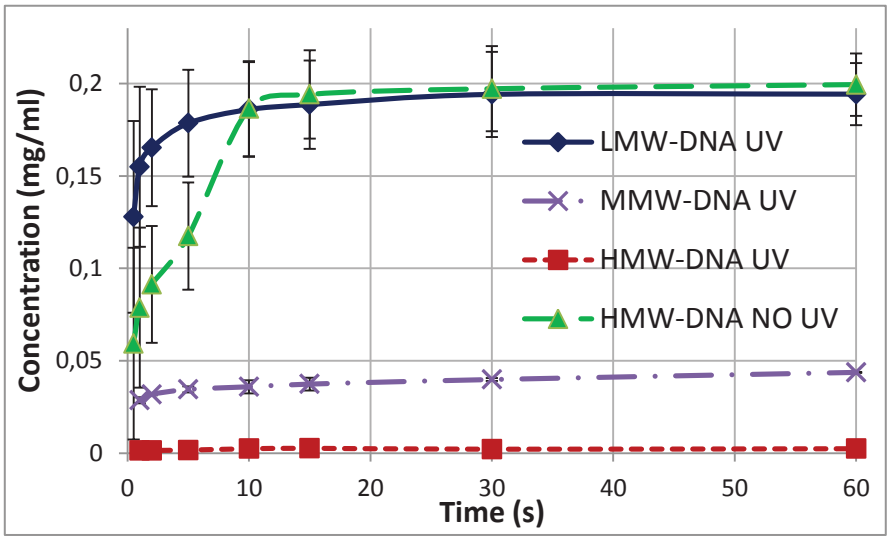


Figure 3.51 - DNA concentration in water during the washing of DNA treated cotton fabrics.

Since the low molecular weight DNA was the most effective in conferring flame retardant features to cotton, as previously described, different combinations of LMW-DNA and HMW-DNA were taken in consideration for the enhancement of washing resistance.

LMW-DNA and HMW-DNA were combined in mixtures or in layers and applied on cotton fabrics with an AO% of about 10:

- Mixtures of LMW-DNA and HMW-DNA in proportion 1:1, 2.5:1 and 4:1 w/w (labelled Mix 1:1, Mix 2.5:1 and Mix 4:1).
- One layer of LMW-DNA with 8% AO covered by one layer of HMW-DNA with 2% AO (labelled Double layer).

For the mixtures, the ratio in DNA quantities was modified on the basis of the behaviour in flammability tests (Table 3.23 and Figure 3.52). in particular, 4:1 ratio between LMW-DNA and HMW-DNA in the solution was necessary to achieve the self-extinction of the samples (Figure 3.52C). In the other cases, the treatment caused a decrease in the burning rate (about 1.20 vs.

1.50 mm/s of untreated cotton) and the formation of a high residue (64 and 49% for 1:1 and 2.5:1 ratios, respectively). HMW-DNA did not show any synergistic effect with LMW-DNA for the enhancement of the flame retardant features. The samples treated with the double layer behaved very similarly to Mix 4:1, showing a burning rate of 1.30 mm/s and a residue of about 80%. In fact, the double layer samples had a 4:1 ratio between LMW-DNA and HMW-DNA, i.e. the same as Mix 4:1 samples.

Table 3.24 – Flammability data of untreated cotton fabrics and cotton treated with LMW-DNA and HMW-DNA in different combinations. (Accuracy ±4%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction	AO(%)
Untreated cotton	66	-	1.50	-	NO	-
Mix 1:1	83	100	1.20	64	NO	10
Mix 2.5:1	85	100	1.17	49	NO	10
Mix 4:1	58	76	1.30	78	YES	10
Double layer	55	71	1.30	81	YES	10

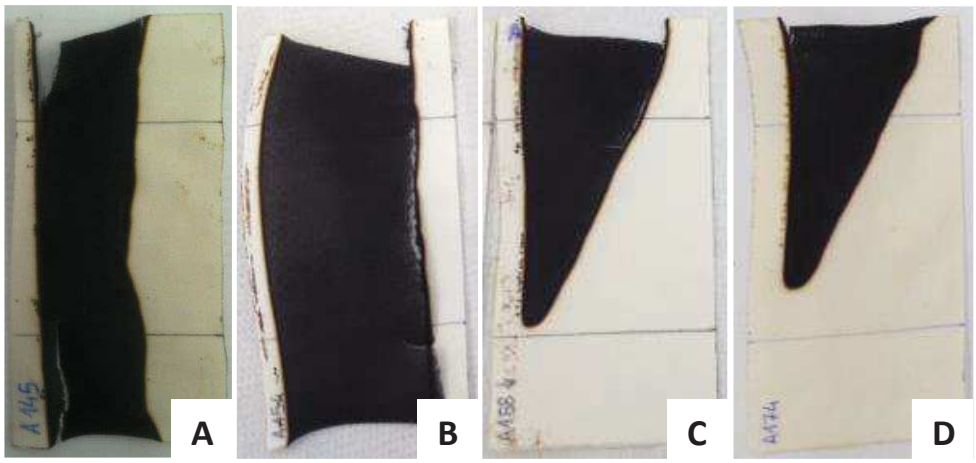


Figure 3.52 – Residues after flame spread tests in horizontal configuration of cotton treated with mixture of LMW-DNA and HMW-DNA in different proportions: A) 1:1 w/w; B) 2.5:1 w/w; C) 4:1 w/w. D) cotton treated with double layer.

The washing tests showed that the presence of HMW-DNA in the mixtures did not improve the possibility of fixing LMW-DNA (Figure 3.53). The DNA release increased as the amount of LMW-DNA in the mixture increased. On the basis of the final concentrations of DNA in water, it was possible to verify that almost all the LMW-DNA on the fabrics was released. A slightly better behaviour was obtained with the Double layer samples: in this case, about 10% of LMW-DNA was blocked on the fabric.

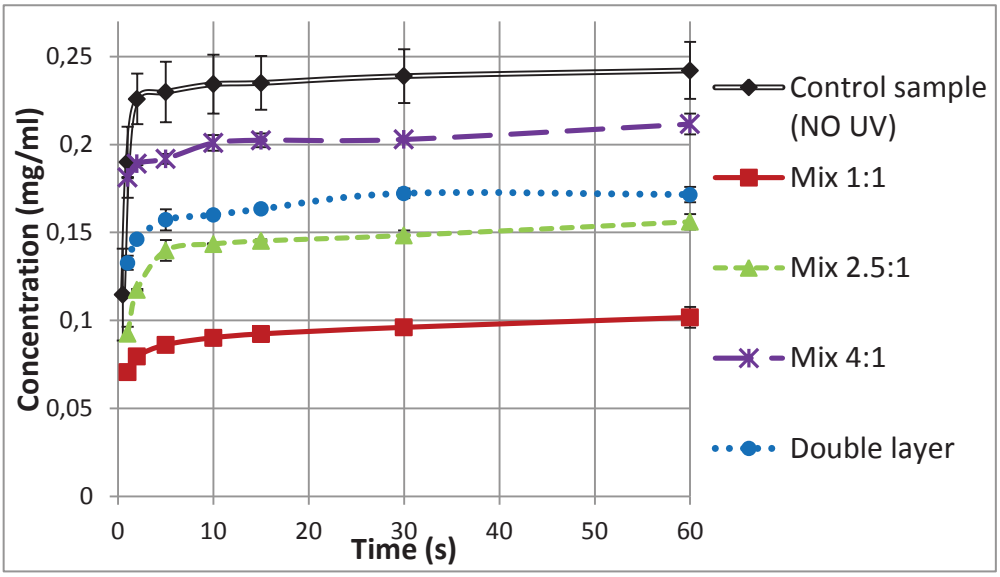


Figure 3.53 - DNA concentration in water during the washing test of fabrics treated with LMW-DNA and HMW-DNA.

Since the improvement of washing resistance of LMW-DNA with the addition of HMW-DNA was not significant enough, other solutions were taken into consideration.

3.4.2 DNA and chitosan

Chitosan is a polysaccharide derived from the deacetylation of chitin, which is easily recovered from crustacean shells from food industry. It is widely known for its high chemical reactivity, biocompatibility and antimicrobial activity, which justify several applications of this biomacromolecule.

Recently, it was demonstrated that chitosan can be grafted to cotton through a radical cross-linking reaction that can be triggered by UV radiation, in the presence of a suitable photoinitiator [212]. This treatment made the finishing resistant to several washing cycles.

With the purpose of improving the washing resistance of the LMW-DNA flame retardant treatment, a combination with chitosan was investigated.

The obtained results were recently published in a paper in 2016 [213].

Several combinations of DNA and chitosan were considered:

- DNA and chitosan were deposited in two separate layers on the fabric, with chitosan over DNA and vice versa;
- DNA and chitosan were mixed together and then applied on fabrics;
- DNA and chitosan were applied following the layer by layer (LbL) method with 30 bilayers and with DNA as final layer in one case and chitosan as final layer in the other one.

The DNA and chitosan combinations are summarized in Table 3.24.

In addition, two different substrates were considered: first, a glass slide was used as a model substrate for assessing the washing fastness. Then, cotton fabrics were treated and subjected to

washing and flammability tests, besides other characterisation analyses such as SEM, FT-IR and antimicrobial tests. For all the fabric samples, an add-on of 8% for DNA and 2% for chitosan was achieved, with the exception of LbL samples, where the total AO% was about 14.

Table 3.25 – DNA-chitosan prepared samples. Adapted from [213].

Sample	Substrate	First layer (in contact with substrate)	Last layer	pH of DNA solution	pH of Chitosan solution
GLASS-CHI-DNApH4	Glass slide	Chitosan	DNA	4.0	4
GLASS-DNApH4-CHI		DNA	Chitosan	4.0	4
Mix DNApH4-CHI		Mixture Chitosan-DNA	-	4.0	4
GLASS-CHI-DNApH8		Chitosan	DNA	8.0	4
GLASS-DNApH8-CHI		DNA	Chitosan	8.0	4
COT-CHI-DNApH4	Cotton fabric	Chitosan	DNA	4.0	4
COT-DNApH4-CHI		DNA	Chitosan	4.0	4
Mix DNApH4-CHI		Mixture Chitosan-DNA	-	4.0	4
LbL-DNA		Chitosan	DNA	7.0	3.5
LbL-CHI		Chitosan	Chitosan	7.0	3.5

3.4.2.1 Washing resistance

The washing resistance was firstly evaluated with glass slide samples. In this case, two pH values of the DNA solutions were considered, namely pH 4 and 8; the chitosan solution was prepared at pH 4 only. From the results of DNA concentration in water during washing (Figure 3.54), it was possible to observe that, using the DNA solution at pH 4, the achieved washing fastness was higher in comparison to the solution at pH 8. Furthermore, the mixture of chitosan and DNA turned out to be the most effective as far as the washing fastness is concerned.

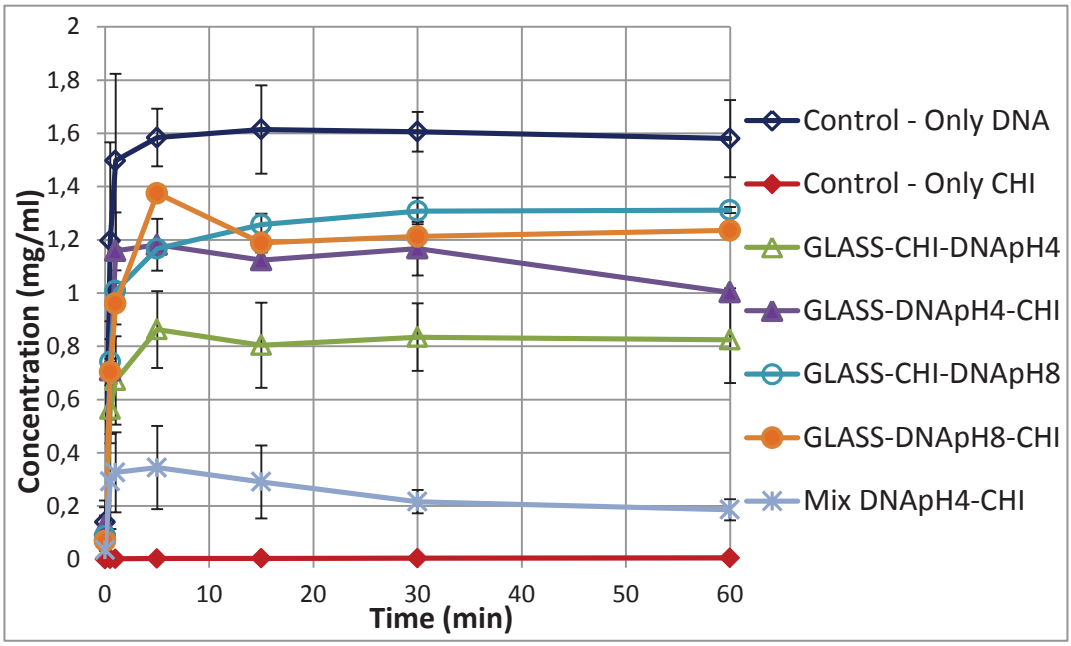


Figure 3.54 – DNA concentration in water during the washing tests performed on glass slides coated with DNA and chitosan. Adapted from [213].

Films of chitosan and DNA at pH 4 and the mixture of chitosan and DNA were investigated by means of FTIR along with a film of the mixture after UV-curing (Figure 3.55), in order to investigate the possible interactions between DNA and chitosan.

Chitosan typical spectra show bands at 3360 cm^{-1} (OH groups), at 3290 cm^{-1} (NH stretching), at 1648 cm^{-1} (C=O) and at 1560 cm^{-1} and 1075 cm^{-1} (NH bending in amide group) [214]. DNA is characterised by bands at 1715 cm^{-1} (guanine/thymine carbonyl stretch vibrations), 1328 cm^{-1} (thymine aromatic amine stretch vibrations), at 1090 cm^{-1} and at 1223 cm^{-1} (symmetric and asymmetric phosphate stretch, respectively) and at 970 cm^{-1} (coupled sugar-phosphodiester) [205].

In the DNA spectrum, the peaks due to phosphates are well defined. However, when chitosan is added to DNA, these peaks cannot be identified, either before or after the UV-curing. This modification in the spectra could be associated to an interaction occurring between the phosphate groups of DNA and the amino groups of chitosan.

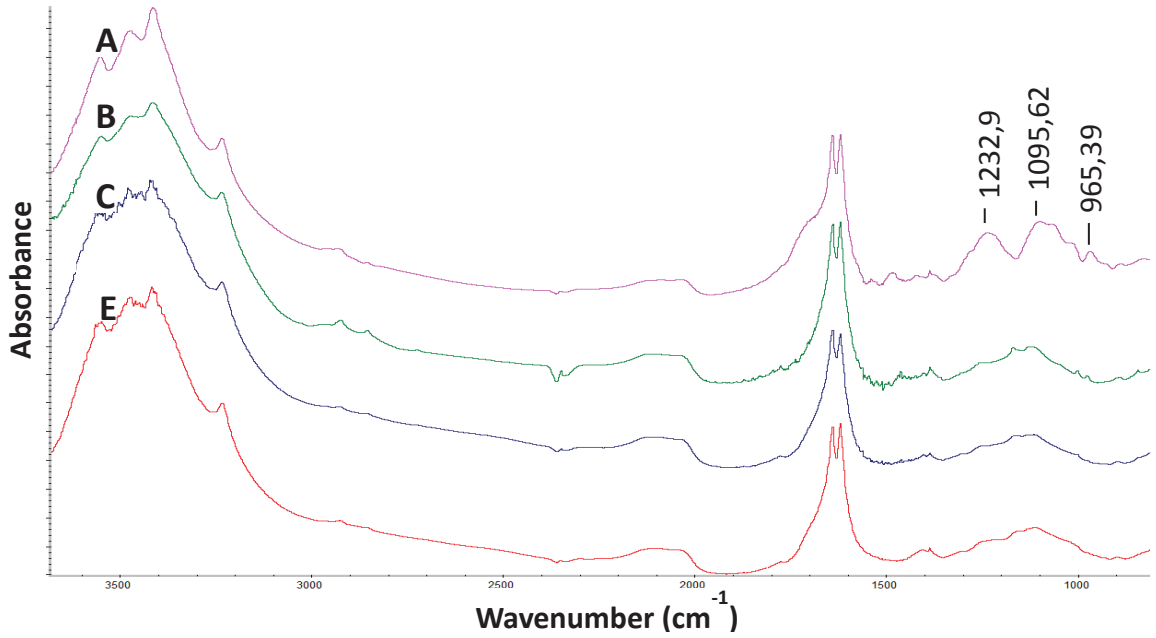


Figure 3.55 - FTIR spectra of chitosan and DNA samples: A) DNA; B) chitosan; C) UV-cured mixture chitosan-DNA; D) mixture chitosan-DNA. Adapted from [213].

Pursuing this investigation, fabric samples were treated with only the DNA solution at pH 4, while the different application methods were all maintained because of the possible interactions of the two biomacromolecules with cotton. In addition, the layer by layer technique was applied to the fabric system.

The results obtained from the washing fastness assessment are shown in Figure 3.56. Fabrics treated with DNA only release almost all the coating in the first 10 min and completed the release within 30 min. The samples with the DNA over the chitosan layer show the lowest washing fastness, with almost 95% of DNA release. The samples with reverse coating combination, with a chitosan layer over a DNA layer, are more stable and half the amount of DNA was blocked on the fabric by the chitosan grafting, which, at the same time, is partially released because of the presence of DNA. The most water resistant systems are the LbL assemblies and the mixture of DNA and chitosan. With the mixture, less than 20% of DNA is released in water, while with the LbL a negligible release was observed, irrespective of the final layer. In this last case, the formation of a series of alternate layers with electrostatic interactions seems to be capable of entrapping the DNA molecules.

The highest standard deviations of Mix DNApH4-CHI samples, in comparison with all the other fabric samples, are probably due to the non-homogeneity of the coating that is responsible for weak interactions between DNA and chitosan molecules.

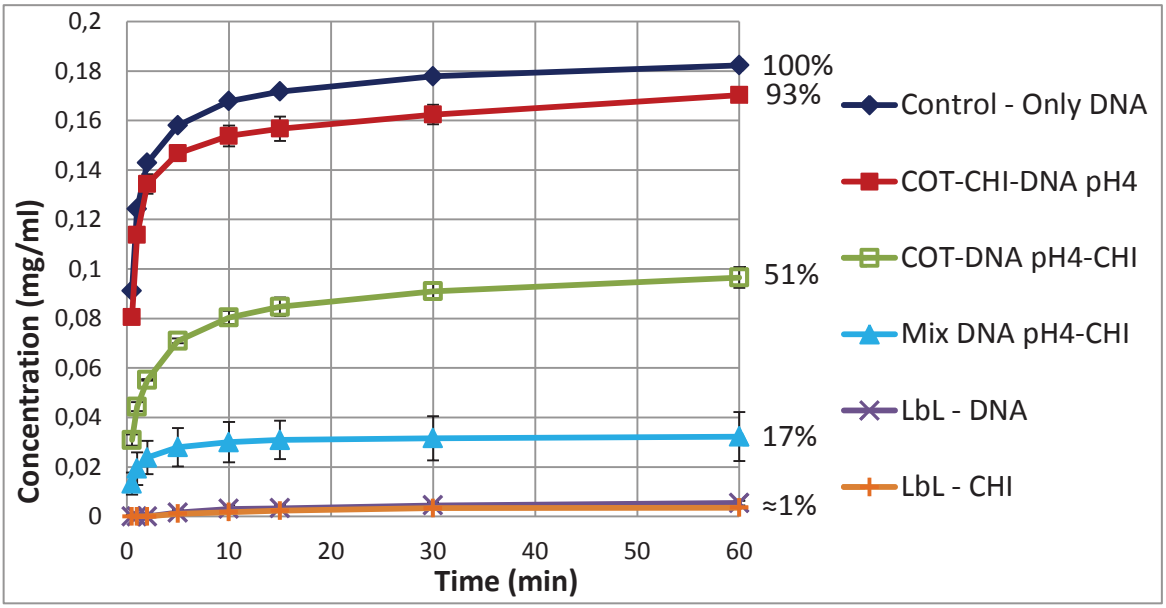


Figure 3.56 – DNA concentration in water during the washing test of fabric samples treated with DNA and chitosan. Adapted from [213].

A confirmation of the samples behaviour in washing tests was obtained with the analysis of the morphology of treated fabrics before and after these tests by means of SEM microscopy (Figure 3.57). In all the cases, the treatment smoothed the fibres surface and, especially in the cases of LbL samples and mixture-treated samples, a homogenous coating was visible on the fibres and filled the gaps in between them (Figure 3.57 C1,D1,E1). After the washing test, a modification in the morphology occurred in the samples treated with the double layers (where a high amount of DNA is released): the fibre surface became rough and irregular (Figure 3.57 A2,B2). In the case of Mix DNApH4-CHI, there was a slight increase in the surface roughness but the coating was still present, also in the fibre interstices (Figure 3.57 C2). Conversely, the morphology of LbL samples did not change and the coating was still covering completely the fibres (Figure 3.57 D2, E2).

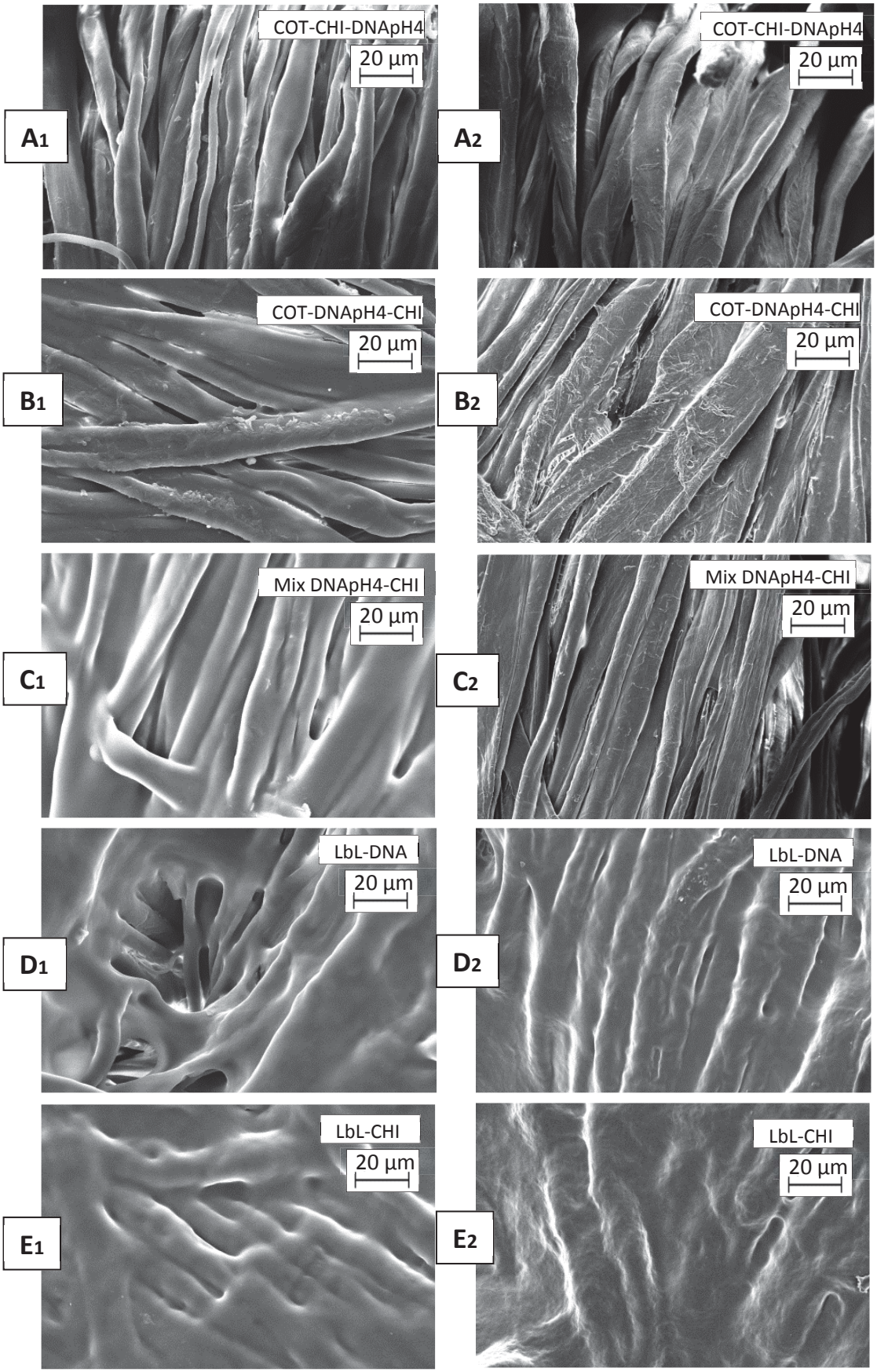


Figure 3.57 - SEM micrographs of samples before(1) and after washing at 55°C(2):A)COT-CHI-DNApH4;B)COT-DNApH4-CHI;C)Mix DNApH4-CHI;D)LbL-CHI;E)LbL-DNA. Adapted from [213].

3.4.1.2 Fire behaviour

Fabric samples treated with DNA and chitosan were subjected to horizontal flame spread tests, before (Table 3.25 and Figure 3.58 and 3.59) and after washing cycle(Table 3.26 and Figure 3.60).

First, cotton fabrics were treated with chitosan alone (8% AO) to verify a possible fire retardant effect. Chitosan caused a decrease in the burning rate (1.12 vs. 1.50 mm/s of cotton), but the treated fabrics burnt completely without leaving a significant residue. A final glowing combustion was also observed.

Before washing, the samples with chitosan covered by a thick layer of DNA were self-extinguishing, with a significant reduction of burning rate (0.98 mm/s) and a high final residue (80%). These samples lost almost all the DNA coating during the washing step. For this reason, they burnt completely in the test performed after washing; however, thay still exhibited some flame retardant features, i.e. a reduction of the burning rate (1.22 mm/s) and the formation of a carbonaceous residue of 39%. The samples with the DNA layer covered by the chitosan layer showed a weak fire retardant behaviour even before washing. In particular, they did not achieve self-extinction, but the burning rate was reduced and a consistent final residue was left; probably the chitosan layer hindered the action of DNA that was not in contact with the atmosphere. The behaviour of these samples did not change significantly after washing, meaning that the presence of half of the DNA did not affect the flame retardant features. A similar result was observed with the mixture of the solutions. In fact, Mix DNApH4-CHI showed a reduction of the burning rate and the formation of a residue of more than 50%, before washing. The residue was less compact and more brittle than that of the other samples, probably due to the lower homogeneity of the coating. A final glowing combustion occurred in some Mix DNApH4-CHI samples, likely owing to the presence of chitosan not in contact with DNA. After washing, the effectiveness of these samples was slightly affected. The best samples were those treated with the LbL technique. They were self-extinguishing both before and after washing and the reduction of the burning rate and the increase of the final residue were significant, irrespective of the type of the final layer. The alternation of thin layers of DNA and chitosan improved the effectiveness of the treatment, since the chitosan could participate as a carbon source in the intumescent process activated by DNA.

Table 3.26 – Flammability data of untreated cotton fabrics and cotton treated with DNA and chitosan in different combinations, before the washing tests. Glowing combustion is in brackets. Adapted from [213]. (Accuracy ±3%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction
Untreated cotton	66	-	1.50	-	NO
COT-CHI-DNApH4	55	54	0.98	80	YES
COT-DNApH4-CHI	82	100	1.22	42	NO
Mix DNApH4-CHI	80 (88)	100	1.25	56	NO
LbL-DNA	23	10	0.44	97	YES
LbL-CHI	44	33	0.75	89	YES
COT-CHI	89 (97)	100	1.12	2	NO

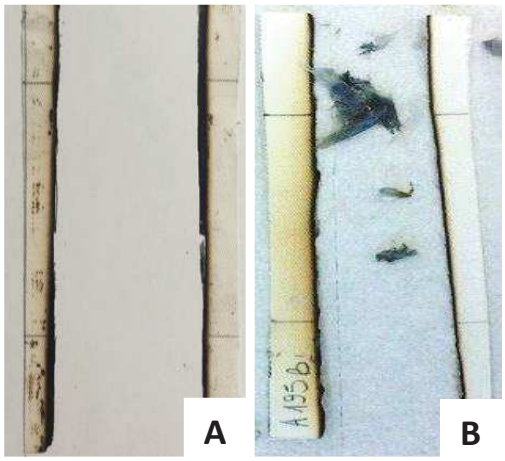


Figure 3.58 – Flame spread tests in horizontal configuration of untreated and cotton treated with chitosan: A) Cotton; B) COT-CHI. Adapted from [213].

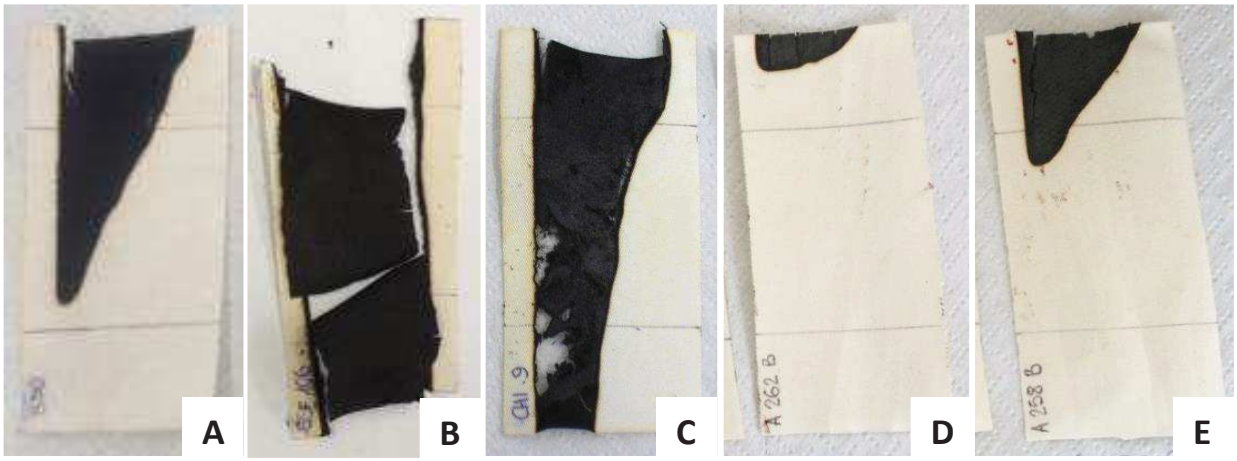


Figure 3.59 - Flame spread tests in horizontal configuration of cotton treated with DNA and chitosan before washing: A)COT-CHI-DNApH4; B)COT-DNApH4-CHI; C)Mix DNApH4-CHI; D)LbL-DNA; E)LbL-CHI. Adapted from [213].

Table 3.27 – Flammability data of untreated cotton fabrics and cotton treated with DNA and chitosan in different combinations, after the washing test. (Accuracy ±3%).

	Total burning time (s)	Char length (mm)	Total burning rate (mm/s)	Residue (%)	Self-extinction
Untreated cotton	66	-	1.50	-	NO
COT-CHI-DNApH4	82	100	1.22	39	NO
COT-DNApH4-CHI	81	100	1.23	40	NO
Mix DNApH4-CHI	78	100	1.28	33	NO
LbL-DNA	75	77	1.03	77	YES
LbL-CHI	39	38	0.96	86	YES

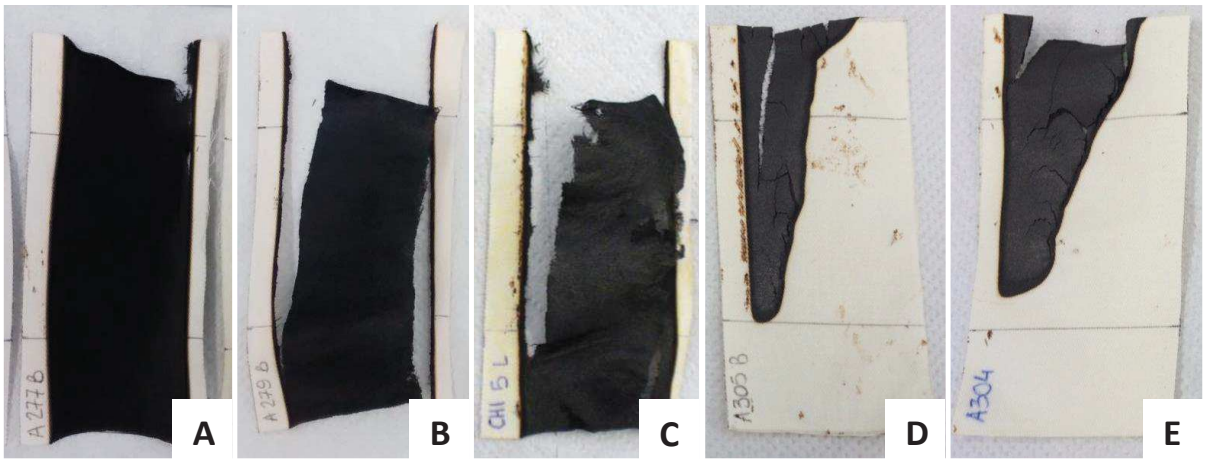


Figure 3.60 – Flame spread tests in horizontal configuration of cotton treated with DNA and chitosan after washing: A)COT-CHI-DNApH4; B)COT-DNApH4-CHI; C)Mix DNApH4-CHI; D)LbL-DNA; E)LbL-CHI.

The investigation of the morphology of fabrics after flammability tests confirmed the intumescent behaviour of DNA and the formation of a stable char during combustion (Figure 3.61). In all the samples, the fabric texture was still perfectly visible and, especially with the Mix DNApH4-CHI and the LbL samples, the coating was still intact and completely covering the fibres (Figure 3.61 C, D and E). Bubbles were present on the fibre surface in any case, with different sizes. On the COT-CHI-DNApH4 samples, bubbles were very small (≈0.1 µm of diameter) and were well spread all over the fibre surface (Figure 3.61 A). On the other samples, there were small and big bubbles, ranging from 0.1 to 10 µm of diameter, with a non-uniform dispersion (Figure 3.61 B). With the mixture, bubbles became bigger (Figure 3.61 C) and with the LbL samples they approached 50 µm diameter, with a homogenous spread on the surface (Figure 3.61 D and E). The presence of chitosan in close contact with DNA enhanced the flame retardant action of this latter.

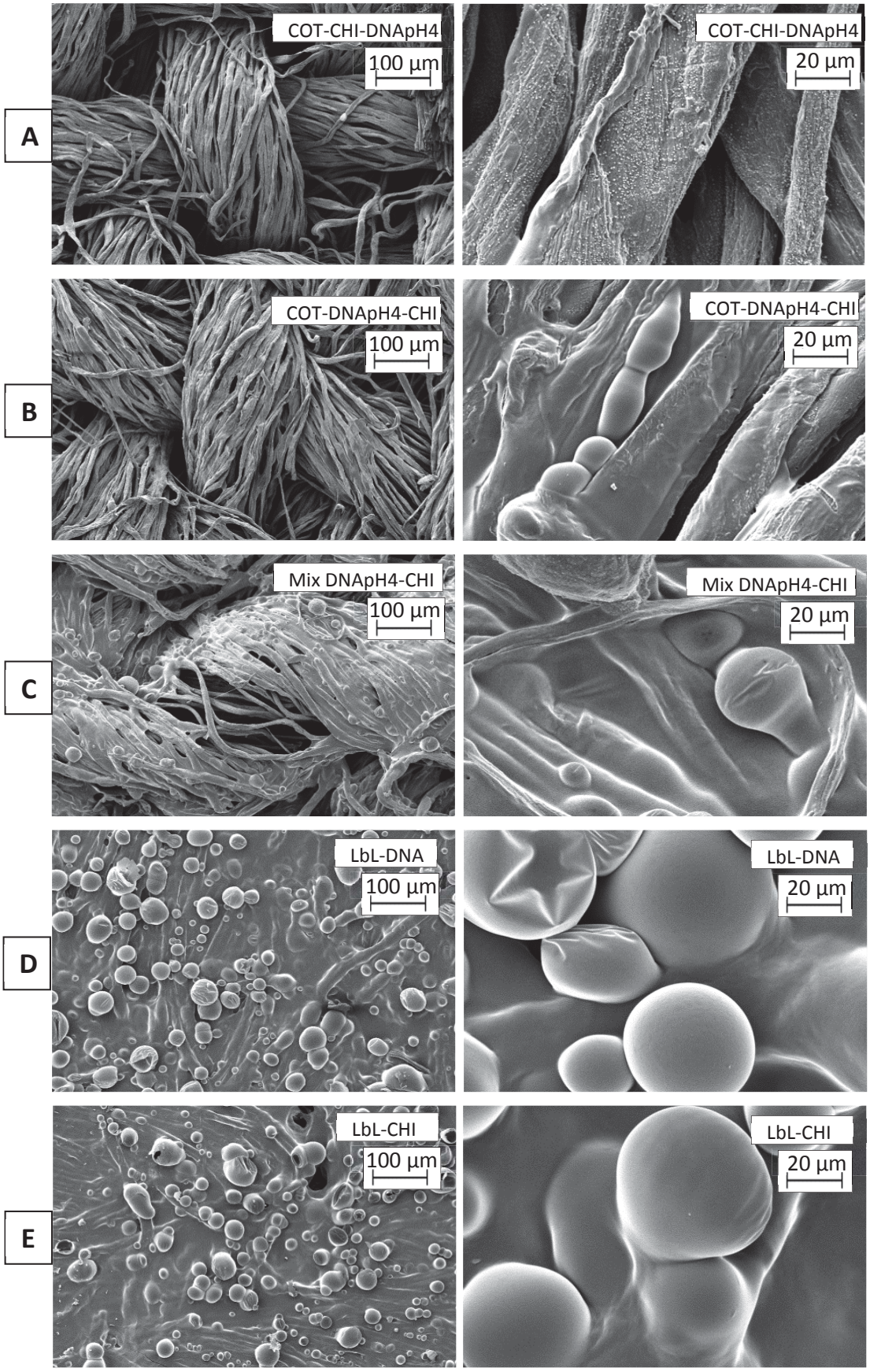


Figure 3.61 - SEM micrographs of fabric samples after flame spread test: A)COT-CHI-DNApH4; B)COT-DNApH4-CHI; C)Mix DNApH4-CHI; D)LbL-CHI; E)LbL-DNA. Adapted from [213].

3.4.2.3 Antimicrobial activity

Chitosan is frequently applied on textiles and other materials to confer antimicrobial activity [215]. This property is associated with the presence of $-NH_3^+$ groups in chitosan chains, which can interfere with the negative charged cell walls and cause the death of microorganisms. In this work, the antimicrobial activity of fabrics treated with DNA and chitosan was assessed against *Staphylococcus aureus* ATCC 6538 according to the ASTM E 2149-01 standard.

The antimicrobial activity was completely absent in the samples with the double layer, regardless of the order of layers (0% microorganism reduction (MR)) (Table 3.27). A slightly higher effect was observed with the mixture, with 6% MR. In these cases, the cationic groups of chitosan were probably hindered or not available because of their interactions with DNA. The best results, concerning the antimicrobial activity, were obtained with the LbL samples, where the chitosan amount was higher than for the other samples. In fact, an MR of about 60% was achieved, irrespective of the final layer.

Table 3.28 – Antibacterial activity against *Staphylococcus aureus* of treated cotton samples. Adapted from [213].

Sample	MR (%)
Untreated cotton	0
COT-CHI-DNApH4	0
COT-DNApH4-CHI	0
Mix DNApH4-CHI	6
LbL-DNA	66
LbL-CHI	57

3.4.3 Caseins and DNA

Finally, some preliminary tests on combinations of LMW-DNA with caseins were carried out. Separately considered, caseins and LMW-DNA turned out to be the most effective biomacromolecules both in flame spread tests and in forced-combustion tests. At the same time, both the treatments had some characteristics that need to be improved. For example, the casein coating strongly affects the flexibility and the comfort of the fabrics, therefore the minimum effective AO% should be preferred. On the other hand, the long smouldering combustion of fabrics with low casein AO% could be a drawback in a fire scenario. As far as DNA is concerned, the high cost could be a big issue for its large scale exploitation. In this context, a reduction in costs, besides the development of a cost-effective and sustainable DNA extraction method from agro-industrial wastes, could be achieved in combination with another effective and cheaper biomacromolecule that could also provide a synergistic effect.

For these reasons, LMW-DNA was applied on cotton fabrics with caseins, with different impregnation sequences. Then, the prepared samples were subjected to preliminary flame spread tests.

Two solutions of caseins and DNA were prepared, with different compositions, namely containing 2.5 wt.% of caseins and 0.5 wt.% of DNA (solution hereinafter coded as CAS-DNA_1), and vice-versa (solution hereinafter coded as CAS-DNA_2). The pH of these solutions was about 12.

In a second set, two different solutions, namely a casein solution at 0.5 wt.% and a DNA solution at 2.5 wt.%, were deposited on fabrics in a three layer assembly (consisting of two casein layers with an interposed DNA layer). A DNA solution at pH 4 and one at pH 8 were tested in this latter case.

For all the prepared samples, 8 wt.% AO was reached.

A comparison of the results of flammability tests from fabric samples treated with caseins and DNA alone and with DNA and caseins together is presented in Table 3.28, Figures 3.62 and 3.63. In all the cases, self-extinction was achieved. The main difference between the samples with DNA and caseins and the samples treated with low molecular weight DNA only was the absence of smouldering in these latter and, therefore, the formation of a substantial black residue, which was not consumed.

The presence of DNA in combination with caseins turned out to enhance the flame retardant features of treated cotton with respect to the casein treatment alone. In fact, the flaming time was halved and the smouldering times was significantly reduced (in between 22-46%), with the shortest time for the 3-layered sample with DNA at pH 4. For the samples treated with the solutions of caseins and DNA together, an increased quantity of DNA improved the results in terms of final residue and smouldering time (307 vs. 325 s smouldering and 74 vs. 68% residue, respectively). Among the samples treated with the three layers, the most effective were those where the DNA solution at pH 4 was applied. These last were also the most performing in general, with a final residue of 85%, a flaming time of 10 s and a smouldering time of 224 s.

From an overall point of view, as already mentioned, both the biomacromolecules exploit the same fire retardant mechanism (mainly in condensed phase). Therefore, the obtained preliminary results, though far from being optimised, seem to indicate that a combination of the two biomacromolecules deserves further investigation.

Table 3.29 – Flammability data of untreated cotton fabrics, cotton treated with caseins and DNA alone and cotton treated with DNA and caseins in different combinations. (Accuracy ±4%).

	Total flaming time (s) (Total flaming + smouldering time (s))	Char length (mm)	Total flaming + smouldering rate (mm/s)	Residue (%)	Self-extinction
Untreated cotton	66	-	1.50 (NO smouldering)	-	NO
LMW-DNA pH4	57	68	1.20 (NO smouldering)	76	YES
LMW-DNA pH8	52	68	1.30 (NO smouldering)	60	YES
CAS-Cotton_8 wt.%	21 (418)	90	0.22	50	YES
CAS-DNA_1	11 (325)	65	0.20	68	YES
CAS-DNA_2	10 (307)	69	0.22	74	YES
3-layered (DNA pH4)	10 (224)	48	0.21	85	YES
3-layered (DNA pH8)	10 (283)	66	0.23	75	YES

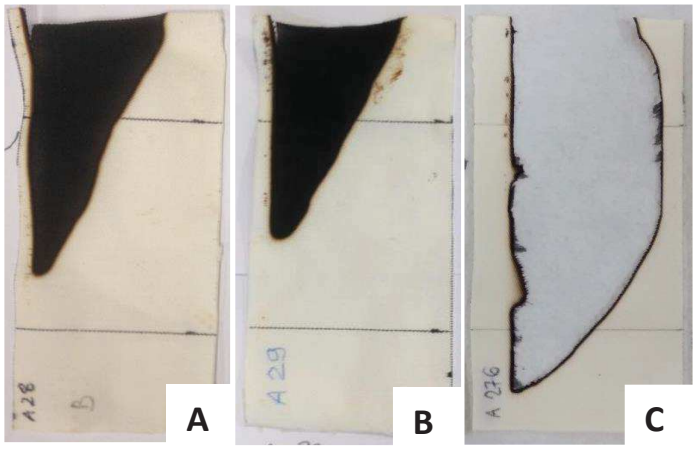


Figure 3.62 – Flame spread tests in horizontal configuration of cotton treated with DNA and caseins alone: A) LMW-DNA pH4; B) LMW-DNA pH8; C) CAS-Cotton_8 wt.%;

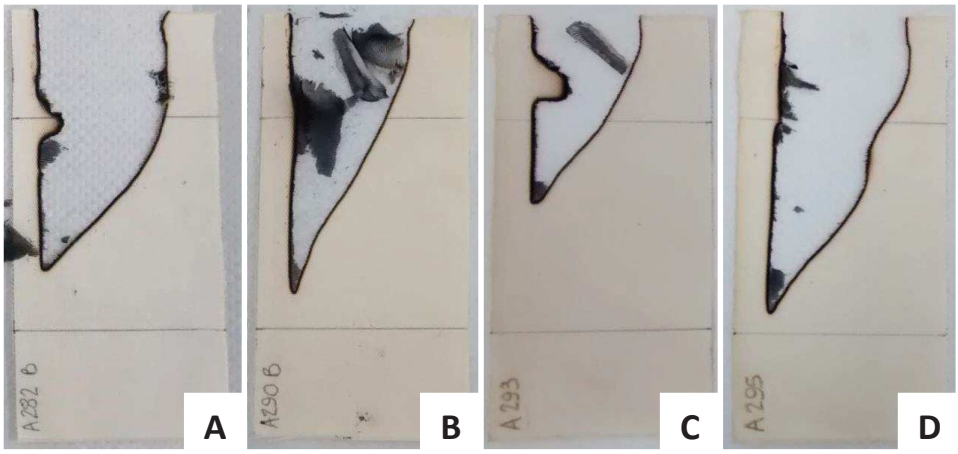


Figure 3.63 – Flame spread tests in horizontal configuration of cotton treated with DNA and caseins in different combinations: A) CAS-DNA_1; B) CAS-DNA_2; C) 3-layered (DNA pH4); D) 3-layered (DNA pH8).

3.5 Biomacromolecules vs. Proban®

Microscale combustion calorimeter, which is an apparatus frequently used to compare the effectiveness of flame retardant treatments, was exploited for comparing the effect of the selected biomacromolecules with that of Proban®, a traditional flame retardant for cotton fabrics. Table 3.1 and Figure 3.64 show the preliminary obtained results. First, the casein coatings behave quite similarly to Proban®, showing comparable values of peak HRR and T peak HRR. Peak HRR is even lower for the fabrics treated with the casein coating with respect to the Proban® treatment (80 vs. 96 W/g).

The peak obtained with the Proban® treatment is very sharp and determines a quite low total heat release (2 kJ/g), unlike the treatments with caseins or DNA (about 5.3 kJ/g). However, this latter total heat release is still half of that of cotton (10.1 kJ/g). This result is further supported by the obtained final residues (40% for Proban®, 28.5% for caseins and 25% for DNA).

Finally, WPI coatings show total heat release and peak HRR values comparable to untreated cotton, hence revealing very low fire retardant performances in MCC tests.

Table 3.30 - MCC results for untreated cotton, cotton fabrics coated with biomacromolecules and cotton treated with Proban®.

	peak HRR (W/g)	T peak HRR (°C)	Total HR (kJ/g)	Residue (%)
Cotton	257 ± 10	396 ± 2	10.1 ± 0.6	10.3 ± 0.5
WPI-cotton	206 ± 12	392 ± 2	10.2 ± 0.3	15.7 ± 2.1
CAS-cotton	80 ± 3	331 ± 2	5.1 ± 0.3	28.5 ± 1.2
LMW-DNApH4-Cotton	151 ± 2	350 ± 2	5.5 ± 0.3	25.0 ± 0.3
Proban®-Cotton	96 ± 3	321 ± 3	2.0 ± 0.1	40.2 ± 0.7

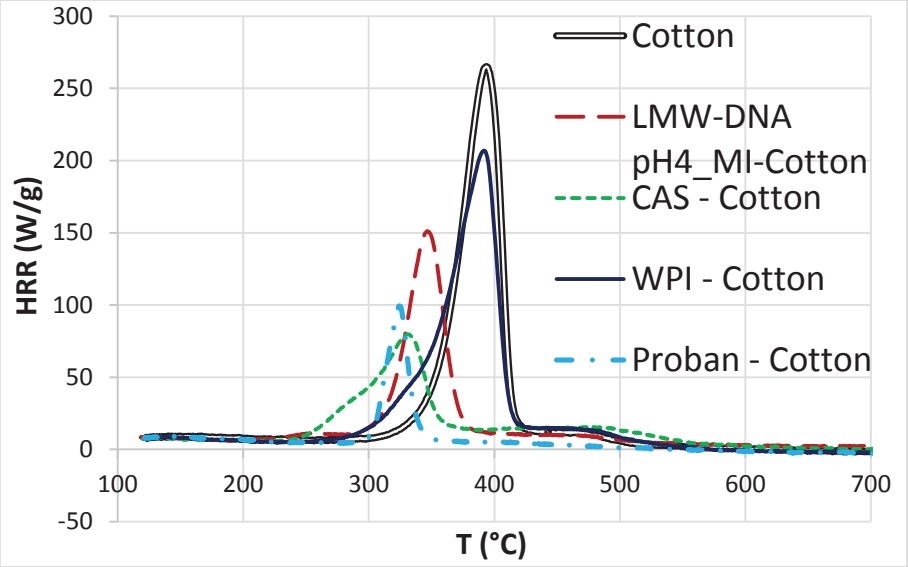


Figure 3.64 - Heat release rate vs. temperature of untreated cotton, cotton fabrics coated with biomacromolecules and cotton treated with Proban®, obtained by MCC.

4. CONCLUSIONS and FUTURE PERSPECTIVES

In this work, an alternative solution to traditional flame retardants was thoroughly studied. In particular, the application of some biomacromolecules as flame retardants for cotton fabrics was investigated.

The selected biomacromolecules belonged to two groups of milk proteins, i.e. caseins and whey proteins, and to DNA. Water solutions were prepared and applied on cotton fabrics with different techniques (impregnation-exhaustion and LbL), aiming at providing flame retardant features. 8% AO was considered for DNA-treated fabrics, 20% for casein-treated fabrics and about 22% for WPI-treated fabrics.

As far as the effectiveness of the treatments is concerned, all the tested biomacromolecules were able to confer flame retardant features to cotton fabrics. However, the low-molecular-weight DNA and the casein coatings turned out to be the most effective in facing the exposure to a flame or to a heat flux. In particular, the low-molecular-weight DNA solution prepared at pH 4 or 8 and applied on cotton with multiple impregnation steps was the most performing among the designed DNA treatments. Both the DNA and the casein coatings were able to provide self-extinction to cotton fabrics in horizontal flame spread tests, with a reduction of the burning rate (-20 and -27% for LMW-DNA and caseins, respectively) and an increase in the final residue (76 and 94%, respectively). Similar reductions in pkHRR and THR were also observed in cone calorimetry tests. Although the casein treatments caused a decrease of almost 50% in TTI, pkHRR was reduced by 44%, similarly to LMW-DNA treatments (-41%), which had almost the same TTI as cotton. Furthermore, caseins and DNA were applied to cotton either in a mixed solution or as consecutive layers. The treated fabrics were subjected to preliminary horizontal flame spread tests. The presence of DNA in combination with caseins enhanced the flame retardant features of cotton with respect to the casein treatment alone. In particular, the flaming time was halved and the smouldering times were reduced, with the best performances for the 3-layered sample with DNA at pH 4. At the same time this designed treatments allowed reducing the DNA content in the flame retardant formulation, hence addressing the use of these flame retardants towards a cost-effective approach.

From the obtained results some hypotheses on the fire retardant mechanism of these biomacromolecules can be made. DNA shows the typical features of intumescent flame retardants: the onset of the degradation is shifted towards lower temperatures compared to the untreated fabric, thus allowing the DNA phosphate groups to decompose and release

phosphoric acid. This species promotes the dehydration of cellulose, with a reduction of combustible volatile species and favouring the formation of a stable char. In addition, the presence of nitrogen is responsible for the swelling of the biomacromolecule coatings and for the formation of bubbles on the fibre surface, as assessed by SEM analyses. As a result, the underlying material can be more effectively protected from heat. In caseins, which contain much lower phosphorous with respect to DNA (i.e. about a tenth), the formation of char is still promoted, but this latter is less stable than that of DNA and is more easily oxidised, as revealed by the prolonged smouldering phenomena. Conversely, whey proteins do not contain phosphorous and their mechanism of action may be mainly related to a dilution of combustible gases and to a charring tendency of the protein itself.

Despite their interesting fire retardant features, the use of the selected biomacromolecules as sustainable and effective flame retardants is currently limited for several reasons. First of all, up to now, the research on this topic has been focused only on the assessment of the flame retardant properties of the fabrics treated with the biomacromolecules. To the best of the author's knowledge, the toxicity and the possible toxicological effects derived from the use of these FR coatings have not been assessed, yet. This issue should deserve further investigation.

Besides, the low washing fastness of the proposed treatments is an important concern, especially for their potential commercial exploitation. As previously discussed, there are possibilities involving the grafting of some biomacromolecules on the fabric substrate, which deserve further investigation, too. In particular, the combination of the DNA coating with chitosan, which could be grafted to cotton through a UV-curing treatment, led to some encouraging results: the piling up of DNA and chitosan in a layer by layer assembly, up to 30 bilayers, turned out to provide self-extinction to the treated cotton fabrics even after a water washing cycle performed at 55°C for 1 h. Furthermore, the combustion behaviour was similar to that provided by DNA only. The layer by layer treatment was much more effective in comparison to the double layer (i.e. chitosan layer + DNA layer) impregnation. Probably, the alternate deposition of thin layers of DNA and chitosan favoured the intumescent process, since chitosan, in close contact with DNA, could better act as carbon source.

Another key issue refers to the comfort of the fabrics after the treatments with the selected biomacromolecules. In fact, very often, the deposited coatings are responsible for an increase of the stiffness of the fabric substrates, which limits their potential use as flame retarded textiles in clothing applications, hence preferably favouring their utilisation in disposable items, furnishing textiles and home decoration, such as curtains and upholstery.

Another limit, especially in the case of DNA, is the cost of the biomacromolecule, which, as reported in Table 4.1, is currently out of market.

Table 4.1 – Cost of the selected commercial biomacromolecules (2016).

Product	**Cost (€/g)**
Herring sperm DNA	***5*** (Sigma Aldrich)
Herring testes DNA	***107*** (Sigma Aldrich)
Whey protein isolates	***0.05*** (Anderson Research)
Caseins	***0.07 – 0.16*** (Sigma Aldrich)

Conversely, this Ph.D. work has shown some clear advantages coming from the use of the selected biomacromolecules. First of all, the proposed treatments are simple, water based and do not need to use particular reagents or equipment designed on purpose. In fact, from an industrial point of view, it is possible to utilize the already existing finishing plants, hence avoiding further investment costs. The layer by layer approach is the only exception, because its exploitation at a large scale has been only recently considered [84].

Besides, the cost-effectiveness of some of the proposed treatments could be significantly ameliorated by recovering the biomacromolecules from exhausted biomasses or wastes from the agro-food industry: in fact, as clearly shown in the present work, the recovery of the nucleic acids and/or proteins can be carried out exploiting sustainable processes, achieving at the same time purity values similar to the commercial products. Obviously, the recovery methods, also including those here discussed, need to be scaled up at least to a pilot scale, which could pave the way to a possible industrialisation.

In conclusion, the proposed bio-based flame retardants for cotton may represent a new “green” alternative to the current phosphorous-based synthetic products.

5. REFERENCES

1. Brushlinsky, N.; Ahrens, M.; Sokolov, S.; Wagner P. (2016). World Fire Statistics. *Centre of Fire Statistics of CTIF*.

2. U.S. Fire Administration/National Fire Data Center (2016). Fire in the United States 2004-2013.

3. Ahrens, M. (2013). Home structure fires. *NFPA report*.

4. Hall, J. R. Jr. (2013). The smoking-material fire problem. *NFPA report*.

5. Ahrens, M. (2015). Home candle fires. *NFPA report*.

6. Department for Business. (2009). A statistical report to investigate the effectiveness of the Furniture and Furnishings (Fire) (Safety) Regulations 1988. *Innovation and Skills (BIS)*.

7. Sette, J. (2014). Cotton Outlook 2015-20, *International Cotton Advisory Committee*.

8. McCall, E. R.; Jurgens, J. F. (1951). Chemical composition of cotton. *Textile Research Journal*, **21**: 19.

9. Needles, H.L. (1986). Textile fibers, dyes, finishes, and processes: A concise guide. *Noyes Publications*, New Jersey, USA.

10. Shafizadeh, F.; Bradbury, A.G.W. (1979) Thermal degradation of cellulose in air and nitrogen at low temperatures. *Journal of Applied Polymer Science*, **23**: 1431–1442.

11. Price, D.; Horrocks, A.R.; Akalin, M.; Faroq, A.A. (1997) Influence of flame retardants on the mechanism of pyrolysis of cotton (cellulose) fabrics in air. *Journal of Analytical and Applied Pyrolysis*, **40**(41): 511–524.

12. Alongi, J.; Camino, G.; Malucelli, G. (2013). Heating rate effect on char yield from cotton, poly(ethylene terephthalate) and blend fabrics*. Carbohydrate polymers,* **92**: 1327-1334.

13. Cullis, C.F.; Hirschler, M.M.; Townsendm, R.P.; Visanuvimol, V. (1983). The pyrolysis of cellulose under conditions of rapid heating. *Combustion and Flame*, **49**: 235–48.

14. Acera Fernández, J.; Le Moigne, N.; Caro-Bretelle, A.S.; El Hage, R.; Le Duc, A.; Lozachmeur, M.; Bono, P.; Bergeret, A. (2016). Role of flax cell wall components on the microstructure and transverse mechanical behaviour of flax fabrics reinforced epoxy biocomposites. *Industrial Crops and Products*, **85**: 93–108.

15. FAO/ICAC. (2013). World Apparel Fibre Consumption Survey.

16. International Wool Textile Organisation (IWTO). (2015). Market Information.

17. Block, R. J. (1939). The composition of keratins: The amino acid composition of hair, wool, horn, and other eukeratins*. Journal of Biological Chemistry*. **128**: 181-186.

18. Alexander, P.; Hudson, R.F.; Earland, C. (1963). Wool: Its chemistry and physics. London, Chapman and Hall.

19. Benisek, L. (1975). Flame retardance of protein fibers. IN: E. Pearce, *Flame-Retardant Polymeric Materials* (pp. 137-191). Springer US.

20. Horrocks, A.R.; Davies, P.J. (2000). Char formation in flame-retarded wool fibres. Part 1. Effect of intumescent on thermogravimetric behaviour. *Fire and Materials*, **24**: 151-157.

21. International Sericultural Commission - http://inserco.org/en/?q=statistics (Accessed November 2016).

22. Kundu, B.; Kurland, N.E.; Bano, S.; Patra, C.; Engel, F. B.; Yadavalli, V.K.; Kundu, S.C. (2013). Silk proteins for biomedical applications: Bioengineering perspectives, *Progress in Polymer Science*, **39**(2): 251-267.

23. Shimura, K. (1983). Chemical composition and biosynthesis of silk proteins. *Experimentia*, **39**(5): 455–461.

24. Price, D.; Horrocks, A.R. (2010). Polymer degradation and the matching of FR chemistry to degradation. 2nd ed. New York, NY, CRC press.

25. The Fiber Year GmbH. (2016). The Fiber Year 2016.

26. Beyler, C.L.; Hirschler, M.M. (2001). Thermal decomposition of polymers. IN P.J. Di Nenno, *SFPE Handbook of Fire Protection Engineering (3rd Edn)*, (pp. 110-131). NFPA, Quincy, MA.

27. Levchik, S.V.; Weil, E.D.; Lewin, M. (1999). Thermal decomposition of aliphatic nylons. *Polymer International*, **48**: 532–557.

28. Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Montaudo, G. (1989). Primary thermal decomposition processes in aliphatic polyamides. *Polymer Degradation and Stability*, **23**(1): 25-41.

29. Achhammer, B.G.; Reinhart, F.W.; Kline, G.M. (1951). Mechanism of the degradation of polyamides. *Journal of Research of the National Bureau of Standards*, **46**(5): 391-421.

30. Chatfield, D.A.; Einhorn, I.N.; Mickelson, R.W.; Futrell, J. H. (1979). Analysis of the products of thermal decomposition of an aromatic polyamide fabric. *Journal of Polymer Science Part A: Polymer Chemistry*, **17**: 1367–1381.

31. Xue, T.J.; McKinney, M.A.; Wilkie C.A. (1997). The thermal degradation of polyacrylonitrile. *Polymer Degradation and Stability*, **58**(1–2): 193-202.

32. Hall, M.E.; Horrocks, A.R.; Zhang, J. (1994). The flammability of polyacrylonitrile and its copolymers. *Polymer Degradation and Stability*, **44**(3): 379-386.

33. McNeill, I.C.; Bounekhel, M. (1991). Thermal degradation studies of terephthalate polyesters: 1. Poly(alkylene terephthalates). *Polymer Degradation and Stability,* **34**(1): 187-204.

34. Levchik, S.V.; Weil, E.D. (2004). A review on thermal decomposition and combustion of thermoplastic polyesters. Polymers for Advanced Technologies. **15**: 691–700.

35. Horrocks, A.R. (1989). Developments in flame-retarding polyester/cotton blends. *Journal of the Society of Dyers and Colourists*, **105**: 346–349.

36. Horrocks, R. (2001). Textiles. IN A.R. Horrocks and D. Price, *Fire retardant materials*. Woodhead Publishing Limited, Cambridge, UK.

37. NIST/BFRL Fire Research Division. (2009). Fire facts, heat flux, temperature, & thermal response. NIST Publications.

38. Walton, W.D.; Thomas, P.H. (1995). Estimating temperature in compartment fires, IN P. J. DiNenno, C.L. Beyler, R.L.P. Custer, W. D. Walton, *SFPE handbook of fire protection engineering*, 2nd Edition (pp. 134-147). National Fire Protection Association, Quincy, MA.

39. Hull, T.R. (2008). Challenges in fire testing: reaction to fire tests and assessment of fire toxicity. IN A. R. Horrocks and D. Price, *Advances in fire retardant materials*. Woodhead, Cambridge, UK.

40. Gann, R.G.; Bryner, N.P. (2008). Combustion products and their effects on life safety. IN *Fire Protection Handbook*, 20th Edition, (pp 11-34). National Fire Protection Association, Quincy, MA.

41. Blomqvist, P.; Hertzberg, T.; Tuovinen, H.; Arrhenius, K.; Rosell, L. (2007). Detailed determination of smoke gas contents using a small-scale controlled equivalence ratio tube furnace method. *Fire and Materials*, **31**(8): 495-521.

42. Rhodes, J.; Smith, C.; Stec, A.A. (2011). Characterisation of soot particulates from fire retarded and nanocomposite materials, and their toxicological impact. *Polymer Degradation and Stability*, **96**(3): 277–284.

43. Hirschler, M.M. (2013). Safety, health and environmental aspects of flame retardants. IN F. Selcen Kilinc-Balci, *Handbook of flame retardant textiles* (pp. 108-173). Woodhead Publishing, Sawston, UK.

44. Horrocks, A.R. (2011). Flame retardant challenges for textiles and fibres: New chemistry versus innovatory solutions. *Polymer Degradation and Stability*, **96**: 377-392.

45. Hornsby, P. (2007). The application of fire-retardant fillers for use in textile barrier materials. IN S. Duquesne, C. Magniez, G. Camino, *Multifunctional Barriers for Flexible Structure* (pp. 3-22). Springer, US.

46. Krylov, O.V. (1970). Catalysis by Non-Metals. *Academic*, London.

47. McKee, D.W. (1981). The Catalyzed Gasification Reactions of Carbon. IN P.L. Walker, P.A. Thrower, *Chemistry and Physics of Carbon*. Dekker, New York.

48. Weil, E.D.; Levchik, S. V. (2009). Flame Retardants for Plastics and Textiles, Practical Applications. Carl Hanser Verlag, Munich.

49. Lewin, M. (2001). Synergism and catalysis in flame retardancy of polymers. *Polymers for Advanced Technologies*, **12**: 215–222.

50. Horrocks, A.R.; Kandola, B.K.; Davies, P.J.; Zhang, S.; Padbury, S.A. (2005). Developments in flame retardant textiles: A review. *Polymer Degradation and Stability*, **88**(1): 3-12.

51. Benisek, L. (1971). Use of titanium complexes to improve the natural flame retardant of wool. *Journal of the Society of Dyers and Colourists*, **87**: 277–278.

52. Shen, K.K.; Kochesfahani, S.; Jouffret, F. (2008). Zinc borates as multi-functional polymer additives. *Polymers for Advanced Technologies,* **19**: 469–474.

53. Lewin, M.; Weil, E.D. (2001). Mechanisms and modes of action in flame retardancy of polymers. IN A.R. Horrocks, D. Price, *Fire Retardant Materials* (pp.31-68). Woodhead Publishing Limited, Cambridge, UK.

54. Ballistreri, A.; Montaudo, G.; Puglisi, C.; Scamporrino, E.; Vitallini, D. (1983). Intumescent flame retardants for polymers. I. The poly(acrylonitrile)–ammonium polyphosphate–hexabromocyclododecane system. *Journal of Applied Polymer Science*, **28**(5): 1743-1750.

55. Oh, S.G.; Rodriguez, N.M. (1993). In situ electron microscopy studies of the inhibition of graphite oxidation by phosphorus. *Journal of Materials Research*, **8**(11): 2879–88.

56. McKee, D.W.; Spiro, C.L.; lamby, E.J. (1984). The inhibition of graphite oxidation by phosphorus additives. *Carbon*, **22**(3): 285–290.

57. Gibov, K.M.; Shapovalova, L.N.; Zhubanov, B. A. (1986). Movement of destruction products through the carbonized layer upon combustion of polymers. *Fire and Materials*, **10**: 133–135.

58. Bourbigot, S. (2008). Flame retardancy of textiles: New approaches. IN A.R. Horrocks, D. Price, *Advances in Fire Retardant Materials* (pp. 9–40). Woodhead Publishing, Cambridge, UK.

59. Broadbent, A. D. (2001). Basic Principles of Textile Coloration. SDC, Bradford, UK.

60. LeBlanc, R.B. (1997). The Durability of Flame Retardant-treated Fabric. *Textile Chemist and Colorist*, **29**(2): 19-20.

61. Muthu, S.S. (2015). Handbook of sustainable apparel production. CRC Press, Boca Raton.

62. Jinping, G.; Guoqiang, C. (2006). Flame retardancy finish with an organophosphorus retardant on silk fabrics. *Fire and Materials*, **30**(6): 415-424.

63. Horrocks, A.R.; Roberts, D. (1998). Minimization of Formaldehyde Emission. IN Proc. Conf. Ecotextile '98: Sustainable Development. Bolton, Woodhead Publishing Ltd., Cambridge, UK.

64. Weil, E.D.; Levchik, S. (2004) Current Practice and Recent Commercial Developments in Flame Retardancy of Polyamides. *Journal of Fire Sciences*, 22(3): 251-264.

65. Samanta, K.K.; Basak, S.; Chattopadhyay, S. K. (2015) Sustainable Flame-Retardant Finishing of Textiles: Advancement in Technology. IN S.S. Muthu, *Handbook of Sustainable Apparel Production* (pp. 51–76). CRC Press.

66. Hall, M.E.; Zhang, J.; Horrocks, A.R. (1994). The flammability of polyacrylonitrile and its copolymers III. Effect of flame retardants. *Fire and Materials*, **18**: 231–241.

67. Davies, P.J.; Horrocks, A.R.; Alderson, A. (2005). The sensitisation of thermal decomposition of APP by selected metal ions and their potential for improved cotton fabric flame retardancy. *Polymer Degradation and Stability*, **88**(1): 114—122.

68. Lewin, M.; Endo, M. (2003). Catalysis of intumescent flame retardancy of polypropylene by metallic compounds. *Polymers for Advanced Technologies,* **14**: 3-11.

69. Wakelyn, P.J. (2008). Environmentally flame resistant textiles. IN A.R. Horrocks, D. Price, *Advances in Fire Retardant Materials* (pp. 188–212). Woodhead Publishing, Cambridge, UK.

70. Bourbigot S.; Flambard X. (2002) Heat resistance and flammability of high performance fibres: a review. *Fire and Materials*, **26**: 155-168.

71. Iler, R.K. (1966). Multilayers of colloidal particles. *Journal of Colloid and Interface Science*, 21(6): 569-594.

72. Li, Y.-C.; Schulz, J.; Grunlan, J.C. (2009). Polyelectrolyte/nanosilicate thin-film assemblies: influence of pH on growth, mechanical behavior, and flammability. ACS Applied Materials & Interfaces, **1**(10): 2338-2347.

73. Laufer, G.; Carosio, F.; Martinez, R.; Camino, G.; Grunlan, J.C. (2011). Growth and fire resistance of colloidal silica-polyelectrolyte thin film assemblies. *Journal of Colloid and Interface Science,* **356**: 69–77.

74. Carosio, F. Laufer, G. Alongi, J. Camino, G. Grunlan, J.C. (2011). Layer-by-layer assembly of silica-based flame retardant thin film on PET fabric. *Polymer Degradation and Stability*, **96**(5): 745-750.

75. Li, Y.-C.; Schulz, J.; Mannen, S.; Delhom, C.; Condon, B.; Chang, S.; Zammarano, M.; Grunlan, J.C. (2010). Flame retardant behavior of polyelectrolyte-clay thin film assemblies on cotton fabric. ACS Nano, **4**(6): 3325-37.

76. Alongi, J.; Carosio, F.; Frache, A.; Malucelli, G. (2013). Layer by Layer coatings assembled through dipping, vertical or horizontal spray for cotton flame retardancy. *Carbohydrate Polymers,* **92**: 114– 119.

77. Carosio, F.; Alongi, J.; Malucelli, G. (2012). Layer by Layer ammonium polyphosphate-based coatings for flame retardancy of polyester-cotton blends. *Carbohydrate Polymers*, 88(4): 1460-1469.

78. Alongi, J.; Carosio, F.; Malucelli, G. (2012). Layer by Layer complex architectures based on ammonium polyphosphate, chitosan and silica on polyester-cotton blends: flammability and combustion behaviour. *Cellulose*, **19**(3): 1041-1050.

79. Li, Y.-C.; Mannen, S.; Morgan, A. B.; Chang, S.; Yang, Y.-H.; Condon, B.; Grunlan, J.C. (2011). Intumescent All-Polymer Multilayer Nanocoating Capable of Extinguishing Flame on Fabric. *Advanced Materials*, **23**: 3926–3931.

80. Pan, H.; Wang, W.; Pan, Y.; Song, L.; Hu, Y.; Liew, K. M. (2015). Formation of self-extinguishing flame retardant biobased coating on cotton fabrics via Layer-by-Layer assembly of chitin derivatives. *Carbohydrate Polymers*, **115**: 516–524.

81. Carosio, F.; Di Blasio, A.; Alongi, J.; Malucelli G. (2013). Green DNA-based flame retardant coatings assembled through Layer by Layer. *Polymer*, **54**(19): 5148-5153.

82. Carosio F.; Fontaine G.; Alongi J.; Bourbigot S. (2015). Starch-Based Layer by Layer Assembly: Efficient and Sustainable Approach to Cotton Fire Protection. ACS Applied Materials & Interfaces, **7**(22): 12158-12167.

83. Apaydin, K.; Laachachi, A.; Ball, V.; Jimenez, M.; Bourbigot, S.; Toniazzo, V.; Ruch, D. (2013). Polyallylamine-montmorillonite as super flame retardant coating assemblies by layer-by layer deposition on polyamide. *Polymer Degradation and Stability,* **98:** 627-634.

84. Mateos, A.J.; Cain, A.A.; Grunlan, J.C. (2014). Large-scale continuous immersion system for layer-by-layer deposition of flame retardant and conductive nanocoatings on fabric. *Industrial & Engineering Chemistry Research*. **53**(15): 6409–6416.

85. Lam, Y.L.; Kan, C.W.; Yuen, C.W.M. (2011). Effect of zinc oxide on flame retardant finishing of plasma pre-treated cotton fabric, *Cellulose,* **18**(1): 151-165.

86. Tsafack, M.J.; Levalois-Grützmacher J. (2006). Plasma-induced graft-polymerization of flame retardant monomers onto PAN fabrics. *Surface and Coatings Technology*, **200**(11): 3503–3510.

87. Tsafack, M.J.; Levalois-Grützmacher J. (2006). Flame retardancy of cotton textiles by plasma-induced graft-polymerization (PIGP). *Surface and Coatings Technology*, **201**(6): 2599–2610.

88. Kamlangkla, K.; Hodak, S.K.; Levaois-Grützmacher, J. (2011). Multifunctional silk fabrics by means of the plasma induced graft polymerization (PIGP) process. *Surface and Coating Technology*, **205**(13–14): 3755–3762.

89. Alongi, J.; Ciobanu, M.; and Malucelli, G. (2012). Sol–gel treatments on cotton fabrics for improving thermal and flame stability: effect of the structure of the alkoxysilane precursor. *Carbohydrate Polymers*, **87**(1): 627–635.

90. Soares, S.; Camino, G.; Levchik, S. (1998). Effect of metal carboxylates on the thermal decomposition of cellulose. *Polymer Degradation and Stability,* **62**(1): 25–31.

91. Davies, P.J.; Horrocks, A.R.; Alderson, A. (2005). The Sensitisation of Thermal Decomposition of APP by Selected Metal Ions and their Potential for Improved Cotton Fabric Flame Retardancy. *Polymer Degradation and Stability*, **88**(1): 114—122.

92. Tian, C.M.; Guo, H.Z.; Zhang, H.Y. ; Xu, J.Z.; Shi, J.R. (1995). Study on the thermal degradation of cotton cellulose ammonium phosphate and its metal complexes. *Thermochimica Acta*, **253**: 243–251.

93. Cireli, A.; Onar, N.; Ebeoglugil, M.F.; Kayatekin, I.; Kutlu, N.; Culha, O.; Celik E. (2007). Development of flame retardancy properties of new halogen-free phosphorous doped SiO_2 thin films on fabrics. *Journal of Applied Polymer Science*, **105**: 3747–3756.

94. Alongi, J.; Ciobanu, M.; Malucelli, G. (2011). Novel flame retardant finishing systems for cotton fabrics based on phosphorus-containing compounds and silica derived from sol–gel processes. *Carbohydrate Polymers*, **85**: 599–608.

95. Hu, S.; Hu, Y.; Song, L.; Lu, H. (2011). Effect of modified organic–inorganic hybrid materials on thermal properties of cotton fabrics. *Journal of Thermal Analysis and Calorimetry*. **103**: 423-427.

96. Yaman, N. (2009). Preparation and flammability properties of hybrid materials containing phosphorous compounds via sol-gel process. *Fibers and Polymers*, **10**: 413-418.

97. Guido, E.; Alongi, J.; Colleoni, C.; Di Blasio, A.; Carosio, F.; Verelst, M.; Malucelli, G.; Rosace G. (2013). Thermal stability and flame retardancy of polyester fabrics sol–gel treated in the presence of boehmite nanoparticles. *Polymer Degradation and Stability*, 98(9): 1609–1616.

98. Alongi, J.; Malucelli, G. (2013). Thermal stability, flame retardancy and abrasion resistance of cotton and cotton–linen blends treated by sol–gel silica coatings containing alumina micro- or nano-particles. *Polymer Degradation and Stability*, 98(8): 1428–1438.

99. Pongprayoon, T., Yanumet, N., O'Rear, E.A., Alvarez, W.E., Resasco, D.E. (2005). Surface characterization of cotton coated by a thin film of polystyrene with and without cross-linking agent. *Journal of Colloid and Interface Science*, **281**(2): 307-315.

100. Siriviriyanun, A.; O'Rear, E.A.; Yanumet N. (2008). Self-extinguishing cotton fabric with minimal phosphorus deposition. *Cellulose*, **15**: 731–737.

101. Siriviriyanun, A.; O'Rear, E.A.; Yanumet N. (2009). The effect of phosphorus content on the thermal and the burning properties of cotton fabric coated with an ultrathin film of a phosphorus-containing polymer. *Polymer Degradation and Stability*, **94**(4): 558–565.

102. Nehra, S.; Hanumansetty, S.; O'Rear, E.A.; Dahiya, J.B. (2014). Enhancement in flame retardancy of cotton fabric by using surfactant-aided polymerization. *Polymer Degradation and Stability*, **109**: 137–146.

103. Bosco, F.; Carletto, R.A.; Alongi, J.; Marmo, L.; Di Blasio, A.; Malucelli, G. (2013). Thermal stability and flame resistance of cotton fabrics treated with whey proteins. *Carbohydrate Polymers*, **94**: 372-377.

104. Alongi, J.; Carletto, R.A.; Bosco, F.; Carosio, F.; Di Blasio, A.; Cuttica, F.; Antonucci, V.; Giordano, M.; Malucelli, G. (2014). Caseins and hydrophobins as novel green flame retardants for cotton fabrics. *Polymer Degradation And Stability*, **99**: 111-117.

105. Alongi, J.; Carletto, R.A.; Di Blasio, A.; Cuttica, F.; Carosio, F.; Bosco, F.; Malucelli, G. (2013). Intrinsic intumescent-like flame retardant properties of DNA-treated cotton fabrics. *Carbohydrate Polymers*, 96: 296-304.

106. Cheng, X.W.; Guan, J.P.; Tang, R.C.; Liu, K.Q. (2016). Phytic acid as a bio-based phosphorus flame retardant for poly(lactic acid) nonwoven fabric. *Journal of Cleaner Production*, 124: 114–119.

107. Cheng, X.W.; Guan, J.P.; Chen, G.; Yang, X.H.; Tang, R.C. (2016). Adsorption and Flame Retardant Properties of Bio-Based Phytic Acid on Wool Fabric, *Polymers*, **8**(4): 122-140.

108. Basak, S.; Samanta, K.K.; Saxena, S.; Chattopadhyay, S.K.; Narkar, R.; Mahangade, R.; Hadge, G.B. (2015). Flame resistant cellulosic substrate using banana pseudostem sap. *Polish Journal of Chemical Technology*, 17(1): 123-133.

109. Drinker, C.K.; Warren, M.F.; Bennett, G.A. (1937). The problem of possible systemic effects from certain chlorinated hydrocarbons. *Journal of Industrial Hygiene and Toxicology*, **19**: 283–311.

110. Jensen, S. (1966). Report of new chemical hazard. *New Scientist*, **32**: 612.

111. Tsuji, M.; Nakano, T.; Okuno, T. (1987). Measurement of combustion products from liquid PCB waste incinerator. *Chemosphere*, **16**(8–9): 1889-1894.

112. Kemmlein, S.; Herzke, D.; Law, R.J. (2009). Brominated flame retardants in the European chemicals policy of REACH—Regulation and determination in materials. *Journal of Chromatography A*, **1216**(3): 320–333.

113. Darnerud, P.O. (2003). Toxic effects of brominated flame retardants in man and in wildlife. *Environment International*, **29**: 841-853.

114. Staskal Wikoff, D.; Birnbaum, L.; (2011). Human Health Effects of Brominated Flame Retardants. IN E. Eljarrat, D. Barceló, *Brominated Flame Retardants* (pp.19-53). Springer Berlin Heidelberg.

115. Yuan, J.; Chen, L.; Chen, D.; Guo, H.; Bi, X.; Ju, Y.; Jiang, P.; Shi, J.; Li, L.; Jiang, Q.; Sheng, G.; Fu, J.; Wu, T.; Chen, X. (2008). Elevated serum polybrominated diphenyl ethers and thyroid-stimulating hormone associated with lymphocytic micronuclei in Chinese workers from an E-waste dismantling site. *Environmental Science & Technology*, **42**: 2195–2200.

116. Costa, L.G.; Giordano, G. (2007). Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology*, **28**(6): 1047-1067.

117. Meerts, I.A.T.M.; Assink, Y.; Cenijin, P.H.; Weijers, B.M.; Van den Berg, H.H.J. (1999). Distribution of the flame retardant tetrabromobisphenol A in pregnant and fetal rats and effect on thyroid hormone homeostasis. *Brominated Flame Retardants P068/. Organohalogen Compounds*, **40**: 375–378.

118. Van der Ven, L.T.; Ven de Kuil, T.; Verhoef, A.; Verwer, C.M.; Lilienthal, H.; Leonards, P.E. Schauer, U.M.; Cantón, R.F.; Litens, S.; De Jong, F.H.; Visser, T.J.; Dekant, W.; Stern, N.; Håkansson, H.; Slob, W.; Van den Berg, M.; Vos, J.G.; Piersma, A.H. (2008). Endocrine effects of tetrabromobisphenol-A (TBBPA) in Wistar rats as tested in a one-generation reproduction study and a subacute toxicity study. *Toxicology*, **260**(1–3): 150–152.

119. Chengelis, C.P. (2001). A 90-day oral (gavage) toxicity study of HBCD in rats. IN WIL-186012, (pp. 1527). Wil Research Laboratories, Inc., Ashland, Ohio, USA.

120. Van der Ven, L.T.M.; Verhoef, A.; Van de Kuil, T.; Slob, W.; Leonards, P.E.G.; Visser, T.J.; Hamers, T.; Håkansson, H.; Olausson, H.; Piersma, A.H.; Vos, J.G. (2006). A 28-day oral dose toxicity study of hexabromocyclododecane (HBCD) in Wistar rats. Toxicological Sciences, **94**: 281–292.

121. Van der Veen, I.; De Boer, J. (2012). Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere*, **88**: 1119–1153.

122. Wakelyn, P. J. (2008). Environmentally flame resistant textiles. IN A.R. Horrocks, D. Price, *Advances in Fire Retardant Materials* (pp. 188–212). Wood-head Publishing, Cambridge, UK.

123. IARC. (1992). Toxicological Profile for Antimony and compounds. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry.

124. US National Research Council. (2000). Toxicological risks of selected flame-retardant chemicals. Sub-committee on Flame-retardant Chemicals of the United States, National Research Council, Washington, DC; National Academy Press, Washington.

125. U.S. Department of Health and Human Services. (2009). Toxicological profile for formaldehyde. Public Health Service Agency for Toxic Substances and Disease Registry.

126. Malucelli, G.; Bosco, F.; Alongi, J.; Carosio, F.; Di Blasio, A.; Mollea, C.; Cuttica, F.; Casale, A. (2014). Biomacromolecules as novel green flame retardant systems for textiles: an overview. *RSC Advances*, **4**: 16.

127. Walstra, P.; Wouters, J.T.M.; Geurts, T.J. (2005). Dairy Science and Technology, Second Edition. CRC Press.

128. Fox, P. F.; Kelly, A.L. (2004). The caseins. IN R. Yada, *Proteins in Food Processing.* Woodhead Publishing, Cambridge, UK.

129. Huang, J.; Wang, L.; Lin, R.; Wang, A.Y.; Yang, L.; Kuang, M.; Qian, W.; Mao, H. (2013). Casein-Coated Iron Oxide Nanoparticles for High MRI Contrast Enhancement and Efficient Cell Targeting, *ACS Applied Materials & Interfaces*, **5**(11): 4632–4639.

130. Liu, Y.; Liu, L.; Yuan, M.; Guo R. (2013). Preparation and characterization of casein-stabilized gold nanoparticles for catalytic applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **417**: 18–25.

131. Bosworth, A.W.; Van Slyke, L.L. (1914). The Phosphorus Content of Casein. *Journal of Biological Chemistry*, **19**(1): 67-71.

132. Etzel, M.R. (2004). Manufacture and Use of Dairy Protein Fractions. *Journal of Nutrition*, **134**(4): 996S-1002S.

133. Madureira, A.R.; Pereira, C.I.; Gomes, A.M.P.; Pintado, M.E.; Malcata F. X. (2007). Bovine whey proteins – Overview on their main biological properties. *Food Research International,* **40:** 1197–1211.

134. Kinsella, J.E.; Whitehead D.M. (1989). Proteins in whey: chemical, physical, and functional properties., *Advances in Food and Nutrition Research*, **33**: 343-438.

135. de Wit, J.N. (1998). Nutritional and Functional Characteristics of Whey Proteins in Food Products. *Journal of Dairy Science*, **81(**3): 597-608.

136. Campos, C.A.; Gerschenson, L.N.; Flores, S.K. (2011). Development of Edible Films and Coatings with Antimicrobial Activity. *Food and Bioprocess Technology*. **4**: 849–875.

137. Gunasekaran, S.; Ko, S.; Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of Food Engineering*, **83**: 31–40.

138. deWit, J. N.; Klarenbeek, G. (1984). Effects of heat treatments on structure and solubility of whey proteins. Journal of Dairy Science. **67**: 2701-2710.

139. Hegg, P.O. (1980). Thermostability of β-lactoglobulin as a function of pH and concentration of sodium dodecyl sulfate. *Acta Agriculturae Scandinavica*, **30**: 400.

140. Wösten, H.A.B.; Scholtmeijer, K. (2015). Applications of hydrophobins: current state and perspectives, *Applied Microbiology and Biotechnology*, **99**: 1587–1597.

141. de Vocht, M.L.; Reviakine, I.; Wösten, H.A.B.; Brisson, A.; Wessels, J.G.H.; Robillard, G.T. (2000). Structural and Functional Role of the Disulfide Bridges in the Hydrophobin SC3. *The Journal of Biological Chemistry*, **275**(37): 28428-28432.

142. Rea, I.; Giardina, P.; Longobardi, S.; Giocondo M.; De Stefano L. (2011). Organic-inorganic Interfaces for a New Generation of Hybrid Biosensors, IN P.A. Serra. *Biosensors - Emerging Materials and Applications*. InTech.

143. Valo, H.K.; Laaksonen, P.H.; Peltonen L.J.; Linder M.B.; Hirvonen J.T.; Laaksonen T.J. (2010). Multifunctional hydrophobin: toward functional coatings for drug nanoparticles. *ACS Nano*, **4**: 1750–1758.

144. Green, A.J.; Littlejohn, K.A.; Hooley, P.; Cox, P.W. (2013). Formation and stability of food foams and aerated emulsions: Hydrophobins as novel functional ingredients. *Current Opinion in Colloid & Interface Science*, **18**(4): 292–301.

145. Cox, A.R.; Aldred, D.L.; Russell, A.B. (2009). Exceptional stability of food foams using class II hydrophobin HFBII. *Food Hydrocolloids*, **23**: 366–376.

146. Zhang, M., Wang, Z.; Wang, Z.; Feng, S.; Xu, H.; Zhao, Q.; Wang, S., Fang, J.; Qiao, M.; Kong, D. (2011). Immobilization of anti-CD31 antibody on electrospun poly(ε-caprolactone) scaffolds through hydrophobins for specific adhesion of endothelial cells. *Colloids and Surfaces B: Biointerfaces*, **85**: 32–39.

147. Zhao, Z.X.; Wang, H.C.; Qin, X.; Wang, X.S.; Qiao, M.Q.; Anzai, J.; Chen, Q. (2009). Self-assembled filmof hydrophobins on gold surfaces and its application to electrochemical biosensing. *Colloids and Surfaces B: Biointerfaces,* **71**: 102–106.

148. Boeuf, S.; Throm, T.; Gutt, B.; Strunk, T.; Hoffmann, M.; Seebach, E.; Mühlberg, L.; Brocher, J.; Gotterbarm, T.; Wenzel, W.; Fischer, R.; Richter, W. (2012). Engineering hydrophobin DewA to generate surfaces that enhance adhesion of human but not bacterial cells. *Acta Biomaterialia*, **8**: 1037–1047.

149. Weickert, U.; Wiesend, F.; Subkowski, T.; Eickhoff, A.; Reiss, G. (2011). Optimizing biliary stent patency by coating with hydrophobin alone or hydrophobin and antibiotics or heparin: an in vitro proof of principle study. *Advances in Medical Sciences*, **56**: 138–144.

150. Lee, S.; Røn, T.; Pakkanen, K.I.; Linder, M. (2015). Hydrophobins as aqueous lubricant additive for a soft sliding contact. *Colloids and Surfaces B: Biointerfaces*, **125**: 264–269.

151. Yang, W.; Ren, Q.; Wu, Y.-N.; Morris, V.K.; Rey, A.A.; Braet, F.; Kwan, A.H.; Sunde, M. (2013). Surface functionalization of carbon nanomaterials by self-assembling hydrophobin proteins. *Biopolymers*, **99**: 84–94.

152. Liu, J. (2014). DNA-stabilized, fluorescent, metal nanoclusters for biosensor development. *TrAC Trends in Analytical Chemistry*, **58**: 99–111.

153. Zhang, F.; Nangreave, J.; Liu, Y.; Yan, H. (2014). Structural DNA nanotechnology: state of the art and future perspective. *Journal of the American Chemical Society*. **136**(32): 11198–11211.

154. Ruiz-Hernández, E.; Baeza, A.; Vallet-Regí M. (2011) Smart Drug Delivery through DNA/Magnetic Nanoparticle Gates. *ACS Nano*, **5**(2): 1259–1266.

155. Ibraheem, D.; Elaissari, A.; Fessi, H. (2014). Gene therapy and DNA delivery systems. *International Journal of Pharmaceutics*, **459**(1–2): 70–83.

156. van den Beucken, J.J.P.; Walboomers, X.F.; Leeuwenburgh, S.C.G.; Vos, M.R.J.; Sommerdijk, N.A.J.M.; Nolte, R.J.M.; Jansen, J.A. (2007). Multilayered DNA coatings: In vitro bioactivity studies and effects on osteoblast-like cell behaviour. *Acta Biomaterialia,* **3**: 587–596.

157. Jiang, Q.-H.; Liu, L.; Shen, J.-W.; Peel, S.; Yang, G.-L.; Zhao, S.-F.; He, F.-M. (2012). Influence of multilayer rhBMP-2 DNA coating on the proliferation and differentiation of MC3T3-E1 cells seeded on roughed titanium surface. *Journal of Biomedical Materials Research*, **100A**: 2766–2774.

158. Tanahashi, M.; Matsuda, T. (1997). Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid. *Journal of Biomedical Materials Research*, **34**: 305–315.

159. Alongi, J.; Carletto, R.A.; Di Blasio, A.; Carosio, F.; Bosco, F.; Malucelli, G. (2013). DNA: a novel, green, natural flame retardant and suppressant for cotton, Journal of Materials Chemistry A, **1**: 4779-4785.

160. Li, Y.-C.; Kim, Y.S.; Shields, J.R.; Davis, R.D. (2014). DNA-based nanocomposite biocoatings for fire-retarding polyurethane foam. *Green Materials*, **2**(3): 144-152.

161. European Commission. Preparatory Study on Food Waste across EU 27. Technical Report-2010-054. Available online: http://ec.europa.eu/environment/eussd/pdf/bio_foodwaste_report.pdf

162. Arancon, R.A.D.; Lin, C.S.K.; Chan, K.M.; Kwan, T.H.; Luque, R. (2013). Advances on waste valorisation: new horizons for a more sustainable society. *Energy Science and Engineering*, **1**(2): 53–71.

163. Baiano A. (2014). Recovery of Biomolecules from Food Wastes - A Review. *Molecules*, **19**: 14821-14842.

164. Arnesen, J.A.; Gildberg A. (2006). Extraction of muscle proteins and gelatin from cod head. *Process Biochemistry*, **41**: 697-700.

165. Batista, I. (1999). Recovery of proteins from fish waste products by alkaline extraction. *European Food Research and Technology*, **210**: 84-89.

166. Gault, N.F.; Lawrie, R.A. (1980). Efficiency of protein extraction and recovery from meat industry by-products. *Meat Science*. **4**(3): 167-90.

167. Santini, M.P.; Renz, D.; Doerfler, W. (2001). A comparison of methods to extract pure DNA from mammalian intestinal contents and from faeces. *Gene Function & Disease*, **1:** 51-57.

168. Suenaga, E.; Nakamura, H. (2005). Evaluation of three methods for effective extraction of DNA from human hair. *Journal of Chromatography B*, 820: 137–141.

169. Rohland, N.; Hofreiter, M. (2007). Ancient DNA extraction from bones and teeth. Nature Protocols, 2: 1756-1762.

170. Mogg, R.J.; Bond, J.M. (2003). A cheap, reliable and rapid method of extracting high-quality DNA from plants. *Molecular Ecology Notes*, **3**: 666–668.

171. Miller, D.N.; Bryant, J.E.; Madsen, E.L.; Ghiorse, W.C. (1999). Evaluation and Optimization of DNA Extraction and Purification Procedures for Soil and Sediment Samples. *Applied And Environmental Microbiology*, 65(11): 4715–4724.

172. Roose-Amsaleg, C.L.; Garnier-Sillam, E.; Harry, M. (2001). Extraction and purification of microbial DNA from soil and sediment samples. *Applied Soil Ecology,* **18**: 47–60.

173. Leff, L.G.; Dana, J.R.; Mcarthur, J.V.; Shimkets, L.J. (1995). Comparison of Methods of DNA Extraction from Stream Sediments. *Applied And Environmental Microbiology*, **61**(3): 1141–1143.

174. Harju, S.; Fedosyuk H.; Peterson K.R. (2004). Rapid isolation of yeast genomic DNA: Bust n' Grab. *BMC Biotechnology*, **4**: 8-13.

175. Hanna, M.; Xiao, W. (2006). Isolation of Nucleic Acids. *Methods in Molecular Biology*, **313**: 15-20.

176. Cheng, Y.-J.; Guo, W.-W.; Yi, H.-L.; Pang X.-M.; Deng, X. (2003). An Efficient Protocol for Genomic DNA Extraction From Citrus Species. *Plant Molecular Biology Reporter,* **21**: 177–178.

177. De Ungria, M.C.A.; Tillett, D.; Neilan, B.A.; Cox, P.T.; Lee, A. (1998). A Novel Method of Extracting Plasmid DNA from Helicobacter Species. *Helicobacter*, **3**: 269–277.

178. Edge-Garza, D.A.; Rowland Jr., T.V.; Haendiges, S.; Peace, C. (2014). A high-throughput and cost-efficient DNA extraction protocol for the tree fruit crops of apple, sweet cherry, and peach relying on silica beads during tissue sampling. *Molecular Breeding*, **34**: 2225–2228.

179. Harry, M.; Gambier, B.; Bourezgui, Y.; Garnier-Sillam, E. (1999). Evaluation of purification procedures for DNA extracted from organic rich samples: interference with humic substances. *Analysis,* **27**: 439–442.

180. Kuske, C.R.; Banton, K.L.; Adorada, D.L.; Stark, P.C.; Hill, K.K.; Jackson, P.J. (1998). Small-scale DNA sample preparation method for field PCR detection of microbial cells and spores in soil. *Applied and Environmental Microbiology*, **64**(7): 2463-2472.

181. Horrocks, A.R.; Price, D.; Tunc, D. (1989) The burning behaviour of textiles and its assessment by oxygen index methods. *Textile Progress*, **18**(1–3): 1–205.

182. Huggett, C. (1980). Estimation of Rate of Heat Release by Means of Oxygen Consumption Measurements. *Fire and Materials*, **4**: 61-65.

183. Nazare, S.; Kandola, B.; Horrocks, A.R. (2002). Use of Cone Calorimetry to Quantify the Burning Hazard of Apparel Fabrics. *Fire and Materials*, **26**: 191–199.

184. NIST (National institute of Standards and Technology) web site - https://www.nist.gov/laboratories/tools-instruments/cone-calorimeter (December 2016).

185. Purser, D.A. (2016). Toxic Combustion Product Yields as a Function of Equivalence Ratio and Flame Retardants in Under-Ventilated Fires: Bench-Large-Scale Comparisons. Polymers, 8(9), 330.

186. Bosco, F.; Casale, A.; Mollea, C.; Terlizzi, M.E.; Gribaudo, G.; Alongi, J.; Malucelli, G. (2015). DNA coatings on cotton fabrics: effect of molecular size and pH on flame retardancy. *Surface & Coatings Technology*, **272**: 86-95.

187. Murray, M. G.; Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, **8**(19): 4321–4325.

188. Choudhary, K.; Mathur, N.; Choudhary O.P.; Pillai, U. (2008). Protocol for isolation of genomic DNA from dry and fresh leaves of vigna species suitable for RAPD and restriction digestion. *Advances in Biological Research*, **2**(5-6): 83-89.

189. Mathew, K.M.; Jose, S.; Rao, Y.S.; Gupta, U.; Thomas, J. (2008). Optimization of genomic DNA extraction from fresh and dry leaves of large cardamom for diversity analysis. *Indian Journal of Biotechnology*, **13**: 221-224.

190. Waldron, C., Lacroute, F. (1975). Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *Journal of Bacteriology*, **122**(3): 855-65.

191. Hatti-Kaul, R.; Mattiasson B. (2003). Isolation and purification of proteins. Marcel Dekker, New York, USA.

192. Barbas, C.F. III; Burton, D.R.; Scott, J.K.; Silverman, G.J. (2001). Phage display: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

193. Parkkinen, E.; Oura, E.; Suomalainen, H. (1974). Effect of storage on the nucleic acid composition of baker's yeast. *Journal of the Institute of Brewing*, **80**: 271–277.

194. Warburg, O.; Christian, W. (1942). Isolation and crystallization of enolase. *Biochemische Zeitschrift*, **310:** 384-421.

195. Tata, J.; Alongi, J.; Carosio, F.; Frache, A. (2011). Optimization of the procedure to burn textile fabrics by cone calorimeter: part I. Combustion behavior of polyester. *Fire and Materials,* **35**(6): 397–409.

196. Nazaré, S.; Kandola, B.; Horrocks, A.R. (2002). Use of cone calorimetry to quantify the burning hazard of apparel fabrics. *Fire and Materials*, **26**: 191–199.

197. Alongi, J.; Malucelli, G. (2015). Thermal degradation of cellulose and cellulosic substrates. In: A. Tiwari and B. Raj, *Reactions and Mechanisms. Thermal Analysis of Advanced Materials* (pp.301–332). Wiley, Beverly, MA.

198. Babrauskas, V. (1995). The generation of CO in bench-scale fire tests and the prediction for real-scale fires. *Fire and Materials*, **19**: 205–213.

199. Sherbet, G. V.; Lakshmi, M. S.; Cajone, F. (1983) Isoelectric characteristics and the secondary structure of some nucleic acids. *Biophysics of structure and mechanism,* **10**: 121-128.

200. Wyatt, G. R. (1951) The purine and pyrimidine composition of deoxypentose nucleic acids. *Biochemical Journal*, **48**(5): 584–590.

201. Chibnall, A. C.; Rees, M. W.; Williams, E. F. (1943) The total nitrogen content of egg albumin and other proteins. *Biochemical Journal*, **37**(3): 354–359.

202. Guillemaut, P.; Marechal-Drouard, L. (1992) Isolation of plant DNA: A fast, inexpensive, and reliable method. *Plant Molecular Biology Reporter*, **10**: 60–65.

203. Echevarría-Machado, I.; Sánchez-Cach, L.A.; Hernández-Zepeda, C.; Rivera-Madrid, R.; Moreno-Valenzuela, O.A. (2005). A simple and efficient method for isolation of DNA in high mucilaginous plant tissues. *Molecular Biotechnology*, **31**: 129-135.

204. Carboni, http://www.funsci.it/indice-esperimenti-scientifici.html, (accessed April 2016)

205. Banyay, M.; Sarkar, M.; Graslund, A. (2003). A library of IR bands of nucleic acids in solution. *Biophysical Chemistry*. **104**: 477–488.

206. Velusamy, V.; Arshak, K.; Yang, C.F.; Yu, L.; Korostynska, O.; Adley, C. (2011). Comparison between DNA immobilization techniques on a redox polymer matrix. *American Journal of Analytical Chemistry*, **2**: 392-400.

207. Liu, J. (2012). Adsorption of DNA onto gold nanoparticles and graphene oxide: surface science and applications. *Physical Chemistry Chemical Physics*, **14**: 10485-10496.

208. van den Beucken, J.J.J.P.; Vos, M.R.J.; Thune, P.C.; Hayakawa, T.; Fukushima, T.; Okahata, Y.; Walboomers, X.F.; Sommerdijk, N.A.J.M.; Nolte, R.J.M.; Jansen, J.A. (2006). Fabrication, characterization, and biological assessment of multilayered DNA-coatings for biomaterial purposes. *Biomaterials*, **27**: 691–701.

209. Lee, J.; Koo, J., Hwang, S.U.; Min, S.; Ahn, S.J.; Roh, Y.; Park, S.H. (2012). Structural and functional stabilities of artificially designed DNA ultra-thin films grown by silica Assistance. *Current Applied Physics,* **12**: 1207-1211.

210. Yamada, M.; Kato, K.; Shindo, K.; Nomizu, M.; Haruki, M.; Sakairi, N.; Ohkawa, K.; Yamamoto, H.; Nishi, N. (2001). UV-irradiation-induced DNA immobilization and functional utilization of DNA on nonwoven cellulose fabric. *Biomaterials*, **22**: 3121-3126.

211. Beukers, R.; Berends, W. (1960). Isolation and identification of the irradiation product of thymine. *Biochimica et Biophysica Acta*, **41**: 550-551.

212. Periolatto, M.; Ferrero, F.; Vineis, C. (2012). Antimicrobial chitosan finish of cotton and silk fabrics by UV curing with 2-hydroxy-2-methylphenylpropane-1-one. *Carbohydrate Polymers*, **88**: 201–205.

213. Casale, A.; Bosco, F.; Malucelli, G.; Mollea, C.; Periolatto, M. (2016). DNA-chitosan cross-linking and photografting to cotton fabrics to improve washing fastness of the fire-resistant finishing. *Cellulose*, **23**: 3963–3984.

214. Sionkowska, A.; Kaczmarek, H.; Wisniewski, M.; Skopinska, J.; Lazare, S.; Tokarev, V. (2006). The influence of UV irradiation on the surface of chitosan films. *Surface Science*, **600**: 3775–3779.

215. Zheng, l.-Y.; Zhu, J.-F. (2003). Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate polymers*, **54**(4): 527-530.

Appendix A

Published papers in peer reviewed journals

- Casale, A.; Bosco, F.; Malucelli, G.; Mollea, C.; Periolatto, M. (2016). DNA-chitosan cross-linking and photografting to cotton fabrics to improve washing fastness of the fire-resistant finishing. *Cellulose*, **23**: 3963–3984.
- Bosco, F.; Casale, A.; Mollea, C.; Terlizzi, M.E.; Gribaudo, G.; Alongi, J.; Malucelli, G. (2015). DNA coatings on cotton fabrics: effect of molecular size and pH on flame retardancy. *Surface & Coatings Technology*, **272**: 86-95.
- Malucelli, G.; Bosco, F.; Alongi, J.; Carosio, F.; Di Blasio, A.; Mollea, C.; Cuttica, F.; Casale, A. (2014). Biomacromolecules as novel green flame retardant systems for textiles: an overview. *RSC Advances*, **4**: 16.

Conference proceedings

- Casale, A.; Mollea, C.; Bosco, F.; Malucelli, G. (2016). Estrazione di biomacromolecole da scarti dell'industria agroalimentare e loro impiego come ritardanti di fiamma per substrati cellulosici. IN Atti del XXII convegno AIM, Edizioni Nuova Cultura (ITA), XXII Convegno Nazionale dell'Associazione Italiana di Scienza e Tecnologia delle Macromolecole, Genova, 11-14 Settembre 2016, pp. 219-222.
- Casale, A.; Mollea, C.; Bosco, F.; Malucelli, G. (2016). Coatings a base di DNA e chitosano foto-reticolato per il conferimento di proprietà di ritardo alla fiamma e antimicrobiche a tessuti di cotone, IN Atti del XXII convegno AIM, Edizioni Nuova Cultura (ITA), XXII Convegno Nazionale dell'Associazione Italiana di Scienza e Tecnologia delle Macromolecole, Genova, 11-14 Settembre 2016, pp. 115-118.
- Casale, A.; Bosco, F.; Mollea, C.; Di Blasio, A.; Alongi, J.; Cuttica, F.; Gribaudo, G.; Malucelli G. (2014). Tessuti cellulosici trattati con DNA: influenza del pH e peso molecolare sulle proprietà di ritardo alla fiamma. IN Atti del XXI convegno AIM, Edizioni Nuova Cultura (ITA), XXI Convegno Nazionale Dell'associazione Italiana di Scienza e Tecnologia delle Macromolecole, Torino 14-19 settembre 2014, p. 448.