

MASTER'S DEGREE ON PHARMACEUTICAL TECHNOLOGY

**Development, characterization, and application of a  
chewing gum to mitigate firefighters' occupational  
exposure**

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**M**

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# **Development, characterization, and application of a chewing gum to mitigate firefighters' occupational exposure**

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*To Carina Duarte*

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# Abstract

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During a fire-event, firefighters are exposed to various pollutants (such as polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants (BFRs)) and may develop pathologies such as cardiovascular, respiratory diseases and cancer over their career. Several mitigation strategies have been implemented to promote firefighters' safety and minimize occupational exposure focusing on improving personal protective equipment and decontamination procedure. This work proposes chewing gum (CG) as an easy-use, cost-effective, and sustainable mitigation technology capable of retaining the lipophilic fire-related pollutants and offer protective capacity against them. Three CG formulations (CGA, CGB, and CGC) incorporating essential mint oil, and dry kiwi berry (DKB), were developed in collaboration with a Portuguese company (Lusiteca Produtos alimentares-PA). For characterization various techniques such as texture profile analysis, rheological and sensory analysis, conducted with a panel of military firefighters of the Special Protection and Relief Unit of the Republican National Guard were used. To assess the CGs capacity for pollutant retention, the various CGs were exposed to commercial saliva contaminated with 18 PAHs and 7 BFRs, which were then extracted and analyzed by liquid and gas chromatography, respectively. This methodology was applied to samples used by firefighters while on-duty. The formulations have similar texture profiles varying slightly on few attributes. Regarding rheology all formulations display pseudoplastic behavior with slight thixotropy. The results of the sensory analysis support those of the texture profile analysis, revealing similarity in perception and good acceptability of the developed CGs. The developed analytical methods promptly demonstrated that all CGs possess capacity to retain several key pollutants (12 PAHs and 7 BFRs). Out of all the three test formulations, CGA appears to be the formulation with the highest capacity for the pollutants tested. Analysis of CG samples used by firefighter while on-duty revealed retention of 2 BFRs. These results reveal the potential of these CGs as possible mitigation strategies for firefighters' occupational exposure. Nevertheless, certain aspects of the methodology still require further refining so as to quantify the pollutant levels found in their oral cavity. It would be interesting to apply and analyze these CGs in other firefighting contexts to study the pollutants present in different environments. In the future, the protective effect of the DKB in the formulations will also be evaluated through in-vitro cell essays.

**Keywords:** Firefighters, Chewing gum, Sensory Analysis, Rheology

## Resumo

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Durante o combate aos incêndios, os bombeiros encontram-se expostos a vários poluentes (p.e., hidrocarbonetos aromáticos policíclicos (PAHs) e retardadores de chama bromados (BFRs)) podendo desenvolver patologias como doenças cardiovasculares, respiratórias e cancro ao longo da sua carreira. Foram implementadas várias estratégias de mitigação para promover a segurança dos bombeiros e minimizar a exposição ocupacional centrando-se na melhoria do equipamento de proteção individual e no procedimento de descontaminação. Este trabalho propõe o uso de pastilha-elástica (CG) como uma estratégia de mitigação conveniente, económica e sustentável, capaz de reter os poluentes lipofílicos libertados durante um fogo e oferecer proteção contra eles. Três formulações de CG (CGA, CGB e CGC) incorporando óleo essencial de menta e kiwi-bebé seco (DKB), foram desenvolvidas em colaboração com uma empresa portuguesa (Lusiteca Produtos alimentares-PA). Para a caracterização das formulações foram utilizadas várias técnicas, tais como análise de perfil de textura, análise reológica e análise sensorial, esta última efetuada com um painel de bombeiros militares da Unidade Especial de Proteção e Socorro da Guarda Nacional Republicana. Para avaliar a capacidade de retenção de poluentes, as várias CGs foram expostas a saliva comercial contaminada com 18 PAHs e 7 BFRs, que foram seguidamente extraídos e analisados através de cromatografias líquida e gasosa, respetivamente. Esta metodologia foi aplicada a amostras utilizadas pelos bombeiros em serviço. As formulações demonstraram ter um perfil de textura semelhante variando ligeiramente em alguns atributos. Todas as formulações apresentaram um comportamento pseudoplástico com ligeira tixotropia. Os resultados da análise sensorial corroboraram os de perfil de textura, revelando semelhanças na perceção e boa aceitabilidade das CGs desenvolvidas. Os métodos analíticos desenvolvidos demonstraram prontamente que todas as CGs possuem capacidade para reter vários poluentes (12 PAHs e 7 BFRs). De todas as formulações testadas, a CGA parece ser a formulação com maior capacidade de reter os poluentes testados. A análise de amostras de CG utilizadas pelos bombeiros em serviço revelou a retenção de 2 BFRs. Estes resultados revelam o potencial destas CGs como possíveis estratégias de mitigação da exposição profissional dos bombeiros. No entanto, alguns aspetos da metodologia requerem ainda um aperfeiçoamento para quantificar os níveis de poluentes encontrados na sua cavidade oral. Seria interessante aplicar e analisar estas CGs noutros contextos de combate a incêndios para estudar os poluentes presentes em diferentes ambientes. No futuro, o efeito protetor do DKB presente nas formulações será também avaliado através de ensaios celulares *in-vitro*.

**Palavras-chave:** Bombeiros, Pastilha-elástica, Análise sensorial, Reologia

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## Abbreviations and acronyms

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|                  |                                           |
|------------------|-------------------------------------------|
| <b>3ITT</b>      | Three interval thixotropy test            |
| <b>Ace</b>       | Acenaphthylene                            |
| <b>Acen</b>      | Acenaphthene                              |
| <b>Ant</b>       | Anthracene                                |
| <b>API</b>       | Active pharmaceutical ingredient          |
| <b>B(a)A</b>     | Benz[a]anthracene                         |
| <b>B(a)P</b>     | Benzo[a]pyrene                            |
| <b>B(b)Ft</b>    | Benzo[b]fluoranthene                      |
| <b>B(g,h,i)P</b> | Benzo[g,h,i]perylene                      |
| <b>B(j)Ft</b>    | Benzo[j]fluoranthene                      |
| <b>B(k)Ft</b>    | Benzo[k]fluoranthene                      |
| <b>BDE</b>       | Brominated diphenyl ether                 |
| <b>BDE100</b>    | 2,2',4,4',6-pentabromodiphenyl ether      |
| <b>BDE153</b>    | 2,2',4,4',5,5'-hexabromodiphenyl ether    |
| <b>BDE154</b>    | 2,2',4,4',5,6'-hexabromodiphenyl ether    |
| <b>BDE183</b>    | 2,2',3,4,4',5',6-heptabromodiphenyl ether |
| <b>BDE28</b>     | 2,4,4'-tribromodiphenyl ether             |
| <b>BDE47</b>     | 2,2',4,4'-tetrabromodiphenyl ether        |
| <b>BDE99</b>     | 2,2',4,4',5-pentabromodiphenyl ether      |
| <b>BFRs</b>      | Brominated flame retardants               |
| <b>CG</b>        | Chewing Gum                               |
| <b>Chry</b>      | Chrysene                                  |
| <b>CIE</b>       | Comission Internationale de l'Eclairage   |
| <b>COPD</b>      | Chronic obstructive pulmonary disease     |
| <b>CRP</b>       | C-reactive protein                        |

|                 |                                             |
|-----------------|---------------------------------------------|
| <b>CVD</b>      | Cardiovascular diseases                     |
| <b>DB(a,h)A</b> | Dibenzo[a,h]anthracene                      |
| <b>DB(a,l)P</b> | Dibenzo[a,l]pyrene                          |
| <b>DKB</b>      | Dry baby-kiwi berry                         |
| <b>DNA</b>      | Deoxyribonucleic acid                       |
| <b>DSC</b>      | Differential scanning calorimetry           |
| <b>ECD</b>      | Electron capture detector                   |
| <b>EMO</b>      | Essential mint oil                          |
| <b>EP</b>       | European pharmacopeia                       |
| <b>Fln</b>      | Fluoranthene                                |
| <b>Flu</b>      | Fluorene                                    |
| <b>G´</b>       | Storage modulus                             |
| <b>G´´</b>      | Loss modulus                                |
| <b>GC</b>       | Gas chromatography                          |
| <b>GNR</b>      | Guarda Nacional Republicana                 |
| <b>HPLC</b>     | High-performance liquid chromatography      |
| <b>IARC</b>     | International Agency for Research on Cancer |
| <b>InP</b>      | Indeno[1,2,3-c, d]pyrene                    |
| <b>LVER</b>     | Linear viscoelastic region                  |
| <b>MCG</b>      | Medicated chewing gum                       |
| <b>Naph</b>     | Naphthalene                                 |
| <b>PAHs</b>     | Polycyclic aromatic hydrocarbons            |
| <b>PBDEs</b>    | Polybrominated diphenyl ethers              |
| <b>Phe</b>      | Phenanthrene                                |
| <b>POP</b>      | Persistent organic pollutants               |
| <b>PPE</b>      | Personal protective equipment               |
| <b>PTSD</b>     | Post-traumatic stress disorder              |

|             |                                             |
|-------------|---------------------------------------------|
| <b>Pyr</b>  | Pyrene                                      |
| <b>SA</b>   | Sensory Analysis                            |
| <b>SCBA</b> | Self-contained breathing apparatus          |
| <b>SPE</b>  | Solid-phase extraction                      |
| <b>TPA</b>  | Texture profile analysis                    |
| <b>UEPS</b> | Unidade de Emergência de Proteção e Socorro |
| <b>USA</b>  | United States of America                    |
| <b>USP</b>  | United states pharmacopeia                  |
| <b>VOC</b>  | Volatile organic compounds                  |
| <b>WUI</b>  | Wildland-urban interface                    |

## Chapter I

### *Introduction*

# 1. Introduction

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## 1.1 Firefighting as an occupation

The role of a firefighter encompasses a variety of tasks carried out both inside and outside the fire station. Inside duties comprise administrative tasks, cleaning, and maintenance, while outside tasks range from operating ladders and diving operations to actual firefighting and rescuing victims, among others (IARC, 2010). Overall, firefighting is a demanding profession, often placing individuals in hazardous situations where they serve as the primary force in combating, controlling, and extinguishing fires. During a response to a fire-event, firefighters engage in various activities, such as driving fire trucks, working on the front line or during the smoldering phase, and acting as team leaders (IARC, 2010). Additionally, these professionals may carry out other daily responsibilities, including transporting chronically ill patients, providing pre-hospital care, conducting rescue operations for civilians and animals, inspecting infrastructure safety, responding to hazardous material leaks, and investigating the origins of fires (such as in cases of arson) (Figure 1).



**Figure 1.** Tasks performed by firefighters: (1) Wildland firefighting; (2) Structural firefighting; (3) Suppression of a chemical leakage; (4) Responding to a medical emergency.

Depending on the localization and the materials present in them, fire-events are usually divided into three fire typologies:

- *Structure fires*

Structure fires occur in urban environments such as residential areas, industrial facilities, oil wells, military bases, and other infrastructures. Firefighting inside these structures is performed within closed spaces, where individuals face extremely elevated temperatures and smoke accumulation which makes breathing nearly impossible in this environment. In order to breathe inside these spaces, firefighters wear what is known as a self-contained breathing apparatus (SCBA), a respiratory device comprised of four parts: cylinder, back plate and harness, regulator, and face piece. This SCBA protects firefighters from nefarious gases and particulate matter (PM) while feeding them clean air from an air bottle placed on their back.

In general, these fires can be divided in two main stages: the “knockdown” that lasts around 5 to 10 minutes, in which firemen perform highly demanding physical activity aiming to extinguish or control fire; and the “overhaul”, a monitorization stage, with no defined duration, where any lingering fire is put out (Firecamp, 1996; IARC, 2010).

- *Wildland fires*

Fires generated in forests, prairies, or plains are considered wildland fires. Firefighting activity in these zones requires that the firefighter’s hike, carrying around various burdensome equipment (20-25 kg) and, sometimes, managing heavy tools like chainsaws. Wildland firefighting, principally in large fires, requires the work for prolonged periods of time, surpassing the regular 8 to 9 hours of work shift while working in irregular terrain and being subject to the elevated temperatures of the surrounding burning environment.

Like structural fires, wildland firefighting activity can be divided into two stages: the “attack” phase, is a very high-energy and time-consuming step, lasting a few hours or, in some cases, extent to days or even weeks to control and suppress the fire; and the “mop-up” stage, where the task is to extinguish any remaining embers or sparks and remove or destroy the material that can act as fuel, preventing re-ignition or spread beyond the established line of fire (Firecamp, 1996; IARC, 2010).

- *Wildland-Urban Interface fires*

Over the last decade, a lot of attention has been paid to fires occurring at the Wildland-Urban Interface (WUI) zones. These are interurban areas, mixed zones where vegetation

collides and mixes with urbanization, forming complex intermediate settings containing bushes, trees, and weeds near residential and/or industrial infrastructures. WUI fires can be seen as more problematic due to the difficulty in their control and extinction, as well as in the variety and content of pollutants in their smoke (Bento-Gonçalves & Vieira, 2020).

## **1.2 Health burden in firefighters**

### **1.2.1 Physical injuries and psychological problems**

The health hazards associated with the occupational activity as a firefighter are extensively well described in the literature, being mostly related to physical and/or psychological stress, dehydration, and exposure to a myriad of health-relevant pollutants present at the workplace and/or released during fires (Guidotti & Clough, 1992; Sousa et al., 2022). High levels of physical activity needed to perform some tasks (e.g., firefighting, operating ladders), coupled with the weight of the necessary equipment to execute the work, contribute to an increase in the risk of physical injury. In the worst scenarios, firefighters may have a work accident that can cause chronic pain, permanent injuries or even disability (Guidotti & Clough, 1992). Research finds that sprains and strains are the prevalent leading injuries that occur as a result of slips, trips and falls, as well as muscle bending, lifting and squatting or muscle stressing (Orr et al., 2019).

Another often overlooked aspect of this profession is the psychological stress that it exerts. The occupation of a firefighter is potentially life-threatening, and professionals must accept and face an elevated level of personal risk, in addition to dealing with their own daily anxieties. Alongside this, the inconstant workflow contributes to mental exhaustion, principally during critical periods of the hot season when firefighters can work during consecutive days with minimum rest. Also, the search and rescue of victims, some in mortal danger coupled with the responsibility for their well-being and safety, might cause psychological stress. The advent of death or a permanent disability to a victim is one of the most stressful aspects of this activity, especially if the victim is a child, relative or work colleague (Guidotti & Clough, 1992).

In recent years, some studies suggested that firemen are at a greater risk of developing certain diseases, such as depression and post-traumatic stress disorder (PTSD), even suggesting a link between them and other recognized occupational diseases namely cardiovascular diseases (CVD), proposing that the presence of PTSD in an individual may increase the risk of developing CVD (Cornell et al., 1999; Zhang et al, 2021).

### 1.2.2 Chronic inflammation

Aside from the physical and psychological damage caused by this occupation, other serious and internal pathologies may arise as these professionals are regularly exposed to several known or suspected carcinogenic compounds, oxidative stressors, and inflammation-inducing chemicals released from the combustion of various materials, primarily via inhalation, dermal contact, and ingestion (**Figure 2**).

The pollutants present in a fire smoke vary tremendously and depend on the type of fuel consumed (e.g., trees, bushes, crops and other biofuels) as well as on the ventilation conditions, task performed and duration of exposure. Fire emissions contain a wide array of pollutants, including PM, carbon monoxide and dioxide, nitrogen oxides, sulfur dioxide, metals, and several of semi-volatile, and volatile organic compounds (VOCs) [alkanes, alkenes, polycyclic aromatic hydrocarbons (PAHs), phenols (2-methoxyphenol, 2,6-dimethoxyphenol and other derivatives)] (Barros et al., 2023). In structural fires, however, the combustive is more complex and may contain polymeric materials including polyethylene, polyvinyl chloride, polyethylene terephthalate, polystyrene and polypropylene, as well as products with brominated flame retardants (BFRs) widely used in various plastics, rubbers, coatings, clothes and electronic equipment, contributing to the release of dioxins, polychlorinated biphenyls, phthalates, polybrominated diphenyl ethers, organophosphate esters, perfluoroalkyl acids, tetrabromobisphenol A, hexabromocyclododecane, hydrogen chloride, etc (Barros et al., 2023; Bessa et al., 2023). Additionally, the strenuous physical activity of these professionals leads to the inhalation of pollutants in larger quantities when compared to normal physical activity levels (IARC, 2010). **Figure 2** summarizes all the main types of fires, routes of exposure and potential health effects related with the occupational activity of a firefighter (Guidotti & Clough, 1992; Sousa et al., 2022).

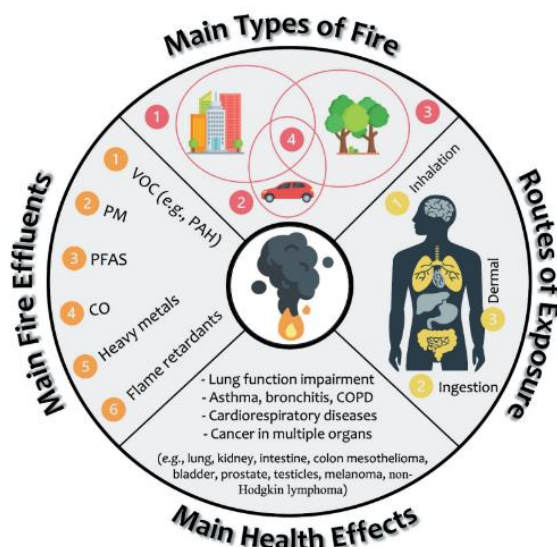


**Figure 2.** Illustrative image summarizing the occupational exposure as a firefighter. Main type of fire: (1) Structure fires, (2) Vehicles fires, (3) Forest fires/wildfires, (4) Fires in the wildland-urban interfaces; Main fire effluents emitted; Main routes of exposure to fire pollutants: (1) Inhalation, (2) Ingestion, (3) Dermal contact; Main health effects that might potentially occur in firefighters after long-term exposure. Adapted from Bessa et al., (2023).

The physical/thermal/psychological stress, dehydration, changes in the circadian rhythm, coupled with the extensive exposure to pollutants, promote the occurrence of reactive oxygen species, imbalance of oxidative/antioxidant activity, and may cause protein and deoxyribonucleic acid (DNA) damage (e.g., DNA methylation), as well as cell homeostasis disruption, kickstarting severe oxidative stress and inflammation, leading to illness (**Figure 3**). Different biomonitoring assays performed on firefighters have already demonstrated the presence of altered levels of compounds or related metabolites released in biological fluids (e.g., urine, blood, saliva, semen, milk, and exhaled breath) (Barros et al., 2023; D'souza & Addepalli, 2018; Sousa et al., 2022). As matter of fact, several studies have described this phenomenon of general or chronic inflammation in firefighters, showing increased levels of certain biomarkers of inflammation (e.g., creatinine or C-reactive protein) responsible for the occurrence of local or acute inflammation that, if persisting, may over time develop into systemic inflammatory response (Melero et al., 2021; Orysiak et al., 2022). Normally, the inflammation process is an essential phase of wound healing and excretion of toxins from the healthy human body. However, in prologued cases such as those of firemen, it can also be seen as one of the main hallmarks of the development of several cardio-respiratory diseases and various cancers (Orysiak et al., 2022).

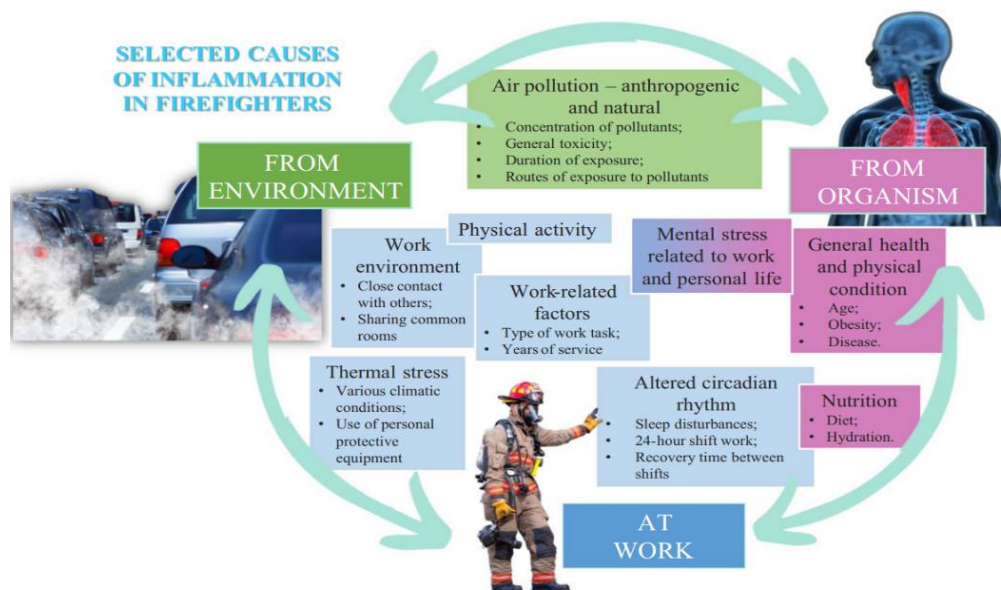


**Figure 3.** Selected causes of inflammation in firefighters (Extracted from Orysiak et al., 2022).

### 1.2.3 Cardiovascular and respiratory deceases

CVD have been identified as a major cause of on-duty death (45% of on-duty facilities) and morbidity among firefighters (Guidotti & Clough, 1992). Alongside this, firefighters are also at risk of developing or even aggravating pre-existing health conditions, such as tachycardia, ischemia, heart failure, and acute myocardial infarction. The causes that promote the development/aggravation of these diseases are multiple but often associated with common stress factors such as e.g., sedentary lifestyle, noise, inhalation of airborne pollutants, shift work, psychological disorders, and dietary habits (Soteriades et al., 2011). As these factors, individually or cumulatively, contribute to promote a complex chain of reactions in the human body that stimulate the adrenal system to produce hormones as cortisol, epinephrine and norepinephrine which may result in higher inflammation and oxidative stress levels that will lead to the impairment of blood vessel dilatation, thus increasing the risk of CVD (Bessa et al., 2023; Gendron et al., 2018; Huang et al., 2013; Soteriades et al., 2011).

Similarly, there is an association between human exposure, via inhalation, to smoke and particles with the onset or aggravation of respiratory pathologies (Guidotti & Clough, 1992). Tobacco smokers are a primary example since the correlation between regular smoking and lung diseases has been thoroughly studied, being a common sense among population that smoking increases the likelihood of developing respiratory diseases such as chronic bronchitis and asthma, emphysema, chronic obstructive pulmonary disease, and

lung cancer (Pietinalho et al., 2009). The release of airborne (particulate and gaseous) pollutants during fires routinely exposes firefighting forces to health-relevant pollutants that may aggravate their respiratory health, even with the use of adequate personal protective equipment (PPE) (Adetona et al., 2013; Rajnoveanu et al., 2022).

#### **1.2.4 Oncological diseases**

Another frequent and no less concerning disease associated with firefighting is cancer. In fact, there is epidemiological evidence which supports a causal relationship between firefighting and cancers in certain organs. This scientific evidence led the International Agency for Research on Cancer (IARC) to recognize bladder cancer and mesothelioma as occupational diseases in firefighters (Demers et al., 2022). Despite the limiting evidence, higher prevalence of other cancers has also been identified, namely on colon, prostate, and testicular cancers, melanoma, and non-Hodgkin lymphoma. Despite this further studies are needed (Demers et al., 2022).

Additionally, available evidence demonstrates that the occupational exposure as a firefighter causes chronic inflammation, induces oxidative stress, genotoxic and epigenetic changes, and disrupts receptor-mediated effects (Demers et al., 2022; Gourd, 2023). Therefore, the IARC also reclassified the occupational activity of firefighting as carcinogenic to humans (Group 1) (Demers et al., 2022).

In the USA, the National Institute for Occupational Health and Safety has also recognized that firefighters have a 9% higher cancer incidence risk and a 14% increased cancer mortality risk when compared to the general U.S. population (National Firefighter Protection Association, 2024). Their research infers that cancer caused 66% of the career firefighter line-of-duty deaths between 2002 and 2019 (Firefighter Cancer Support Network, 2024).

### **1.3 Available mitigation strategies**

The serious consequences of occupational exposure have driven researchers globally to explore and develop new methods for reducing exposure to various pollutants. The focus and effectiveness of these strategies varies significantly. Some, aimed at a broader level, seek to prevent fire incidents and revise administrative procedures. Others, focused on the individual level, involve improving PPE and creating new cleaning products to decontaminate firefighters' skin and gear (Horn et al., 2022).

### **1.3.1 Reduction of fires events**

While complete elimination of accidental fires is not achievable, reducing the risk of fire events is an feasible goal. Public education programs may raise attentiveness of local occupants for flammable products, and risky behaviors, which may result in less fires and, consequently, lower exposure to pollutants for the responding firefighters (Horn et al., 2022). Devices such as smoke alarms may alert the inhabitants present in a building, offering an opportunity for escape, eliminating the need for rescue at the fire incident and preventing the spreading of fires (Horn et al., 2022). Another useful technology that can be implemented are the automatic fire sprinklers, which release water and help control fires inside structures, eliminating the occurrence of larger and more complicated fires that create increased risk for exposure to carcinogens (Horn et al., 2022). Finally, careful management of various forms of fuels (e.g., vegetation or gas bottles) around various establishments may also reduce the risk of accidental ignitions, preventing fires from spreading and reducing the need for firefighter intervention (Cohen, 2000).

### **1.3.2 Personal protective equipment**

PPE is a crucial safeguard apparel to firefighters from the various hazards encountered in the unpredictable conditions of emergency scenarios. Typical risks include environmental heat, water, and physical abrasion. However, because of the recent evidence for the health risks from exposure to PM and gases in smoke, the effectiveness of PPE in protecting against these elements is under review (Horn et al., 2022; Kesler et al., 2021). Systems like SCBA are obligatory in urban environments and help to prevent contaminants inhalation. The proper use of this equipment during all stages of a fire, especially during overhaul, is the key to enhancing protection (Horn et al., 2022). Improving the fit of gear around typically exposed areas, such as the neck and wrists, or using particle-blocking hoods, are common PPE enhancements aimed at increasing protection and limiting exposure (Horn et al., 2022; Kesler et al., 2021).

### **1.3.3 Equipment decontamination and safety procedures**

The environments in which firefighters act upon are normally full of fume, so contamination may occur at all stages of fire response (Horn et al., 2022; Wolffe et al., 2023). Every time firefighters engage in firefighting activities, one assumes they are contaminated in some way and since PPE is the outermost layer of protection, the chances it returns heavily contaminated are high (Krzemińska & Szewczyńska, 2022). As result, it is necessary to implement some form of decontamination procedure across various stages of fire response to remove pollutants and lower the risk of possible cross-contamination.

Decontamination should start swiftly to avoid cross-contamination between the PPE, surrounding structures, and firefighters' skin (Horn et al., 2022). Immediately after a fire event, while still on site, brushing the apparel is an effective way to remove contaminants. Fent et al (2017) demonstrated that decontamination using dish soap, water, and brushing was able to reduce the PAHs levels on turnout jackets by 85%. Additionally, the authors also demonstrated that even dry brushing, although less effective, can remove 23% of the PAHs from gear, remaining a viable option (Fent et al., 2017).

Inversely, other studies that evaluated the efficacy of washing firefighter gear using a water-only method, found it ineffective since it ended up resulting in an overall 42% increase in PAHs contamination (Calvillo et al., 2019). However, the authors attributed this increase to the difference in pre- and post- washing sample collection sites in the gear. When taking both studies into consideration, it is clear that detergents are more effective in removing PAHs from PPE, which can be explained by the presence of surfactants in the soap formulations that aid to remove lipid soluble PAHs (Librando et al., 2003). The slight effectiveness of dry brushing may be explained by the greater mechanical force applied by a brush when compared to that of normal hose rinsing. Ideally, firefighters should keep the SCBA on during cleaning procedures, being the last part of the equipment removed (Horn et al., 2022).

#### **1.3.4 Skin cleaning and rehabilitation**

Unfortunately, despite the PPE's robustness, contaminants still penetrate the equipment, reaching their innermost layers and even the firefighters' skin. Therefore, to deodorize, sanitize and, most important, detoxify firefighters' skin, the professionals should wash their hands, face, neck, and skin areas exposed with soap, water or disposable wipes (Horn et al., 2022). Personnel should clean themselves immediately after a fire-event and before rehabilitation in living quarters where they eat or drink. This practice prevents the exposure through dermal contact and ingestion by diminishing the risk of cross-contamination (FCSN, 2022; Horn et al., 2022).

To aid and incentivize these procedures, companies such as Sootsoap Supply Co. and Responder Wipes commercialize a bundle of different personal hygiene products such as skin wipes, shampoos, and shower gels among others, specifically designed for firefighters. These products are intended to be used immediately after a fire service for the decontamination of skin and hair (Keir et al., 2023; Mayer et al., 2019). Some of the products contain activated charcoal in their formulations, taking advantage of charcoal's ability to adsorb chemicals (Hossain et al., 2023). However, the cleaning capacity of charcoal-based detergents appears similar to regular detergents, although this was only proven to be case

for the removal of PAHs from firefighters PPE (Hossain et al., 2023). The concentration of detergent used is a determinant factor when it comes to removing PAHs as concentrations around 10 mL per 90 mL of water, presented good cleaning efficacy against most of the PAHs and, after this point, the cleaning efficacy increased slowly (Hossain et al., 2023).

Once they return to the living quarters, it is recommended that firefighters shower as soon as possible and avoid contact with any food, or equipment. An unorthodox exposure reduction strategy that could remove levels to PAHs is a treatment that consists in subjecting fighters to an infrared sauna bath after a fire-event (Burgess et al., 2020).

Harrison et al., (2018) analyzed the behavior of firefighters towards these equipment decontamination measures and found that firefighters demonstrated receptive attitudes and beliefs about decontamination norms, but showering after a fire intervention was the only decontamination process that was regularly performed. Inversely, measures such as field decontamination, use of cleansing wipes, and routine gear cleaning, among others, were by comparison, rarely executed. Firefighters blame time and concerns over wet gear as the main obstacles.

As resumed in **Figure 4**, various investigators have devised different and interesting strategies to mitigate the occupational exposure risk firefighters fall victim to. Some procedures employ products that can provide moderate protection as they are able to prevent exposure and remove pollutants with a certain degree of efficacy. Regardless of the success of some of the ideas presented herein, there is still room for improvement, as the available strategies are not completely effective and so the demand for new scientific technologies that can offer greater protection to the firemen persists.

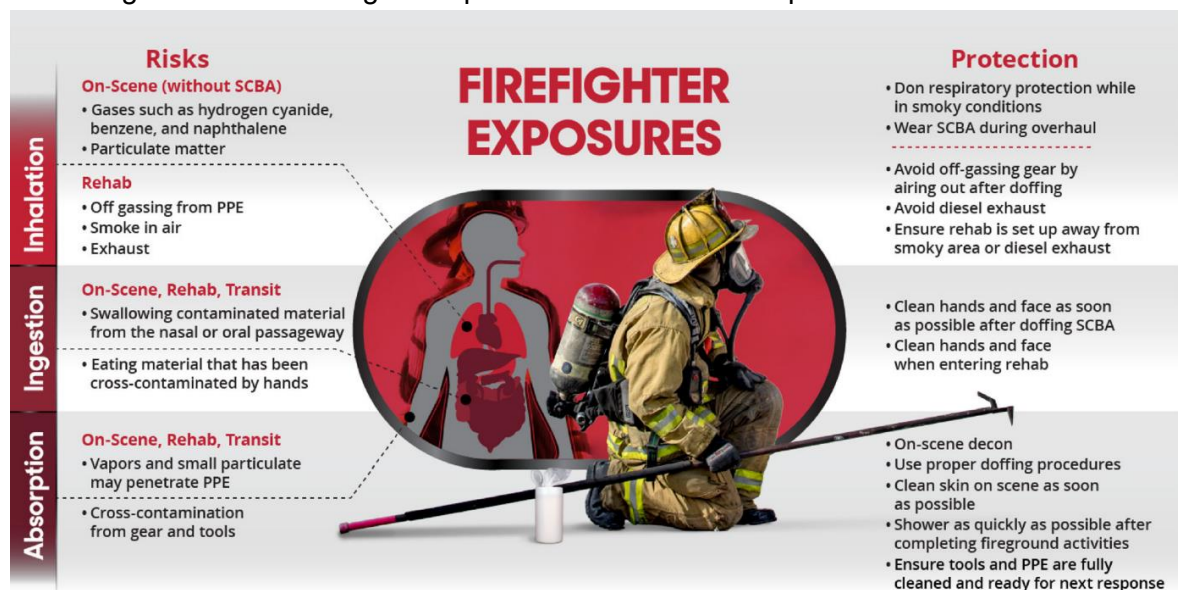


**Figure 4.** Occupational exposure risks and potential protection strategies. (Extracted from Horn et al., 2022).

## 1.4 Chewing gum as a dosage form

### 1.4.1 A brief history of chewing gum

Nowadays, chewing gum (CG) is among the most popular forms of confectionery in the world, although the idea of using non-food materials for chewing has been around since ancient times (**Figure 5**). Early civilizations such as the Egyptians, the Mayans and American Indians have used various resins extracted from different trees (Imfeld, 1999). For instance, the Mayans are known to have chewed in a material known as *chicle* a white gummy substance present in the bark of the sapodilla tree (*Manilkara zapota*). Other civilizations like the native Americans used spruce tree (*Picea* species) sap to produce a resin for chewing purposes. Nevertheless, the first available CG was developed by John B. Curtis and commercialized as “State of Maine Pure Spruce Gum”, in 1849. Curiously, in 1869, the first official patent for a CG was issued to a dentist, Dr. William F. Semple of Mount Vernon, Ohio (Aslani & Rostami, 2015).

However, the modern flavored CG, as we know it, was first developed when in the 1870s, General Antonio Lopez de Santa Anna (a deposed Mexican dictator exiled to the United States) brought to North America a large amount of the chicle resin. He introduced this product to Thomas Adams, his secretary, photographer, and inventor who tried to use it as a substitute for rubber. Chicle did not succeed in this application field. However, the chicle was cut into strips and marketed as “Adams New York Chewing Gum” in 1871. Thomas Adams eventually founded the American Chicle Company and, in 1915, introduced Chiclets to the market. The brand quickly spread through the world, becoming emblematic.

In Portugal, the first brand of CG brand available on the market were the “Chiclet” gum which their debut in 1934. The impact that it has become synonymous with CG product, so much so that the term “chiclete” is now used to refer to any CG products (Briefing, 2014). Since then, various CG products have been commercialized under various brands like Trident or Oral-B.

## Brief history of Chewing gum

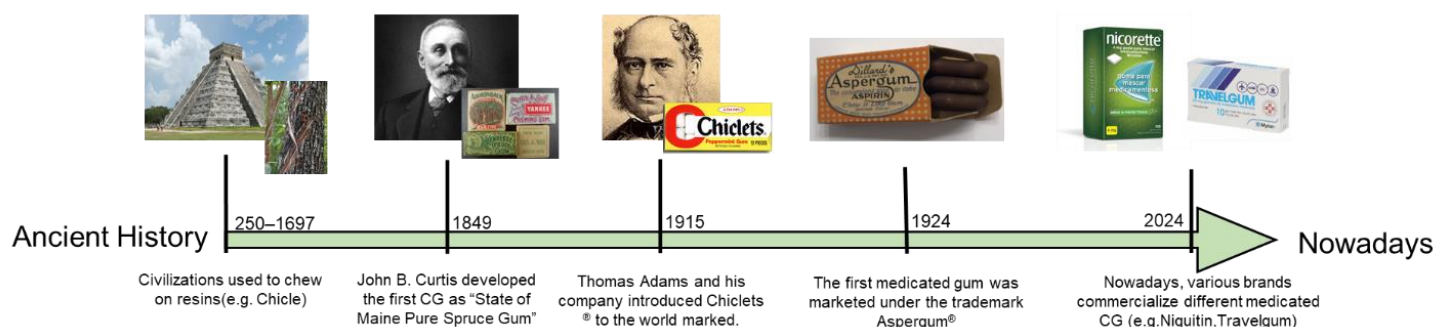


**Figure 5.** Brief chronology of CG development.

### 1.4.2 Medicated chewing gum

As a drug delivery system, the first medicated chewing gum (MCG) was released to the market in 1924 under the trademark Aspergum®, (**Figure 5**) an analgesic CG containing acetyl salicylic acid as active pharmaceutical ingredient (API) (Aslani et al., 2015).

Nowadays, MCG are defined in a monograph of the European Pharmacopoeia (EP), although the term "chewing gum" was first included in the guidelines as a pharmaceutical dosage forms in 1991 (Aslani & Rostami, 2015). The United States Pharmacopeia (USP) and EP, define MCG as "solid, single-dose preparations with a base consisting mainly of gum that are intended to be chewed but not swallowed". They incorporate one or more API which is meant to be dispersed or dissolved in the saliva during mastication. MCG are intended to be used in the local treatment of mouth diseases and/or systemic delivery after absorption through the buccal mucosa or from the gastrointestinal tract (Council of Europe et al., 2022).

Nowadays, several kinds of MCG have flourished in the pharmaceutical market, aiming to treat, or aid in treatment of an array of health conditions as described in **Table I**. Popular brands like Nicorette and Niquitin market MCG containing nicotine, designed to aid in smoking cessation, which is the most popular therapeutic application of MCG, alongside teeth health (Aslani & Rostami, 2015; Imfeld, 1999; Keservani et al., 2010). Nevertheless, other brands have explored the potential of this system for releasing other kinds of API, incorporating stimulants like caffeine to boost alertness or incorporating antihistamine drugs such as dimenhydrinate to treat motion sickness.

As of September 2024, as stated in Infomed, there are 15 authorized medicines in Portugal, containing either chlorine-hexedine + benzocaine or nicotine, but only 9 are being marketed, all of which, containing nicotine (2 of them are generics).

**Table I.** Commercially available brands of CG containing drug/API.

| <b>Therapeutic indication/use</b> | <b>Drug/Ingredient</b>                      | <b>Brand name(s)<sup>®</sup></b>             |
|-----------------------------------|---------------------------------------------|----------------------------------------------|
| Smoking cessation                 | Nicotine                                    | Nicorette, Nicoquitin, Nicontrol, Nicotinell |
| Analgesic action                  | Aspirin, Benzocaine                         | Aspergum, Chex                               |
| Preventing tooth decay            | Chlorhexidine, Fluor, Sodium fluoride       | Oral-B, Fluogum, Flourette                   |
| Nutrient supplementation          | Vitamins A, E, B1, B12, Biotin, Folic acid, | Vitaminize                                   |
| Alertness, Appetite control       | Caffeine, Guarana                           | MEG, Stay Alert, Go Gum, Buzz gum            |
| Teeth whitening/ health           | Calcium Phosphate                           | Happydent, Orbit                             |
| Anti-acid action                  | Calcium Carbonate                           | Chooz                                        |
| Motion sickness/nausea control    | Dimenhydrinate                              | Travelgum, Superpep, Trawell                 |

Despite not being among the most popular drug dosage forms on the market, research on the potential of MCG is still on-going in the scientific world. Researchers have been experimenting with different kinds of API, opening new therapeutic possibilities or exploring further established therapies for this dosage form, as can be seen in **Table II** (Aslani & Rostami, 2015; Banakar et al., 2022; Keservani et al., 2010; Sauer et al., 2007).

**Table II.** Potential therapeutic uses of CG explored in literature.

| <b>Therapeutic indication/use</b>                       | <b>API</b>                        | <b>Reference</b>                        |
|---------------------------------------------------------|-----------------------------------|-----------------------------------------|
| Smoking cessation                                       | Nicotine                          | (Jarvis et al., 1982)                   |
| Opioids withdraw                                        | Methadone                         | (Christrup et al., 1990)                |
| Analgesic                                               | Aspirin                           | (Woodford & Lesko, 1981)                |
| Preventing tooth decay                                  | Chlorhexidine                     | (Imfeld, 2006)                          |
| Alertness or stimulant                                  | Caffeine                          | (Moradi et al., 2022)                   |
| Antifungal activity                                     | Miconazole, Sulfathiazole         | (FOX et al., 1945; Rindum et al., 1993) |
| Teeth Whitening/Health                                  | Potassium aluminum sulphate       | (C J Kleber; & M S Putt, 1995)          |
| Antioxidant, Anti-inflammatory, antiseptic, and healing | Aloe vera and green tea           | (Aslani et al., 2015)                   |
| Otitis media treatment                                  | Xylitol                           | (Azarpazhooh et al., 2016)              |
| Plaque removal                                          | Zirconium silicate, Zinc sulphate | (Svatun et al., 1990)                   |

### 1.4.3 The composition and manufacturing of medicated chewing gum

Although formulations may change from product to product, MCG usually consists of a mixture of water-insoluble and water-soluble portions in appropriate ratio (Jhanwar et al., 2017). MCG are made with a masticatory gum base without taste composed by natural or synthetic elastomers (Council of Europe et al., 2022). This gum base is the indigestible, inert, non-nutritive part, which is not dissolved while chewing (Patel et al., 2011). Gum bases are the most important portion of a CG (constituting 15-20% of the total CG) since they not only act as bases for other compounds such as sweeteners, flavoring agents, and other water-soluble ingredients; but are also responsible for the mechanical properties and texture of the CG, which are considered the leading quality parameters in CG (Kaveh et al., 2023).

The formulation of gum base may vary, they are usually composed by a mix of elastomers, fillers, softeners, sweetening agents, flavoring substances, stabilizers, plasticizers and authorized coloring matter (Council of Europe et al., 2022; Jhanwar et al., 2017). Despite companies keeping secrecy regarding the formulation of their products, the majority of gum base ingredients are listed and regulated by law agents such as the Food and Drug Administration (FDA). Common ingredients that are generally considered safe by the FDA and USP are chicle, paraffin wax, polyisoprene, polyisobutylene, isobutylene isoprene copolymer, polyvinyl acetate, polyethylene and polystyrenes. The API is usually mixed in with the insoluble gum base material (Jhanwar et al., 2017).

Elastomers are polymers with high elongation properties and viscoelasticity capable of withstanding tension without breaking or cracking (Kaveh et al., 2023). They are responsible for gum bases' cohesiveness, elasticity and rubbery texture. When hydrated, elastomers become a slippery mass restricting the CG adherence to surfaces (Kaveh et al., 2023). Other advantageous properties of elastomers include low permeability to water, air and other gases, good thermal and electrical insulation, and adhesion to various metals, fibers, and rigid plastics (Kaveh et al., 2023). Depending on their origin, these can be divided into two categories: **natural elastomers**, such as chicle, latex, rosadinha, nispero, which are extracted from various trees; and **synthetic elastomers**, when they result from chemical synthesis, as is the case with polyvinyl acetate and polyisobutylene (Patel et al., 2011). Nowadays, natural elastomers have been substituted by synthetic ones such as polyisobutylene and butyl rubber, although sometimes they may be used in conjunction, composing 10-45% of the overall chewing gum mass (Kaveh et al., 2023).

To refine design, increase flexibility, workability or distensibility until optimal, elastomeric plasticizers such as fat or resins are often considered during the manufacture of bubble gum, these ingredients also act as binding agents between elastomers and texturizers (Jhanwar et al., 2017; Kaveh et al., 2023). Plasticizers are typically used as softeners. They are characterized as compounds with a high boiling point, low molecular weight, non-volatile, and 14-40 linear or circular carbon chains that assist in the improvement of the gum bases' texture. Additionally, by changing the glass temperature of the gum base, they increase the flexibility and elongation break (Kaveh et al., 2023).

Also present in gum bases are fillers or texturizers, which are ingredients that modify overall texture helping to reinforce it and facilitate blending and other processing steps during manufacturing process, reducing the cost of production (Kaveh et al., 2023). The amount of texturizer in a formulation influences its cud size, chewing ability, and stretchability. Fillers are usually divided into two categories: reinforcing and non-reinforcing. The reinforcing fillers aid in the enhancement of gum strength, which depends on the structure, size, and surface activity of filler particles, while the non-reinforcing fillers are usually employed to reduce manufacturing cost (Kaveh et al., 2023).

The water-soluble part of the CG is comprised of other compounds that modify certain sensorial attributes and organoleptic characteristics while simultaneously aiding to stabilize the formulation (Aslani & Rostami, 2015). Although all medication may be evaluated based on its effectiveness regarding treatment, when discussing oral/buccal dosage forms, patients will not comply with a certain medication willingly unless it is appealing. The willingness to take a certain medication is psychological and depends upon the reactions of the individual to its color, odor and feel (Pokharkar et al., 2022). In other words, oral medication must be appealing to the senses, it must possess appropriate taste and texture, especially if targeted to specific population groups such as children or the elderly (Pokharkar et al., 2022).

According to the USP, sweetening agents are *“compounds or blends of compounds of natural or synthetic origin that have the ability to elicit a sweet taste to the drug product formulation”*. Sweeteners are additives responsible for conferring sweetness to a formulation, enhancing its taste (Chattopadhyay et al., 2014). Because they increase palatability and acceptability, they are often used in some food and pharmaceutical products to mask bitter taste (Aslani & Rostami, 2015; Jhanwar et al., 2017). Natural sweeteners (e.g. corn syrup, hydrogenated starch, and sorbitol) help to retain moisture and freshness of the final product. They may function as a plasticizer, softening agent or binding agent (Aslani & Rostami, 2015).

High-intensity artificial sweeteners provide sweetness, but possess a lower caloric value, due to the partial absorbance in the intestine, which makes them a very popular choice. They are known to be greatly sweeter than sucrose, so lower doses of these ingredients are needed to produce a sweet flavor (*High-Intensity Sweeteners* | FDA, 2014). Due to this fact, high-intensity artificial sweeteners have gained a lot of popularity in recent times, and it is estimated that more than 50% of commercially available CG contain artificial sugar substitutes rather than conventional sweeteners, and so, are considered to be 'sugar-free' gums (Kaveh et al., 2023).

A flavoring agent is defined by the USP as a “*single chemical entity or a blend of chemicals of natural or synthetic origin that can elicit a taste or aroma (i.e., fragrance) response when orally consumed or smelled*”. In a pharmaceutical product, the purpose of an flavoring agent is to provide all or part of the taste and aroma of the product taken into the mouth. They are employed in oral disintegrating tablets, solutions and suspensions to mask poor drug taste and increase a formulations' palatability, thus promoting patient compliance (Aurand et al., 1987). Usually, the flavoring agents found in CG formulations are essential oils, such as peppermint oil or clove oil (Council of Europe et al., 2022).

Coloring agents, color additives or food colorants, are generally described as dyes, pigments, or compounds that, when added to a drug or to the human body, will impart a particular color (Amchova et al., 2015). They are frequently used in almost all the kinds of pharmaceutical dosage forms because of technical (e.g., allowing consumers to identify products on sight, especially drugs, photolabile components of the dosage form) or aesthetic reasons (e.g., compensation of color loss due to exposure to stress factor or storage conditions or enhancing of natural colors to make the food more attractive) (Amchova et al., 2015). According to the USP, these substances are subdivided into soluble dyes, lakes (insoluble forms of a dye that result from its irreversible adsorption onto a hydrous metal oxide), inorganic pigments (substances such as titanium dioxide or iron oxides), and natural colorants (colored compounds not considered dyes per se, such as riboflavin).

Anti-tack agents aim to eliminate self-adhesiveness of gum formulations (Aslani & Rostami, 2015). They are especially useful in the plastic and rubber industries, where products tend to stick together when placed near each other or particularly when they are stacked and cannot be separated easily without damaging the articles (Kern Etal & Composition, 1961). The same effect applies to polymeric materials, such as elastomers, when they are rolled up or folded, and the surfaces of the same article are in mutual contact. In the pharmaceutical field the anti-tack agents such as glyceryl monostearate and talc,

have been used in tablet coatings (Kriangkrai et al., 2014; Sauer et al., 2007). In the case of CG, anti-tack agents may help reduce fragmentation of the gum while chewing and prevent the product from attaching to the chewer's teeth (Aslani & Rostami, 2015). Anti-tack agents differ from lubricants in that they are designed to reduce inherent tackiness while lubricants are designed to reduce friction between any two surfaces.

The term "anti-caking agent" designates the ability of a compound to prevent/reduce caking problems (Aslani & Rostami, 2015). The phenomenon known as "Caking" is the agglomeration of the individual particles that make up a powder, such phenomenon is commonly associated with the presence of moisture (Ortiz de Zarate, 2014). Anti-caking agents are thus used to prevent caking and the generation of lumps. These materials improve flowability, rehydration and help package the product. They have been used to increase hardness in CG formulations but are also advantageous during manufacturing to avoid deformation (Guillaume Ribadeau-Dumas et al., 2015; Ortiz De Zarate, 2014).

Due to the low water content, preservatives are usually not added to the formulations. However, there may be cases in which, certain antioxidants may be added to protect certain API from oxidation (Aslani & Rostami, 2015; Jhanwar et al., 2017).

#### **1.4.4 Manufacturing process of chewing gum**

Although CG manufacturing methods may vary in certain steps depending on the manufacturer and the target product, in general they can be divided into 3 main methods:

- **Fusion or melting method**

Firstly, the gum base is well grounded, melted (around 60°C) and mixed in a recipient, until a soft base is formed. Afterwards, other ingredients such as sugar, glycerin, sweeteners are added to the softened base, lately the flavoring agent is also added to the mixing. The mixture is then allowed to cool down to be sized and cut into slices of desired shapes and sizes. To make a coated gum, a coating agent should be sprayed to form a uniform surface (Aslani & Rostami, 2015; Jhanwar et al., 2017).

- **Freezing, grinding method**

Another method for producing CG is to mix gum base with favorable and suitable ingredients, and then refrigerate it utilizing a freezing device or by contact with a coolant fluid, until a temperature of around -15°C. The mix is then pulverized with a cutter or grinding apparatus resulting in fine particles which are heated to a temperature that makes them adhere to one another, forming a consistent bulk. If there is no evident self-adherence, low pressure would be applied manually or mechanically before they are warmed to the

normal room temperature to thereby promote self-adhesion. The minute particles may be coated by edible substances or premixed with powdery materials. Finally, the resulting mix is screened and subjected to compression (Aslani & Rostami, 2015; Jhanwar et al., 2017).

- **Direct compression method**

Depending on the compressibility of the powdered ingredients, CG can also be made in a similar manner to tablets, which are usually produced via direct compression (Jhanwar et al., 2017).

In this method, a granulating agent and lubricant are added to the formulation before compression to facilitate the process. The first step is the mixing of the dry gum base, granulating agent and then adding API, and other ingredients to the mixture. The formulation of free-flowing materials is then directly compressed into a CG with a tablet form. This method provides a CG which dissociates into various particles, that after a few seconds of chewing, adhere together and form a uniform and homogenous mass (Aslani & Rostami, 2015).

#### **1.4.5 Advantages and limitations of CG as dosage forms**

Despite not being a popular drug dosage form, there are many reasons for choosing CG over other more conventional drug delivery systems such as tablets or capsules, as summarized in **Table III**. Firstly, MCG can be used to achieve a good topical effect when it comes to diseases or health conditions that directly affect the oral cavity and throat, especially if they affect teeth (**Figure 6**). Alongside this, human buccal mucosa allows a direct passage of API into the bloodstream, meaning that API released during the mastication process may be directly absorbed, bypassing the liver first pass effect and leading to a faster onset of therapeutic effect, while simultaneously reducing the API dosage required to produce that effect. Some medication is associated with xerostomia, commonly known as dry mouth, but MCG stimulates salivary secretion up to 10-fold, helping to keep the mouth moist during treatment (Jhanwar et al., 2017). Besides this, CG is highly palatable, pleasant, and acceptable by children, while also being useful in the treatment of nausea and dysphagia constituting a convenient self-administration dosage form without the need of water intake. Finally, CG is normally easier to store and less susceptible to microbial contamination, due to its low water content.

**Table III. Main Advantages and disadvantages of MCG.**

| Medicated Chewing Gum                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Advantages                                                                                                                                                                                                                                                                                                                           | Limitations                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| <ul style="list-style-type: none"><li>✓ It has a very good local effect due to a long time of chewing and sustained delivery</li><li>✓ Can be administered without water</li><li>✓ Capable of both systemic and topical drug delivery</li><li>✓ Increased release of API over time</li><li>✓ CG can be removed at any time</li></ul> | <ul style="list-style-type: none"><li>✗ Certain sugars may cause flatulence and diarrhea</li><li>✗ CGs may adhere to the dental enamel or dentures.</li><li>✗ Involuntary swallowing may remove the API from its site of action.</li><li>✗ Salivary dilution reduces API concentration, causing variability in treatment effectiveness.</li><li>✗ The chewing pattern influences the speed and release profile of the formulation.</li></ul> |

Unless they are swallowed, CG does not get direct exposure to the gastrointestinal mucosa, reducing the side effects of most excipients and their risk of intolerance by the gastric mucosa. The amount of saliva produced during the mastication process reduces the acidity of gastric fluids because of its buffering properties. The duration of action may be prolonged due to the drug reaching the stomach continuously through the saliva.

Despite its numerous advantages, MCG also possesses some disadvantages. The possibility of accidental or involuntary swallowing may remove the formulation from its desired site of action as well as possibly leading to choking. The amount of saliva released during mastication may dilute the API, lowering its concentration within the mouth, adding unexpected variability to the treatment. Since the mastication process varies across individuals, drug release of MCG may also vary according to the individual's chewing pattern.

CG may cause some problems in the oral cavity. Although controversial, some experts believe that the excessive CG consumption may lead to overuse of the mandibular muscles, which may, over time, result in jaw muscle imbalances and temporomandibular joint disorder, a condition characterized by pain, limited movement, and joint sounds in the jaw (Al Sayegh et al., 2020; Tabrizi et al., 2014). Another problem is the usually high adherence of CG, which may adhere to dentures and other dentifrice materials, leading to tooth and material decay (Wada et al., 2020).

It is worth mentioning that, when addressing the CG formulation, certain sweetening agents may cause different health complications. Inside human body, aspartame is metabolized into formaldehyde and methyl alcohol which are carcinogenic and poisonous, respectively (Jhanwar et al., 2017). Sucralose consumption has been linked with anemia, infertility, abortions in animals. Lastly, sorbitol and xylitol may cause, stomach irritation,

flatulence, abdominal discomfort, and laxative effects as side effects in individuals' susceptible to gastric imbalances (Lenhart & Chey, 2017).

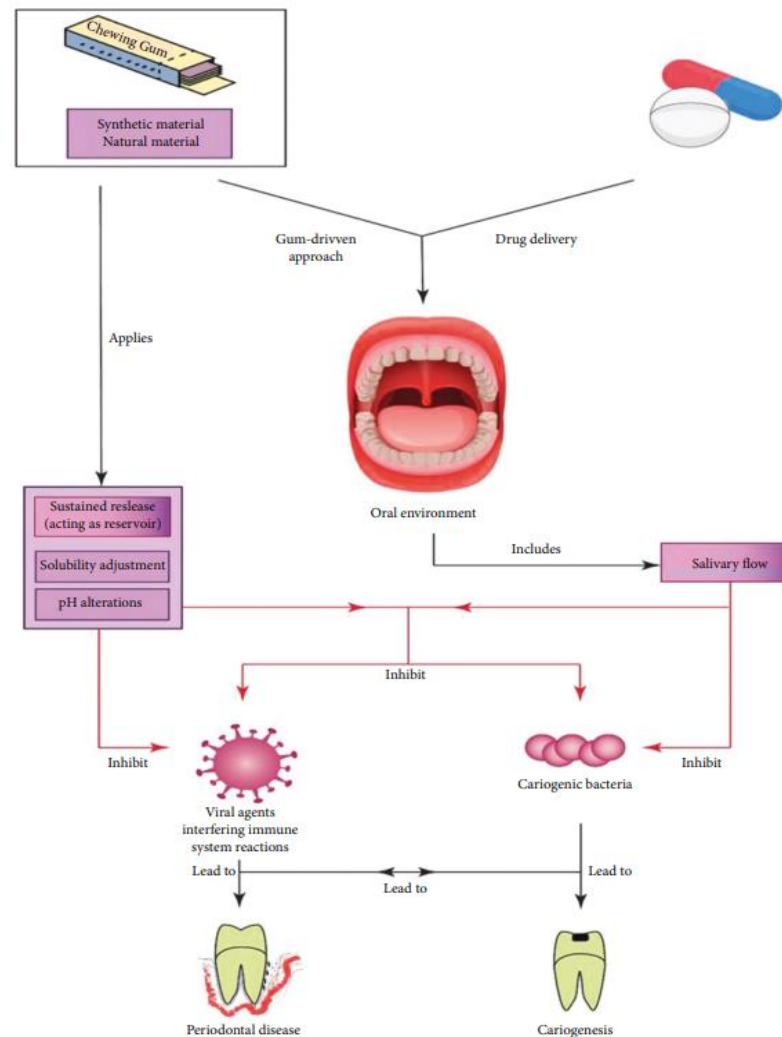


**Figure 6.** Schematic diagram of the gum drug delivery approach and factors affecting it. (Extracted from Banakar et al., 2022).

Finally, some authors highlight that CG provides a false food intake signal to the body, stimulating the secretion of enzymes and acids (Aslani & Rostami, 2015). Nevertheless, some evidence suggests that chewing CG does not increase the gastric fluid secretion to a degree where it could cause aggravation on stomach mucosa and lead to ulcer development (Hansen et al., 2005).

## Chapter II

### *Overview and objectives of this work*

## 2. Overview and objectives of this work

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### 2.1. CG as a possible mitigation strategy

As was previously shown, the occupational activity as a firefighter is multitasked and complex, involving several risks that at short, but principally long-term, become a burden for their overall health. Firefighters demonstrate increased mortality and morbidity rates as well as an increased prevalence of cardiovascular diseases and cancer. Literature highlights the contribution of firefighting activities to aggravate the firefighter's health condition. Therefore, mitigation procedures and preventive tools are urgently needed to promote firefighters' safety and health.

As a possible answer to this serious problem, this work aims to investigate an easy-use, cost-effective and sustainable mitigation device through the development of a CG containing 2 key ingredients: essential mint oil (EMO) and dry kiwiberry (DKB).

Essential oils are concentrated plant extracts which maintain the natural smell and flavor of their origin, produced via processes of mechanical pressing or distillation (Pezantes-Orellana et al., 2024). Theoretically, the EMO in the CG may have the capacity of retaining some pollutants that are emitted from the combustion of materials during fire-events, such as PAHs and BFRs as they are known to be lipophilic (Sjodin et al., 1999). This oil is incorporated and mixed with the hydrophobic polymers, remaining in the insoluble part of the CG that is not ingested and can be discarded from the mouth easily after a fire intervention. Alongside this, these oils have shown multiple beneficial effects as a result of their antimicrobial and anti-inflammatory properties (Pezantes-Orellana et al., 2024).

*Actinidia arguta* (**Figure 5**), known commonly as kiwiberry, baby kiwi, hardy kiwi, mini kiwi or bower *Actinidia* is a vine species belonging to the *Actinidiaceae* family, a small family of flowering plants that includes nearly 360 species. Its fruit as well as its subproducts (such as stems, leaves and roots) are rich in bioactive compounds such as phenolic acids, anthocyanins, flavonoids, sugars, various vitamins and carotenoids, which have potent antioxidant and anti-inflammatory properties, in addition to other benefits (Macedo et al., 2024). However, due to its small size, fruits are easily damaged during processing and storage, generating large quantities of waste that did not commercialize that results in waste (Silva et al., 2023). The inclusion of the DKB in the different CG formulations may provide protection against the oxidative damage caused by different pollutants.

Additionally, it allows for the reuse and application of potentially wasted kiwiberry through a sustainable solution.



**Figure 7.** Baby kiwiberry subproducts (Extracted from Silva et al.,2023)

## 2.2. Main objectives

Based on the available literature and ambitioning to reply to firefighters' needs, the specific objectives of this work are:

- 1) To develop CG formulations capable of retaining and protect against persistent organic pollutants released during fires.
- 2) To characterize the organoleptic properties of the CG formulations and perform a sensorial analysis within firefighters.
- 3) To develop an analytical methodology to analyze persistent organic pollutants in the CG.
- 4) To evaluate the potential of CG formulations to mitigate firefighters' exposure.

## Chapter III

### *Methodology*

### 3. Methodology

In the second chapter of this thesis, the methodology as well as the materials and equipment employed in the: development and characterization of the different CG formulations; analytic methodology for the analysis of pollutants and data treatment of the generated results is presented.

#### 3.1. Development of chewing gum formulations

The formulations utilized in this research project were developed in collaboration with Lusiteca Produtos-alimentares, SA. Since 1968, the company's main purpose has been the development and manufacturing of different confectionary products with high quality that provide consumers with sensations of well-being, fun, taste, and sweetness. In order to provide optimal products, Lusiteca establishes relationships of confidence with not only consumers, but also scientific researchers, stimulating creative discussions and scientific research that ultimately enhance the product's quality.

Their commercially available "Gorilla" mint CG was used as a reference gum (CGR). Each commercial Gorilla CG has an established weight of 5 grams and is composed of the following ingredients: sugar, glucose syrup, gum base, mint aroma, and glycerin. Using the commercial CG formulation as base, Lusiteca developed three different experimental CG formulations (CGA, CGB, and CGC) containing different concentrations of EMO and DKB, as described in **Table IV**.

**Table IV.** Main composition of the developed CG formulations

| Formulations |     | CGR                                                                                 | CGA                                                                                  | CGB                                                                                   | CGC                                                                                   |
|--------------|-----|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Appearance   |     |  |  |  |  |
| Composition  | EMO | 6g/kg of EMO                                                                        | 6g/kg of EMO                                                                         | 8g/kg of EMO                                                                          | 8g/kg of EMO                                                                          |
|              | DKB | Not present                                                                         | 2g/kg of DKB                                                                         | Not present                                                                           | 20g/kg of DKB                                                                         |

## 3.2. Characterization of the chewing gum formulations

### 3.2.1. Transparency and homogeneity

Transparency (also known as pellucidity or diaphaneity) is the physical property of permitting the passage of light through a material without substantial scattering. If light passes through a material, it is possible for the naked human eye to visualize past such material. Usually, the transparency of an object can be described in four easily understandable categories: 'opaque', 'translucent', 'transparent' and 'water-clear' (Al-Hamdani, 2018).

In the field of physics, a homogeneous material or system has the same properties at every point and, as such, it is uniform (*Homogeneity* | *Encyclopedia MDPI*, 2022). In the context of this work, it is related to the material surface and whether it is accordant or displayed inconsistencies, such as visible separation of ingredients. Homogeneity was determined through visual inspection.

To determine the CG formulations transparency, each CG unit was visually inspected as it was exposed against natural light exuded from the laboratory window. This process was repeated thrice for all formulations and the results were noted.

### 3.2.2. Dimensions

The gorilla CG produced by Lusiteca are known for their large, rectangular shape, with two long rectangular surfaces separated by two smaller rectangular on the sides.

To determine the width and length of CG, a generic ruler was used. However, when determining the thickness of the CG formulations, a digital micrometer gauge (Fervi Tools, Italy) was employed (**Figure 8**). A Micrometer is a measuring device ingrained with a calibrated screw, which can accurately measure the thickness of small components. They can be either analog or digital. Each unit of CG was measured in three different points. The process was repeated 3 times, with different units for every CG formulation.



**Figure 8.** Digital Micrometer (Fervi Tools, Italy).

### 3.2.3. Uniformity of mass

To determine the mass of each CG formulation, one unit of gum was unwrapped from its paper package, placed on the center of a Mettler Toledo® PM4600 Balance (Mettler Toledo, EUA) as seen in **Figure 9**, and the final value was noted. This process was repeated 3 times to determine the mean and standard deviation of all CG.



**Figure 9.** Mettler Toledo® PM4600 Balance (Mettler Toledo, EUA).

### 3.2.4. Water content

Water content or humidity can be defined as the quantity of liquid, namely water, contained within a food, medicine, cosmetics, or products sample. An infrared moisture balance (also known as an IR moisture balance), as seen in **Figure 10**, is a laboratory instrument which measures the moisture content of a sample. This device uses an infrared lamp that produces heat that dries the samples placed on an aluminum, and the difference in weight lost during this drying process is considered as the water weight. IR moisture balances are commonly used in industries such as food, pharmaceutical, and chemical, to determine the moisture content of certain materials, as well as in research and development applications to investigate the characteristics of certain materials and optimize production processes.

CG were divided in 3 samples; each sample was cut into 8 small pieces that were placed on an aluminum plate, subsequently placed inside a steel pan of a Infrared Moisture Determination Balance AD-4713 (A&D Company limited, Japan). The samples were heated until the temperature inside the balance reached 90 °C and remained at that temperature for 10 minutes. Afterwards, the resulting value was displayed on the scale in percentage.



**Figure 10.** *Infrared Moisture Determination Balance AD-4713 (A&D Company limited, Japan).*

### 3.2.5. Surface pH analysis

pH can be defined as the negative logarithm of the hydrogen-ion activity, measuring the amount of hydrogen ions ( $[H^+]$ ) present in a solution or medium. pH is classified on a scale from 0 - 7 (exclusive) as acidic, 7 as neutral and >7 - 14 as alkaline (M.J.Singer & A.George, 2009).

The Skin-pH-meter (Courage & Khazaka, Germany), as seen in **Figure 11**, is a device that uses the potentiometry method through a special flat glass electrode, which allows the direct measurement of this variable on the surface sample and allows sensitivity in the order of 0.1 of the pH unit, providing quickly measurements (1 s). As stated by the manufacturer, both glass  $H^+$  ion sensitive electrode and additional reference electrode are placed in one housing that is connected to a probe handle containing the measurement electronics (Courage+ Khazaka electronic GmbH, 2022). This device is commonly used in cosmetic and pharmaceutical research fields to determine skin or scalp pH.



**Figure 11.** Skin-pH-meter® (Courage & Khazaka, Germany).

Each unit of CG was measured by placing the head of the probe of the pH meter in contact with three different regions on the surface of the CG, resulting in three measurements per unit of gum. This process was repeated 3 times to calculate the mean pH surface value of each formulation.

### 3.2.6. Texture profile analysis

Texture is an important property being considered a key quality factor of most food/cosmetic/pharmaceutical products that are consumed nowadays. Consequently, research in this field has blossomed to understand food physics and develop new products with novel ingredients that cater to different tastes and specific groups, such as elderlies or obese individuals. Although the definition of texture may vary among authors, Bourne, 2002, provides a suitable one, defining texture as “... *the response of the tactile senses to physical stimuli that result from contact between some part of the body and the sample. The tactile sense (touch) is the primary method for sensing texture but kinesthetics (sense of movement and position) and sometimes sight (degree of slump, rate of flow), and sound (associated with crisp, crunchy, and crackly textures) are also used to evaluate texture*”.

Texture is apprehended sensorially as a result of multiple sensory stimuli working in a synchronized way. Nevertheless, the texture of any sample is derived from its structure or microstructure (from the molecular level to the microstructure and macroscopic level), which dictate how the structure deforms and breaks when handled or eaten. Therefore, any factor that may impact the structural properties (e.g., ingredient interactions, processing conditions, storage, and packaging, etc.) will result in a texture change (Bourne, 2002).

To evaluate the texture of a vast spectrum of foods, Dr. Alina Szczesniak, developed, through extensive research utilizing different foods and various trained sensory panels, a

method that displayed good correlation between instrumental values and subjective evaluation based on several key universal parameters (Friedman et al., 1962). This method is known as Texture profile analysis (TPA) and often called the "two bite test". TPA has become a standard procedure in the quality control of the foods and in the field of pharmaceuticals (Moskowitz & Kapsalis, 1975).

There are five basic independent mechanical parameters (hardness, cohesiveness, adhesiveness, viscosity, and springiness) and three dependent parameters (brittleness, chewiness, and gumminess) in this assay. These parameters can be precisely calculated, as seen in **Figure 12**, and can be described as follows (Friedman et al., 1962; *Texture Profile Analysis | Texture Technologies*, 2015):

- **Hardness:** An indicator of the most direct response to taste, directly affecting chewiness, gumminess, and cohesiveness.
- **Adhesiveness:** After submitting the sample to pressure deformation, if the surface is sticky, a negative force will be generated. In the food field, it can be interpreted as a sticky mouthfeel.
- **Cohesiveness:** Tensile force is a manifestation of cohesion. If a better sample is condensed, the display probe is also easier to keep clean. Cohesion is usually tested as a lesser parameter than chewiness, and adhesion.
- **Resilience:** Resilience is an indicator of how a sample recovers from deformation, including both speed and strength. Attention should be paid to the recovery of the sample during the measurement. Generally, a slower test speed is used to achieve sufficient time for the sample to recover. It also ensures the accuracy of this feature.
- **Springiness:** The height at which food can recover between the end of the first bite and the beginning of the second mouth.
- **Gumminess:** One of the characteristics of semi-solid foods is low hardness and high cohesion. Therefore, this indicator should be used to describe the taste of semi-solid foods.
- **Chewiness:** It can be interpreted as the energy required to chew solid food. It is difficult to measure accurately because chewing involves compression, shearing, puncture, comminution, tearing, cutting, etc., and is also related to oral conditions (saliva secretion, body temperature). This parameter is mainly used in the description of the solid, semi-solid mouthfeel.

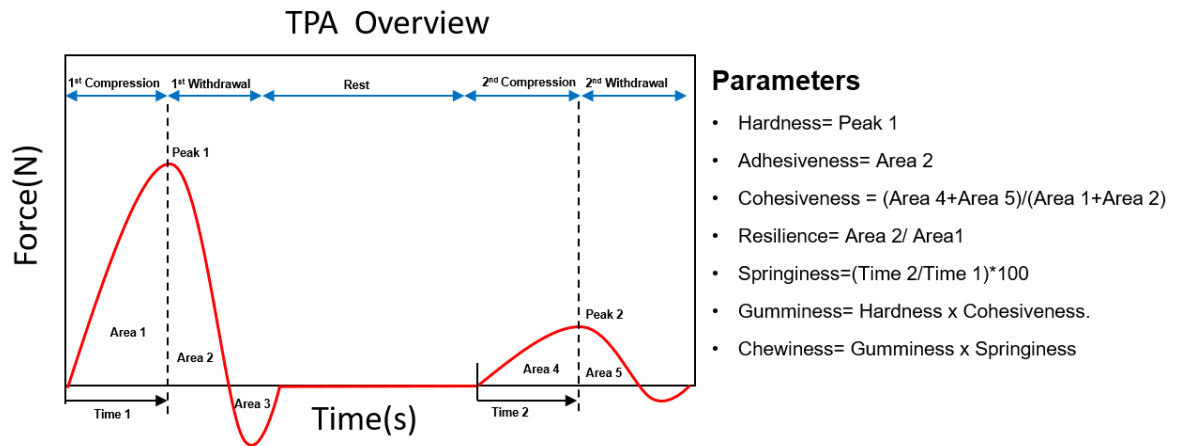


**Figure 12.** Typical TPA graph and parameter overview.

To simulate the mastication process utilized for TPA, a hand-plier was employed. All CG formulations were subjected to strains for 20 minutes, inside a porcelain mortar, filled with 10 mL of deionized water, at room temperature. Afterwards, the CGs were left to rest for 1 hour in a beaker filled with water at room temperature.

For this work, TPA was performed on a texture analyzer TA-XT2i, (Stable Micro Systems, United Kingdom), represented in **Figure 13**, utilizing a stainless-steel probe (4 mm diameter) and a 5 kg load cell. The CGs were taken out of the wrapping and placed in the center of a platform. The speed of the pre-test, test, and post-test was 1 mm/s. The distance from the platform was 8 mm, with double compression performed in intervals of 5 seconds between two compressions. The TPA produced a graph of force *versus* distance and measured all the parameters as described above.

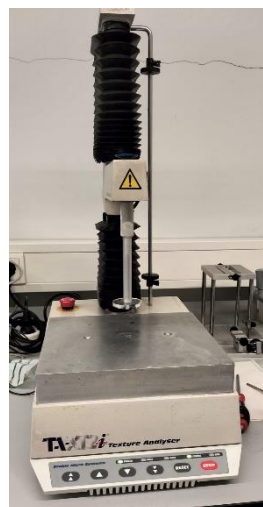


**Figure 13.** Texture analyzer TA-XT2i (Stable Micro Systems, United Kingdom).

### 3.2.7. Rheology Analysis

Rheology is the study of the flow behavior of materials, which strongly depends on the internal structure of the material and how that changes with time, stress, and shear rate. This is usually visualized by a flow curve in a diagram that depicts the relationship between shear stress and shear rate.

Over the years, many tests have been developed and employed to examine the rheology of a material, but in the context of this work only four were employed, as they can examine diverse parameters that are commonly associated with most semi-solid materials. These characteristics are the flow behavior, thixotropy, the linear viscoelastic region (LVER) and yield strength.

The LVER is important in rheology and dynamic mechanical analysis because it represents a region of strains where the viscoelastic parameters of the material are strain independent (Kevin, 2024). After this point, superior strains result in a change in the microstructure of a sample. Oscillatory sweep tests are used to examine this region. In these tests the measuring system moves backwards and forwards at a certain frequency and amplitude (*Joe Flow - Oscillatory Measurements | Anton Paar*, n.d.). The given deformation and the measured time-delayed shear stress response are used to calculate the two parameters: the storage modulus ( $G'$ ) and loss modulus ( $G''$ ). The  $G'$  is a measure of the deformation energy stored in the system. It reflects the solid character (the elastic part) of the sample. The  $G''$  is a measure of the lost deformation energy and therefore characterizes the liquid part (the viscous part) of the sample (*Joe Flow - Oscillatory Measurements | Anton Paar*, 2024).

Thixotropy is interpreted as the continuous decrease of viscosity with time, when flow is applied to a sample that has been previously at rest, and the subsequent time-dependent recovery of viscosity when that flow is discontinued. Three interval thixotropy test (3ITT) can be a useful tool to perform instant shear stress/shear rate deformation to simulate effects of pumping and instant stirring during processing and handling steps of food materials. As the name suggests, it is composed of three intervals or phases: the 1<sup>st</sup> phase (reference), 2<sup>nd</sup> phase (high shear) and 3<sup>rd</sup> phase (regeneration). In the first interval, shear conditions are practically at rest; therefore, low shear is applied in the LVER. In the 2<sup>nd</sup> phase, high-shear conditions are performed outside the LVER to break the internal structure of the food materials in order to simulate conditions normally occurring during processing and handling of materials. In the 3<sup>rd</sup> phase the same low-shear conditions are again applied in order to study the regeneration of the structure (Mezger, 2011).

In rheology, yield stress is defined as the minimum applied stress at which irreversible plastic deformation is first observed across the sample.

To determine the rheological properties of CG, the mastication process was simulated by maceration utilizing hand-pliers for 20 minutes and a porcelain mortar. Afterwards, CG was placed in beaker with water, where it remained submersed for 1 hour. Finally, a sample was taken from CG and placed in the center of a plate of a Kinexus Lab rheometer (Malvern, USA) (**Figure 14**) and excess material was trimmed with a spatula. Complex viscosity was determined using a semisolid probe with a 2 mm gap between the probe and sample. All essays were performed at 37 °C.

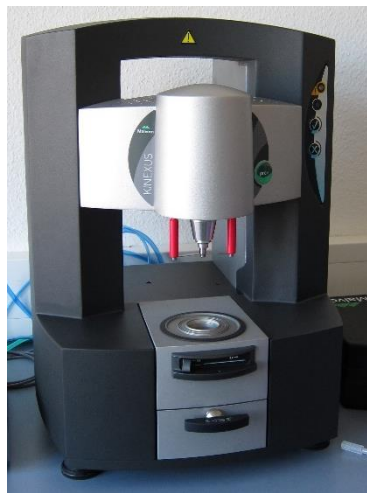


**Figure 14.** Rheometer Kinexus Lab (Malvern, USA).

### 3.2.8. Colorimetry

The definition of color is variable across disciplines. Color is not an innate property of material objects; it is a perception taken by vision based on the materials interaction with light (Ohno, 2000). The way in which an object's surface absorbs, reflects, and emits back an emission spectra that is captured and processed by the eyes and brain forms the color of the material. In physics, colors are associated specifically with electromagnetic radiation of certain range of wavelengths visible to the human eye (Nassau, 2024). Radiation of such wavelengths constitutes that portion of electromagnetic spectrum known as the visible spectrum or visible light (Nassau, 2024).

Colorimetry is the science and technology used to quantify and describe physically the human color perception (Ohno, 2000). The basis for colorimetry was established by the *Comission Internationale de l'Eclairage* (CIE) in 1931 based on visual experiments. Even though limitations are well recognized, the CIE system remains the only internationally

agreed metric for color measurement. Since its inception in 1931, other systems have been developed based on it, such as the CIELUV and CIELAB.

The CIELAB color was also created by the CIE in 1976 to allow a more accurate specification of object colors and color differences (Ohno, 2000). In this model, there is 3 coordinates which represent the perceptual lightness of the color ( $L^*$ ), its position between red and green ( $a^*$ ) and its position between yellow and blue ( $b^*$ ) (**Figure 15**).

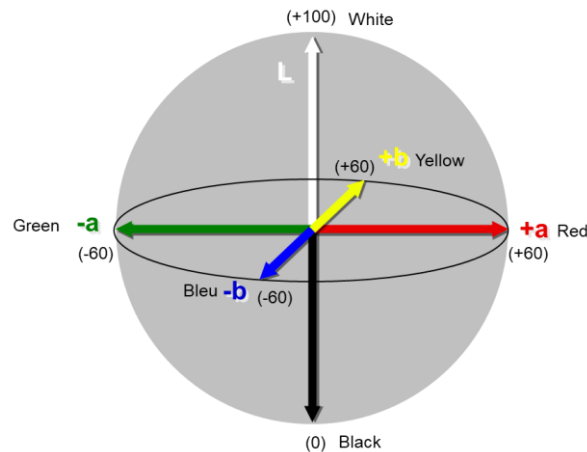


**Figure 15.** CIELAB Color model.

In this work, the color of the CG formulations was evaluated utilizing the colorimeter CR-400 Chroma Meter (Konica Minolta, Japan) (**Figure 16**), a handheld, portable measurement instrument designed to evaluate the color of objects, particularly with smoother surface conditions or minimal color variation. It accurately identifies color characteristics in objects, determines color differences between objects, and can be used to determine if a sample meets the defined standard, making it suitable for color inspections of food, building materials, cosmetic and medical products within quality control, quality assurance, and R&D fields.

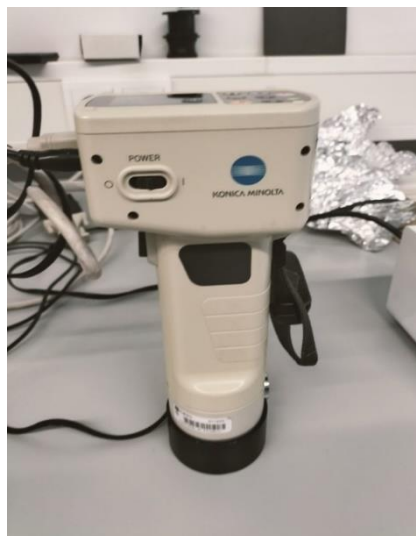


**Figure 16.** CR-400 Chroma Meter (Konica Minolta, Japan).

To measure CG formulations color, a unit was placed on top of the lens of the colorimeter, which was then covered with a carton protection. Each unit of gum was measured in three different spots. This process was repeated three times with three units per formulation, and the results were expressed according to the color space CIE 1976  $L^*a^*b$ .

### 3.2.9. Differential Surface Calorimetry

Calorimetry is a branch of thermodynamics that studies the thermal properties of materials to establish a correlation between temperature and specific physical properties of substances, being the only method for direct determination of the enthalpy associated with the process of interest (Gill et al., 2010). The most common technique employed in calorimetry is the differential scanning calorimetry (DSC).

DSC is a thermal analysis measuring how physical properties of a sample change, along with temperature against time. In other words, the device is a thermal analysis instrument that determines the enthalpy or heat flow associated with specific material transitions as a function of temperature or time. During changes in temperature, the DSC measures the heat quantity, which is radiated or absorbed excessively by the sample based on a temperature difference between the sample and the reference material (Gill et al., 2010). The simpleness and versatility of this technique makes it widely applicable across different fields such as chemistry, pharmacology, and recently, in nanoscience to measure thermodynamic properties of biomolecules and nano-sized materials.

Each unit of CG was cut into small samples weighing about 27-30 mg and sealed in small aluminum crucible (**Figure 17**) with the aid of a steel seal press. DSC curves were

obtained at a temperature rate of  $10\text{ }^{\circ}\text{C min}^{-1}$  from 0 to  $200\text{ }^{\circ}\text{C}$ . Nitrogen was used as a protective gas and flow were kept at  $100\text{ mL/min}$  and  $50\text{ mL/min}$ , respectively.



**Figure 17.** Aluminum crucibles (NETZSCH Premier Technologies, USA) containing CG samples.

A blank aluminum crucible was applied as a reference standard, and Netchze software version 1.54 was used for the data analysis. Raw data was extracted, and graphs were generated in Microsoft Excel. The process was repeated with 3 different units for every CG formulation. A DSC 200 F3 Maia (NETZSCH Premier Technologies, USA) was used to characterize all formulations (**Figure 18**).

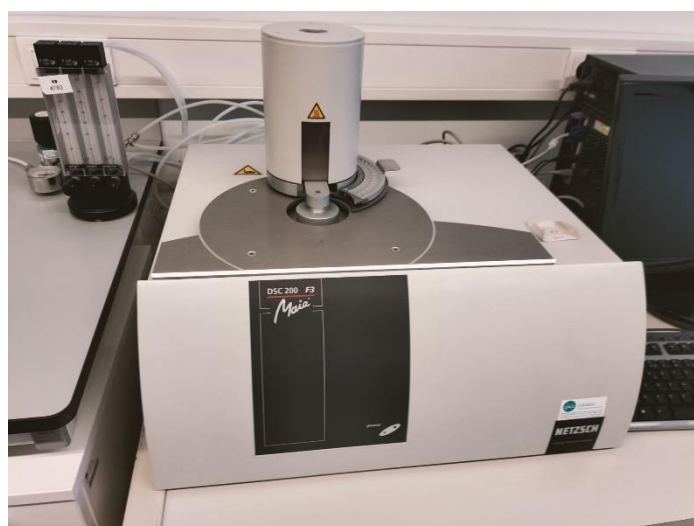


**Figure 18.** DSC 200 F3 Maia (NETZSCH Premier Technologies, USA).

### 3.3. Selection of Participants

In total, 12 firefighters serving at two different stations located in the Aveiro district, Portugal, volunteered to participate in the project's activities. All firefighters filled an personal questionnaire to collect individual characteristics (age, weight, height, existence of diagnosed chronic disease and current health status, and smoking habits) and professional information (years of service as a firefighter and recent participation in fire events). The collected data assesses exposures to pollutants (e.g., dietary habits, tobacco consumption, and recent environmental exposure to tobacco smoke). All participants read and signed an consent form which was previously approved by the Ethic Committee of the University of Porto.

### 3.4. Sensorial Analysis

Sensory analysis (SA) examines the characteristics (texture, flavor, taste, appearance, smell, etc.) of a product or food through the senses (sight, smell, taste, touch, and hearing) of the panel of individuals involved. This form of analysis has been used to evaluate the acceptability of food and pharmaceutical products. Over the years, this kind of methodology has evolved and is now considered as one of the most important essays in product development and innovation (Ruiz-Capillas & Herrero, 2021). To perform a sensorial analysis, a test panel, composed of trained individuals or general consumers, evaluates an products organoleptic qualities using traditional sensory techniques, such as discriminatory, descriptive evaluations, preference, and hedonic tests (Ruiz-Capillas & Herrero, 2021).

The test panel of this sensorial analysis was composed by military firefighters of the Emergency Protection and Relief Unit (Unidade de Emergência de Proteção e Socorro - UEPS) of the Republican National Guard (Guarda Nacional Republicana - GNR), aged between 34 and 42 years old. The chewing gum was evaluated with the following order: CGR, CGA, CGB, and CGC. To cleanse the palate, participants consumed dry toast and drank water in between each formulations. The attributes evaluated were color, odor, hardness, adhesiveness, aroma, flavor, homogeneity, and disintegration before chewing the CGs, 10 seconds and 10 minutes after chewing each of the CGs. Most parameters were evaluated on a hedonic scale from 1 to 5, except the attribute of disintegration and homogeneity that were evaluated from 1 to 3. Besides these attributes, participants were also requested to pick a preferred formulation and questioned about their CG consumption with 3 different questions. The sensory test, including the evaluated attributes and the respective scales, are present in **Appendix X** in greater detail.

### 3.5. Assessing the chewing gum capacity for pollutant retention

Once the main characteristics and palatability of the different formulations were determined, it was necessary to assess the CG's capacity to retain different pollutants that can be released during fire events.

To achieve this, two analytical methods that simulated mastication of the product during conditions of exposure were explored to analyze the retention of PAHs and BFRs which are two common pollutants that result from fire emissions. These methods were based on high-performance liquid chromatography (HPLC) and gas chromatography (GC), respectively.

#### 3.5.1. Polycyclic aromatic hydrocarbons

HPLC, is a sophisticated form of column chromatography which is commonly used in biochemistry and analysis to separate, identify, and quantify active chemicals in isolation or in a mixture (Sadaphal & Dhamak, 2022). A typical HPLC setup is outlined in **Figure 19**. Firstly, a solvent (also known as mobile phase) flows under high pressures into the system from a recipient pushed by a pump mechanism, creating a mobile phase stream. The samples are introduced into this stream in small quantities via an injector. They are then pushed along a column containing a packing material (known as stationary phase), through which the various compounds will be separated and eluted depending on their specific chemical or physical interactions with the material of the column. At the end of the system, there is a detector which captures the molecule's retention times and producing an electrical signal that is converted by the computer into data which can be analyzed. The retention time of a compound is influenced by the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) utilized.

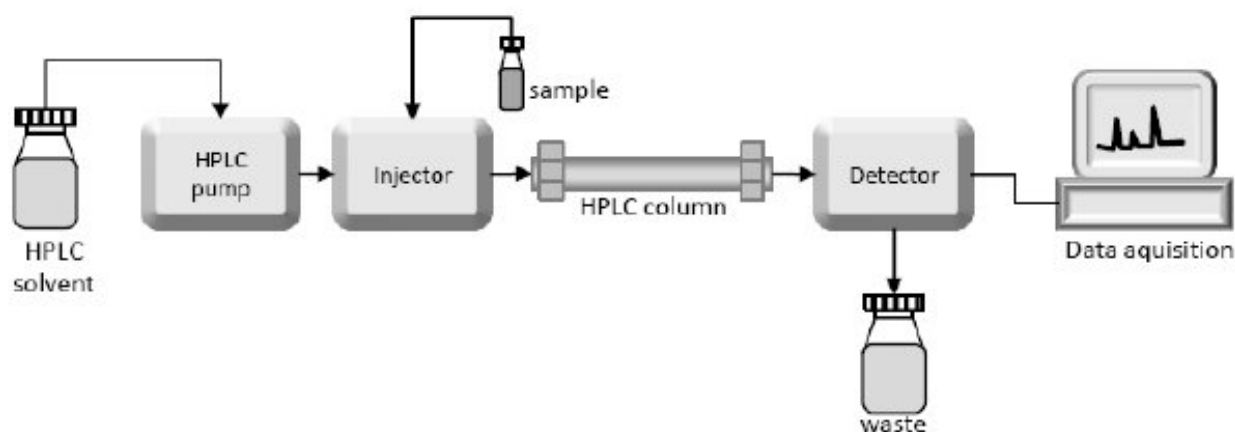


**Figure 19.** Diagram of HPLC setup. Adapted from (Adapted from Unacademy, 2023)

Regarding the procedure, firstly, a small portion of CG (0.5g) was weighted and fractioned into 8 pieces and placed inside that test tube along with 1mL of artificial saliva for pharmaceutical research (Sigma-Aldrich, EUA) and 100uL of PAHs standard solution. The test tube is vortexed for 1 min, after which saliva and CG were separated into 2 individual tubes.

In the case of the test tube containing the saliva, 5ml of acetonitrile was added to the vial. The flask is vortexed for 5 minutes and centrifuged for 5 minutes at 45000 rpm. The mixture was then dried with 1 gram of anhydrous sodium sulfate (Pronalab, Mexico). The supernatant is transferred to a evaporation tube and taken to a Rotavapor R-200 (Buchi, USA) (**Figure 20**). The extract is then reconstituted in 500 uL and filtered through a 0.45  $\mu$ m filter into a vial, using a syringe.



**Figure 20.** Rotavapor R-200 (Buchi, USA).

In the case of the test tube containing the CG itself, 5ml of acetonitrile (VWR Chemicals, USA) was added to the vial. The flask is vortexed for 5 minutes and centrifuged for 5 minutes at 45000 rpm. This process is repeated 3 times. Any remaining CG is dissolved utilizing 3ml of *n*-hexane (Carlos Erba reagents, Italy) and 5 ml of a acetonitrile and centrifuged for 5 minutes at 45000 rpm.

The total 20ml of extraction acetonitrile is then transferred to an evaporation tube and taken to a rotavapor R-200. The extract is then reconstituted in 500  $\mu$ L and filtered through a 0.45  $\mu$ m filter into a vial, using a syringe.

The extracts of the CG and saliva were then injected into the HPLC system (**Figure 21**) and analyzed. This system has a mobile phase composed of a mixture of acetonitrile and water and a stationary phase which is a Nucleosil 100-5 C-18 PAH column (Macherey-

nagel, Germany). Finally, the chromatograms were collected, analyzed and the resulting peak area and retention times were measured, and the final data was transferred to Ms. Excel.



**Figure 21.** HPLC system (Shimadzu, Japan).

The PAHs standard solution contained the 18 following compounds: *Naphthalene* (Naph), *Acenaphthene* (Acen), *Acenaphthylene* (Ace), *Fluorene* (Flu), *Phenanthrene* (Phe), *Anthracene* (Ant), *Fluoranthene* (Fln), *Pyrene* (Pyr), *Benz[a]anthracene* (B(a)A), *Chrysene* (Chry), *Benzo[b]fluoranthene* (B(b)Ft), *Benzo[j]fluoranthene* (B(j)Ft), *Benzo[k]fluoranthene* (B(k)Ft), *Benzo[a]pyrene* (B(a)P), *Dibenzo[a,l]pyrene* (DB(a,l)P), *Dibenzo[a,h]anthracene* (DB(a,h)A), *Benzo[g,h,i]perylene* (B(g,h,i)P), *Indeno[1,2,3-c,d]pyrene* (InP)

### 3.5.2. Brominated flame retardants

GC is a term used to describe the group of analytical separation techniques used to analyze volatile substances in the gas phase. In gas chromatography, the components of a sample are dissolved within a liquid solvent and vaporized in order to separate the analytes by distributing the sample between two phases: a stationary phase and a mobile phase. A typical GC setup can be seen in **Figure 22**. The mobile phase is a chemically inert gas which carries the analyte through a heated column. GC is one of the sole forms of chromatography that does not utilize the mobile phase for interacting with the analyte. The stationary phase is either a solid adsorbent, termed gas-solid chromatography, or a liquid on an inert support, termed gas-liquid chromatography. Gas chromatography is an instrumental technique used forensically in drug analysis, arson, toxicology analyses of other organic compounds. In the context of this work, the GC was utilized in order to quantify the amounts of brominated diphenyl ethers (BDE), which are a class of BFRs, present the contaminated CGs.

The used GC machinery is equipped with an electron capture detector (ECD), a device invented in 1957 by James Lovelock, capable of detecting atoms and molecules in a gas through the attachment of electrons via electron capture ionization. It is commonly used in the analysis of halogen compounds (Snow, 2022).

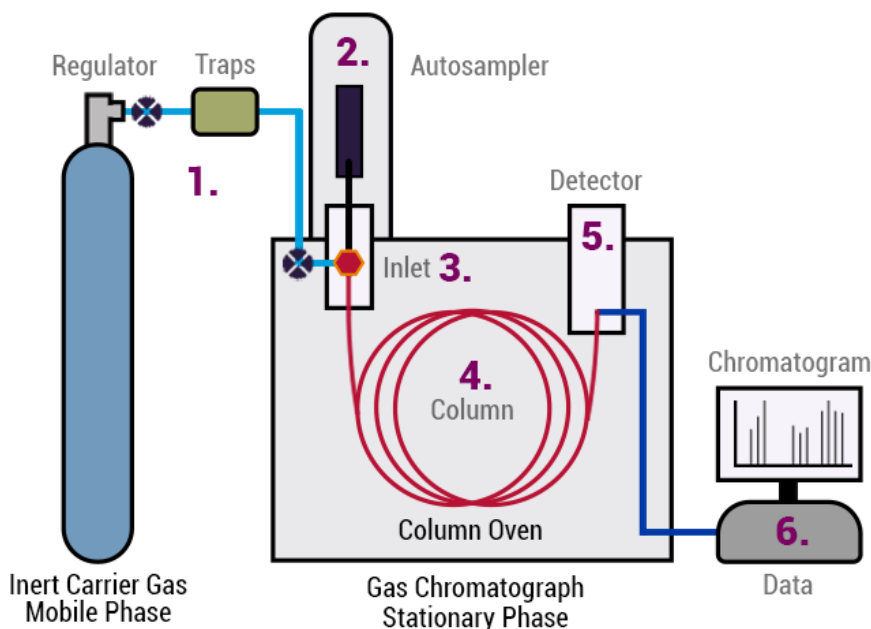


**Figure 22.** Different sections of the GC (Extracted from How Does Gas Chromatography Work? PerkinElmer Blog, 2022)

Regarding the exact procedure, firstly, 100 $\mu$ L of BFRs standard solution (500  $\mu$ g/L) was added to the tube and then evaporated with nitrogen flow. A small portion of CG (0.5g) was weighted, fractioned into 8 pieces and placed inside that test tube along with 1mL of Artificial saliva for pharmaceutical research (Sigma-Aldrich, EUA). The test tube was vortexed for 1 min and saliva and CG were separated into 2 individual tubes. 1.5 ml of n-Hexane are added to each tube. In the case of the test tube containing saliva, it is vortex for 5 mins. Afterwards, the supernatant is transferred to a dispersive SPE 2ml cartridge (Agilent Technology, USA) and centrifuged for 5 mins at 10000 rpm. Finally, 300  $\mu$ L are transferred to a vial.

In the case of the test tube containing the CG, 1ml of ultra-pure water was also added to the vial. The flask is vortexed for 5 minutes and centrifuged for 5 minutes at 45000 rpm. The supernatant is transferred to a dispersive SPE 2ml cartridge (Agilent Technology, USA), as seen in **Figure 23**, which is then centrifuged for 5 minutes at 100000 rpm. Finally, 300  $\mu$ L are transferred to vial.



**Figure 23.** Extract inside the SPE cartridge.

The extracts were then injected into the GC-2010 (Shimadzu, Japan) system with ECD and analyzed (**Figure 24**). This system has a mobile phase composed of nitrogen (Linde, Germany) and a stationary phase which is a column Zebron ZB-5ms GC Columns (Phenomenex, USA). Finally, the chromatograms were collected, analyzed and the resulting peak area and retention times were measured, and the final data was transferred to excel.



**Figure 24.** GC-2010 (Shimadzu, Japan).

The BFRs standard solution contained the 7 following compounds: 2,4,4'-tribromodiphenyl ether (BDE 28), 2,2',4,4'-tetrabromodiphenyl ether (BDE 47), 2,2',4,4',6-pentabromodiphenyl ether (BDE 100), 2,2',4,4',5-pentabromodiphenyl ether (BDE 99), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE 154), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE 153), 2,2',3,4,4',5,6'-heptabromodiphenyl ether (BDE 183).

The methodology described was reproduced to analyze the different pollutants present in the CG samples used by members of the GNR-UEPS during 2024 firefighting events.

### **3.6. Data Treatment**

To draw the graphs used for discussion, raw data was extracted from all the equipment used and was later plotted utilizing Ms.Excel and PowerPoint. To analyze the significance of the results obtained in the characterization of the CG and compare different formulations between each other, one-way Analysis of Variance (ANOVA) with Turkey HSD post-hoc test was performed utilizing the program IBM SPSS Statistics® (Version 28.0).

ANOVA is a crucial tool which helps researchers to carry out studies on more than two experimental groups and control groups. This test gives a significant result, which identifies if there is a significant difference between the groups tested. However, the test does not inform on the pattern of differences between the means of groups (Nanda et al., 2021).

Tukey HSD ("honestly significant difference" or "honest significant difference") test is a post hoc statistical tool used to determine if the relationship between two sets of data is statistically significant – that is, whether there's a strong chance that an observed numerical change in one value is causally related to an observed change in another value and is generally used after ANOVA. In simpler terms, the Tukey test is a way to test an experimental hypothesis and can be used to compare different means. In the context of this work this test was performed to compare the different CG formulations against each other (Nanda et al., 2021).

## Chapter IV

### *Results and Discussion*

## 4. Results and Discussion

In the current chapter, the resulting data obtained from the assays described in Chapter II are presented and discussed. The results are divided in three sections: the first section (3.1) is dedicated to the outcomes related with the characterization of the CG formulations, while the second one (3.2) pertains to the analytical methodology developed to extract and analyze the pollutants retained by the CG systems and its application in real-life wildland firefighting scenarios.

### 4.1. Chewing gum characterization

The CG physical and chemical characteristics are described in **Table V**.

*Table V. Physical and chemical characteristics of the CG formulations.*

| Attributes          | Formulations             |                          |                          |                          |
|---------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                     | CGR                      | CGA                      | CGB                      | CGC                      |
| Transparency        | Opaque                   | Opaque                   | Opaque                   | Opaque                   |
| Homogeneity         | Homogenic                | Heterogenic              | Homogenic                | Heterogenic              |
| Mean Length (cm)    | 3.00 ± 0.05 <sup>a</sup> | 3.20 ± 0.05 <sup>a</sup> | 3.20 ± 0.05 <sup>a</sup> | 3.20 ± 0.13 <sup>a</sup> |
| Mean Width (cm)     | 2.00 ± 0.00 <sup>a</sup> | 2.10 ± 0.21 <sup>a</sup> | 1.90 ± 0.12 <sup>a</sup> | 1.90 ± 0.13 <sup>a</sup> |
| Mean Thickness (mm) | 6.71 ± 0.49 <sup>a</sup> | 7.90 ± 0.14 <sup>a</sup> | 6.87 ± 0.22 <sup>a</sup> | 7.15 ± 0.20 <sup>a</sup> |
| Mean Mass (g)       | 5.17 ± 0.42 <sup>a</sup> | 7.10 ± 0.09 <sup>a</sup> | 6.36 ± 0.53 <sup>a</sup> | 6.42 ± 0.70 <sup>a</sup> |
| Water content (%)   | 0.34 ± 0.01 <sup>a</sup> | 0.39 ± 0.07 <sup>a</sup> | 0.40 ± 0.03 <sup>a</sup> | 0.27 ± 0.03 <sup>a</sup> |
| Surface pH          | 5.07 ± 0.04 <sup>a</sup> | 5.80 ± 0.35 <sup>a</sup> | 5.36 ± 0.36 <sup>a</sup> | 5.11 ± 0.10 <sup>a</sup> |

<sup>a</sup> Standard Deviation (STD)

#### 4.1.1. Transparency and homogeneity

As previously stated, transparency is the physical property of allowing light to pass through material without scattering. All CG formulations were classified as opaque. The variation in ingredients across formulations (EMO and DKB) did not affect the apparent transparency of the formulations. Most of the commercially available CG are opaque, since their ingredients do not allow for the passage of light.

Regarding homogeneity, it is possible to observe that CGR and CGB display a homogenous surface appearance, while CGA and CGC have heterogeneous aspect, with

brown spots dispersed over the surface. These results suggest that the differences in appearance were related to the presence of DKB. In addition, CGC, which as a higher concentration of DKB, has noticeably more spots than CGA.

#### **4.1.2. Dimensions**

The formulations displayed a rectangular shape analogous to the one of the reference CG, despite some differences were found regarding thickness.

The average length of the CG formulations varied between 3 and 3.2 cm, without significant differences ( $p>0.05$ ). Similarly, the mean width of the CG formulations does not vary greatly, ranging from 1.90 to 2.10 cm, despite significant differences were observed ( $p<0.05$ ).

However, when measuring the mean thickness of all formulations, the results ranged from 6.71 to 7.9 mm. The mean thickness of CGA varied significantly from the rest, having almost 8 mm.

#### **4.1.3. Uniformity of mass**

As stated by Lusiteca®, the average mass of each commercial gorilla CG unit is 5 grams. Unsurprisingly, the CGR did not vary much from the alleged weight, presenting a mean mass of 5.17 g. However, the test formulations vary significantly from it, displaying higher average masses. CGB and CGC did not have significant differences ( $p>0.05$ ) among them, displaying an average mass of 6.17g and 6.42 g, respectively. Finally, CGA varied significantly from all formulations, having an additional weight of almost 2 grams than the reference gum. This mass increase is probably correlated with the verified increase in dimensions. As these were experimental batches, the differences may be a consequence of the established procedure and the formula with the added ingredients.

#### **4.1.4. Water content**

The water content typically found in commercial gum is below to 2% (Rudolph & Hynson, 1977). The water content of all CG formulations was generally low and within the expected values. CGB showed the highest amount, while CGR, CGA and CGB do not vary significantly from each other ( $p>0.05$ ). The CGC formulation presented a significantly lower water content ( $p<0.05$ ) when compared to CGA and CGB, which may be related with the presence of a very high concentration of DKB.

The low moisture displayed by the formulations may be beneficial as it may result in a rather long shelf-life. According to the International Chewing Gum Association, CG retains its quality for so long that, in most countries, it is not required by law to be labeled with an expiration date. If CG remains in a stable environment, the gum may become brittle or forsake its flavor over time, but it will never be unsafe to eat. If it is exposed to moisture, water migration might occur over time, resulting in a soggy CG (ICGA, 2005).

#### **4.1.5. Surface pH**

The formulations displayed acidic surface pH values ranging between 5.07 and 5.80. CGR, CGB and CGC do not vary significantly among them ( $p>0.05$ ), while CGA showed the highest pH. The addition of DKB and EMO resulted in a pH increase, reducing the acidity of the CGs. The literature available regarding surface pH of CG is scarce. To the best of our knowledge, only one study evaluated the mean pH of several commercially available CG and reported that the pH of six popular sugar gums ranged between 2.70 and 3.56, while seven sugar-free gums varied between 6.58 and 7.57 (Patel et al., 2010). The authors concluded that acidic gums may damage tooth enamel and dentine erosion, being not this increase detrimental to the CGs quality (Patel et al., 2010). The results achieved in the present study are slightly higher than expected, probably due to the presence of sugar.

#### **4.1.6. Texture Profile Analysis**

As previously stated, TPA simulates the successive penetrations (Bites) that occur when a material is subjected to mastication, producing a graph of force (N) in function of time (sec), as displayed in **Figures 25-26**. The textural profiles of the experimental CG formulations when dry or unchewed (**Figure 25**) are identical, and statistical analysis revealed that the differences between them were non-significant ( $p>0.05$ ). However, in some textural attributes, the experimental CGs differed significantly ( $p<0.05$ ) from the reference gum. Common to all profiles is the difference in force required for penetration between the first and second bite.

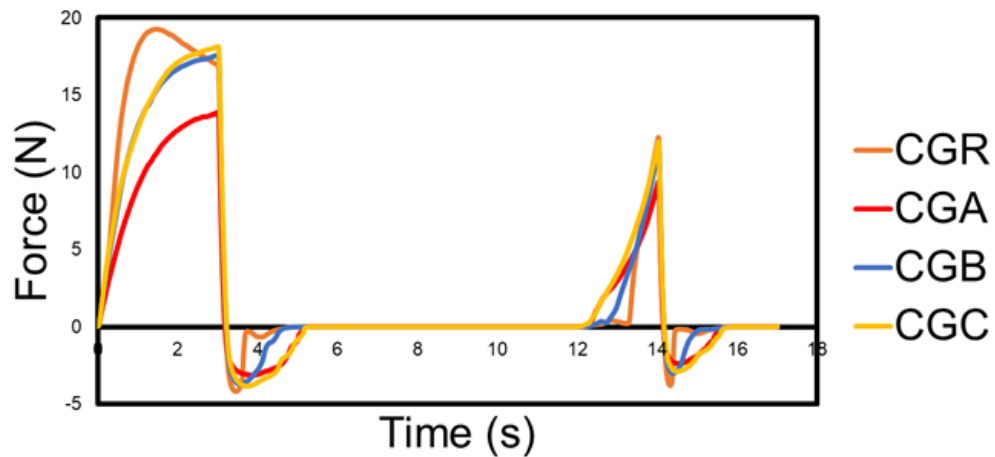


**Figure 25.** Texture profile analysis of dry CG formulation.

After CG formulations are subjected to a mastication process (**Figure 26**), their textural profiles become more similar. The CGB and CGA profiles were nearly identical, without significant variation ( $p>0.05$ ). As was seen before, the textural profile of CGR was noticeably different than the remaining formulations, except regarding hardness, represented by the peak of the first penetration. Overall, when comparing the textural profiles of all CG formulations, before and after mastication, the first apparent change is a pronounced drop in the force required for each penetration. Alongside this, after chewing there is less differences between the first and second penetration, unlike what was observed for the dry profiles.

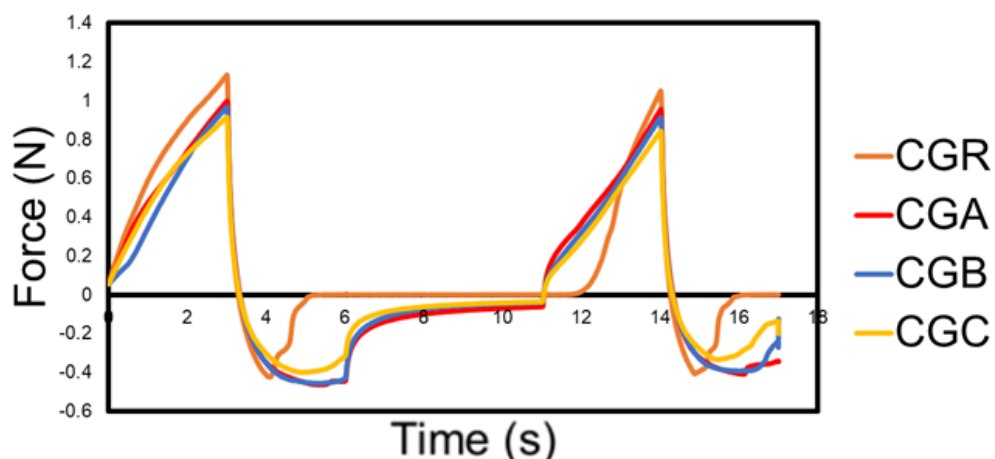


**Figure 26.** Texture profile analysis of chewed CG formulations.

Utilizing the displayed TPA graphs as base, several different attributes can be calculated by the equipment software, as expressed in Chapter 2. These attributes allow for a more exact description (utilizing values) of the textural behavior of CG formulations. In general terms, when comparing the attributes of the CG formulations before and after

mastication, some were attenuated by the chewing process, while others were enhanced. For instance, attributes such as hardness and adhesiveness decreased, while parameters like resilience, cohesion, springiness, gumminess, and chewiness increased.

The first parameter examined was hardness, which is determined by the highest peak force during the first “bite” of the probe (**Figure 27**). CGR revealed the highest hardness value. TPA indicates that the experimental formulations possessed lower hardness values than the reference gum. To better understand the causes of these outcomes, comparisons between the different formulations were made. The comparison between CGR and CGB suggested that an increase in EMO solely may result in a softer gum. In an opposite way, the comparison of CGC and CGB suggested that the presence of a high quantity of DKB slightly increased the formulation hardness. Considering this, the lower hardness value found in CGA seems like an anachronism since, being the formulation with the lowest concentrations of both ingredients, the drop in hardness should not be as significant as was demonstrated, especially when compared to the others.

After chewing, hardness decreased pronouncedly to almost 1 N in all formulations, without significant ( $p>0.05$ ) variation between them. This was expected since the system is hydrated and the bonds between the polymers that make up the material were broken by offering less resistance to penetration. Another possible explanation is that, as the product is chewed, the ingredients are released to water, leaving only the insoluble gum base that is similar for all formulations.

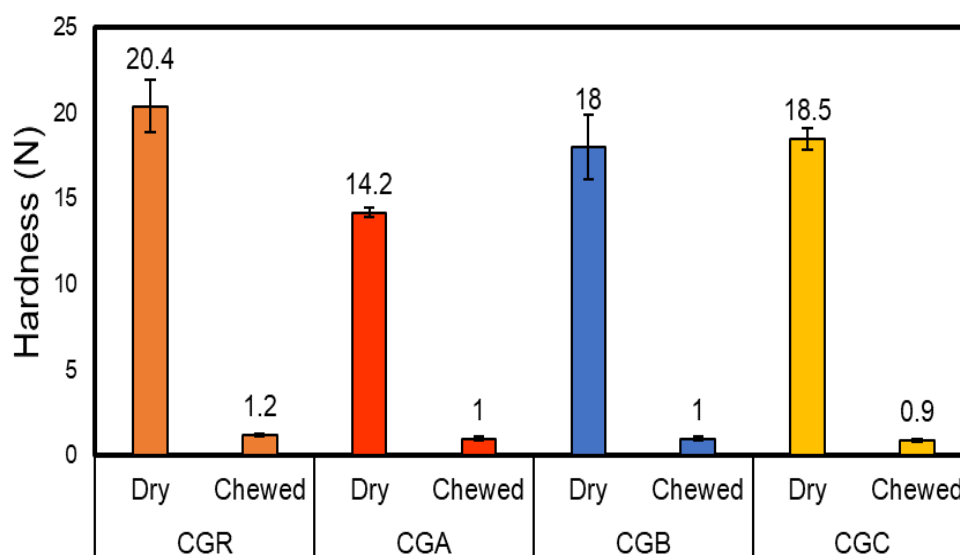


**Figure 27.** Hardness of formulations as determined by TPA.

After the first descent in which the testing material is penetrated, the probe is lifted away from the sample. During this ascent, if the surface of the sample is sticky, a negative

force will be generated as resistance to the upward movement; this resistant force represents the attribute of adhesiveness. As can be seen in **Figure 28**, CGC demonstrated the highest adhesiveness, while CGR showed the lowest adhesiveness. Comparing CGB and CGR it is possible to conclude that the presence of EMO increases the adhesiveness of the formulation. This could occur since CG systems are mainly composed by hydrophobic polymers that repel water and attract oil (Kaveh et al., 2023). The adhesiveness observed results from CG hydrophobic nature, as the gum can form bonds and adhere when contacts with oily surfaces. Moreover, since CG are heterogeneous systems, especially when unchewed, the extra EMO present in the experimental formulations may contribute to the higher adhesion of gums to the probe.

CGB and CGC had the same concentration of oil, while DKB is only present in CGC, leading to higher adhesiveness. Additionally, CGA, which has less EMO than CGB and DKB, also achieved higher adhesiveness values. These two comparisons suggests that DKB increases the CG adhesiveness.

After mastication the adhesiveness decreases for all formulations. Test formulations display approximate adhesiveness values which are higher than that of the reference gum. As the different ingredients are blended into one cohesive mass and the hydrophilic components are released into the aqueous phase, the resulting CG became less prone to adhere to the probe.

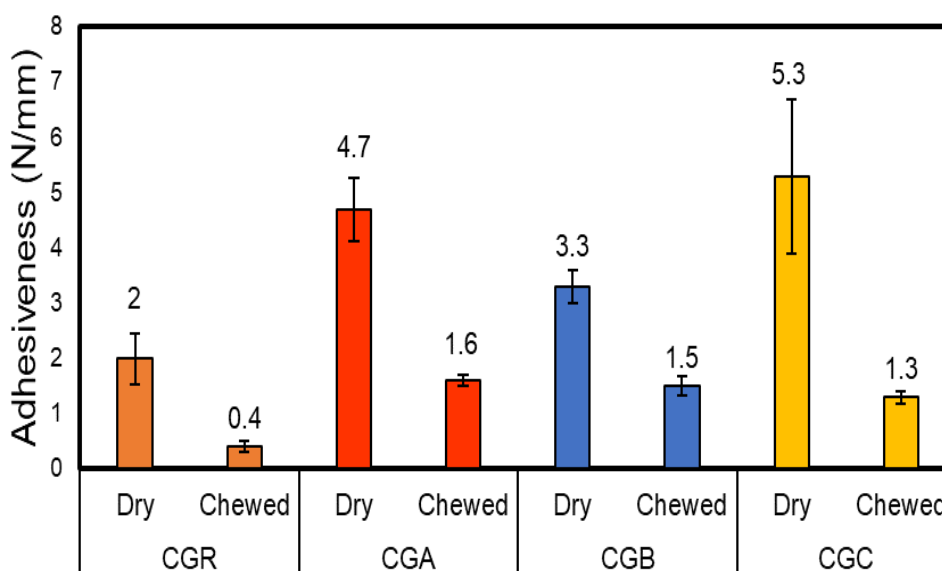


**Figure 28.** Adhesiveness of formulations as determined by TPA.

Cohesiveness is a parameter that reflects the capacity of a material to remain together when stress is applied. A product is cohesive when it adheres to itself under some compressive or tensile stress. As can be seen in **Figure 29**, the dry CG formulation

presented low cohesiveness values that greatly increase after the mastication process. This is only natural as mastication allows for a greater mixture of the ingredients and separation of the hydrophilic phase of the gum, resulting in a more homogenous and mailable capacity of withstanding stress without rupture. These results are expected since CGs are known for their elastic behavior that allow them to withstand stress without rupturing easily. The experimental formulations displayed significantly higher cohesiveness values than CGR.

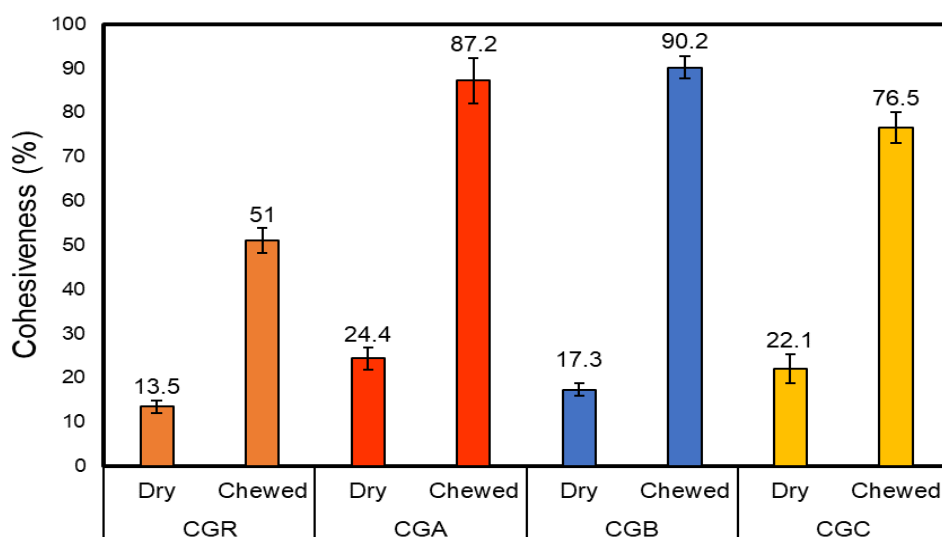


**Figure 29.** Cohesion of formulations as determined by TPA.

Resilience evaluates how a CG sample recovers its original shape after been deformed. As shown in **Figure 30**, the percentual resilience values are quite low across all formulations and the results are not significantly different ( $p>0.05$ ) between experimental formulations when dry. However, they are significantly higher than the CGR result. It is possible to attest that after being chewed, there is a slight increase in resilience, but the resulting values are still low. This may be beneficial since it is well known that low resilience values are linked to good sensorial chewiness property.

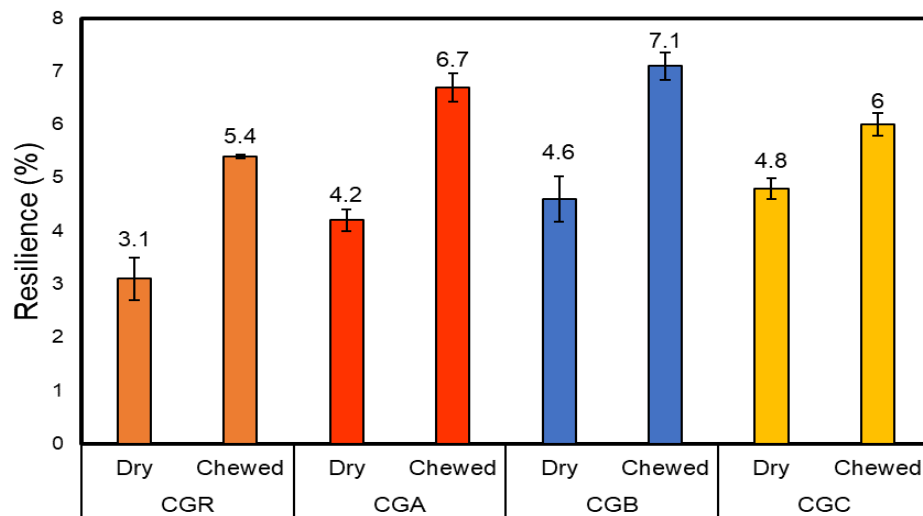


**Figure 30.** Resilience of formulations as determined by TPA.

The height at which food can recover between the end of the first penetration and the beginning of the second one is known as springiness (**Figure 31**). When dry, CG displays similar springiness values, recovering between 55.3 and 59.5% of their original height. Springiness increases greatly after chewing in CGA, CGB and CGC as they can recover almost 100 percent of their initial height. However, the CGR springiness did not increase significantly ( $p>0.05$ ) as the remaining formulations after mastication.

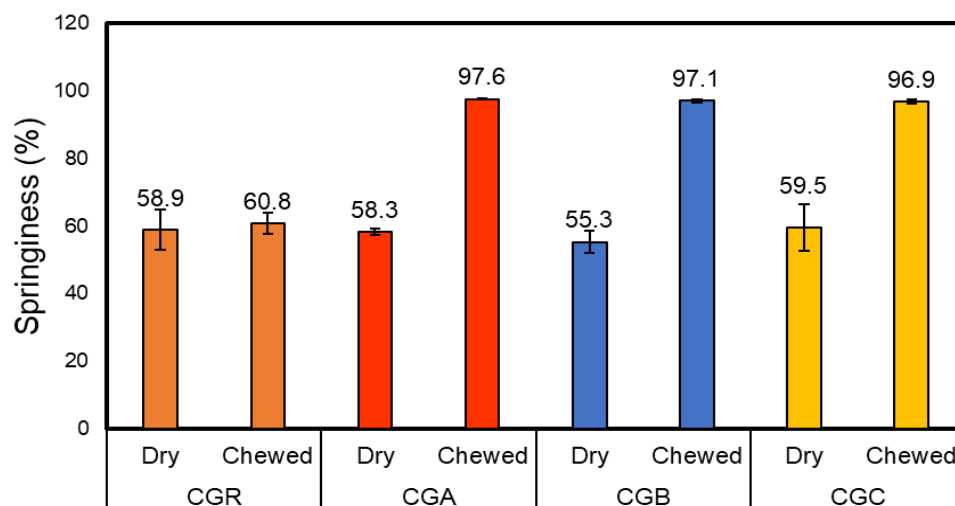


**Figure 31.** Springiness of formulations as determined by TPA.

Gumminess can be interpreted as the energy needed to disintegrate a semisolid food until it is ready to swallow, while chewiness is the energy required to chew a solid food until it is ready for swallowing. Since CG is considered either a solid or semi-solid both

parameters can be considered. As shown in **Figures 32-33**, both chewability and gumminess results were very similar.

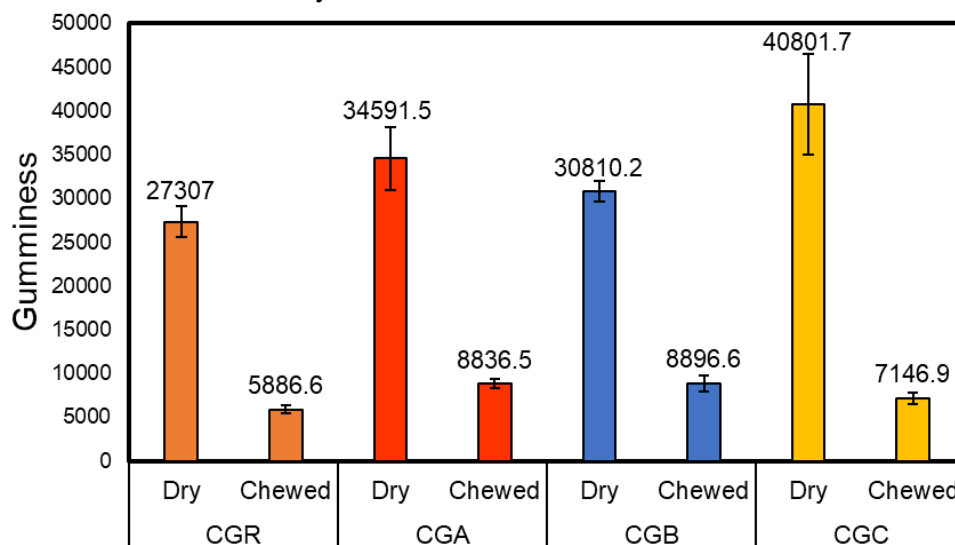


**Figure 32.** Gumminess of formulations as determined by TPA.

For both parameters, the values obtained with the dry CG decreased pronouncedly when the formulations were chewed. These results indicate that as the CG systems are subjected to mastication less energy is required to further chew or disintegrate them. In the case of chewiness, the difference in values between the dry and chewed formulations are not as pronounced as the ones reported for gumminess.

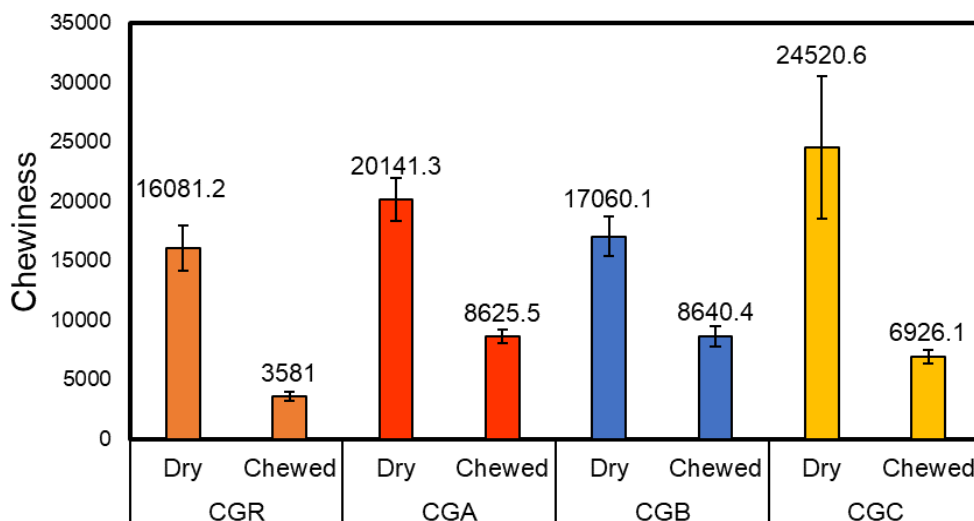


**Figure 33.** Chewiness of formulations as determined by TPA.

#### 4.1.7. Rheological analysis

As previously stated, CG is typically classified as a semi-solid which, by definition, implies it possesses some properties also associated with liquids. One characteristic frequently associated with liquids is the ability to deform and flow when put under shear

stress. To better understand the rheological profile of CG, several tests were performed to evaluate its flow behavior, study thixotropy and determine its LVER.

To determine the CG behavior, an amplitude sweep test was performed and the results (**Figure 34**) revealed sheer thinning as the predominant flow behavior across all CG formulations since the sheer viscosity of every formulation decreases as shear rate increases. This was expected since CG become more malleable and easier to flow after subjected to a force exerted by the act of mastication. CGC showed a more similar profile to the commercial CG.

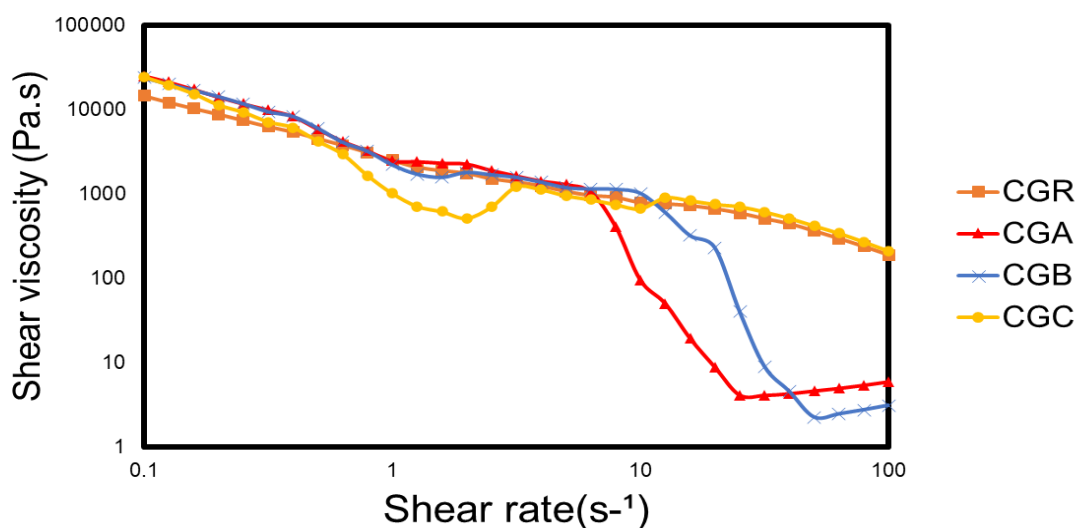


**Figure 34.** Amplitude Sweep test of the CG formulations.

Regarding the tested formulations, an interesting phenomenon occurs around 0.8-5s shear rate in the case of CGC and between the 8-10s<sup>-1</sup> mark for CGB and CGA. This phenomenon is known as “Wall slip” and is characterized by a “slip” or sudden detachment of the CG material from the surface of the probe, resulting in a sudden loss of viscosity usually perceived in high shear rates. This phenomenon normally arises from static, hydrodynamic, viscoelastic, chemical, and gravitational forces, acting on a dispersed phase immediately adjacent to solid boundaries (Chen, 1994). This event cannot be seen in CGR and maybe it is related to the EMO present in the experimental formulations, since certain oils have a lubricant effect that facilitate the probe’s movement.

In the future, to overcome this phenomenon and achieve more feasible results, the same tests should be repeated with adjusted conditions such as different sample gaps, rougher geometry probes that possess physical differences (*i.e.* Crosshatches or bumps) or chemical conditioning to provide more adhesion to the CG samples (Chen, 1994).

As determined by the amplitude sweep test, sheer thinning is the prominent behavior of all CG formulations, despite not meaning that this behavior is linear across all shears. To

investigate the shear regions in which this behavior is linear, oscillatory tests were performed.

As can be seen in **Figure 35**, all CG formulations display similar profiles. At low shear strains, the predominant behavior is that of an apparent elastic behavior represented by the module  $G'$ . The LVER region is narrow and extends up 0.32% shear rates. However, when the shear rates reach 0.38%,  $G'$  begins to decrease slowly as the system is becoming permanently deformed. Eventually, around 3 to 10% shear strain, the system suffers an inversion as the  $G''$  module surpasses the  $G'$ . After this point, the formulations begin to resemble a liquid displaying a viscous behavior.

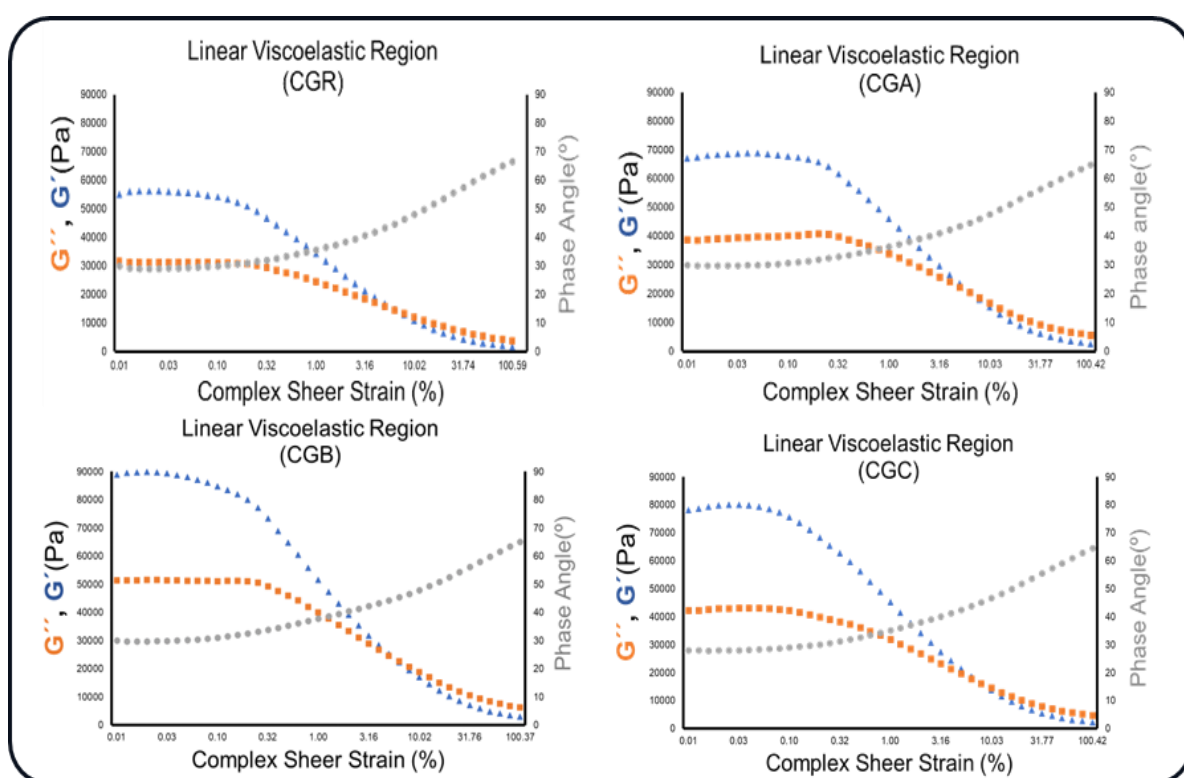


**Figure 35.** Linear viscoelastic region determination of all chewing gum formulations.

Thixotropy is the continuous decrease of viscosity with time when flow is applied to a sample, and the subsequent recovery of viscosity when flow is discontinued. The first test has already shown that CG viscosity is lower when the systems was exposed to high shear rates. However, the test did not indicate if samples can recover their original viscosity after been disturbed or if that recovery is time dependent. Therefore, to evaluate if CG formulations display thixotropy a three-step shear (3 ITT) rate test was performed.

The results of this assay (**Figure 36**) reveal that all formulations possess slight thixotropic behavior since they cannot recover the initial viscosity instantaneously, and instead, require a period of about 30 seconds after being subjected to high shear strains to

recover the initial viscosity. After this point, the recovery of viscosity slows down, increasing slightly for the remainder of the test.

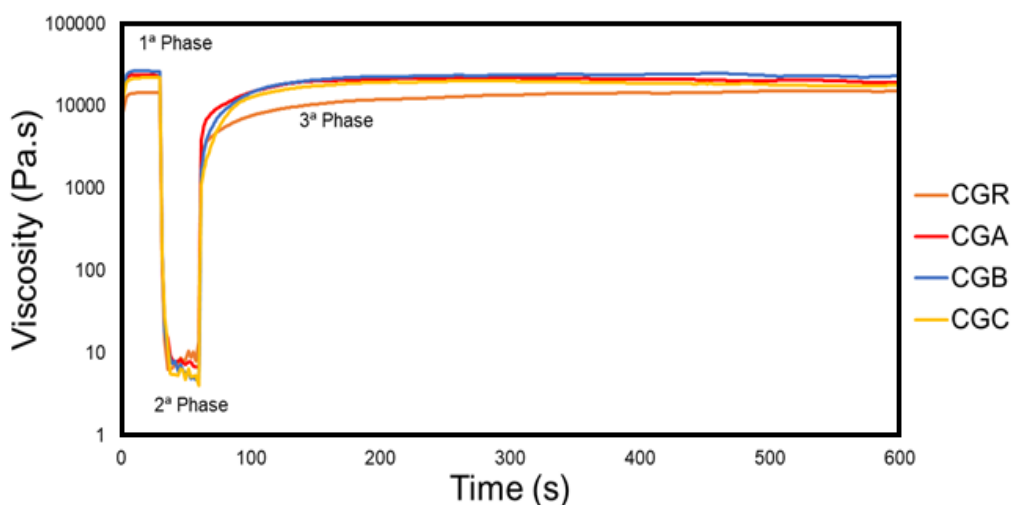


**Figure 36.** Three step shear rate tests of all formulations.

When comparing the initial shear viscosity obtained in 1<sup>st</sup> phase with the one observed at the end of the 3<sup>rd</sup> phase, the recovery can be calculated, being the average recovery (**Figure 37**) very high across all CG formulations. Even though the CGR displayed a higher viscosity recovery, this higher value is not statistically significant ( $p>0.05$ ). The product could not recover 100% its initial viscosity as the second phase destabilizes the microstructure of the system beyond the LVER resulting permanent deformation.

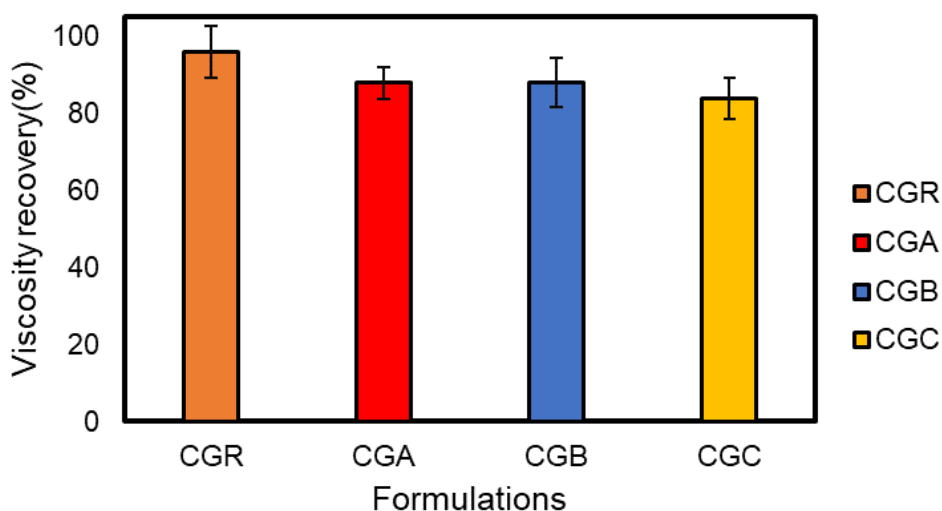


**Figure 37.** Average recovery of shear viscosity of the CG formulations.

Finally, the last assay performed was the stress ramp test to determine the yield point. The results (**Table VI**) revealed that all the formulations presented yield points ranging

between 196.4 and 186.9 Pa. Statistical analysis reveals that there is no significant difference ( $p>0.05$ ) between the formulations. The comparison between CGB and CGC shows that the addition of DKB led to a slight decrease in the yield point of the CG system.

**Table VI.** Yield point of the formulations.

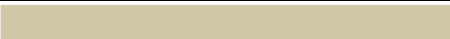
| Formulations | Yield point (Pa)               |
|--------------|--------------------------------|
| CGR          | 196.4 $\pm$ 3,28 <sup>a</sup>  |
| CGA          | 191.6 $\pm$ 7,85 <sup>a</sup>  |
| CGB          | 194.2 $\pm$ 5,38 <sup>a</sup>  |
| CGC          | 186.9 $\pm$ 14,28 <sup>a</sup> |

<sup>a</sup> Standard Deviation (STD)

#### 4.1.8. Colorimetry

The resulting information emitted by the colorimeter is represented in **Table VII** using the 3 different coordinates that compose the CIELAB color model ( $L^*$ ,  $a^*$  and  $b^*$ ). These coordinates were digitally combined forming a digital “pseudo color”, that is represented beside each formulation.

**Table VII.** Color space representation of the CG formulations.

| Formulation | CIELAB Color Space |                   |                   | Pseudo Color                                                                         |
|-------------|--------------------|-------------------|-------------------|--------------------------------------------------------------------------------------|
|             | $L^*(D65)$         | $a^*(D65)$        | $b^*(D65)$        |                                                                                      |
| CGR         | 80.87 $\pm$ 0.546  | -1.43 $\pm$ 0.053 | 17.96 $\pm$ 0.122 |  |
| CGA         | 75.77 $\pm$ 0.328  | -1.40 $\pm$ 0.065 | 16.41 $\pm$ 0.172 |  |
| CGB         | 75.60 $\pm$ 0.465  | -1.47 $\pm$ 0.061 | 15.12 $\pm$ 0.170 |  |
| CGC         | 70.75 $\pm$ 0.345  | -0.58 $\pm$ 0.082 | 21.56 $\pm$ 0.216 |  |

<sup>a</sup> Standard Deviation (STD)

The  $L^*$  values represent perceptual lightness, which ranges from black to white. The CGR displayed the highest  $L^*$  value, which means it has the brightest color. On the other hand, CGC, the formulation with the highest concentration of ingredients, displayed the lowest  $L^*$  value. CGA and CGB have similar  $L^*$  values.

The comparison between CCB and CGR reveals that an increase in the concentration of EMO results in a lower  $L^*$  value, suggesting that increases in the concentration of this ingredient darkens the final product. Similarly, comparing CGB and CGC it is possible to

observe that the addition of DKB darkens the color of the final product. CGA and CGB had not significant differences ( $p>0.05$ ).

Regarding  $a^*$  values, which represent a color range between green and red, it is possible to observe that the values of this coordinate were slightly negative, but very close to zero, representing a middle ground between the two colors. Statistical analysis determined that formulations CGR, CGA and CGB do not vary significantly from each other ( $p>0.05$ ), having a lower  $a^*$  value and, therefore, a more greenish color. On the other hand, CGC has a significantly higher  $a^*$  value than the rest ( $p<0.05$ ).

The  $b^*$  values represent a color range between blue and yellow. All formulations reveal positive  $b^*$  values, which represent a yellow color. CGC has the highest  $b^*$  value, displaying the yellowest color, while CGB revealed the lowest  $b^*$  result and, consequently, a weaker yellow color. Statistical analysis shows that all formulations differ significantly ( $p>0.05$ ) from each other.

Overall, the findings obtained via colorimetry correlate well with the ones achieved in the attribute “Color” evaluated in the sensory analysis trials, as the CGC is evidently different from all other formulations being considerably yellower and darker. Additionally, both assays attested that formulations CGA and CGB have a similar color. Regarding CGR, colorimetry shows small differences in its color when compared to the test formulations, which was not perceived by participants in the SA.

#### 4.1.9. Differential scanning calorimetry

As previously stated, during temperature changes, the DSC measures the heat flow or enthalpy, represented in by the ordinate (Y-axis), which is radiated or absorbed excessively by the sample and it is associated with material phase transition, in function of a temperature variation represented in the abscissa (X-axis) (Gill et al., 2010).

The resulting DSC plots (**Figure 38**), reveal several phenomena common to all formulations. The first apparent phenom is a glass transition, represented by a light shift in the base line, with onset around 30 °C, where the CG system shifts from a solid-like glassy state to a rubbery like state, which was expected as all CGs were made with a high content of amorphous polymers. These systems are noted for having glass transitions temperatures ranging from 0 °C to 37 °C, so the results obtained are within the commonly described in literature (Wittorff, 2005).

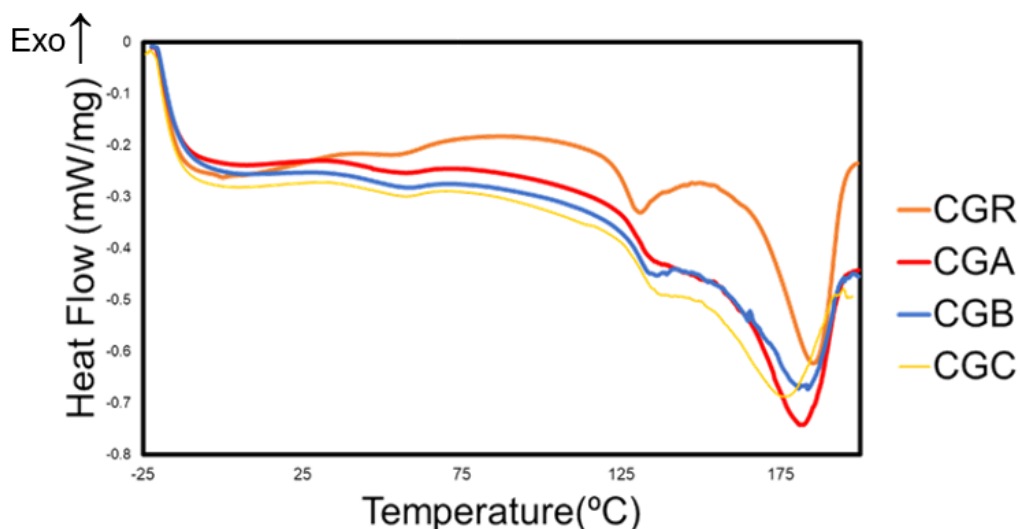


**Figure 38.** DSC thermochemical profile of all formulations.

Afterwards, another phenomenon becomes visible around 130 °C, which is most likely a melting point. Regarding CGR, it clearly visible an endothermic peak, while in the test formulations the peak distorted. This may be explained by the added ingredients that can interfere with melting, resulting in an imperfect peak. According to the information available in literature, the CG melting, namely the gum base, is around 115 °C. In this case, since the exact composition of the gum base is unknown, the comparison with other materials is impossible. Finally, an endothermic peak with an onset around 160 °C, being a possible indication of the thermal degradation of the CG system.

## 4.2. Sensory analysis

The different parameters evaluated in the SA are described in a radar chart or web chart, represented in **Figure 39**. Overall, all the initial parameters decreased in intensity after mastication, correlating with the TPA results. Parameters such as odor, smell, disintegration, and homogeneity can be evaluated alone. The remaining parameters were analyzed together.

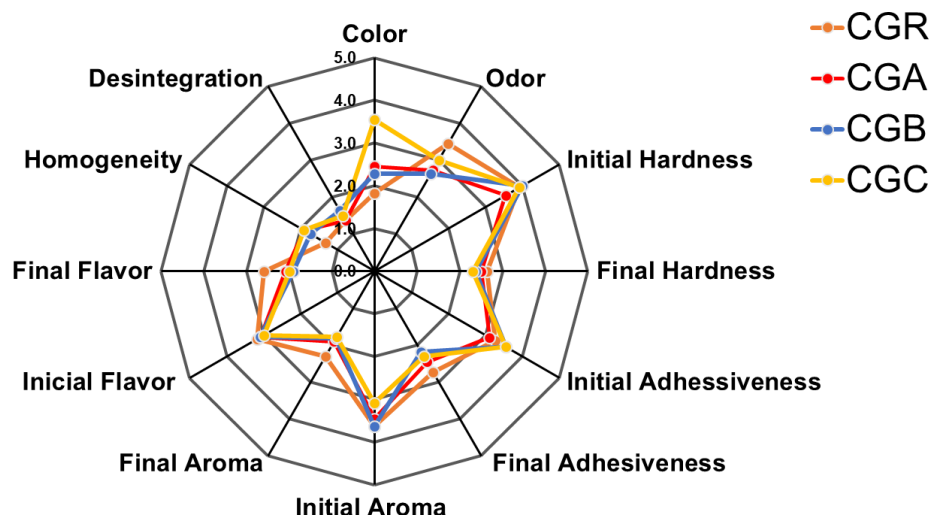


**Figure 39.** Sensory analysis.

Hardness is considered as the strength required to bite down a formulation. Initially, the CG formulations were quite hard as they took “considerable force” to bite down. CGR, CGA and CGC displayed the highest hardness values. After 10 minutes of mastication, the overall hardness of the CGs decreased, requiring “moderate force” to chew. This is expected since as the CG system becomes more hydrated, the elastomers become more flexible and elastic, requiring less force to chew. The findings obtained in the SA correlate well with hardness parameter evaluated in the TPA.

The adhesiveness of a material can be perceived by the force required to separate the teeth after biting down upon a piece of CG. Initially, CGC was perceived as the formulation with the highest adhesiveness. Inversely to what was revealed in the TPA, after chewing, the formulation with the highest perceived adhesiveness is CGR.

The parameter “homogeneity” refers to the texture of CG in the mouth, more concretely it is intended to evaluate if CG display any lumps or granules on their surface that can be perceived when chewing. CGA and CGC were classified with the highest average rating (1.9), which corresponds to a slightly rugose surface, while CGR achieved the lowest value (1.3) corresponding to a smoother texture. The rougher texture of the experimental CGs may be related to the presence of DKB, as there are numerous granules over their surface.

Flavor is the perceived sweetness of the CG formulations. It is a consequence of the sugars and syrup present in the formulations. Since the concentrations of these ingredients were constant across all CGs, their flavor should not vary significantly. The initial SA results reveal exactly that, as all formulations were perceived similarly with a “moderate” flavor.

However, after 10 minutes of chewing, the flavor of CGR was more intense than the remaining formulations, decreasing slightly when compared to its initial value.

Regarding odor, CGR was the highest rated formulation with an average score of 3.5, standing between “moderate” and “intense”. The experimental formulations demonstrated less intense odor than CGR, with scores between 2.6 and 3, between “less intense” and “moderate”. This was unexpected as the higher concentration of EMO should, in theory, result in a more intense mint odor.

Color was perceived by the sensory panel as the only attribute that changed significantly between formulations, with CGC being significantly different from all others. This result was also verified through colorimetry.

Aroma is related to the mint taste of the CG in the mouth. It was expected that an increased concentration of EMO lead to a higher aroma perception. However, the CGR that has the lowest EMO concentration was perceived as the formulation with the strongest aroma. In the first instances of chewing, the mint aroma was classified as “moderate”, while after 10 minutes of chewing the aroma decreases pronouncedly for all formulations, going from “moderate” to “less intense”. At the end, all formulations displayed similar aroma, being perceived as less intense than the reference gum.

In terms of disintegration, the CG formulations display similar behaviors. On average, the score given by participants was 1.5, which places the formulations in the middle of the 2 classifications, suggesting that perhaps some small particles might separate from the chewing gum mass. This could be explained by the presence of DKB as it is not homogeneously dispersed through the CG. Nonetheless, since some disintegration was also perceived in the reference gum, the phenomenon is most likely related with the base formulation of the CG.

Lastly, the sensory panel was asked to respond to final questions to determine the preferred formulation, chewing gum habits and receptibility to this mitigation technology. In terms of preference, CGA was the formulation that appealed to most of the participants in the SA panel. Besides that, 42% of the participants also expressed a preference for CGC (**Figure 40(1)**). No preference was shown for CGB. Despite this, none of the formulations were considered inedible as most of the participants (75%) showed interest in the project and answered that given the chance would consume any of the experimental formulations.

SA are usually performed with a panel of trained individuals who have either a background and specialty in the field or are trained for weeks for the purpose of appraising a certain item. However, this SA was performed with a panel of volunteers from the UEPS-GNR, which are the target population for this mitigation technology. Although they can give an opinion on the palatability of the formulation and choose their preferred formulations, it is not possible to ensure that they possess sufficient skills or have any background on the matter. Alongside this, 58% of the participants do not chew gum very often and even the ones that do, do it a few times in a week or even few times in a month (**Figure 40(3)**).

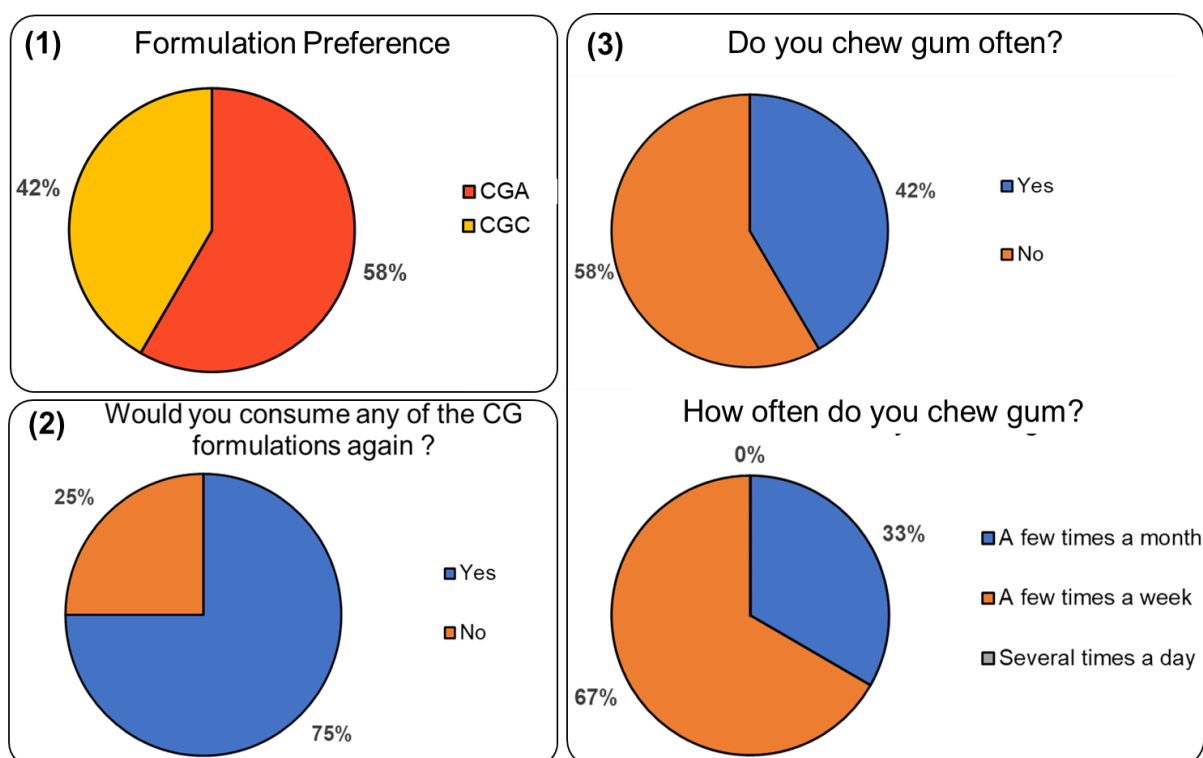


**Figure 40.** Final appraisal results: (1) Formulation preference according to the SA panel; (2) Willingness to use the mitigation device; (3) CG consumption and its frequency.

### 4.3. Assessing the chewing gum capacity for pollutant retention

In this section, the results pertaining to the CGs capacity to retain different POPs are discussed and examined alongside the described methodologies employed to achieve this (section 2.5).

#### 4.3.1. Polycyclic aromatic hydrocarbons

The first methodology was developed utilizing HPLC to assay the PAHs. The different CGs were contaminated with a standard mixture solution containing 18 PAHs. The recoveries of some of these compounds across all formulations are summarized in **Table VIII** of **Appendix II**. The graphs describe the recoveries found in the CGs themselves, the saliva and the sum total of both (**Figure 41**). To achieve the different compound recoveries a calibration curve was drawn, as seen in **Figure 46** of **Appendix II**, and the linear equation was used to calculate the PAHs masses that were compared to the expected masses.

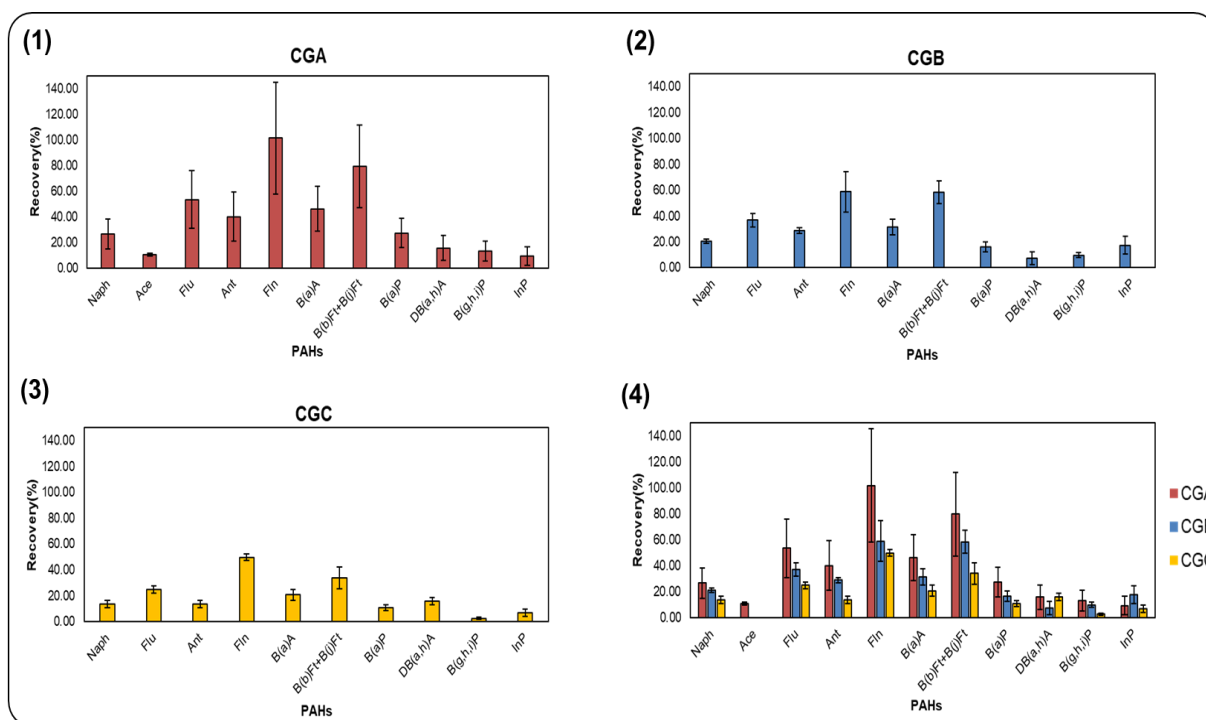


**Figure 41.** PAHs recoveries found on: (1) formulation A; (2) formulation B; (3) formulation C; (4) Side-by-side comparison of all CG recoveries.

Firstly, it is possible to observe that the different formulations were able to recover between 11 (in the cases of CGB and CGC) and 12 (in the cases of CGA) compounds out of the total 18. Higher recoveries were seen in the case of middle weight PAHs compounds, namely Flt, B(b)Ft and B(j)Ft, across all CGs.

Side-by-side comparison between the pollutant recoveries across all the CGs formulations, as represented in **Figure 41(4)**, allows for a better perception of each formulations capacity to retain the different PAHs. As can be seen, CGA displayed the highest average recoveries (from 9.2 to 101.7%) across most compounds, with the exception of InP. Nevertheless, the high standard deviations found make it difficult to draw

certain conclusions. CGB exhibited lower, but more precise average recoveries (from 7.3 to 58.8%) in most compounds. Finally, CG<sub>C</sub> displayed the lowest recoveries (from 2.5 to 49.7%) across all compounds, with the exception of DB(a,h)A.

In the case of saliva, in all formulations it was impossible to recover the majority of compounds, with the exception of very low trace amounts as CGA and CGC.

Overall, the total recoveries obtained employing this methodology were quite lower than expected. Considering the absence of compounds found in the different formulations saliva's, it is highly probable that the different PAHs may be lost during the procedure itself. This is likeliest explanation, as the PAHs are known to be semi-volatile substances and so may evaporate during the course of procedure (Wilson et al., 2021). However, this loss of compounds is problematic as it hinders the understanding of the full potential of absorption of each CG, compromising the method.

Another adversity to the assessment of pollutants in the CG is the, as already elucidated, varied composition of their base. This variety results in a matrix that is, ultimately, very complex to analyze. This is clearly visible in **Figure 42**, as several of the compounds present in the blank CG samples are eluted at the same time as some of the expected pollutants, and thus their peaks become superimposed, producing very high or large signal responses. In these cases, the exacerbated responses made peak identification and measurement either impossible or inaccurate. Certainly, future improvements to the method should strive to obtain extracts with less interference. Perhaps, employing a cleaning step similar to the one employed when analyzing the different BFRs which utilize an SPE cartridge to remove unwanted.

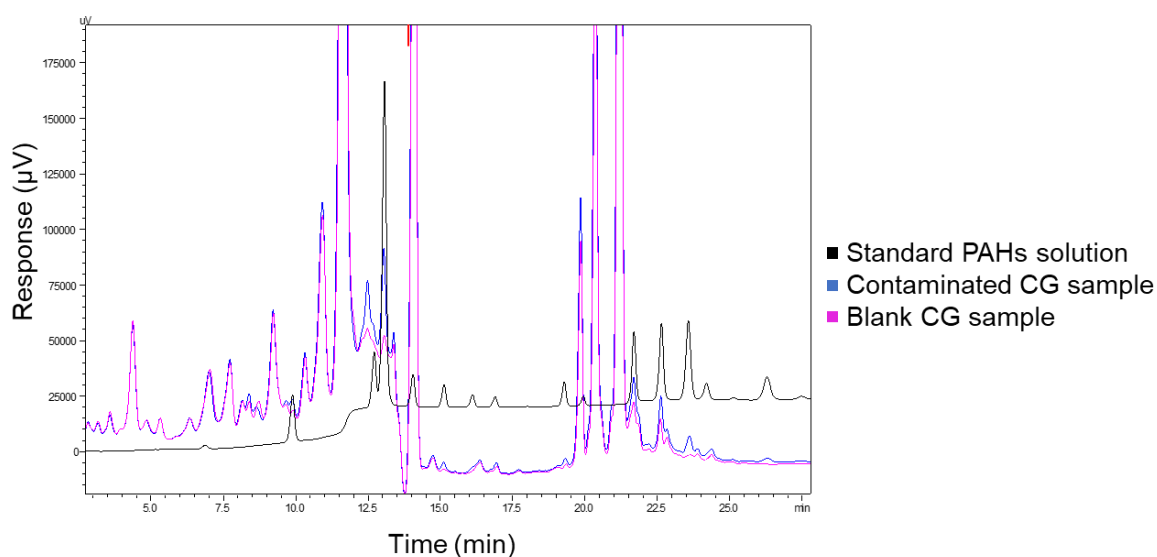


**Figure 42.** Example of the PAHs obtained in the HPLC analysis.

Nevertheless, these results demonstrate that all different CGs possess the capacity to retain pollutants to some extent and thus maybe have a potential as mitigation strategies to firefighters' occupational exposure. In the future, the methodology should be refined to recover higher quantities of PAHs with more precision so as to accurately quantify the different pollutants.

#### 4.3.2. Brominated flame retardants

Unlike what was found with the HPLC results above described, the GC method revealed to be more promising as it allows to recover all compounds with near 100% of recoveries as described in **Table IX** of **Appendix II** and **Figure 43**. The recoveries were calculated through a comparison between the peak areas of the compounds found on the contaminated CGs and those found on the standard solutions.

The comparison between the pollutant recoveries across all the CGs formulations, as shown by **Figure 43**, allows for a better perception of each formulation capacity to retain the different BFRs. Once again, CGA displayed highest recoveries (from 51.9 to 97.6%) on most of the compounds tested, except for BDE-99. CGB and CGC displayed very similar recoveries, differing most on the latter compounds BDE-153 and BDE-184. These findings correlate with those of the HPLC, despite being somewhat unexpected as CG<sub>A</sub> has the lowest EMO concentration of all formulations and should, in theory, be less capable of retain pollutant.

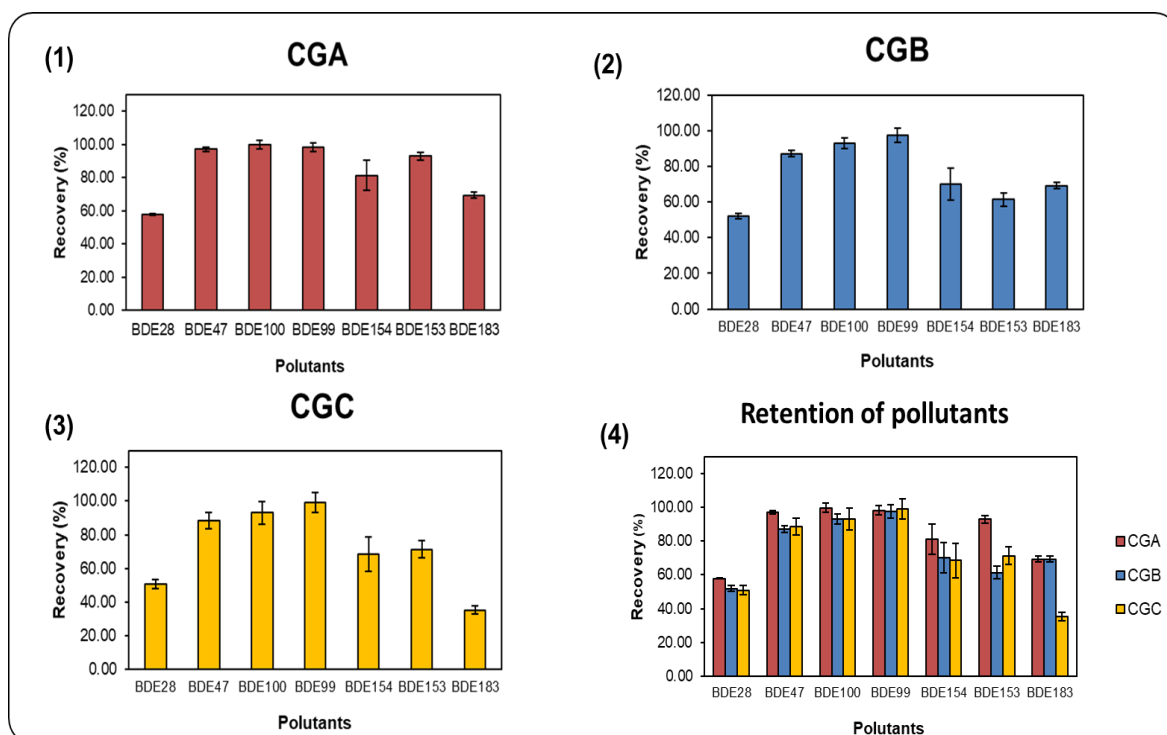


**Figure 43.** BFRs recoveries found on: (1) formulation A; (2) formulation B; (3) formulation C; (4) Side-by-side comparison of all CG recoveries.

In opposition to what was observed for the HPLC method, various compounds were identified in saliva. In the case of formulation CGA (**Figure 43(1)**), very low recoveries were found (from 0.9 to 6.3%). CGC, displayed the higher pollutant recoveries (from 12.8 to 31.8%). CGB saliva recoveries stood between the other two (from 8.4 to 18.5%).

The total recoveries are quite high for the majority compounds. The compounds with the lowest total recoveries were BDE-28 and BDE-184, showing that little amounts of pollutants are loss during the handling procedures.

Another favorable aspect of analyzing BFRs through GC was that the CG matrix did not hinder peak analysis, as the resulting chromatograms, exemplified in **Figure 44**, were very clear and the pollutant peaks were easily distinguished and measured unlike that which was found in the HPLC analysis. This clearness results from the treatment through dispersive SPE 2ml cartridges, which contain sorbent mixture of PSA, C18 silica and magnesium sulfate which pull out interfering matrix materials from the CG extracts, such as polar organic acids, some sugars, more lipids and sterols. A similar procedure could be applied in the analysis of PAHs to obtain extracts with less interference.

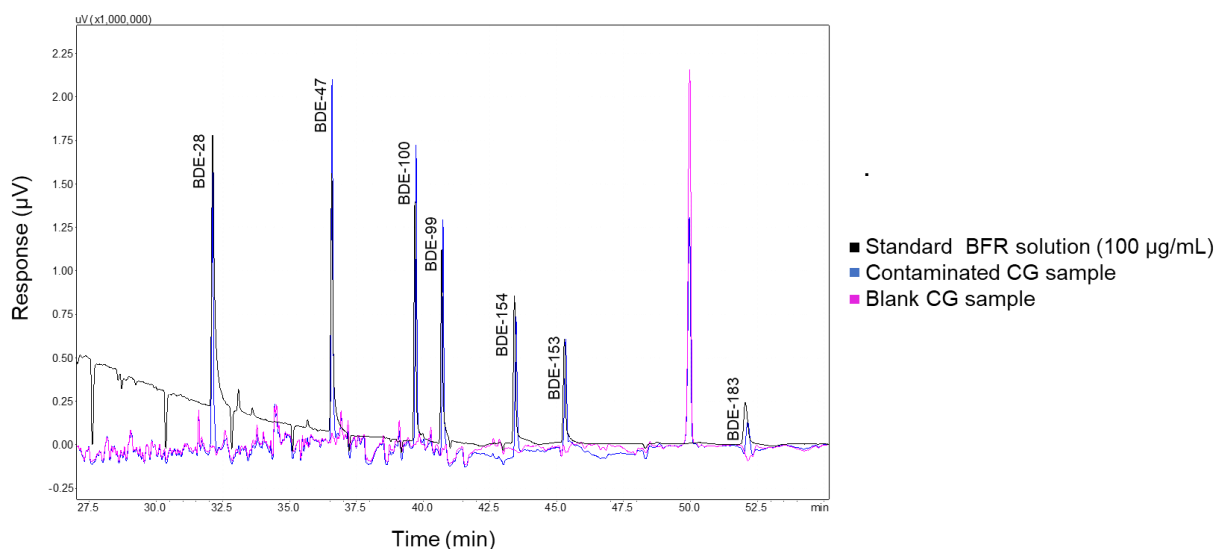


**Figure 44.** Example of the 7 BFRs detected.

Once more, the obtained results prove the CGs capacity to retain certain POPs, namely the 7 BFRs tested, and demonstrated the possibility of recovering these compounds through the methodology described. To quantify the different compounds and obtain more rigorous

measurements, a calibration curve should be performed and plotted like what was done with the HPLC.

#### 4.3.3. Analysis of chewing gum samples used in fires

As the CGs capacity for BFRs retention was successfully demonstrated through preliminary lab tests, an attempt at identification of these compounds in CG samples that were used by firefighting members of the GNR-UEPS during 2024 was made.

As can be seen by **Figure 45**, through a comparative analysis between a standard solution of BFRs and the CG samples, it is clear that two of the total expected BDEs could be identified on the resulting CG sample chromatograms. The two compounds identified were BDE-100 and BDE-99, represented by small but visible peaks within the expected retention times which were 39.85 and 40.72 min, respectively. These results were consistent across the five samples examined. However, this is merely an qualitative analysis which allows for identification of compounds but does not allow for exact quantification of the identified compounds. Nevertheless, it is apparent by the difference in peak area that the concentrations found in the CG samples are lower than 25  $\mu\text{g}/\text{mL}$  present in the standard solution. In the future, it would be interesting to draw a calibration curve with different standard solutions of known concentration in order to assess the exact quantity of pollutants present in the samples.

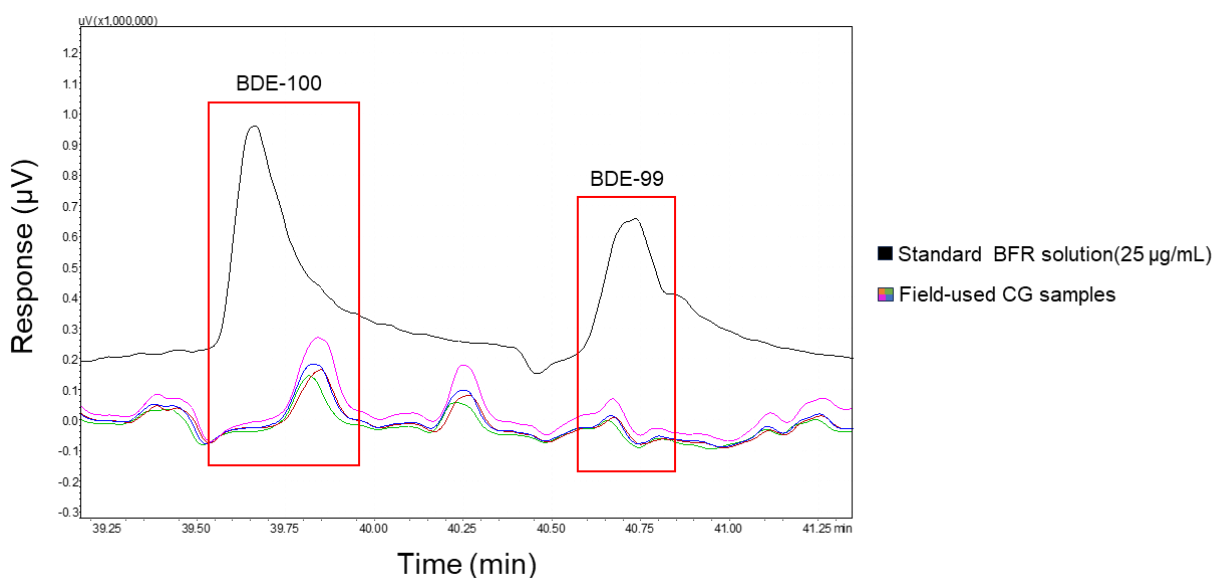


**Figure 45.** Comparison between standard BFRs solution and field-used sample.

Regarding the low amounts of pollutants found in these field used CGs, the most plausible justification for this may be the fact that the GNR-UPS firefighting activities usually

take place within wildland areas, in which the materials that are subjected to combustion include bushes, vegetation and other organic matter that do not contain BFRs. These compounds are more prevalent in certain kinds of electronics, plastics and kitchen equipment typically found in urban environments. Trace amounts of these compounds could be found as they are present in firefighters' PPE, however this was not the case of the samples analyzed.

In the future, it would be interesting to apply these formulations in urban or WUI firefighting scenarios so as to observe whether or not the proposed formulations can effectively retain these compounds during fire-events, particularly during the mop-up phase. However, the CG device could also be used as a control to measure the SCBAs alleged hermetic capacity during the attack phase.

Chapter V:  
*Conclusion and future prospects*

## 5. Conclusions and future prospects

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Overall, the different tests carried out on the CGs developed showed great similarities between the different experimental formulations. All CG formulations exhibit very similar texture profiles, especially once chewed, varying slightly on some texture attributes (i.e. Hardness and Adhesiveness). Regarding rheology, all formulations display sheer thinning behavior with slight thixotropy. They possess a narrow LVER (0-0.38%), exhibiting an apparent elastic behavior at low complex sheer strains and an apparent viscous behavior at high sheer strains. DSC plots display glass transition temperature around 40 °C and melting onset around 130 °C and finally thermal degradation around 160 °C. The SA results are important as, to a certain extent, they support the TPA and colorimetry findings, showing that the small differences between the formulations were barely perceived by the test panel (except for the parameter of color). Most importantly, the SA results demonstrated that, in terms of palatability, all formulations were considered acceptable on the part of the target public.

The developed analytical methods promptly demonstrated that all CGs possess the capacity to retain several key pollutants. Out of all the three test formulations, CGA appears to be the formulation with the highest capacity for the pollutants tested. Notwithstanding, the analytical procedures described herein should be refined, especially the HPLC analysis of PAHs, to obtain higher recoveries and accurately quantify the different pollutants. The CG was shown to be a difficult matrix to analyze because of its varied composition which interferes in the analysis. of these pollutants. Future improvements to this methodology should be focused on obtaining a “cleaner” extracts.

GC analysis of field-used fire fighter samples revealed the presence of two of the expected BFRs, demonstrating that the developed inventions can retain the pollutants when applied in real firefighting scenarios. One must keep in mind that these results were obtained in wildland fire samples where BFRs are not abundant. In the future, it would be interesting to apply and analyze all formulations in other firefighting contexts to obtain a better grasp of the pollutants that are present in the different environments. Additionally, the pollutants found in the samples could be quantified using a calibration curve to accurately assess the concentration present in the firefighter's oral cavity.

Another aspect that would be interesting to evaluate is the potential protective effect of the DKB within formulations CGA and CGC. This could be done through in-vitro assays utilizing different cell models.

Altogether, the results obtained through this research display the CGs potential as possible mitigation strategies for firefighters' occupational exposure. In spite of this fact, their potential may not only be limited to firefighters, being also applied to smokers or other professions where individuals are exposed to different pollutants examined. These professions include cooks, carpenters, steelworkers, mechanics, coal workers and many more. Additionally, once the DKB effects have been properly studied, these inventions could also be commercialized as nutraceuticals as they are full of various bioactive compounds that can provide several health benefits to consumers.

## Chapter VI

### *References*

## 6. References

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## Chapter VII

### *Appendixes*

# BOOK OF ABSTRACTS



Organização



Apoio



## 21727 | Incidence rates and mortality caused by oral cancer in firefighters

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**Background and Aim:** Firefighters (FFs) are exposed to various hazardous pollutants that promote the development of cancer and aggravate pre-existing cardio-respiratory diseases [1-2]. Oral cancer (OC) is a common malignancy among population and FFs may be at increased risk of developing OC due to their occupation [3]. This study aims to characterize the incidence and mortality rates of OC in FFs. **Methods:** A search for studies reporting incidence and mortality of OC in FFs was conducted across several databases (PubMed, Google Scholar, and Web of Science ISI). Only the studies reporting data on cancer in the buccal cavity of FFs were selected. A total of 28 studies published between 1977 and 2023 were selected and included in the analysis. Data was extracted, compiled, and analysed with different statistical tools. **Results:** Published studies characterize FFs from North America, Europe, and Australia. In Europe, the available information is still lacking as FFs from most countries, including Portugal, remain uncharacterized. About 35% of the studies presented increased incidence rates of oral cancer in FFs with standard incidence ratios ranging from 0.14 to 4.10. Additionally, some authors reported increased mortality values with standard mortality ratios ranging from 0.18 to 5.26 in 61.5% of studies. Despite being limited, the available literature suggests an increased risk of developing OC among firefighters. **Conclusions:** More epidemiologic research needs to be conducted, including a higher number of FFs from different geographical regions to explore the relation between firefighting activity and the risk of developing oral cancer.

**Keywords:** Occupational exposure, Firefighters, Oral Cancer

### Acknowledgments

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#### References:

- [1] M.J. Bessa, et al., *J. Toxicol. Env. Health*, 26,4 (2023): 238-255.
- [2] G. Sousa, et al., *Int. J. Env. Res. Public Health*,4, 19 (2022):12677.
- [3] P.H. Montero, S.G. Patel *Surg. Oncol. Clin. N. Am.*,24(3) (2015):491-508.
- [4] K.W. Fent KW, et al., *J. Occup. Environ. Hyg.* 14(10) (2017):801-814.

## Appendix II. International Conference of Food Sustainable Foods Abstract

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## DEVELOPMENT OF A CHEWING GUM WITH *ACTINIDIA ARGUTA* EXTRACT AS AN INNOVATIVE MITIGATION STRATEGY FOR FIREFIGHTERS OCCUPATIONAL EXPOSURE

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Firefighting activity has been classified as carcinogenic to humans, since fires emit a complex mixture of pollutants, such as, polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants (BFRs). [1,2]. Several mitigation strategies have been implemented to promote firefighters' safety and minimize the occupational exposure, including better quality and fire resistance of personal protective equipment (PPE) and decontamination procedures of PPE, among others [3]. This work aims to explore a chewing gum (CG) as a convenient, affordable, and sustainable mitigation technology to retain the lipophilic fire-related pollutants and offer protective capacity against them. Three CG formulations (CGA, CGB, and CGC) with different proportions of natural excipients, namely absorbent mint oil, and *Actinidia arguta* extract, were developed in close collaboration with a renowned Portuguese company, based upon available scientific and technological knowledge. The formulations were characterized regarding texture profile, rheometry and sensory analysis (SA) conducted with a panel of military firefighters of the Special Protection and Relief Unit of the Republican National Guard. To evaluate the pollutants retention, a CG fraction was placed in contact with commercial saliva previously contaminated with 18 PAHs and 5 BFRs that were analyzed via HPLC and gas chromatography (GC), respectively. Overall, the formulations have similar texture profiles. Attributes such as hardness and adhesiveness decreased after chewing. Rheometric analysis reveals that all formulations have pseudoplastic behavior with slight thixotropy. The results of the SA support those found in the texture profile. Moreover, HPLC results attested the retention of 10 PAHs (with recoveries ranging from 26% to 76%), while the GC analysis demonstrated the retention of 5 BFRs (with recoveries from 35.1% to 99.6%).

**Keywords:** Chewing gum, Firefighters, Fruit extract, Occupational exposure.

### References

- [1] IARC Monographs on The Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer (2022)132, Lion, France.
- [2] G. Sousa, et al., Int. J. Env. Res. Pub. Health. 2022, 4;19(19):12677.
- [3] Horn, Gavin P et al. Journal of occupational and environmental hygiene vol. 19,9 (2022): 538-557.

### Acknowledgments

This work received support from UIDB/50006/2020, UIDP/50006/2020, LA/P/0008/2020, and <http://doi.org/10.54499/PCIF/SSO/0090/2019> by FCT-MCTES. M. Oliveira and F. Rodrigues are thankful for the scientific contracts <https://doi.org/10.54499/CEECIND/03666/2017/CP1427/CT0007> and <https://doi.org/10.54499/2020.01886.CEECIND/CP1596/CT0001>, respectively. The authors thank Lusitana and the agents of UEPS-GNR for their collaboration and support.



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The integration of *Actinidia arguta* fruit extract in chewing gums has an innovative strategy to adsorb fire pollutants during firefighting activities, contributing to mitigate firefighters' occupational exposure in the oral cavity and promoting occupational health (SDG 3, 8).



## ORGANOLEPTIC AND TEXTURAL CHARACTERIZATION OF A CHEWING GUM TO ABSORBE POLLUTANTS RELEASED DURING FIRE EVENTS



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### INTRODUCTION

- During occupation, firefighters are exposed to various pollutants (e.g., polycyclic aromatic hydrocarbons and heavy metals), via ingestion, inhalation or dermal contact [1].
- In 2022, IARC has classified firefighting as carcinogenic to humans (Group 1)[1].
- Recently, investigators has been focusing on innovative mitigation strategies to reduce exposure [2].

### AIM

- Characterization of various chewing gum (CG) formulations.
- Development of an easy-use, cost-effective, and sustainable mitigation technology.

### METHODOLOGY

#### Texture Profile Analysis (TPA)



#### Rheology



#### Differential Scanning calorimetry (DSC)



#### Colorimetry

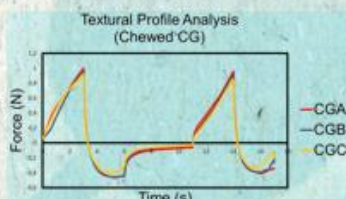
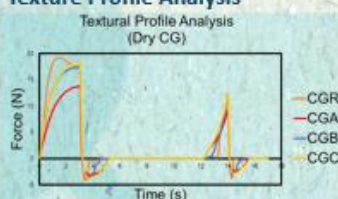


#### Sensorial Analysis



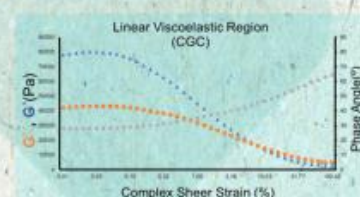
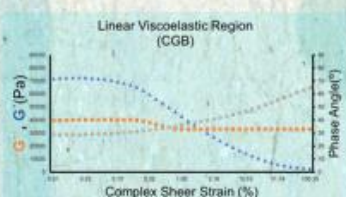
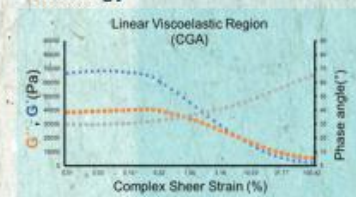
### RESULTS

#### Texture Profile Analysis



| Formulation         | CGR   |        | CGA  |        | CGB  |        | CGC  |        |
|---------------------|-------|--------|------|--------|------|--------|------|--------|
|                     | Dry   | Chewed | Dry  | Chewed | Dry  | Chewed | Dry  | Chewed |
| Hardness (N)        | 19    | 13     | 1    | 18     | 1    | 18     | 1    | 1      |
| Adhesiveness (N/mm) | 2     | 1      | 2    | 3      | 2    | 5      | 1    | 1      |
| Resilience (%)      | 4     | 4      | 7    | 5      | 7    | 5      | 6    | 6      |
| Cohesion (%)        | 13    | 16     | 87   | 17     | 90   | 22     | 77   | 77     |
| Springiness (%)     | 54    | 50     | 98   | 55     | 97   | 59     | 97   | 97     |
| Gumminess           | 249   | 203    | 87   | 304    | 90   | 398    | 69   | 69     |
| Chewiness           | 13419 | 10099  | 8511 | 16838  | 8758 | 23653  | 6672 | 6672   |

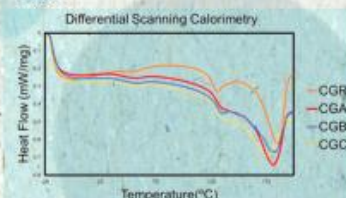
#### Rheology



#### Sensorial Analysis

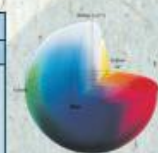


#### DSC



#### Colorimetry

| Formulations | CIELAB color space |         |         |
|--------------|--------------------|---------|---------|
|              | L*(D65)            | a*(D65) | b*(D65) |
| CGR          | 80,87              | -1,43   | 17,96   |
| CGA          | 75,77              | -1,40   | 16,41   |
| CGB          | 75,60              | -1,47   | 15,12   |
| CGC          | 70,75              | -0,58   | 21,56   |



### CONCLUSIONS

- SA and TPA show similar hardness and adhesiveness results.
- After chewing, CGA, CGB and CGC show similar profiles.
- CG have a predominately elastic behavior within LVER.
- Complex shear strains above 0,10% resulted in permanent deformation of CG formulations.
- DSC plots show glass transition temperature ( $T_g$ ) around 40°C and melting onset around 160°C.

### REFERENCES

- 1) P. Demers, et al. The Lancet Oncology, 23 (6) (2022), 989-999.
- 2) G. Horn, et al. Journal of occupational and environmental hygiene vol. 10 (9) (2022), 535-557.

### Acknowledgments

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### Funding

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## Development of a chewing gum to mitigate firefighters' occupational exposure to fire-related pollutants

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### Introduction

- Firefighters are exposed to various pollutants, released during fire-events (e.g., polycyclic aromatic hydrocarbons and heavy metals), via ingestion, inhalation or dermal contact [1].
- Recently, IARC has classified firefighting as a Group 1 occupation (carcinogenic to humans) [1].
- Consequently, investigators have been focusing on innovative mitigation strategies to reduce exposure [2].
- One possible strategy, is the use of chewing gum containing oils capable of absorbing lipophilic pollutants.

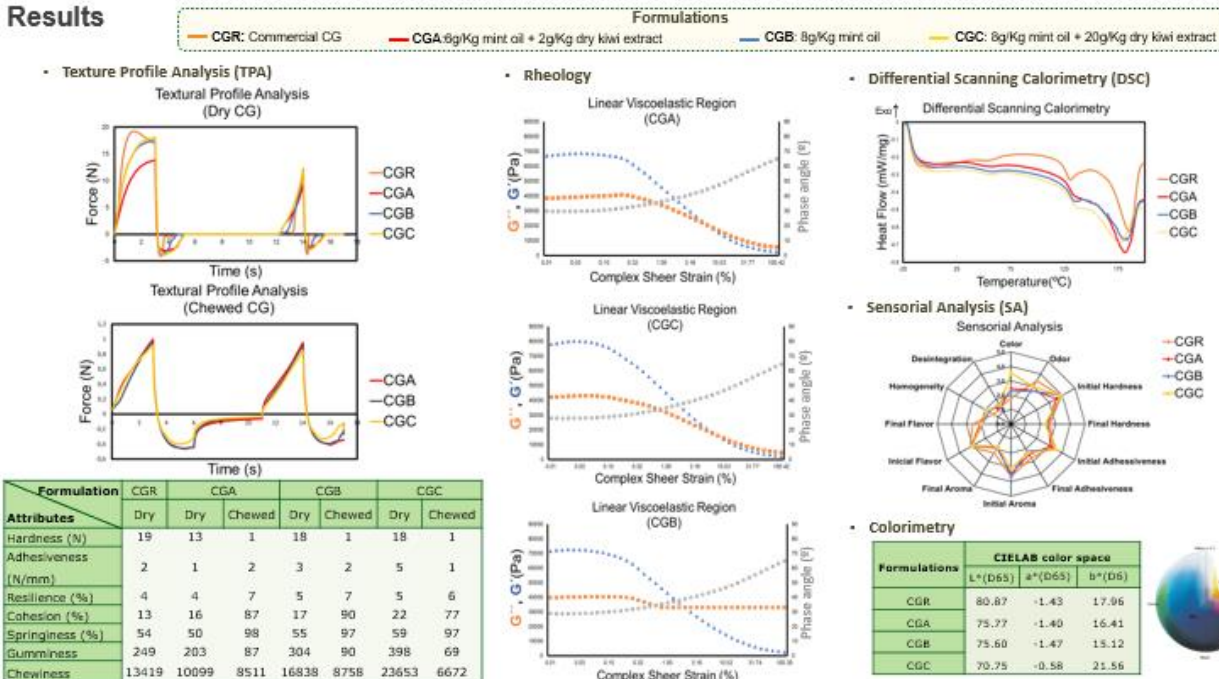
### Aim

- Characterization of various chewing gum (CG) formulations.
- Development of an easy-use, cost-effective, and sustainable mitigation technology.

### Methodology



### Results



### Conclusions

- SA and TPA show similar hardness and adhesiveness results.
- After chewing, CGA, CGB and CGC show similar texture profiles.
- CG have a predominately elastic behavior within the linear viscoelastic region.
- Complex shear strains above 0.10 to 0.32% resulted in permanent deformation of CG formulations.
- DSC plots show glass transition temperature (T<sub>g</sub>) around 40°C, 125°C and melting onset around 160°C.

### References

- [1] P. Dornon, et al., The Lancet Oncology, 23 (8) (2022), 985-988
- [2] G. Horn, et al., Journal of occupational and environmental hygiene vol. 10,9 (2022): 538-567

### Acknowledgements

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XXVII Luso-Galego Chemistry Meeting  
23th of November 2023  
Porto, Portugal

## Development of a chewing gum as a device capable of retaining fire-related pollutants

Pedro Almeida<sup>1</sup>, V. Fernandes<sup>1</sup>, C. Delerue-Matos<sup>1</sup>, Paulo Costa<sup>2,3</sup>, Francisca Rodrigues<sup>1</sup>, Marta Oliveira<sup>1\*</sup>

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### Background

- The occupational activity as a firefighter causes cancer [1-2].
- There is a scientific and technological demand to prevent and mitigate occupational exposure to fire emissions.

### Objectives

- Development and characterization of chewing gum (CG) formulations capable of absorbing fire-related pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants (BFR).

### Methods



The SA was conducted with a panel of military firefighters from the Special Protection and Relief Unit of the Republican National Guard.

A methodology to extract PAHs and BFR from CG is being developed. Extracts will be analyzed by Gas chromatography (GC) with mass detection.

### Funding

This work was supported by project <http://doi.org/10.54499/2022.05381.PTDC> funded by Fundação para a Ciência e a Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior (FCT-MCTES) through national funds.

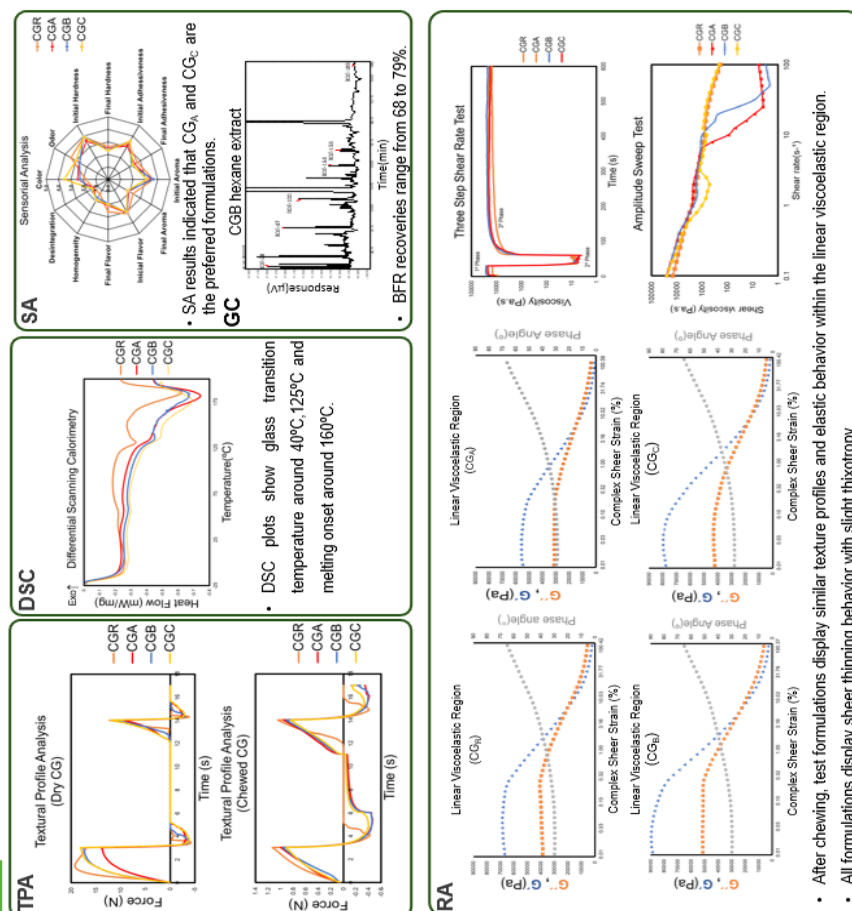
### Acknowledgements

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### References

- [1] IARC: Volume 132: IARC Monographs on The Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, June 7–14, Lyon, France (2022)
- [2] G. Sousa, et al. International Journal of Environmental Research and Public Health. 2022 Oct 4;19(19):12677.

### Results



• After chewing, test formulations display similar texture profiles and elastic behavior within the linear viscoelastic region.

• All formulations display shear thinning behavior with slight thixotropy.

### Conclusions

- The analytical method described requires further optimization to obtain higher recoveries and investigate the retention of PAHs.
- Accepted and optimal CG formulations will be used by participants during 2024 firefighting activities to evaluate the presence of fire-related pollutants in the oral cavity of military firefighters.
- In the future, the protective capacity of the dry fruit extracts, present in CGA and CGC, will be evaluated through *in-vitro* cell assays.

# Appendix VII. 11th Meeting of the SPQ Analytical Chemistry Poster



## Development of a methodology to analyze fire-released pollutants in chewing gum

Pedro Almeida<sup>1</sup>, Virgínia Cruz Fernandes<sup>1</sup>, Cristina Delerue-Matos<sup>1</sup>, Paulo Costa<sup>2,3</sup>, Francisca Rodrigues<sup>1</sup>, Marta Oliveira<sup>1\*</sup>

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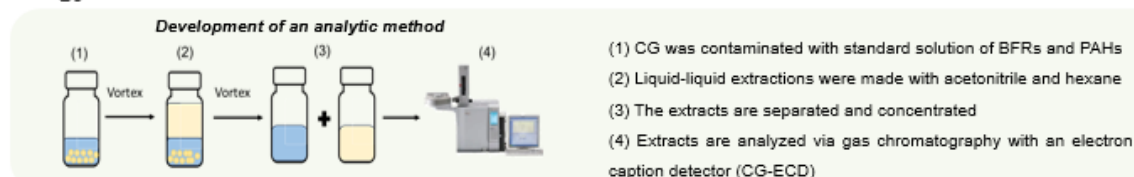
### Introduction

- During firefighting activities, firefighters are exposed to various pollutants which may cause cancer and cardio-respiratory diseases [1-2]
- As a possible mitigation technology, several chewing gum (CG) formulations enriched with an essential oil capable of retaining lipophilic fire-released pollutants, were developed in collaboration with a renowned Portuguese company.

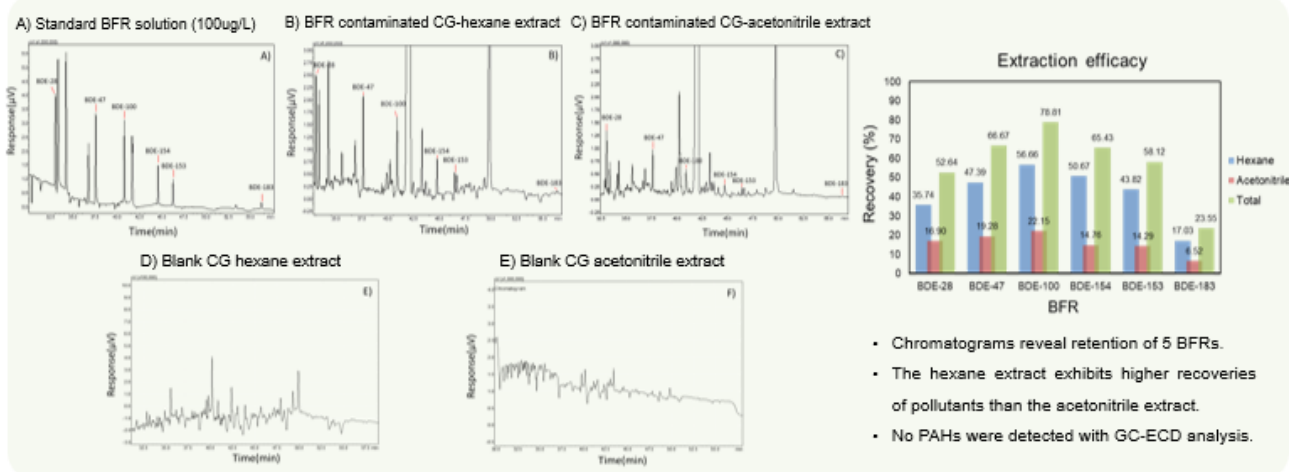
### Aim

- Development of an analytic methodology for extraction and analysis of pollutants such as Polycyclic aromatic hydrocarbons (PAHs) and Brominated flame retardants (BFRs) retained inside the CG.

### Methodology



### Results



### Conclusions

- Overall, the current methodology was capable of extraction and detection of several BFRs, retained in the CG system, with acceptable recoveries (the highest ranging from 68 to 79%).
- Nevertheless, the analytical method described still requires further optimization to achieve higher recoveries and examine the retention of PAHs, which should be analyzed using GC-MS or HPLC-FL.
- Afterwards, the optimal methodology will be employed in the analysis of CG used by members of the National Republican Guard during fire events.

### Funding

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### Acknowledgements

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### References

- [1] IARC: Volume 132. IARC MONOG EVAL CARC, June 7–14, Lion, France (2022)
- [2] G. Sousa, et al., IERPH. 2022 Oct 4;19(19):12677.



Certifica-se que

Pedro Miguel Soares de Almeida

obteve o primeiro (1º) lugar, na categoria 2º Ciclo de Estudos, no Concurso de Pósteres Científicos, integrado no XVIII Congresso Científico da Associação de Estudantes da Faculdade de Farmácia da Universidade do Porto (AEFFUP), que decorreu nos dias 10 e 11 de novembro de 2023, na sede da Delegação Norte da Associação Nacional das Farmácias.

Porto, 11 de Novembro de 2023

P'la Direção da AEFUP,

 ASSOCIAÇÃO DE ESTUDANT  
DA FACULDADE DE FARMÁC  
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## Appendix IX. International Conference of Food Sustainable Foods Award



**ReadyToPub Award**

Awarded to the best student oral communication presented at the

**International Conference on Sustainable Foods**

**July 24-25, 2024, Bragança - PORTUGAL**

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## Appendix X. Sensory analysis questionnaire

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*Gum4Fires - Uma nova tecnologia para mitigar a exposição ocupacional dos bombeiros*

### Instruções para avaliação sensorial

Por favor, leia com atenção as seguintes informações e o questionário, pelo menos uma vez antes de começar o ensaio. Muito obrigado!

**Parâmetros a avaliar:**

- 1. Primeira fase (Pré-mastigação)**
  - Cor (Cor da pastilha elástica)
  - Odor (Intensidade do odor libertado pela pastilha)
- 2. Segunda fase (após 10 segundos de mastigação)**
  - Dureza inicial (Força necessária para comprimir a pastilha entre os dentes)
  - Adesividade inicial (Força necessária para separar os dentes)
  - Homogeneidade (Avaliação da textura da pastilha na boca)
  - Aroma inicial (Intensidade do aroma a mentol)
  - Sabor Inicial (Intensidade do sabor doce)
- 3. Terceira fase (após 10 minutos de mastigação)**
  - Dureza final (Força necessária para comprimir a pastilha entre os dentes)
  - Adesividade final (Força necessária para separar os dentes após trinca)
  - Desagregação (Libertação de partículas pela pastilha elástica)
  - Aroma remanescente (Intensidade do aroma a mentol)
  - Sabor remanescente (Intensidade do sabor doce)
- 4. Avaliação final**

## **Questionário sensorial**

**Código participante:** \_\_\_\_\_

**Unidade a que pertence:** \_\_\_\_\_

**Idade:** \_\_\_\_\_ **É Fumador:** \_\_\_\_ Sim \_\_\_\_ Não

**Data:** \_\_/\_\_/\_\_ **Amostra:** \_\_\_\_\_

### **Planificação do ensaio**

- 1) Pastilha A
- 2) Quebra de sabor e limpeza palato
- 3) Pastilha B
- 4) Quebra de sabor e limpeza do palato
- 5) Pastilha C
- 6) Final do ensaio sensorial

Após se familiarizar com o significado dos diferentes parâmetros, classifique as pastilhas elásticas colocando um X em cada quadrícula, numa escala de 1 a 5 ou 1 a 3 conforme o parâmetro em análise.

## Questionário sensorial

| Características                                  | Pastilha 1 |   |   |   |   | Pastilha 2 |   |   |   |   | Pastilha 3 |   |   |   |   | Controlo |   |   |   |   |
|--------------------------------------------------|------------|---|---|---|---|------------|---|---|---|---|------------|---|---|---|---|----------|---|---|---|---|
|                                                  | Avaliação  |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
|                                                  | 1          | 2 | 3 | 4 | 5 | 1          | 2 | 3 | 4 | 5 | 1          | 2 | 3 | 4 | 5 | 1        | 2 | 3 | 4 | 5 |
| 1. Primeira fase (Pré-mastigação)                |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Cor                                              |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Odor                                             |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| 2. Segunda fase (após 10 segundos de mastigação) |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Dureza inicial                                   |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Adesividade Inicial                              |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Homogeneidade                                    |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Sabor inicial                                    |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Aroma inicial                                    |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| 3. Terceira fase (após 10 minutos de mastigação) |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Dureza final                                     |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Adesividade final                                |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Desagregação                                     |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Sabor remanescente                               |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |
| Aroma remanescente                               |            |   |   |   |   |            |   |   |   |   |            |   |   |   |   |          |   |   |   |   |

| 4. Avaliação final                                                               |                                                |                                           |
|----------------------------------------------------------------------------------|------------------------------------------------|-------------------------------------------|
| Após ter experimentado as três pastilhas, qual delas achou ser a mais agradável? |                                                |                                           |
| <input type="radio"/> Pastilha 1                                                 | <input type="radio"/> Pastilha 2               | <input type="radio"/> Pastilha 3          |
| Se voltassem a ser disponibilizadas, consumiria alguma das pastilhas?            |                                                |                                           |
| <input type="radio"/> Sim                                                        | <input type="radio"/> Não                      |                                           |
| Recomendaria alguma das pastilhas a outras pessoas?                              |                                                |                                           |
| <input type="radio"/> Sim                                                        | <input type="radio"/> Não                      |                                           |
| Costuma mascar pastilha elástica?                                                |                                                |                                           |
| <input type="radio"/> Sim                                                        | <input type="radio"/> Não                      |                                           |
| Se sim, com que frequência?                                                      |                                                |                                           |
| <input type="radio"/> Várias vezes ao dia                                        | <input type="radio"/> Algumas vezes por semana | <input type="radio"/> Poucas vezes ao mês |

| Cor                          | Sabor                  | Dureza                        | Homogeneidade                    |
|------------------------------|------------------------|-------------------------------|----------------------------------|
| 1. Quase branca              | 1. Muito pouco doce    | 1. Praticamente força nenhuma | 1. Apenas a pastilha             |
| 2. Ligeiramente amarela      | 2. Pouco doce          | 2. Força ligeira              | 2. Algumas partículas e pastilha |
| 3. Amarela                   | 3. Moderadamente doce  | 3. Força moderada             | 3. Apenas partículas             |
| 4. Consideravelmente amarela | 4. Doce                | 4. Força considerável         |                                  |
| 5. Quase castanha            | 5. Muito doce          | 5. Muita força                |                                  |
| Odor                         | Aroma                  | Adesividade                   | Desagregação                     |
| 1. Muito pouco intenso       | 1. Muito pouco intenso | 1. Praticamente força nenhuma | 1. Lisa                          |
| 2. Pouco intenso             | 2. Pouco intenso       | 2. Força ligeira              | 2. Ligeiramente rugosa           |
| 3. Moderado                  | 3. Moderado            | 3. Força moderada             | 3. Rugosa                        |
| 4. Intenso                   | 4. Intenso             | 4. Força considerável         |                                  |
| 5. Muito intenso             | 5. Muito intenso       | 5. Muita força                |                                  |

## Appendix XI. HPLC and GC recovery tables and calibration curves

**Table VIII.** PAHs recoveries of each formulation through the HPLC method.

| PAHs          | T ret (min) | Recovery (%)              |                       |                          |                          |        |                          |                         |                        |                         |
|---------------|-------------|---------------------------|-----------------------|--------------------------|--------------------------|--------|--------------------------|-------------------------|------------------------|-------------------------|
|               |             | CGA                       |                       |                          | CGB                      |        |                          | CGC                     |                        |                         |
|               |             | CG                        | Saliva                | Total                    | CG                       | Saliva | Total                    | CG                      | Saliva                 | Total                   |
| Naph          | 9.551       | 26.52±11.83 <sup>a</sup>  | 2.9±1.63 <sup>a</sup> | 29.5±13.46 <sup>a</sup>  | 20.72±1.71 <sup>a</sup>  | NF     | 20.72±1.71 <sup>a</sup>  | 13.47±2.87 <sup>a</sup> | NF                     | 13.47±2.87 <sup>a</sup> |
| Ace           | 12.405      | 10.58±1.24 <sup>a</sup>   | NF                    | 10.6±1.24 <sup>a</sup>   | NF                       | NF     | NF                       | NF                      | NF                     | NF                      |
| Flu           | 12.728      | 53.5±22.59 <sup>a</sup>   | NF                    | 53.5±22.59 <sup>a</sup>  | 36.77±5.08 <sup>a</sup>  | NF     | 36.77±5.08 <sup>a</sup>  | 24.72±2.77 <sup>a</sup> | 3.2±1.97 <sup>a</sup>  | 27.94±4.74 <sup>a</sup> |
| Ant           | 14.756      | 40.04±19.26 <sup>a</sup>  | NF                    | 40.0±19.26 <sup>a</sup>  | 28.74±2.00 <sup>a</sup>  | NF     | 28.74±2.00 <sup>a</sup>  | 13.6±3.01 <sup>a</sup>  | 0.9±0.90 <sup>a</sup>  | 14.51±3.91 <sup>a</sup> |
| Fln           | 15.700      | 101.67±43.73 <sup>a</sup> | NF                    | 101.7±43.73 <sup>a</sup> | 58.77±15.66 <sup>a</sup> | NF     | 58.77±15.66 <sup>a</sup> | 49.6±2.60 <sup>a</sup>  | 1.21±1.21 <sup>a</sup> | 50.85±3.81 <sup>a</sup> |
| B(a)A         | 18.849      | 46.28±17.61 <sup>a</sup>  | NF                    | 46.3±17.61 <sup>a</sup>  | 31.33±6.05 <sup>a</sup>  | NF     | 31.33±6.05 <sup>a</sup>  | 20.69±4.39 <sup>a</sup> | 0.25±0.07 <sup>a</sup> | 20.94±4.46 <sup>a</sup> |
| B(b)Ft+B(j)Ft | 21.241      | 79.63±32.26 <sup>a</sup>  | NF                    | 79.6±32.26 <sup>a</sup>  | 58.30±8.77 <sup>a</sup>  | NF     | 58.30±8.77 <sup>a</sup>  | 33.97±8.40 <sup>a</sup> | NF                     | 33.97±8.40 <sup>a</sup> |
| B(a)P         | 23.008      | 27.29±11.47 <sup>a</sup>  | NF                    | 27.3±11.47 <sup>a</sup>  | 16.29±3.97 <sup>a</sup>  | NF     | 16.29±3.97 <sup>a</sup>  | 10.78±2.39 <sup>a</sup> | NF                     | 10.78±2.39 <sup>a</sup> |
| DB(a,h)A      | 24.248      | 15.67±9.56 <sup>a</sup>   | NF                    | 15.7±9.56 <sup>a</sup>   | 7.32±5.13 <sup>a</sup>   | NF     | 7.32±5.13 <sup>a</sup>   | 15.95±2.95 <sup>a</sup> | NF                     | 15.95±2.95 <sup>a</sup> |
| B(g,h,i)P     | 25.428      | 13.23±8.00 <sup>a</sup>   | NF                    | 13.2±8.00 <sup>a</sup>   | 9.82±1.79 <sup>a</sup>   | NF     | 9.82±1.79 <sup>a</sup>   | 2.53±0.89 <sup>a</sup>  | NF                     | 2.53±0.89 <sup>a</sup>  |
| InP           | 26.590      | 9.24±7.16 <sup>a</sup>    | 2.8±2.80 <sup>a</sup> | 12.0±9.96 <sup>a</sup>   | 17.47±6.92 <sup>a</sup>  | NF     | 17.47±6.92 <sup>a</sup>  | 6.71±2.77 <sup>a</sup>  | NF                     | 6.71±2.77 <sup>a</sup>  |

<sup>a</sup> Standard Deviation (STD)

NF-Not found

**Table IX.** BFRs recoveries of each formulation through the HPLC method.

| BFRS   | T ret (min) | Recovery (%)             |                        |                          |                         |                         |                          |                          |                         |                          |
|--------|-------------|--------------------------|------------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|
|        |             | CGA                      |                        |                          | CGB                     |                         |                          | CGC                      |                         |                          |
|        |             | CG                       | Saliva                 | Total                    | CG                      | Saliva                  | Total                    | CG                       | Saliva                  | Total                    |
| BDE28  | 32.598      | 57.76 ±0.31 <sup>a</sup> | 6.25±0.42 <sup>a</sup> | 64.02±0.72 <sup>a</sup>  | 51.92±1.54 <sup>a</sup> | 18.48±0.51 <sup>a</sup> | 70.40±2.05 <sup>a</sup>  | 50.90±2.69 <sup>a</sup>  | 23.82±0.78 <sup>a</sup> | 74.72±3.47 <sup>a</sup>  |
| BDE47  | 36.547      | 96.96±1.19 <sup>a</sup>  | 5.11±0.31 <sup>a</sup> | 102.07±1.50 <sup>a</sup> | 87.11±1.85 <sup>a</sup> | 17.05±0.53 <sup>a</sup> | 104.16±2.39 <sup>a</sup> | 88.51±4.90 <sup>a</sup>  | 27.26±0.52 <sup>a</sup> | 115.77±5.42 <sup>a</sup> |
| BDE100 | 39.664      | 99.63±2.59 <sup>a</sup>  | 3.57±0.16 <sup>a</sup> | 103.20±2.76 <sup>a</sup> | 92.94±3.14 <sup>a</sup> | 16.97±1.55 <sup>a</sup> | 109.91±4.69 <sup>a</sup> | 92.99±6.57 <sup>a</sup>  | 30.59±0.76 <sup>a</sup> | 123.57±7.33 <sup>a</sup> |
| BDE99  | 40.734      | 98.17±2.75 <sup>a</sup>  | 2.62±0.20 <sup>a</sup> | 100.78±2.95 <sup>a</sup> | 97.59±3.99 <sup>a</sup> | 13.76±0.52 <sup>a</sup> | 111.34±4.51 <sup>a</sup> | 99.06±5.85 <sup>a</sup>  | 26.69±0.76 <sup>a</sup> | 125.76±6.61 <sup>a</sup> |
| BDE154 | 43.557      | 81.20±9.03 <sup>a</sup>  | 1.66±0.15 <sup>a</sup> | 82.86±9.18 <sup>a</sup>  | 70.14±8.82 <sup>a</sup> | 11.74±0.71 <sup>a</sup> | 81.88±9.53 <sup>a</sup>  | 68.57±10.19 <sup>a</sup> | 24.64±0.74 <sup>a</sup> | 93.21±10.93 <sup>a</sup> |
| BDE153 | 45.247      | 92.78±2.08 <sup>a</sup>  | 0.94±0.01 <sup>a</sup> | 93.72±2.09 <sup>a</sup>  | 61.39±3.64 <sup>a</sup> | 8.39±0.84 <sup>a</sup>  | 69.78±4.48 <sup>a</sup>  | 71.26±5.08 <sup>a</sup>  | 12.78±1.30 <sup>a</sup> | 84.04±6.38 <sup>a</sup>  |
| BDE183 | 52.197      | 69.27±1.85 <sup>a</sup>  | 1.46±0.07 <sup>a</sup> | 70.73±1.92 <sup>a</sup>  | 69.28±1.68 <sup>a</sup> | 15.36±0.53 <sup>a</sup> | 84.65±2.21 <sup>a</sup>  | 35.21±2.40 <sup>a</sup>  | 31.84±0.51 <sup>a</sup> | 67.05±2.91 <sup>a</sup>  |

<sup>a</sup> Standard Deviation (STD)

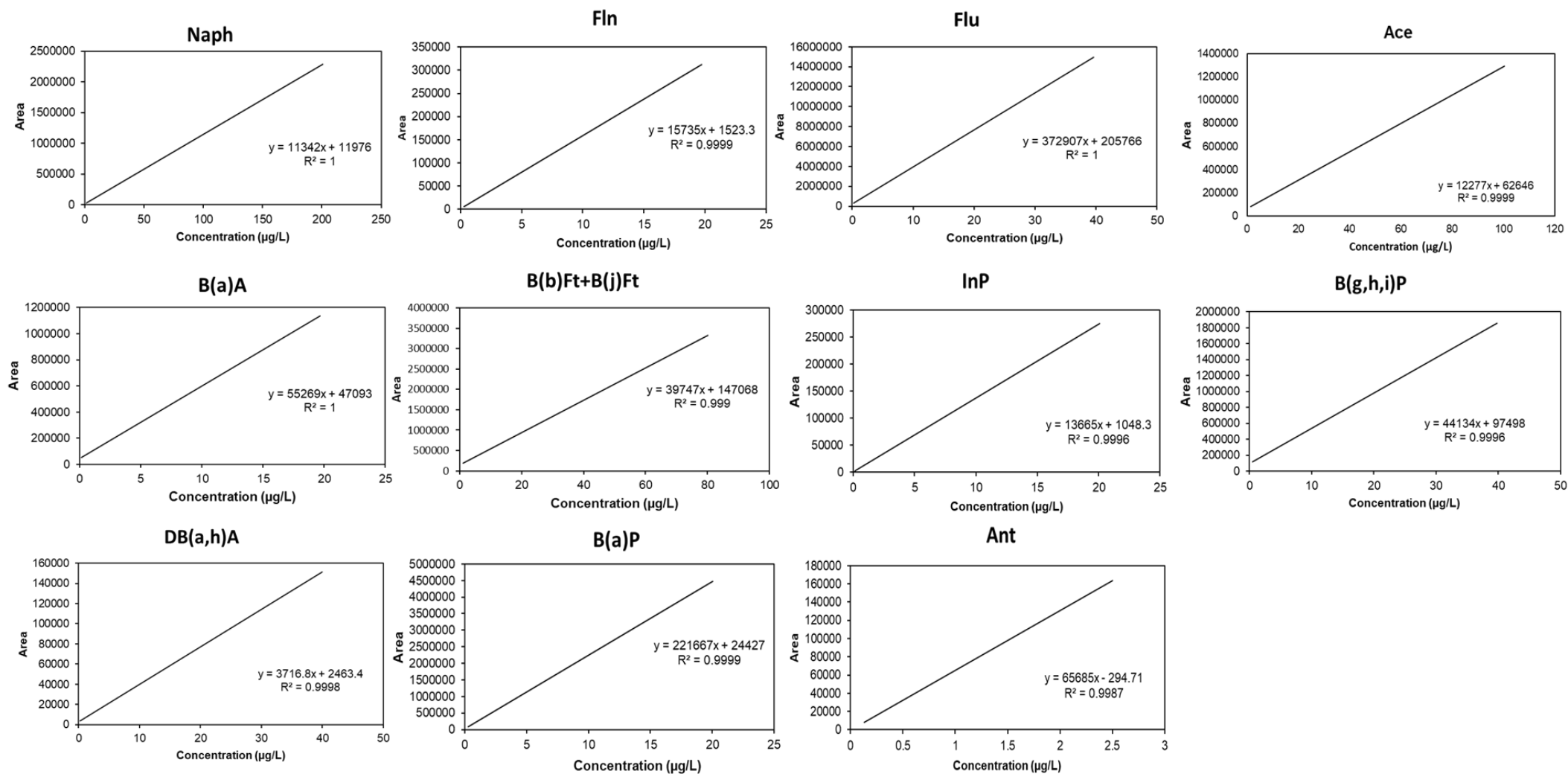


**Figure 46.** Calibration curves of various PAHs examined.

