Development of a Honey and Aloe vera Sports Gel

Ahmed Rami Najjar

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Supervised by

Dr. Bruno Melgar Castañeda

Dr. Tayse F.F. da Silveira

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Index

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

Abstract

Sports supplements, designed to enhance athletic performance and recovery, have seen significant market growth due to increased awareness of exercise benefits. This trend has led to the development of sports gels, offering a digestive advantage for endurance athletes during high-intensity exercise. This study aimed to develop a novel sports gel with natural ingredients, addressing the demand for innovative sports nutrition solutions. The formulation includes mineral water for electrolytes, honey (fructose and glucose) as a primary carbohydrate source, maltodextrin for sustained energy release, and aloe vera for antioxidant properties. The combination of simple and complex carbohydrates was strategically chosen to provide efficient and sustained energy delivery through distinct metabolic pathways. The product development process focused on optimizing gelling agent formulations to create a sports gel with rheological properties comparable to those of a commercial control product. Initial analyses of the control gel established key reference parameters: firmness (26.5 ± 0.49 g), consistency (247 ± 5.44 g/s), cohesiveness (-19.6 ± 1.48 g), index of viscosity (-79.40±8.06 g/s) and viscosity (133.7 ± 14.84 mPa/s). To achieve similar properties, five gelling agents—carrageenan (IC), gellan gum (GG), guar gum (GU), locust bean gum (LB), and xanthan gum (XG)—were evaluated at concentrations ranging from 0.5% to 3%. These agents were screened based on their influence on rheological properties (firmness, consistency, cohesiveness, viscosity, and index of viscosity), as well as their time-stability, visual appeal, and cost-effectiveness. The goal was to identify gums that closely matched the reference values of the control product while maintaining high quality and market feasibility. Based on the results of the parameter evaluations, concentrations between 1.25% and 2.25% were shown to be the most appropriate. The gelling agents were ranked from 1st to 5th place, with xanthan gum ranking first, followed by GG and GU, so these three gelling agents were selected for further studies. Finally, a mixture design experiment was conducted to refine the formulation, identifying optimal combinations of Xanthan Gum, Gellan Gum, and Guar Gum to meet the target rheological properties. The regression models generated demonstrated strong predictive accuracy, with high R^2 values (>0.7) and no significant lack of fit ($p>0.05$). Simultaneous optimization of the rheological parameters, aligned with the properties of the control gel, yielded an optimal gelling agent formulation consisting of 0.75% xanthan gum and 0.25% gellan gum. Validation experiments confirmed the reliability of the predictive models, showing strong agreement between predicted and experimentally observed values across all measured parameters. The final product formulation comprised 47.40% honey, 43.14% water, 7.46% maltodextrin, 1% aloe vera, 0.75% xanthan gum, and 0.25% gellan gum. Compared to the control sports gel, the final product

demonstrated similar firmness (27.4 g vs. 26.5 g) and consistency (258.8 g\s vs. 247 g\s), cohesiveness (-10.99 vs -13.93 g), while showing a lower viscosity index (-57.7 g\s vs. -79.4 g\s) and viscosity (36.71 mPa\s vs 57.87 mPa\s). Chemical and physicochemical analyses revealed a reduced moisture content (41.2% vs. 66.5%), likely attributed to the inclusion of honey. The sugar content, comprising glucose (15 g/100 mL) and fructose (30 g/100 mL), aligned with the desired amount, while the pH (4.1) was within an acceptable range. The final product exhibited antioxidant activity, measured at 10.42 µl Trolox equivalents using the DPPH assay. The DPPH assay for aloe vera alone indicated a significant antioxidant potential (47.33 µl Trolox eq), highlighting its promising contribution to the overall antioxidant properties of the gel. These findings suggest that increasing the aloe vera content in future formulations could further enhance the antioxidant profile of the product. Additionally, Aloin concentrations derived from aloe vera were measured at 0.83 mg/L, which is below the legal limit of 10 mg/L, ensuring the product's safety for consumption. Overall, the results confirm the feasibility of developing a sports gel with optimized texture, stability, and natural ingredients. Future research should prioritize optimizing aloe vera concentration to enhance antioxidant benefits and further evaluate the functional advantages of the final formulation.

Key-words: Sports gel, rheological parameters, gelling agents, carbohydrates, minerals, sport supplement.

Resumo

Suplementos desportivos, concebidos para melhorar o desempenho atlético e a recuperação, têm registado um crescimento significativo no mercado devido à maior consciencialização dos benefícios do exercício. Esta tendência levou ao desenvolvimento de géis desportivos, oferecendo uma vantagem digestiva para atletas de resistência durante exercícios de alta intensidade. Este estudo visou desenvolver um novo gel desportivo com ingredientes naturais, respondendo à procura de soluções inovadoras em nutrição desportiva. A formulação inclui água mineral para eletrólitos, mel (frutose e glucose) como fonte primária de hidratos de carbono, maltodextrina para liberação sustentada de energia e aloe vera pelas suas propriedades antioxidantes. A combinação de hidratos de carbono simples e complexos foi estrategicamente escolhida para fornecer energia de forma eficiente e sustentada através de vias metabólicas distintas. O processo de desenvolvimento do produto focou-se na otimização das formulações de agentes gelificantes para criar um gel desportivo com propriedades reológicas comparáveis às de um produto comercial de controlo. As análises iniciais do gel de controlo estabeleceram parâmetros de referência chave: firmeza (26,5 ± 0,49 g), consistência (247 ± 5,44 g/s), coesividade (-19,6 ± 1,48 g) index de viscosidade (-79,40 ±8.06 g/s) e viscosidade (133,7 ± 14,84 mPa/s). Para alcançar propriedades semelhantes, cinco agentes gelificantes—carragenina (IC), goma gelana (GG), goma guar (GU), goma de alfarroba (LB) e goma xantana (XG)—foram avaliados em concentrações variando de 0,5% a 3%. Estes agentes foram selecionados com base na sua influência nas propriedades reológicas (firmeza, consistência, coesividade, viscosidade e índice de viscosidade), bem como na sua estabilidade ao longo do tempo, apelo visual e custo-efetividade. O objetivo era identificar gomas que se aproximassem dos valores de referência do produto de controlo, mantendo alta qualidade e viabilidade de mercado. Com base nos resultados das avaliações dos parâmetros, concentrações entre 1,25% e 2,25% mostraram-se as mais apropriadas. Os agentes gelificantes foram classificados do 1º ao 5º lugar, com a goma xantana em primeiro, seguida por GG e GU, sendo estes três agentes gelificantes selecionados para estudos posteriores. Por fim, foi realizado um experimento de design de mistura para refinar a formulação, identificando combinações ótimas de Goma Xantana, Goma Gelana e Goma Guar para atender às propriedades reológicas alvo. Os modelos de regressão gerados demonstraram forte precisão preditiva, com altos valores de R^2 (>0,7) e sem falta de ajuste significativa (p>0,05). A otimização simultânea dos parâmetros reológicos, alinhada com as propriedades do gel de controlo, resultou numa formulação ótima de agente gelificante consistindo em 0,75% de goma xantana e 0,25% de goma gelana. Experimentos de validação

confirmaram a confiabilidade dos modelos preditivos, mostrando forte concordância entre os valores previstos e os observados experimentalmente em todos os parâmetros medidos. A formulação final do produto compreendeu 47,40% de mel, 43,14% de água, 7,46% de maltodextrina, 1% de aloe vera, 0,75% de goma xantana e 0,25% de goma gelana. Em comparação com o gel desportivo de controlo, o produto final demonstrou firmeza (27,4 g vs. 26,5 g) e consistência (258,8 g\s vs. 247 g\s) semelhantes, coesividade (-10,99 vs -13,93 g), enquanto apresentava um índice de viscosidade (-57,7 g\s vs. -79,4 g\s) e viscosidade (-36,71 mPa\s vs -57,87 mPa\s) mais baixos. Análises químicas e físico-químicas revelaram um teor de humidade reduzido (41,2% vs. 66,5%), provavelmente atribuído à inclusão do mel. O conteúdo de açúcar, compreendendo glucose (15 g/100 mL) e frutose (30 g/100 mL), estava alinhado com a quantidade desejada, enquanto o pH (4,1) estava dentro de uma faixa aceitável. O produto final exibiu atividade antioxidante, medida em 10,42 µl equivalentes de Trolox usando o ensaio DPPH. O ensaio DPPH apenas para aloe vera indicou um potencial antioxidante significativo (47,33 µl eq Trolox), destacando sua promissora contribuição para as propriedades antioxidantes gerais do gel. Estes resultados sugerem que aumentar o conteúdo de aloe vera em formulações futuras poderia melhorar ainda mais o perfil antioxidante do produto. Adicionalmente, as concentrações de Aloína derivadas da aloe vera foram medidas em 0,83 mg/L, o que está abaixo do limite legal de 10 mg/L, garantindo a segurança do produto para consumo. No geral, os resultados confirmam a viabilidade de desenvolver um gel desportivo com textura otimizada, estabilidade e ingredientes naturais. Pesquisas futuras devem priorizar a otimização da concentração de aloe vera para aumentar os benefícios antioxidantes e avaliar ainda mais as vantagens funcionais da formulação final.

Palavras-chave: Gel desportivo, parâmetros reológicos, agentes gelificantes, hidratos de carbono, minerais, suplemento desportivo.

1. Introduction

Over the past few decades, humans have markedly evolved from simply meeting caloric needs to a more nuanced understanding of nutrition and its influence on overall health (Cannataro et al., 2022). Public interest in what they eat came after recognizing the health issues related to diet, such as heart disease, diabetes, and obesity. People are now more informed about macronutrients and the benefits of whole food. This shift placed greater emphasis on quality over quantity, leading people to explore how nutrients could enhance performance, recovery, and overall health (Margaritelis et al., 2024).

The growing interest of people in health maintenance also made them increasingly include physical activity in their daily lives. This has led to a rising interest in sports-related products, such as sports supplements, to improve performance, aid recovery, and meet nutritional needs during training. These supplements comprise sports and isotonic drinks, protein powders, energy or protein bars, and sports gels. They often include ingredients like protein, creatine, and amino acids, which help with muscle recovery, strength, and endurance, making them essential for intense workouts or long-duration activities. Some supplements also contain caffeine to increase focus and beta-alanine to help reduce muscle fatigue. For endurance athletes, sports supplements typically provide fast-acting carbohydrates and electrolytes to boost energy levels and replace minerals lost through sweat, helping to maintain hydration and minimize the risk of cramps.

The demand for sports supplements has grown, and this sector has been under pressure to provide new, natural ingredients and products that can meet consumers' daily nutritional, functional, and organoleptic requirements (Lam et al., 2022).

1.1. Sports gel

Energy gels have become increasingly popular among endurance athletes due to their convenience and effectiveness in providing quick energy during prolonged physical activities. This trend has led to developments in the energy gel market and consumption patterns among athletes (Cannataro et al., 2022).

Sports gel (SG) is designed to boost athletic performance or aid in recovery during physical activity. It is consumed orally and composed of carbohydrates, electrolytes, and sometimes caffeine or other ingredients to provide quick energy and hydration to athletes during long activities like running and cycling, athletes usually use sports gel to refill glycogen stores and keep hydration levels balanced during prolonged exercise (Kayshar et al., 2024). This form

is interesting since it combines hydration, energy, and essential nutrients in a compact, portable packet. Unlike whole foods, sports gel is easy to carry, and its semi-liquid consistency makes it simple to consume without causing digestive discomfort. It is ideal for athletes looking to sustain energy levels and optimize performance without interrupting their activity (Baroyi et al., 2023).

Sports gels come in several varieties, each designed to meet specific athlete needs. Classic energy gels provide quick-release, simple carbohydrates for immediate energy, but often require water for optimal absorption. Isotonic gels, also known as electrolyte gels, contain a balanced water-to-electrolyte ratio and are thinner in consistency, making them easier to consume without additional water. Both electrolyte-enhanced gels and isotonic gels contain electrolytes to support hydration during exercise, their formulations and usage can differ. Isotonic gels are specifically designed to match the body's blood concentration, allowing for easy absorption without additional water. Electrolyte-enhanced gels, however, may require water consumption if they are not isotonic, to ensure proper hydration and prevent potential digestive issues. Caffeinated gels include caffeine to boost alertness and energy levels, particularly beneficial during longer events. However, athletes should use caffeinated gels cautiously, as individual responses to caffeine during exercise can vary (Patterson & Gray, 2007). Some gels also incorporate additional components like amino acids or multiple transportable carbohydrates to enhance performance and digestibility. The choice of gel type depends on individual tolerance, specific nutritional needs, and the nature of the athletic activity (Campbell et al., 2008).

1.2 Composition of sports energy gel

The composition of sports gels can vary depending on the specific use or brand. They are composed commonly of simple carbohydrates like glucose, fructose, or maltodextrin. Carbohydrates provide a source of energy for muscles during exercise. Sodium, potassium, magnesium, and other electrolytes are usually included in sports gel to help replace those lost into sweat and maintain proper hydration levels (Cermak & van Loon, 2013). They play an important role in regulating fluid balance and muscle function. Water is an essential component in sports gels since it facilitates consumption and absorption during exercise. They often include flavorings such as fruit extracts or artificial flavors. Preservatives may also be included to extend shelf life and maintain product stability. Sports gel may contain other additives such as vitamins, amino acids, or herbal extracts to further enhance performance or recovery. These additives can vary widely between brands and formulations (Baroyi et al., 2023).

1.2.1. Carbohydrates

Athletes are advised to follow a high-carbohydrate (CHO) diet, consume CHO before exercise, take enough CHO during exercise, and restore CHO levels quickly after. The International Olympic Committee (IOC) states that a high-CHO diet before competition improves performance, especially for exercise lasting over 60 minutes. Athletes should match their CHO intake to the energy needs of their training (Jeukendrup et al., 2006).

The form in which carbohydrates (CHO) are consumed during exercise, whether solid or liquid, does not appear to influence their performance-enhancing effects. Hargreaves & Spriet,(2020) found that eating a candy bar containing 43 g of CHO, 9 g of fat, and 3 g of protein led to a 46% improvement in sprint capacity after 4 hours of exercise. Similar findings were later confirmed by other studies, showing that both liquid and solid CHO sources improved exercise performance equally (Lugo et al., 1993).

Studies have shown that a fructose-to-glucose ratio of approximately 0.8:1 maximizes energy utilization and endurance performance during prolonged exercise. Consuming beverages with a 0.5–1.0:1 fructose-to-glucose/maltodextrin ratio at a rate of 1.3–2.4 g/min supports optimal endurance over 2.5–3 hours, outperforming single saccharide solutions of equal caloric content (Rowlands et al., 2008).

This enhancement is due to their distinct absorption mechanisms in the small intestine. Glucose is absorbed via the sodium-glucose linked transporter 1 (SGLT1), which is sodium-dependent and actively transports glucose into intestinal cells (Mikušová et al., 2022). Fructose, in contrast, is absorbed through the GLUT5 transporter, which uses facilitated diffusion and does not depend on sodium (Pfeiffer et al., 2012). This allows glucose and fructose to be absorbed simultaneously without competing for the same transporters, leading to more efficient uptake. Sucrose, a disaccharide, is hydrolyzed into glucose and fructose by the enzyme sucrase in the small intestine, providing both sugars in a form that can be readily absorbed. Maltodextrin, a polysaccharide made of glucose units, is rapidly broken down into glucose, which is then absorbed by the SGLT1 transporter (Fuchs et al., 2019).

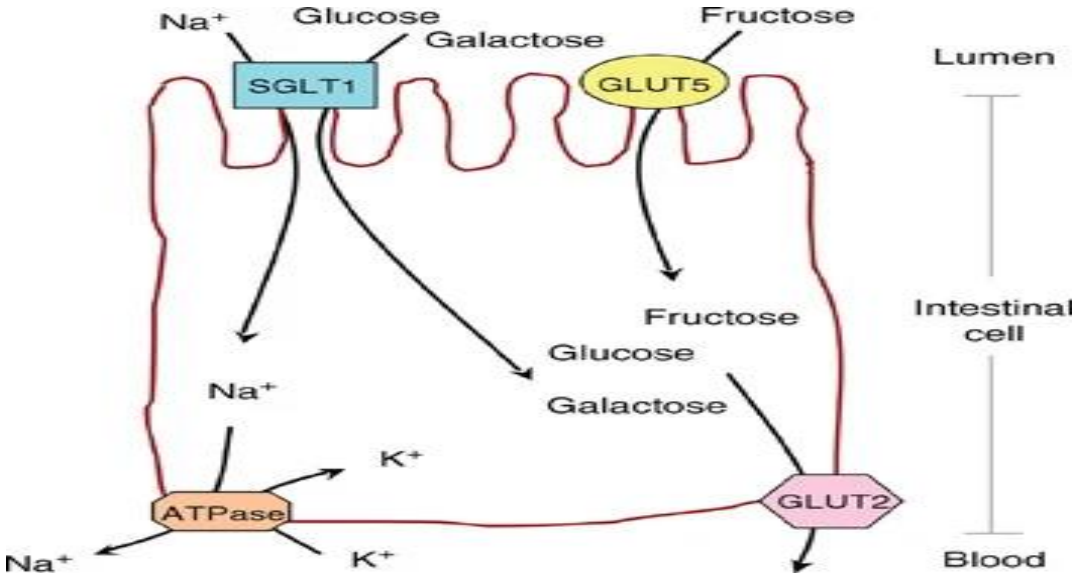

Figure 1. 1. Fructose and Glucose absorption via sodium-dependent glucose transporters (SGLT1), and GLUT5 transporters (Rowlands et al. (2015).

1.2.2. Electrolytes

Intensive exercise may lead to sweat rates of 1 L/h, while exercising at high temperatures may increase sweat loss of >2 L/h. Progressive dehydration is likely in those sweating over long periods that can produce metabolic or physiological effects such as reduced blood flow, impaired heat exchange, reduced oxygen supply to muscle cells, increased stress hormone levels, and enhanced glycogen breakdown rates in the liver and muscle (Evans et al., 2009).

Sodium is the most abundant electrolyte lost in sweat during exercise and is vital in maintaining fluid balance. It helps regulate blood volume and pressure by controlling the amount of water in and around cells (Watson & Austin, 2018). Intake of salt is diet-dependent (Sadowska, 2020). It aids in nerve signal transmission and muscle contraction (Porfírio et al., 2019). Potassium, another essential electrolyte, is critical for muscle function and nerve signaling. It works alongside sodium to maintain proper fluid balance, but its primary role is in muscle contraction. Potassium helps transmit electrical impulses that trigger muscle contractions, making it vital for coordinated movement and preventing cramping during exercise (Evans and al., 2009).

Other important electrolytes include calcium and magnesium, which play critical roles in muscle contraction and nerve function and help prevent cramps and muscle stiffness during and after intense exercise (Leiper, 1998). In addition to these primary electrolytes, other ions like chloride and phosphate, working in tandem with sodium, also contribute to the overall fluid and acid-base balance during exercise.

1.2.3. Thickeners

Gelling agents, when added to an aqueous solution, typically absorb water and undergo structural changes that lead to the formation of a gel network. This network traps water molecules, creating a semi-liquid texture. It is often made from polysaccharides or proteins and reacts with water, ions, and heat, causing polymer chains to bond and form cross-linked structures. This process affects the rheological properties of the solution, making it thicker and more stable (Yang et al., 2022). The gel formation and stability depend on the concentration of the gelling, pH, temperature, presence of other compounds, and the chemical structure (Fu et al., 2024).

Gelling agents, such as guar gum, carrageenan, and arrowroot powder play an important role in various formulations across different industries, especially in food pharmaceuticals, and cosmetics. Xanthan gum (XG), derived from the fermentation of sugars by the bacterium *Xanthomonas campestris*, is widely used for its exceptional thickening and stabilizing properties, enhancing texture and mouthfeel in food products like sauce, café, and dairy alternatives. Guar gum (GU), obtained from the guar plant's seeds, is an effective thickening agent and is usually utilized in gluten-free baking. Locust bean gum (LB) is usually used in conjunction with XG to create synergies in thickening and stabilizing (Zafeiriadis et al., 2024). It is important to control the gel structure and mechanical properties for different applications. This can be done by adjusting the gelling agent type, composition, and concentration. The way the gel behaves mechanically also depends on the characteristics of particles and how their surfaces are modified (Yang et al., 2022).

The gelling process of different polysaccharide-based hydrogels follows a "Coil → Helix → Gel" transition as shown in **Figure 1.2,** which contains distinct conformational changes and molecular interactions. Initially, the polysaccharide chains exist in a disordered coil conformation in solution. As the temperature decreases or other stimuli are present, these coils transition to ordered helical structures. This coil-to-helix transition is typically driven by intramolecular hydrogen bonding and hydrophobic interactions (Karoyo & Wilson, 2017).

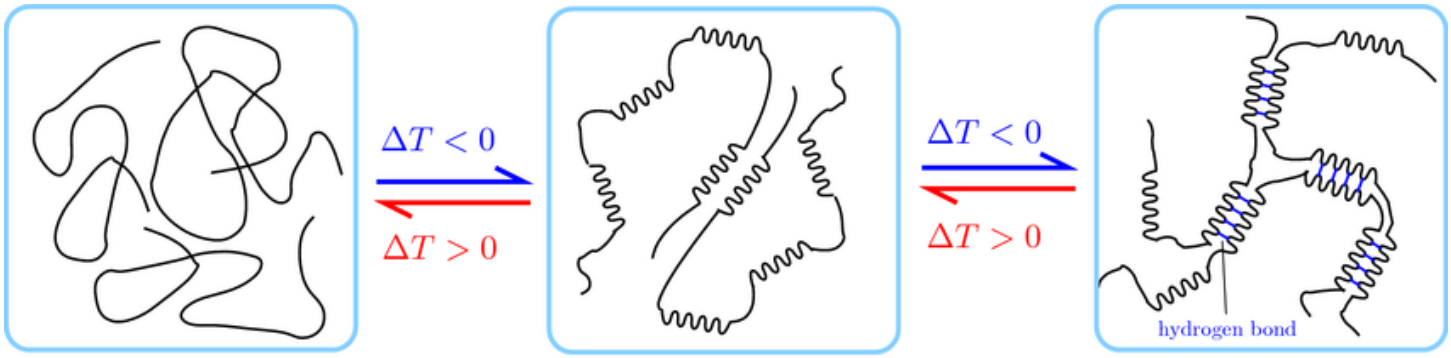

Figure 1. 2. Formation of a gel network Coil → Helix → GeL (Kanesaka et al., 2004)

The helices then aggregate and associate, forming junction zones through intermolecular interactions. These junction zones act as physical crosslinks, creating a three-dimensional network structure that characterizes the gel state(Hayashi et al., 1980). The helix formation and subsequent aggregation are crucial steps in the gelation process, as they provide the structural framework necessary for gel formation. This mechanism has been observed in various polysaccharides, including agarose, carrageenan, and gellan gum (Nitta et al. 2001). The transition temperatures and specific molecular interactions involved can vary depending on the polysaccharide type, molecular weight, and environmental conditions such as ion concentration and pH (Karoyo & Wilson, 2017).

1.3. Texture parameters

Sports gels stand out from other sports supplements due to their unique rheological properties, characterized by their distinct texture and consistency. This texture is primarily a result of the gelling agents incorporated into their formulation, and lists as a main quality parameter for this product category.

Gelling agents significantly influence the rheological properties of polysaccharide-based hydrogels, affecting their mechanical strength, viscoelastic behavior, swelling characteristics, gelation kinetics, and shear-thinning properties. The degree of cross-linking and network formation directly impact the gel's stiffness and structural integrity, while the viscoelastic nature reflects the material's response to stress and deformation (Mikušová et al., 2022).

The visual representation in **Figure 1.3** represents the graph given by the texture parameters. To analyze the texture parameters such as firmness, cohesiveness, consistency, and

index of viscosity in the diagram provided, it is important to understand how a texture analyzer works and how these parameters are calculated from the force-time curve.

Firmness is the maximum force required to deform a product, it is presented at the peak of the positive force curve in the diagram, this corresponds to the highest point of the curve on the red area. A higher peak indicates a firmer texture, which means the material resists more before it starts deforming (Wu et al., 2021).

Consistency measures the overall resistance to deformation, giving an idea of how the material behaves under continuous stress, it is related to the area under the first positive force curve (red area). A larger area under this curve means the material offers more resistance to the applied force, indicating a stronger consistency (Zawada et al., 2018).

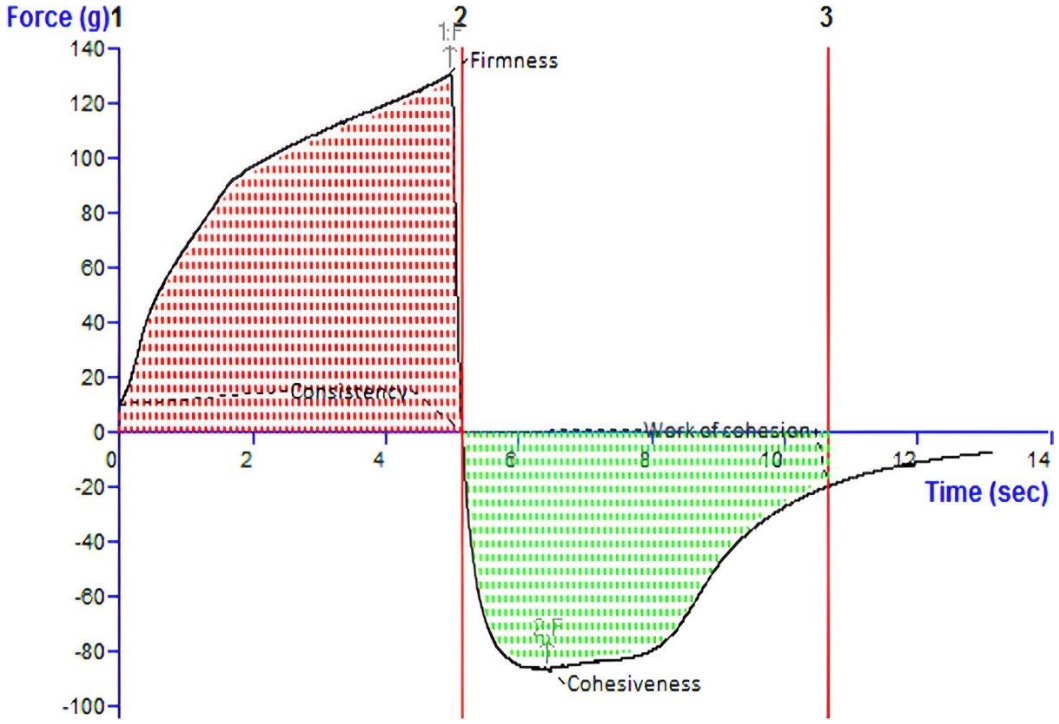

Figure 1.3. Force-time curve from texture profile analysis of control sports gel (Lis et al., 2021).

Cohesiveness measures the internal strength of the material after deformation. It indicates how well the material sticks together; it is associated with the negative area (green area) under the force-time curve after the first deformation. The larger this negative area, the more cohesive the material is. The index of viscosity (work of cohesion figure 1.3) represents the behavior of the material under stress over time, particularly during and after deformation, the index of viscosity would be inferred from both the area under the positive curve and the

negative area, it is most derived from the relationship between the two curves (Nishinari et al., 2019).

Viscosity measures the resistance to flow of a material, indicating how easily it deforms under applied stress. This property is crucial in determining how the gel will behave during application and consumption. It is measured using a viscometer to quantify the gel's flow characteristics and resistance to deformation.

The rheological and textural properties of hydrocolloids significantly influence oral perception and mouthfeel of food products. Viscosity, firmness, and cohesiveness play crucial roles in texture perception during oral processing. Higher viscosity is associated with increased thickness and creaminess, while firmness affects the force required for mastication and contributes to chewiness sensations. Cohesiveness influences how well the food holds together during oral processing and can contribute to smoothness or graininess perceptions. Shear-thinning behavior, common in many hydrocolloids, affects how they spread in the mouth and can contribute to slipperiness or creaminess sensations (Deblais et al., 2021).

1.4. How to consume sports gel

Sports gel consumption recommendations vary based on the timing relative to exercise and the duration of physical activity. Manufacturers suggest using sports gels 15 to 30 minutes before exercise to allow for nutrient absorption. During endurance activities lasting more than an hour, consumption frequency typically ranges from every 30 to 45 minutes, with most sources recommending 1-2 gels per hour. This aligns with the American College of Sports Medicine (ACSM) guidelines, which advise a carbohydrate intake of 30-60 g/hour for exercise lasting longer than one hour. A study in the Journal of Strength and Conditioning Research found that cyclists taking gels every 30 minutes performed 5-7% better than those taking them every 45 minutes (Kozlowski et al., 2021). The general guideline translates to about 1-3 gels per hour, depending on the brand and composition.

Factors influencing optimal frequency include activity duration, individual tolerance, gel composition, and environmental conditions. It is crucial to start gel intake early (around 45-60 minutes into the activity), stay hydrated, and practice your fueling strategy during training (Nobari et al., 2023). While some research suggests higher carbohydrate intake rates may benefit certain types of prolonged, intense exercise, it is important to note that the human body typically absorbs about 60 grams of carbohydrates per hour, and exceeding this may lead to digestive discomfort without additional benefits (Kozlowski et al., 2021).

While less common than pre- and during-exercise use, some athletes utilize gels post-exercise to replenish glycogen stores. This practice can contribute to the rapid restoration of muscle glycogen, which is critical for recovery, especially after prolonged or intense exercise. The timing of gel consumption post-exercise is particularly important, as there is a window of opportunity for optimal glycogen replenishment within the first 30-60 minutes after activity cessation. By following these guidelines, athletes can maximize the benefits of energy gels while minimizing potential digestive issues, thereby supporting both performance and recovery.

1.5. Honey

Honey is a natural substance produced by honeybees (*Apis mellifera*). They collect flower nectar, plant secretions or excretions of plant-sucking insects from plants and transform it into honey (Palma-Morales et al., 2023). Honey´s composition consists of carbohydrates (around 75-80%), water (15-17%), and proteins (0.1-0.4%). The most important carbohydrates in honey are fructose and glucose (monosaccharides), but it has also sucrose, maltose, and trehalose in minimal amounts. It also contains enzymes, organic acids, vitamins, minerals, and phenolic compounds, which contribute to its sensory and functional characteristics (Palma-Morales et al., 2023; Yusof et al., 2018). Honey has been related with antioxidant, anti-inflammatory, antibacterial, and antiviral properties. These beneficial effects of honey have been mainly attributed to phenolic compounds, primarily flavonoids and phenolic acids (Palma-Morales et al., 2023).

In addition to the mentioned bioactivities, studies suggest that consuming honey before or during exercise may improve performance in endurance activities and taking it post-exercise can replenish glycogen stores and aid muscle recovery. In a 64-km simulated cycling time trial, dextrose and honey supplementation resulted in faster completion times than placebo. Notably, the dextrose group performed better than the placebo group in the final 16 km, suggesting that maintaining glucose concentration helps prevent fatigue in the latter stages of prolonged exercise (Davey et al., 2018). Another study found that honey supplementation improved performance in a 20-minute time trial following exhaustive exercise compared to water. These findings indicate that the carbohydrate content in honey can play a comparable role to other carbohydrate sources in supporting prolonged exercise performance, highlighting the potential benefits for endurance athletes (Yusof et al., 2018).

Abbey and Rankin, (2009) conducted a study to evaluate the impact of a honey-sweetened beverage versus a commercial sports drink and a placebo on soccer-specific performance metrics. Although the results showed no significant differences in performance

among the three groups, the research indicated that honey could serve as an effective alternative to commercial sports drinks without adversely affecting performance. These findings suggest that honey is a viable natural option for athletes, providing similar benefits in terms of energy sustenance and performance enhancement during exercise (Abbey & Rankin, 2009).

1.6. Aloe *Barbadensis Miller*

Aloe Barbadensis Miller or aloe vera (AV) is a tropical or subtropical plant native to northern Africa, and currently widely distributed throughout the world. The plant's main characteristics are its sharp tips and rough edges and fleshy and thick leaves, often grouped in rosettes, the leaves are normally green and get red when it's in an aggressive climate (too much sun and insufficient water). The plant adapts to sunny conditions and prefers wet soil, it can be cultivated indoors as well (Khaldoune et al., 2024). There are more than 360 identified aloe species, Although *Aloe barbadensis Miller* stands out as the most known and cultivated (Bendjedid & Benouchenne, 2023).

In AV, there are two main liquid sources found, a clear gel (called mucilage) and the aloe latex, a yellowish exudate existing between the rind (outer green skin protecting the gel) and the inner gel (Figure 1.4). The primary compounds of the yellowish latex are anthraquinone *C*- and *O*-glycosides, anthrones, and other hydroxyanthraquinone derivatives. Among these, aloin and aloe-emodin predominate, which at certain concentrations have been reported to be genotoxic in bacterial mutation and mammalian cell assays in vitro (Kim et al. 2023). A study examining the dose-dependent effects of aloin on intestinal health revealed its potential to disrupt gut microbiota balance. The research found that aloin exhibits antibacterial properties against certain beneficial bacteria, leading to decreased butyrate production—a crucial short-chain fatty acid for intestinal well-being. While lower concentrations showed minimal impact, higher doses (500 μM) significantly compromised intestinal epithelial cell barrier integrity in an in vitro rat model. These findings suggest that excessive aloin intake could lead to adverse effects such as inflammation or increased susceptibility to gastrointestinal disorders by altering normal bacterial metabolism and intestinal permeability. The research underscores the importance of cautious consumption of aloin-containing products, balancing potential benefits against possible risks to gut health (Gokulan et al., 2019).
Aloe vera gel contains a variety of bioactive compounds, with acemannan being its primary polysaccharide. Acemannan is an acetylated glucomannan known for its immunomodulatory and wound-healing properties. It also supports antiviral activity and digestive health. These components contribute significantly to the therapeutic effects of Aloe vera gel (Hamman, 2018).

Due to concerns related to the potential genotoxicity and carcinogenicity risk associated with the substances in the latex, aloe vera gel production has undergone pre-treatments for removing it (Kim et al., 2023). The International Aloe Science Council (IASC) has established not more than 10 ppm aloin for all Aloe vera leaf juice ingredients for use in products intended for oral consumption (Brown et al., 2014). The European Union has set a limit for the detection of aloin in food and food supplements at 10 ppm, as outlined in Regulation (EU) 2021/468. This threshold is considered the lowest level that can be reliably quantified in laboratories across the EU, ensuring a harmonized risk management approach. Products exceeding this level are prohibited from being marketed within the EU due to potential health risks associated with these compounds (Sánchez-Machado et al., 2017).

On the hand, AV gel and extracts have been related to wound healing, anti-ulcer effects, anti-inflammatory effects, antioxidant activity, anticancer activity, antidiabetic effects, treating gastrointestinal disorders, lowering low-density lipoprotein (LDL), increasing high-density lipoprotein (HDL), decreasing blood glucose level (Araya-Quintanilla et al., 2021). This beneficial property has made AV a burgeoning industry for producing cosmetics, functional food, and pharmaceuticals.

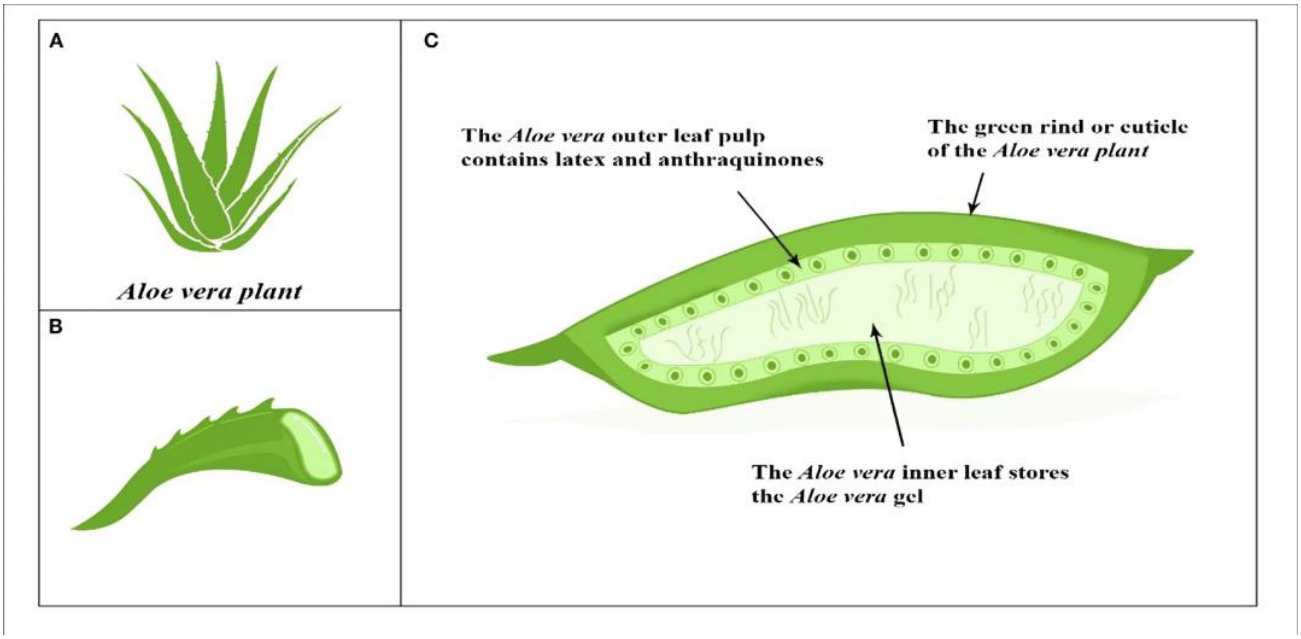

Figure 1.4. Aloe vera leaf composition (Kim et al., 2023)

AV has up to 200 distinct kinds of compounds (Davis 1997), while approximately 98% of the AV leaf gel is water (Bozzi et al., 2007). AV gel has a total solid concentration of 0.66% and soluble solids of 0.56%, with a notable seasonal variation. Polysaccharides make up about 55% of the dry matter content of aloe gel, followed by sugars (17%), minerals (16%), proteins

(7%), lipids (4%), and phenolic compounds (1%). Many vitamins, including vitamins A, C, and E, are present in aloe vera gel. There is also folic acid, choline, vitamin B2 (riboflavin), niacin, and vitamin B1 (thiamine).

Additionally, several writers proposed trace levels of vitamin B12, or cyanocobalamin, typically found in animal sources (Baruah et al., 2016). AV gel also contains 20 of the 22 amino acids and seven of the eight essential amino acids the human body needs.

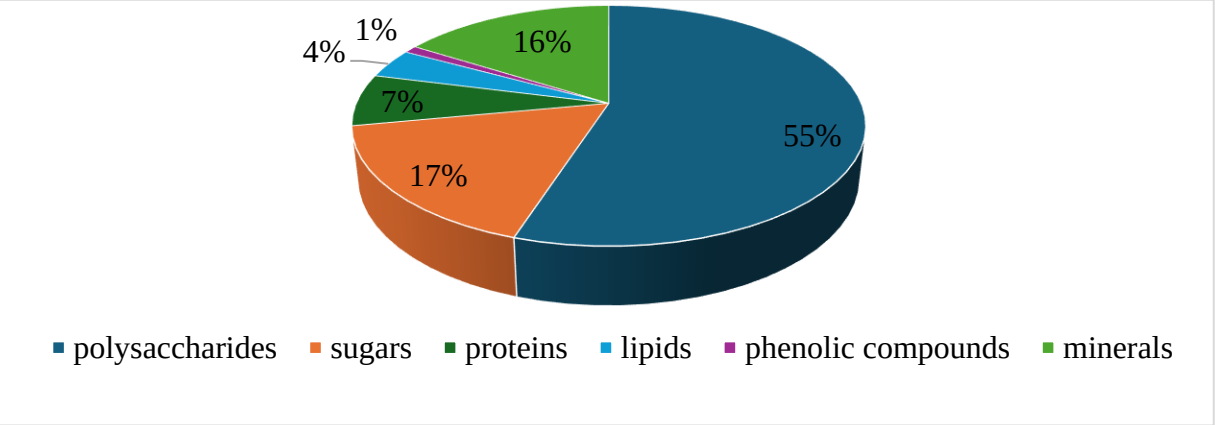

Figure 1. 5. Composition of aloe vera gel (Khaldoune et al., 2024).

It's important to note that while aloe vera has gained significant popularity in various food and beverage products, there is a notable gap in its application to sports gels. Despite the widespread use of aloe vera in skincare, beverages, and general wellness products, its potential as an ingredient in sports gels remains largely unexplored. This presents an interesting opportunity for innovation in the sports supplement industry.

1.7. Mixture design

Mixture design of experiments (DOE) is a statistical methodology specifically tailored for optimizing formulations where the response of interest depends on the relative proportions of components within a mixture. Unlike factorial or response surface designs, which allow independent variation of factors, mixture designs are governed by a compositional constraint where the sum of all component proportions equals a fixed total, such as 1 or 100% (Thorsteinsdottir, 2021).

The primary advantage of mixture design is its ability to systematically explore and model the relationships between component proportions and the system responses, by using advanced regression techniques, mixture designs provide predictive models that describe the behavior of the system, allowing for the identification of optimal compositions and insights into

how interactions among components influence key attributes. These models not only aid in understanding the effects of individual components but also elucidate synergistic or antagonistic interactions, which are often crucial for achieving desired properties (N Politis et al., 2017).

In mixture designs, the simplex triangle is a geometric representation of all possible combinations of components in a three-component system, constrained by the requirement that the sum of the proportions equals 100%. This visualization is essential for interpreting the experimental domain and understanding how different mixtures influence the responses being studied.

Each vertex of the triangle represents a formulation composed entirely of one component at 100%, with the other two components absent. These are referred to as *pure blends* (or pure components). The edges of the triangle illustrate *binary mixtures*, where two components vary in proportion while the third remains at zero. For example, as one moves along the edge between two vertices, the proportion of one component increases while the other decreases, with the third component excluded from the mixture.

The interior of the simplex represents *ternary mixtures*, where all three components are present in varying proportions. Specific points within the simplex are selected for experimentation, known as *design points*, and include pure blends, binary blends, and additional interior points strategically placed to capture interactions and trends. The placement of these points is shown in **Figure 1.6**. follows statistical algorithms designed to maximize the amount of information obtained from the least number of experiments, ensuring a comprehensive understanding of the system's behavior.

In practical terms, the simplex triangle provides a framework for understanding the relationship between component proportions and the responses of interest (Kasemiire et al., 2021a). Design points are often augmented with *axial points* or *center points*, depending on the complexity of the system and the chosen experimental design. These additional points provide more data for generating accurate predictive models, allowing to map response surfaces within the triangle.

Moreover, mixture designs are cost-effective and efficient. By strategically reducing the number of experimental runs while maximizing the information obtained, they minimize resource use without compromising the reliability or robustness of the results, which makes them an essential tool in research and formulation development for new products.

In the context of this study, the mixture DOE considers its application to develop a natural sports gel with rheological properties comparable to commercial formulations. The focus is on the optimizing of textural and viscous properties of the gel by adjusting the proportions of hydrocolloids, which act as thickeners. Through this approach, contour plots or response surfaces can then be overlaid on the simplex to illustrate how the responses, such as rheological parameters, vary across the mixture space, identify optimal combinations, and create predictive models that guided the formulation process (Kasemiire et al., 2021).

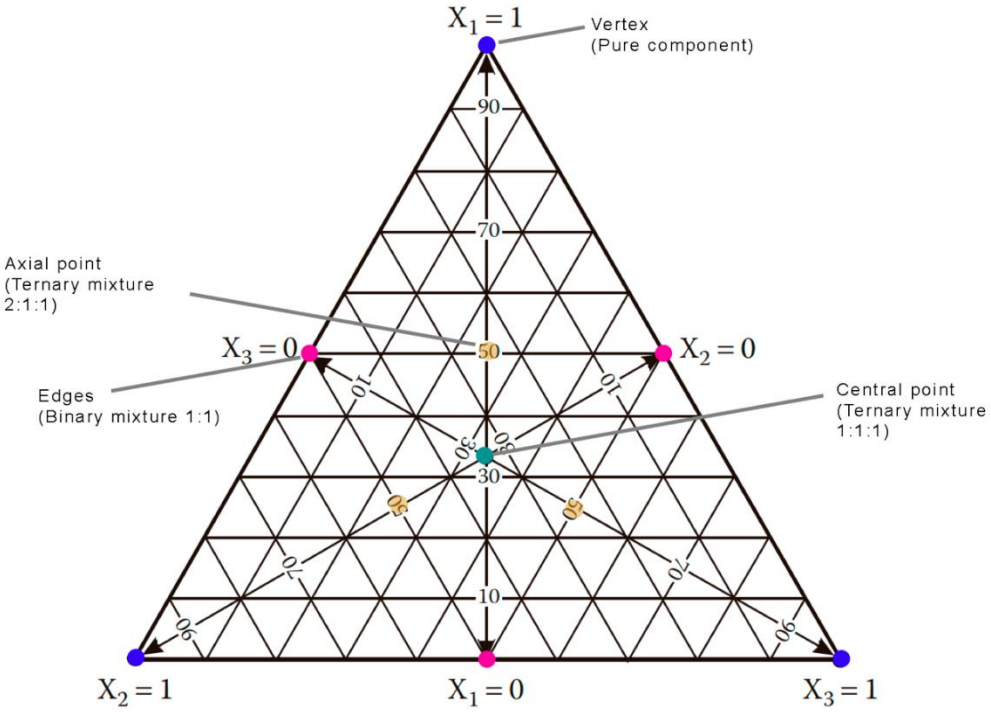

Figure 1. 6. Simplex triangle for a three-component mixture design, showing vertices (pure components), edges (binary mixtures), and interior points (ternary mixtures).

2. Objectives

General objectives:

To develop and optimize an innovative energy gel formulation utilizing mineral water, honey, aloe vera, and a blend of gelling agents, to achieve optimal rheological properties comparable to commercial products while providing an ideal sugar supply for the human body during sports activities.

Specific objectives:

- Modeling the rheological properties of thickeners and determine the best thickener type to apply in a new sports gel formulation

- Optimize the thickeners composition and concentration in the formulation

- Characterize the developed sports gel for chemical and physicochemical properties, namely, texture, rheological properties, moisture, pH, sugar content, antioxidant activity and aloin content.

3. Material and method

3.1. Chemicals, standards, and raw materials

All reagents used in the study were of analytical grade, except for those employed in chromatographic analyses, which were of chromatographic grade. These chromatographic reagents were sourced from European suppliers of high standards, from different companies. The standards used for the analyses included Aloin A and B (Extraynthese, Genay, France), glucose and fructose (for sugar analysis), and Trolox (for antioxidant analysis).

The raw materials utilized in the formulation of the sport gel were selected for their natural origin and local sourcing Aloe vera was freshly harvested from Bragança, Portugal, specifically for each analysis. Honey was obtained from the Montesinho region of Bragança and Vinhais, also in Portugal. The mineral water used was commercial water from Pedras Salgadas, sourced from Vila Real, Portugal. This water was degassed prior to use and was chosen for its mineral composition, which includes 2.3 mg/L of bicarbonate, 620 mg/L of sodium, 65 mg/L of calcium, 35 mg/L of magnesium, and 30 mg/L of other minerals.

Additionally, the following hydrocolloids were incorporated into the formulation for their gelling properties: Gellan gum (GG) was sourced from Thermo Scientific (Germany), while i-carrageenan (IC), guar gum (GU), locust bean gum (LB), and xanthan gum (XG) were purchased from Sigma-Aldrich (St. Louis, USA).

Figure 3 1. Materials sourced for the development of the energy gel.

3.2. Rheological properties analysis

The rheological properties in this document were assessed using two instruments: a texture analyzer and a viscometer, which together provided measurements of five key responses: firmness, consistency, cohesiveness, index of viscosity, and viscosity.

Texture analysis was conducted using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Surrey, United Kingdom). The instrument was calibrated with a 5 kg weight, and the height calibration was set to a return distance of 100 mm, with a return speed of 10 mm/sec and a contact force of 1 g. An extrusion rig and probe were used in the texture analyzer, following a predefined back extrusion method to obtain accurate textural measurements.

Viscosity was measured using a B-One Plus Viscometer (Lamy Rheology, Champagne-au-Mont-d'Or, France). The LV2 probe was selected for the measurements, with the device set at a speed of 60 rpm for 25 seconds. Temperature was carefully controlled at room temperature during all measurements. The solution was placed into a standardized container, ensuring that the probe did not come into contact with the sides of the beaker. Each measurement was carefully positioned at the same level to maintain consistency and precision across all analyses.

Figure 3.2. Apparatus used for rheological analysis: (left) TA.XT Plus Texture Analyzer for texture measurements and (right) B-One Plus Viscometer for viscosity measurements.

3.3 Sports gel exploratory market research and benchmarking

The exploratory market research for the sports gel product was conducted through a comprehensive review of existing literature and an online search of similar products available in the market. This process involved collecting a wide range of sports gel products to analyze their compositions, key characteristics, and ingredients. The goal was to identify trends, common formulations, and industry standards for sports gels, which could serve as a basis for the development of a new, innovative product. Various commercially available sports gels were evaluated, considering factors such as ingredient composition, nutritional values, packaging, and product claims.

Following the initial collection of data, a filtration process was applied to narrow down the products to those that are most closely aligned with the intended formulation and target market. Products that were frequently cited in the literature and online sources as popular or widely accepted were given higher consideration. This led to the identification of a product from the brand SIS (Science in Sport) as the final selection for comparison. The SIS sport gel was chosen due to its established status as a standard in the market, which made it a suitable benchmark for the development of the new product. Once selected, laboratorial evaluations of the product's rheological characteristics were conducted, allowing for direct comparison of all experiments against this standard product. By focusing on a well-established product, the research aimed to ensure that the final sport gel formulation would meet industry expectations and consumer demands.

Figure 3. 3. Selected commercial energy gel from the brand SIS (Science in Sport), chosen as a benchmark for product comparison in the exploratory market research.

3.4. Screening analysis of the gelling agents

The objective of the screening analysis was to assess the responses of various gelling agents and identify those that align with the key parameters observed in the standard commercial sport gel selected. These parameters —firmness, consistency, cohesiveness, index of viscosity, and viscosity— were measured using a viscometer and texture analyzer as mentioned before to provide a comprehensive profile of the jelly solutions properties. The gelling agents tested in this analysis were i-carrageenan (IC), gellan gum (GG), guar gum (GU), locust bean gum (LB), and xanthan gum (XG).

The analysis was conducted in a chronological and sequential manner, with a clear step-by-step approach aimed at systematically evaluating the behavior of the selected gelling agents. The first phase involved identifying the concentration ranges that have been previously reported for the gelling agents and will be used in this study. At this stage, the responses of the commercial control product were compared with those of the selected gelling agents, enabling the identification of concentration ranges that could yield desirable gel characteristics. The concentrations tested for each gelling agent were 0.5%, 1.25%, 2.25%, and 3%. This phase was critical in narrowing down the ranges within which each gelling agent exhibited the desired rheological properties, ensuring that the tested formulations closely resembled the characteristics of the selected standard sport gel.

As the initial results indicated that the gelling agents did not exhibit a linear increase in gel strength, the next step focused on understanding the more complex behavior of these gelling agents. To gain deeper insights, mathematical models were applied to describe the behavior of each gelling agent across the tested concentrations. Linear regressions and second-order polynomial regressions were used to analyze the relationship between concentration and gel strength for each gelling agent. This modeling approach allowed for a more accurate representation of the gelling agents performance across the entire concentration range, providing a clearer understanding of how each gum behaved within the four tested points. The use of these models was essential to fine-tune the correct range of concentrations for future experiments.

In the final phase of the screening analysis, a temporal analysis was conducted to assess the stability and evolution of the rheological properties of the gelling agents over time. This step was essential for understanding how the gels would behave during a quick storage, as their properties could change after preparation. Although the timespan is very narrow, the concept for this test was to observe the immediate behavior of gums at different concentrations, trying

to spot syneresis or precipitation phenomena. Additionally, the test aimed to identify any structural changes that might occur under significant temperature variations, a condition commonly experienced by commercial products. The gelling agents were prepared and measured at time point 0, followed by refrigeration at 4°C. Every 24 hours, the gels were removed from the refrigerator and allowed to return to room temperature for 4 hours before re-evaluating their properties, ensuring that they reached a stable ambient temperature (24 °C) before measurement. The rheological properties were recorded at four distinct time points: 0, 24, 48, and 72 hours. This time-based evaluation was crucial to monitor any changes or degradation in the gel properties, providing important information on the stability behavior of the gelling agents under refrigerated storage conditions.

3.5. Matrix for selection of gelling agents

A decision matrix was developed to select the three most suitable gelling agents for the following experimental mixture design phase, based on 4 desired characteristics: rheological properties, integrity properties, commercial price and visual appeal. The selection was based on data from the screening analysis, identifying gelling agents that achieved similar responses to the control product at the lowest concentrations. For each gelling agent, the two concentrations closest to the target response values were selected —one higher and one lower— to determine the best-performing concentrations at the lowest levels while maintaining desired gel characteristics.

Each gelling agent was ranked based on the selected concentration, with the lowest concentration achieving the target response receiving the best score. A scoring scale from 1 to 5 was used, where 1 was the best value and 5 the worst. The rheological properties were considering the most important of four properties that were taken into account within the matrix, thus, rheological properties were given a weight of 4 which is the multiplier of the score received, therefore, the worst gum with value of 5 (score) will be multiplied by 4 (weight), giving this gum the value of 20 for the rheological properties' characteristic.

Next, the integrity of the gel properties over time was considered, based on the temporal analysis monitoring rheological characteristics over four days. The rankings system was maintained, with the lowest score reflecting the best performance (least change). These rankings were multiplied by a weight of 3.

The commercial price of the gelling agents was also taken into account, with the lowest-priced gelling agent receiving the top score (1), multiplied by a weight of 2. Finally, the visual

appeal of the gel was evaluated, and given lower priority due to packaging considerations. This factor was assigned a weight of 1, and the most visually appealing gel was ranked with the lowest value.

The weighted scores from all factors were summed for each gelling agent, and the three gelling agents with the lowest total scores were selected for the next phase, the experimental mixture design to optimize the formulation from a rheological perspective. This matrix provided an objective, systematic approach to selecting the most promising gelling Agents for further development.

3.6. Mixture design for selection of best gums to apply in the final product

A D-optimal, simple lattice randomized mixture design without blocks, comprising 32 experiments (runs in triplicate plus centroids), was employed to evaluate the effects of three selected thickening agents (Xanthan, Gellan, and Guar gum) on the critical rheological properties of a new sports gel formulation. This experimental design approach was chosen due to its efficiency in identifying the complex relationships between the mixture components and the desired responses while minimizing the number of required experimental trials.

The proportions of the three gums were set as the independent variables, with each component allowed to vary between 0 and 1 (0% to 100%), subject to the constraint that the total sum of the proportions of the gums (A + B + C) must equal 1.0, and the remaining base formulation (D) was held constant at 99.0% using water for this experiment, but which will be replaced in the final formulation. The selected rheological responses included Firmness (g), Consistency (g/s), Cohesiveness (g), Index of Viscosity (g/s), and Viscosity (mPa/s). The experimental runs were conducted in a randomized order, and all samples were analyzed in triplicate to ensure the reliability of the data. In the following **Table 3.1**, the standard run and combinations if displayed:

Table 3. 1. Experimental runs

Run	A: Xanthane	B: Gellan	C: Guar	D: Base
1	0.75	0.25	0	99
2	1	0	0	99
3	0	1	0	99
4	0	1	0	99
5	0.50	0.50	0	99
6	0.50	0.50	0	99

7	0.17	0.67	0.15	99
8	0.75	0	0.25	99
9	0.33	0.33	0.35	99
10	0	0.50	0.50	99
11	0	0.50	0.50	99
12	0	0.50	0.50	99
13	0.49	0	0.51	99
14	0.49	0	0.51	99
15	0.18	0.15	0.68	99
16	0.00	0	1	99
17	0	0.50	0.50	99
18	0.33	0.33	0.35	99
19	0.17	0.67	0.15	99
20	0	0.50	0.50	99
21	1	0	0	99
22	0.50	0.50	0	99
23	0	1	0	99
24	0.50	0.50	0	99
25	0.75	0	0.25	99
26	0.49	0	0.51	99
27	0.49	0	0.51	99
28	0.18	0.15	0.68	99
29	0	0	1	99
30	0	0.50	0.50	99
31	0	1	0	99
32	0.75	0.25	0	99

The experimental data obtained (**Table S-1**) was then analyzed using the Design-Expert® software to develop mathematical models describing the relationships between the gum proportions and the measured rheological properties. Model fitting was performed using multiple regression analysis including always the hierarchical terms, and the adequacy of the models was assessed through various statistical parameters, including Model significance through ANOVA, Lack of fit, R^2, adjusted R^2, predicted R^2, and adequate precision.

The validated models were subsequently used to generate response surface plots and contour maps to visualize the effects of the gum combinations on the rheological responses. These graphical representations facilitated the identification of optimal gum ratios that could

achieve the desired product characteristics, as informed by the control sample and the project's specific requirements (**Figures S16-S20**).

Finally, a numerical optimization procedure was conducted to determine the specific gelling agent's formulation that best satisfied the target values from the commercial product and importance levels assigned to each rheological response. The selected optimal solution was then subjected to experimental validation to confirm the model's predictive accuracy using the software Stat-Ease (Minneapolis, Minnesota).

3.7. Preparation of the ingredients for sports gel formulation

3.7.1. Aloe vera gel extraction and aloin content

The aloe vera gel extraction was carried out following the procedure outlined by Khaldoune and his colleagues (2024) with modifications to improve the efficiency of the process. The extraction began with the careful removal of the upper and lower bases of the aloe vera leaves using a sharp knife, followed by cutting the spikes along the sides of the leaf. To eliminate aloin, the leaves were soaked in water for 20 minutes, during which the water turned yellow, indicating the leaching of aloin from the leaf. After this, the outer rinds were removed using a sharp knife, ensuring no scratches were made on the rind to prevent contamination of the gel with any remaining aloin in the rind's inner layer. The inner aloe vera gel was then extracted and transferred to a beaker.

It was subsequently ground for 20 minutes using an Ultra Turax (IKA T25, Staufen im Breisgau, Germany), with the grinder speed adjusted between 7000 and 12000 rpm to accommodate the consistency of the aloe vera gel. The grinding process aimed to achieve a homogeneous gel texture. After grinding, the aloe vera gel was filtered using a 125 mm paper filter to separate any remaining solid particles, which is taken afterward for the determination of aloin isomers.

Figure 3. 4. Aloe vera gel extraction process. The image shows the step of cutting the aloe vera leaves and some gel parts soaking them in water to remove aloin.

The aloin content in the grinded aloe vera gel was measured using High-Performance Liquid Chromatography (HPLC) coupled with diode array detection (DAD), as described in section 3.7.1.

3.7.2. Honey sugar content analysis and carbohydrates ratio adjustments

For sugar analysis, a solution was prepared by diluting 2.5g of honey in 20 mL of deionized water, and 12.5 ml of methanol, in a 50ml volumetric flask, afterwards an aliquot of the sample was taken and filtered in 0.2 μm nylon filters before injecting it into the chromatograph.

The chromatography system used consisted of a pump (Knauer, Smartline 1000 system), a degassing (Smartline 5000), an automatic sampler (AS-2057 Jasco), and an RI (refractive index) detector (Knauer Smartline 2300). Data analysis was performed with clarity 2.4 (chromeleon) software. For chromatographic separation, a 100-5 25 NH2 Eurospher column (4.6 x 250 mm, 5 mm, Knauer) was used operating at 30 ºC (Grace 7971 R oven). As a mobile phase, a mixture of acetonitrile/water 80:20 (v/v) was used, with a flow rate of 1.3 mL.min-1. The identification of sugars was obtained by comparing the retention times of the peaks of the samples with those of standards, namely fructose, glucose. For each of these standards, a calibration line was established by the internal standard method, using a range of concentrations according to the expected levels for each sugar.

Figure 3. 5. HPLC system used for analysis of fructose and glucose in honey.

To determine the required sugar content in the final formulation, a target value of 45 g sugars per 100 g of product was established, based on values reported in the literature (Kozlowski et al., 2021) The aim was to use honey as the primary source of sugars while

ensuring adherence to a glucose-to-fructose ratio of 1:0.8. Data from previous studies conducted within our research group, using the same honey source, indicated that 100 g of honey contains 42.2 g of fructose and 37.0 g of glucose.

From these values, the quantity of honey required to meet the fructose needs of the formulation was first calculated. To achieve 20 g of fructose (1:08 G/F ratio), approximately 47.4 g of honey was necessary. This amount of honey also provided 17.5 g of glucose. Since the total sugar content in the formulation was set at 45 g, with the glucose-to-fructose ratio fixed at 1:0.8, this meant the formulation required 25 g of glucose and 20 g of fructose.

While the fructose requirement was fully met with honey, an additional 7.46 g of glucose was necessary to meet the glucose requirement. To achieve this, maltodextrin was incorporated into the formulation, choosing this oligosaccharide in order to add also some slow released carbohydrates. Maltodextrin was added in the calculated amount of 7.46 g to ensure both the total sugar content, and the desired glucose-to-fructose ratio were satisfied.

3.7.3. Mineral water preparation

In the formulation of sports gels, the standard practice has been the use of water and synthetic minerals to provide a solvent and essential electrolyte to the body. The objective of this study was to eliminate synthetic minerals by utilizing the naturally occurring electrolytes present in mineralized water of natural origin. Since this water is carbonated, the first step involved complete degasification to prepare it as a solvent containing the appropriate electrolytes. Subsequently, the degassed mineral water was utilized for the preparation of the McIlvaine buffer. Instead of preparing separate solutions of disodium phosphate and citric acid and mixing them under the required conditions to form the buffer, the components of the buffer were used directly in the degassed mineral water, ensuring a final solution at pH 4. This pH 4 is not only optimal for preventing microbial growth but also aligns with the typical pH range of commercial sports gels.

To determine the required quantities of disodium phosphate and citric acid, stoichiometric calculations were performed. Using McIlvaine buffer tables for pH 4.0, the formulation required 7.71 mL of Na_2HPO_4 [0.2 M] and 12.29 mL of citric acid [0.1 M] for a final volume of 20 mL. For Na_2HPO_4, the necessary mass was calculated by multiplying its molecular weight (141.96 g/mol) by the desired concentration (0.2 M) and then by the required volume (7.71 mL), resulting in 218.9 mg. Similarly, for citric acid, its molecular weight (192.124 g/mol) was multiplied by the concentration (0.1 M) and volume (12.29 mL), yielding

236.12 mg. These components were weighed accurately and dissolved directly into 20 mL of degassed mineral water, achieving the required concentrations of 0.2 M for Na_2HPO_4 and 0.1 M for citric acid, achieving the resulting buffer with a final pH of 4. Therefore, the preparation was conducted by direct weighing of the solid components, bypassing the need for stock solutions. The 20 mL preparation was then scaled to larger quantities as needed through proportional calculations.

3.8. Development of the new sport gel formulation

The formulation of the new sport gel represents the culmination of prior experimental findings and serves as a critical step toward achieving the desired product characteristics. This section details the step-by-step preparation process of the final product, which will subsequently undergo testing to verify compliance with all expected properties.

The formulation began by adding 43.14 mL of pH-adjusted mineral water to a flask equipped with a high-speed digital overhead stirrer fitted with a stainless-steel cross-blade. Stirring is maintained at 1200 rpm at room temperature. Subsequently, as determined in the previous section, the calculated amount of honey (47.4 g) is incorporated as the primary sugar source. To complement the sugar profile, 7.46 g of maltodextrin was added. Stirring continues until the complete dissolution of the sugars is visually observed.

Once the sugar components are fully dissolved, liquid aloe vera is added at a concentration of 1% (w/v), and stirring is maintained until complete homogenization is achieved. Finally, the required amounts of gelling agents are incorporated to achieve the desired texture, based on the results of the mixture experimental design. Specifically, 0.75 g of xanthan gum and 0.25 g of gellan gum are added to the solution. The mixture is stirred for approximately 30 minutes to ensure all components are fully dissolved and homogenized.

The final formulation is then stored under refrigeration to prevent any alterations before characterization, ensuring accurate evaluation of its properties.

3.9. Characterization of the final product

3.9.1 Moisture and pH

The moisture content of the formulated product was determined using a moisture analyzer (ADAM 163, Kingston, UK). Prior to measurement, the device was powered on and allowed to stabilize for 5 minutes as per the manufacturer recommendations. The sample pan and the machine were cleaned thoroughly to ensure accurate results. The pan was placed inside

the analyzer, and the tare function was activated to zero the weight. Subsequently, 2 grams of the product were evenly distributed across the pan to facilitate uniform drying. Once the lid was closed, the analyzer continuously monitored the sample's weight during the drying process, automatically halting once a stable weight was achieved. The moisture content was then displayed on the screen and recorded.

The pH of the samples was measured using a calibrated digital pH meter (HI 99161, Hanna Instruments, Woonsocket, RI, USA). The device was calibrated using standard buffer solutions with pH values of 4.01, 7.01, and 10.01 to ensure accuracy. The electrode was rinsed with distilled water and gently dried with a lint-free tissue between measurements to prevent cross-contamination.

For the analysis of sugars in the final product, the same methodology mention in the section 3.5.2. for sugar detection in honey was used.

3.9.2. Aloin analysis

Aloin extraction was conducted according to (Sánchez-Machado et al., 2017). Two g of each sample was dissolved in PBS pH 3 (adjusted with hydrochloric acid 0.3 M), and the volume was made up to 10 mL. The samples were filtered through 0.22 μm pore size nylon membrane filters (GVS Filter technologies, Indianapolis, IN, USA) and injected into the UHPLC.

Chromatographic analysis was performed in a Dionex Ultimate 3000 UHPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector (DAD), a quaternary pump, an auto-sampler (kept at 10 °C), a degasser and an automated thermostatted column compartment (Figure 3.6). Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20–25% B (10 min), 25–35% B (10 min), 35–50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 mL/min. Detection was carried out in the DAD using 330 nm as the preferred wavelength. The identification of aloin was performed using standard compounds, by comparing their retention times and UV–Vis spectra. For quantitative analysis, an aloin calibration curve was constructed based on the UV signal at 330 nm ($y=8892x-5756.1$, $R^2=0.9975$).

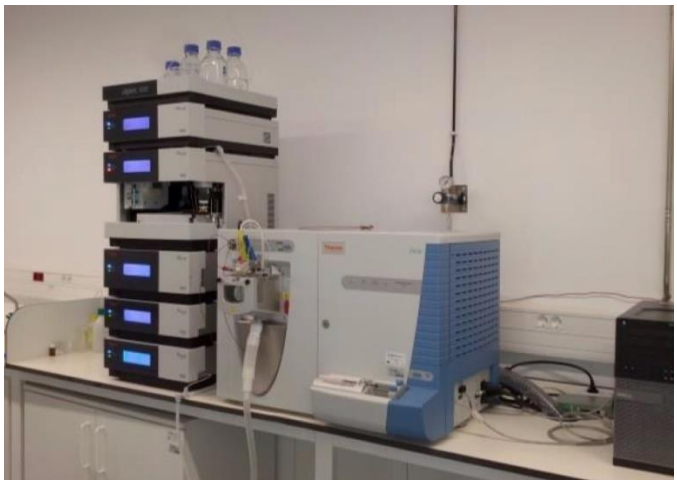

Figure 3 6. HPLC system used for analysis of aloin isomers A and B in *Aloe vera* and in the final product.

3.9.3. Antioxidant activity through DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The preparation of the methodology was according to Brand-Williams, (1995) with some modifications. Briefly, 0.5 L solution of DPPH at a concentration of 6×10^{-5}M, was prepared for the test, along with a calibration curve, from a stock solution of Trolox [0.5 mg/ml]. This stock solution was then diluted in half to get a concentration of 0.25 mg/ml. The dilution process was repeated 12 times until reaching a final concentration of 0.0005 mg/ml. In a 96-well plate, 30 µL of each Trolox dilution or sample were added to individual wells, replicated 3 times to ensure accuracy. 30 µL of methanol was employed as a blank (negative control). Subsequently, 270 µL of DPPH solution was added to each well, the plate was then allowed to rest for 60 mins in a dark spot to minimize light exposure, facilitating the reaction between DPPH and the antioxidants in the samples. After the incubation, the plate was placed in the microplate reader (SPECTROstar nano microplate, Ortenberg, Germany) to measure the absorbance values at a wavelength of 517 nm. The absorbance recorded from the DPPH assays were transformed according to Eq 1, in order to express the final values as Trolox equivalent by using calibration curve.

$$\%DPPH\ scavenge = [\frac{(Abs\ sam - AbsNC)}{(\text{Abs PC} - \text{Abs NC})}] \times 100 \quad \textbf{(Eq 1)}$$

To interpolate the data, a 4 parameters logistic regression was model with the Trolox values, and after transforming the absorbance values of samples (Eq. 1), the % of DPPH scavenge values were interpolated to obtain the Trolox equivalents.

3.10. Data treatment and statistical treatments

All experimental data obtained throughout the study were converted into tabular data files. The data were primarily processed and analyzed using R Studio, except for a longitudinal analysis that was conducted in Python. The excel files containing the tabular data, the scripts, as well as all the packages utilized in the analyses, are included in the repository https://github.com/Bruno-melgar/SportGel to ensure clarity in the execution of the data treatments and to enable replicability.

The thesis employs multiple data transformation techniques such standardization when necessary, including the generation of exploratory data analyses to understand and visualize the data. Furthermore, a variety of regression models were utilized, ranging from linear regressions in chromatographic analyses to second-order polynomial regressions for understanding the behavior of the gums, four-parameter logarithmic regressions in dose-response analyses such as DPPH, and response surface methodologies in the experimental design.

Once the visualizations were completed, and the final data were synthesized, the corresponding statistical tests were executed, always verifying the statistical assumptions to determine the need for parametric or non-parametric approaches. In all cases, the decision was made to analyze more than two populations, and in the longitudinal analysis, non-parametric comparison and post-hoc tests were performed to identify the exact locations of significant differences.

4. Results and discussion

4.1. Rheological properties of control sports gel

The rheological properties of the selected control sports gel (SIS brand) were evaluated as a benchmark for the development of the natural sports gel formulation. Measurements were conducted to assess the key textural and viscosity-related parameters, firmness, consistency, cohesiveness, index of viscosity, and viscosity. These metrics were analyzed using a TA.XT Plus Texture Analyzer and a B-One Plus Viscometer follow the previous section's methodologies.

The measurements shown in **Table 4.1** display the texture parameters of the commercial energy gel used through the experimental process. These baselines are essential for assessing the effects of future development for sport gel formulation. By comparing experimental results to these reference values, we can monitor gel texture changes across different experimental conditions, ensuring that any alterations improve or maintain the product performance attributes.

Table 4. 1. Summary of final texture parameters for SIS sports gel

	SIS sports gel
Firmness (g)	26.50±0.49
Consistency (g/s)	247.00±5.44
Cohesiveness (g)	-19.60±1.48
Index of viscosity(g/s)	-79.40±8.06
Viscosity (mPa/s)	133.70±14.84

Consequently, the control gel rheological properties provide a baseline for understanding textural and flow behaviors desirable in sports gels, guiding the optimization of the natural formulation to achieve comparable or improved performance characteristics.

4.2. Screening analysis of gums

4.2.1. Rheological properties of gelling agents

An initial investigation of the gelling agents was conducted by measuring the selected rheological parameters (firmness, consistency, cohesiveness, index of viscosity and viscosity) of gel solutions produced at the concentrations of 0.5, 1.25, 2.25 and 3%, and comparing the

obtained results with the benchmark energy gel. In order to simplify the presentation of results, some steps were followed, first, firmness was selected as the representative parameter for this analysis, while the other responses, including consistency, cohesiveness, index of viscosity, and viscosity, were similarly analyzed but are presented in the supplementary material for completeness.

Accordingly, the **Figure 4.1** display a dashboard composed of three section that described the process for understanding the firmness response, where the panel of **Figure 4.1a.** illustrates the values (in grams) of the gelling agents at four concentrations tested. The green dashed line represents the firmness value of the control commercial gel, serving as the reference point. The results show significant variability, with some gelling agents exhibiting a steep increase in firmness at higher concentrations (i.e. guar gum), while others display minimal changes across the tested range, or even locust bean, which was unable to reach control values in any of the concentrations tested, exhibiting the importance of concentration in modulating gel firmness and its non-linear behavior. This panel also highlights gums by different colors, and at the same time only colored the two bars close to the reference point without considering magnitudes.

To facilitate cross-comparison and better contextualize the results, the data were transformed into a percentage of similarity relative to the control gel's firmness (**Figure 4.1b**). This transformation was applied using the formula:

$$\text{Percentage of Similarity} = \left(\frac{\text{Original Response}}{\text{Control Reference Value}}\right) \times 100 \qquad \text{Eq 4.1}$$

The density plot in **Figure 4.1b** illustrates the distribution of *percentages of similarity* across all responses, gelling agents and concentrations, revealing that most data points fall between 0% and 200%, this magnitude at the same time was considered reasonable to the screening process. Consequently, the analysis was refined to focus on this interval, and the results were re-plotted accordingly (**Figure 4.1c**), presenting now the control value (target) as 100%, while extreme negative or positive values could only reach 0% or 200%, respectively.

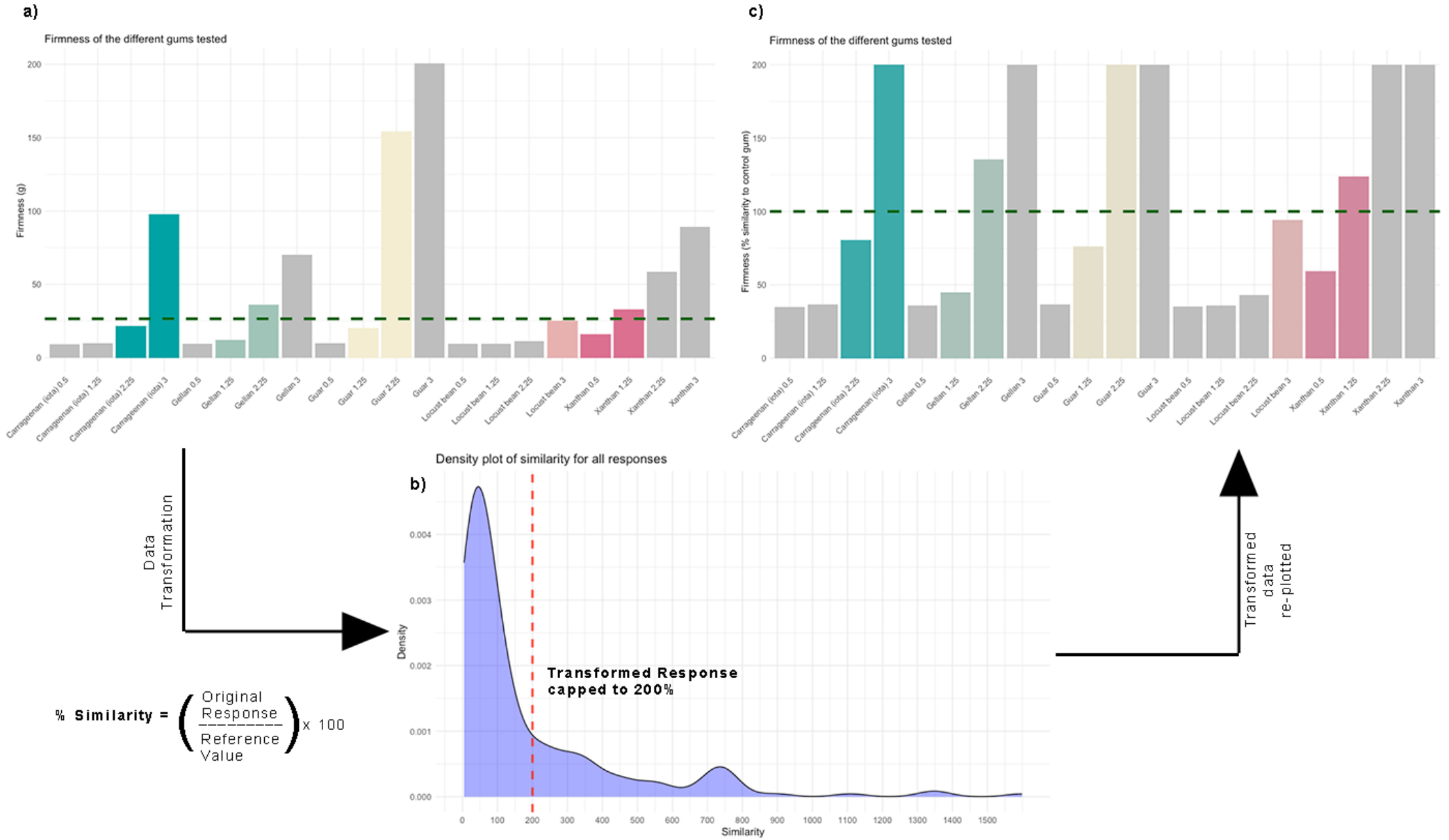

Figure 4.1. Firmness of gelling agents at various concentrations. (a) Firmness values (g) with the control gel as a reference (green dashed line). (b) Density plot of percentage similarity data capped at 200% (red dashed line). (c) Transformed firmness data as percentage similarity relative to the control, enabling standardized comparisons.

This transformation and visualization approach provides a standardized metric for evaluating the performance of different gelling agents relative to the commercial benchmark. And also allows for a more intuitive interpretation of the data and facilitates the identification of promising candidates for subsequent formulation stages.

Therefore, the heatmap presented in **Figure 4.2** summarizes the rheological behavior of the gelling agents across all tested concentrations for the five key rheological responses. The color gradient indicates the percentage similarity, where darker shades represent higher similarity to the reference gel. Notably, responses exceeding 200% similarity are trimmed to ensure visual clarity and maintain focus on values within the practical formulation range. This visualization provides an integrated overview of how each gum performs relative to the control, facilitating a direct comparison across multiple dimensions of gel properties.

The plot highlights that at higher concentrations (2.25–3.0%), several gums, such as Guar and Xanthan, exhibit strong similarities (>200%) to the commercial benchmark across multiple responses, particularly in cohesiveness, firmness, and consistency, which points out to the use of lower concentrations. In contrast, iota Carrageenan demonstrates a less consistent performance, with high percentages of similarity in certain parameters (e.g., viscosity) but relatively lower changes between 0.5 and 1.25% concentrations, suggesting to avoid concentrations lower than 1%. Similarly, Gellan shows moderate performance in most responses but lacks the high values observed in Guar or Xanthan.

Locust bean gum emerges as a low strength agent, achieving low percentages of similarity in key parameters across all concentration levels. Its performance profile suggests as iota caragenin, to avoid working concentrations lower than 2.25% where start showing some changes, although, for this specific comparison the gum was not able to reach control values in any response, except for viscosity.

The impact of concentration is evident across all gums, with higher concentrations generally leading to improved similarity percentages. However, the diminishing returns observed in certain responses at the highest concentrations indicate that optimal levels must be carefully determined to avoid excessive thickening or undesirable texture changes.

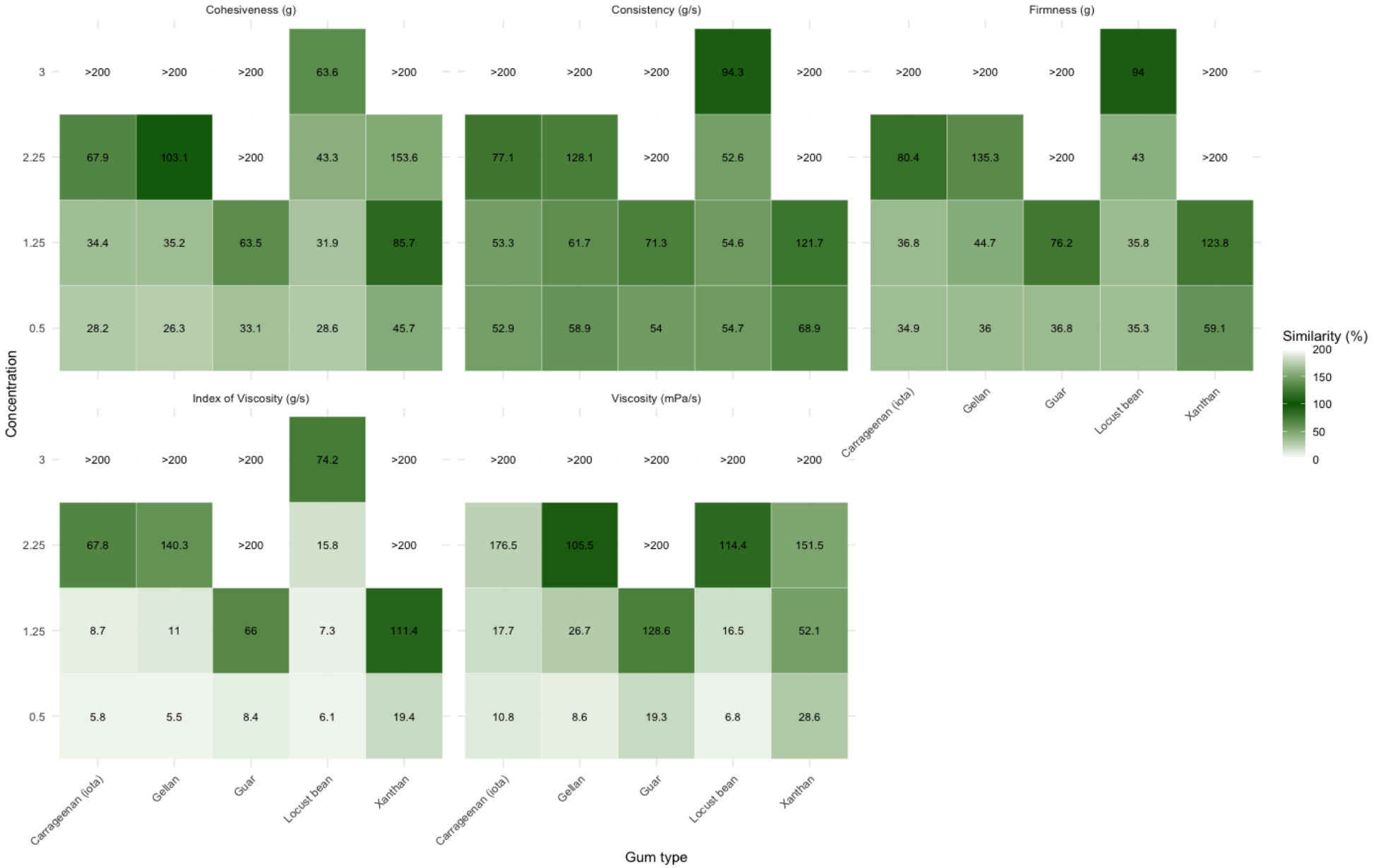

Figure 4. 2. Summary of properties of gum tested at different concentrations (0.5-3%). Darker green indicates higher similarity.

These findings underscore the importance of selecting gums and concentration levels based on the desired balance of rheological properties for the final product and highlighting the critical influence of both gum type and concentration on gel performance. Additionally, the results reveal that the behavior of the gums is not linear, as their performance across rheological properties does not consistently scale with concentration.

Although a mixture experimental design will be conducted later, the purpose of the regressions is to refine the understanding of each gum behavior at the concentrations required to achieve the characteristics of the control. As shown in **Table 4.2**, the overall data revealed distinct trends for each gum, with second-order polynomial regressions providing a better fit in most cases. Xanthan gum displayed predominantly linear behavior, indicating a simpler relationship between concentration and responses. However, for gellan gum, locust bean gum, guar gum, and carrageenan, the viscosity response showed clear non-linear trends, emphasizing their more complex gelation mechanisms.

Alongside the equations, **Figure 4.3** presents a visual representation of the regression analysis for all gums, focusing on viscosity as the displayed response, while other responses are provided in the supplementary material. Viscosity was selected due to measurement challenges encountered at higher values, as the equipment used in the lab could not measure viscosities exceeding 1000 mPa/s due to spindle limitations. Values reported as "too high" by the equipment were standardized to 1000 mPa/s, the maximum measurable value, to facilitate regression analysis.

Despite this adjustment, the regression models captured the distinct patterns of each gum. For example, carrageenan guar and locust bean gums exhibited steep increases in viscosity at higher concentrations, suggesting strong swift between concentrations 2.25% and 3%, while gellan and xanthan gums showed the most uniform increase for polynomic behavior. Viscosity strength goes from higher to lower starting with guar *i*-carrageenan and locust bean gums (1000 mPa/s), followed by xanthan and gellan gum (300 mPa/s). These variations underscore the importance of matching the gum type and concentration to the desired viscosity profile fo the control (133.70 ± 14.84 mPa/s).

Table 4. 2. Compilation of regression equations for the gums tested and coefficients of determination within brackets

	I-carrageenan	Gellan gum	Guar gum	Locust bean gum	Xanthan gum
Firmness	$y = 51.55 - 91.10x + 36.86x^2$ ***(R^2=0.88)***	$y = 15.28 - 16.55x + 9.65x^2$ ***(R^2=0.97)***	$y = -10.13 + 9.62x + 31.29x^2$ ***(R^2=0.91)***	$y = 14.79 - 12.86x + 6.10x^2$ ***(R^2=0.96)***	$y = - 0.63 + 26.1x$ ***(R^2=0.97)***
Consistency	$y = 430.06 - 608.94x + 243.05x^2$ ***(R^2=0.88)***	$y = 215.64 - 165.89x + 77.47x^2$ ***(R^2=0.93)***	$y = -36.55 + 135.41x + 185.04x^2$ ***(R^2=0.91)***	$y = 185.98 - 92.63x + 39.80x^2$ ***(R^2=0.86)***	$y = 39.8 + 194x$ ***(R^2=0.98)***
Cohesiveness	$y = -20.57 + 34.11x - 13.49x^2$ ***(R^2=0.91)***	$y = -10.97 + 13.29x - 6.22x^2$ ***(R^2=0.91)***	$y = 16.95 - 25.05x - 13.38x^2$ ***(R^2=0.89)***	$y = - 9.41 + 8.23x - 3.46x^2$ ***(R^2=0.78)***	$y = -1.38 - 13.1x$ ***(R^2=0.97)***
Index of viscosity	$y = -114.17 + 234.33x - 92.59x^2$ ***(R^2=0.9)***	$y = -65.83 + 138.36x - 60.10x^2$ ***(R^2=0.94)***	$y = 140.30 - 127.78x - 134.62x^2$ ***(R^2=0.91)***	$y = - 50.64 + 97.30x - 38.52x^2$ ***(R^2=0.81)***	$y = 67.8 - 134x$ ***(R^2=0.99)***
Viscosity	$y = 368.33 - 779.70x + 324.52x^2$ ***(R^2=0.94)***	$y = 37.13 - 79.68x + 52.59x^2$ ***(R^2=0.98)***	$y = -405.96 + 830.45x - 115.23x^2$ ***(R^2=0.93)***	$y = 213.22 - 451.14x + 208.13x^2$ ***(R^2=0.83)***	$y = 42 - 23.52x + 33.34x^2$ ***(R^2=0.95)***

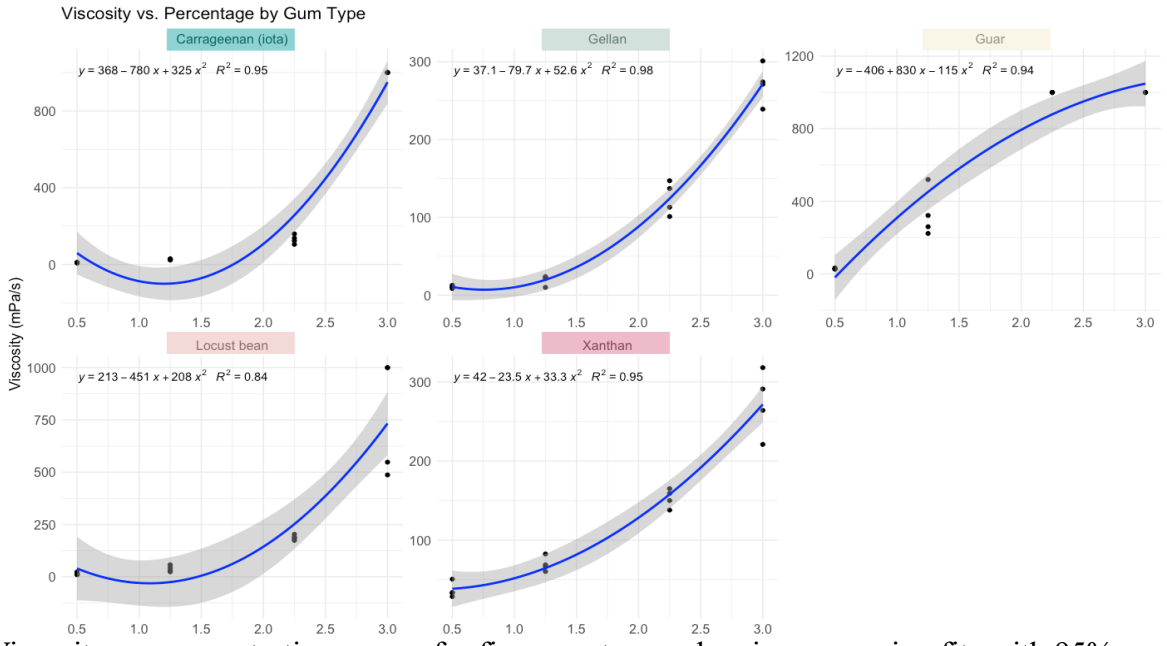

Figure 4. 3. Viscosity vs. concentration curves for five gum types, showing regression fits with 95% confidence intervals.

While this analysis narrows down the understanding of gum behavior, further exploration through mixture design experiments will be essential to optimize the concentration ranges and fully align the formulations with the control product properties. Supplementary material includes the complete regression visualizations with equations and R^2 coefficients for all rheological responses and gelling agents.

The final phase of the screening analysis involved a temporal evaluation to assess the stability and evolution of the rheological properties of the gelling agents over time. This analysis was crucial to understanding how the gels behave during short-term storage, as their properties can change after preparation. Although the timespan was narrow, this test aimed to observe immediate behaviors of gums at various concentrations, particularly focusing on syneresis or precipitation phenomena. Additionally, it sought to identify structural changes under significant temperature variations, a condition often encountered by commercial products.

In **Figure 4.4** the consistency response over time is presented as an example of the rheological behavior with T0 = 0h measurement made directly after making the gel solution, T1 = 24h, T2 = 48h, and T3 = 72h. Consistency was chosen here to present a different response from those previously discussed, providing a broader perspective on the screening analysis. The trends depicted in the figure showcase the varying stability of the gums and their concentrations, as visualized through the parallel plot. The first dimension on the left distinguishes the gums (represented by different colors) and their respective concentrations, while the subsequent dimensions to the right illustrate the consistency values recorded at the different time points.

The parallel plot reveals variations in consistency (g/s) among gums and concentrations over time. Carrageenan shows the highest initial consistency at 3% concentration (853.85 g/s), followed by a steady but small decline, particularly at later times, while gellan gum exhibits moderate consistency, with values peaking at 3% (592.40 g/s). Guar gum displays the highest overall consistency at 3% concentration (1972.25 g/s at Time 1), though it also decreases significantly over time. Locust bean gum demonstrates relatively low and stable consistency values across concentrations, while xanthan gum maintains high consistency with a more gradual decline compared to other gums. These trends highlight the influence of gum type and concentration on consistency stability, with xanthan gum offering better performance over time, particularly at higher concentrations.

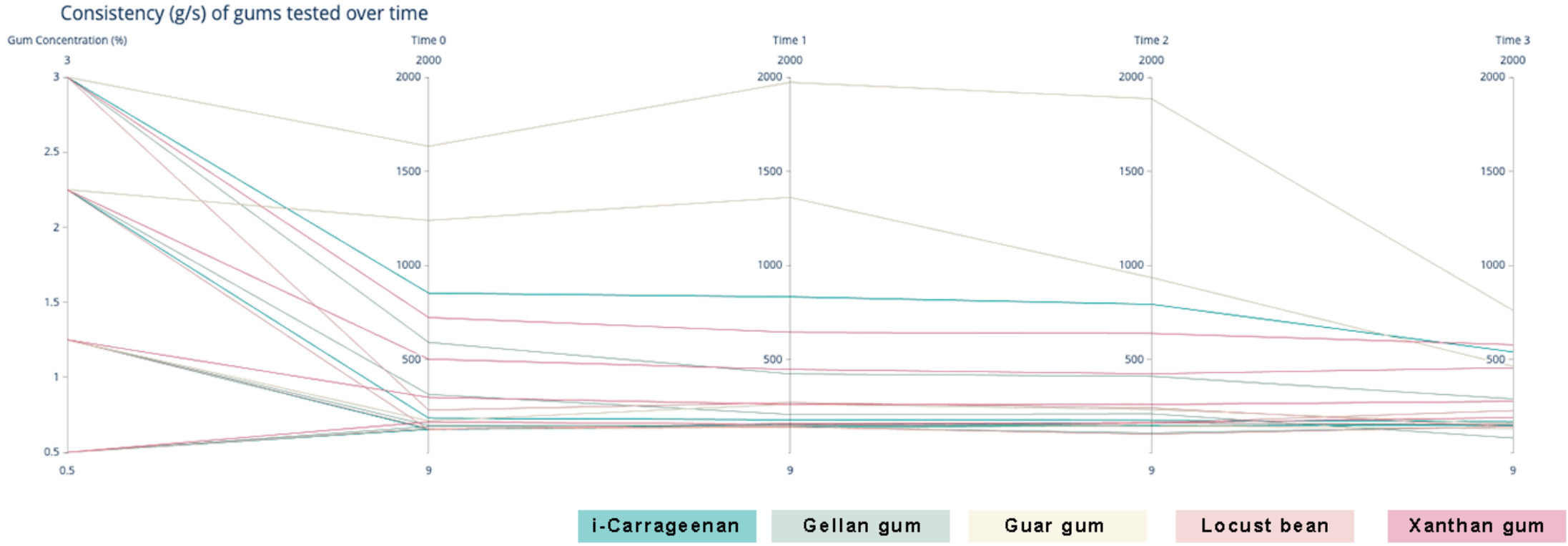

Figure 4. 4. Behavior of gums in the consistency response along the time test. T0 = 0h, T1= 24h, T2 = 48h, and T3 = 72h.

This time-based evaluation provides valuable insights into the stability behavior of the gelling agents under refrigerated storage or frequent temperature changes, helping to identify formulations that maintain their desired properties throughout the storage period. Therefore, in order to provide strong evidence, the corresponding statistical analyses was performed. The analysis revealed significant patterns and interactions, as evidenced by the Kruskal-Wallis's test results shown in **Table 4.3**. Due to the limited number of replicates, non-parametric statistical analysis was employed to ensure robust conclusions.

Table 4. 3. *P*-values from Kruskal-Wallis (not normality found) analysis on texture attributes

Kruskal-Wallis					
			***p*-values**		
Factor	**Firmness**	**Consistency**	**Cohesiveness**	**Index of Viscosity**	**Viscosity**
Gum	$1.94x10^{-10}$	$7.44x10^{-11}$	$8.35x10^{-12}$	$7.23x10^{-13}$	$4.71x10^{-06}$
Concentration (%)	$1.00x10^{-34}$	$6.66x10^{-26}$	$1.35x10^{-29}$	$4.09\ x10^{-30}$	$1.83\ x10^{-37}$
Time (days)	0.954	0.706	0.089	0.143	0.630
Gum x Concentration (%)	$9.8\ x10^{-38}$	$1.0\ x10^{-30}$	$8.3x10^{-35}$	$8.1\ x10^{-36}$	$7.8\ x10^{-36}$
Gum x Time (days)	$2.8\ x10^{-05}$	$4.8\ x10^{-06}$	$3.1\ x10^{-07}$	$9.1\ x10^{-08}$	$2.0\ x10^{-02}$
Concentration (%) x Time (days)	$1.0\ x10^{-26}$	$4.8\ x10^{-20}$	$2.0\ x10^{-23}$	$1.2\ x10^{-23}$	$1.3\ x10^{-29}$

According to Table 4.3, the results from the Kruskal-Wallis test demonstrate that both the type of gum and concentration had highly significant effects on all rheological responses (Firmness, Consistency, Cohesiveness, Index of Viscosity, and Viscosity). This non-parametric method was employed because the data violated the normality assumption required for parametric ANOVA, providing robust analysis despite non-normal distributions or ordinal characteristics in the rheological measurements. The extremely low p-values for concentration suggest that this factor has the strongest influence on the rheological properties, indicating that the textural characteristics of the system are highly dependent on the hydrocolloid concentration regardless of the type of gum used.

Interestingly, time as an independent factor did not show significant effects (lower $p = 0.089$

for Cohesiveness) on any of the rheological parameters, suggesting initial stability in the gum systems over the four days. Some values within the time analysis had changed as seen in **Figure 4.4** and **Supplementary Material S5-8**, but the majority of the observations had the same trend, therefore, considering all observations at once, the ones that change are the minority. However, the significant interactions between time and other factors reveal more complex behavior. The interaction between gum type and time indicates that different hydrocolloids exhibit distinct stability patterns over the observation period, although with a relatively modest level of significance compared to other effects.

Of particular importance is the strong interaction between gum type and concentration (lower p = 9.8E-38 for Firmness), which suggests that different hydrocolloids respond differently to concentration changes as expected and shown previously in the results. This interaction is crucial for formulation optimization as it indicates that each gum has its optimal concentration range for achieving desired rheological properties. Similarly, the significant interaction between concentration and time reveals that the stability of the rheological properties over time is concentration-dependent, especially at higher concentrations and for specific gums, a critical consideration for product shelf-life and stability.

The similar trends of p-values across all rheological responses suggest that these parameters are closely interrelated and respond similarly to changes in the experimental factors. This finding is particularly valuable for formulation development as it indicates that optimizing one rheological parameter is likely to have predictable effects on others.

4.3. Selection of the best gelling agents for experimental design

To determine the most suitable gelling agents for the subsequent experimental mixture design phase, a decision matrix was developed based on four key criteria: rheological properties, integrity over time, commercial price, and visual appeal. The evaluation prioritized achieving the closest performance to the control product at the lowest gum concentrations. Each gum was scored across the criteria without any ties, and weighted scores were calculated to ensure systematic selection.

The rheological properties were assigned the highest weight (4), as they are the most critical factor. Guar gum achieved the best score due to its ability to closely replicate the control's rheological responses at the lowest concentrations. Conversely, locust bean gum received the worst score in this category due to its higher concentration requirements to reach the target responses. Although i-carrageenan showed lower average rheological response values

than gellan gum, it received a worse score because it forms overly hard gels, similar to gelatin, which is not desirable for this application.

Table 4. 4. Decision matrix for selecting top 3 gums for experimental design (Lower values means better gum).

Gum	Visual Rank (Weight=1)	Price per kg (€)	Rank price (Weight=2)	Properties integrity Rank (Weight=3)	Firmness (% of gum needed)	Consistency (% of gum needed)	Cohesiveness (% of gum needed)	Index of Viscosity (% of gum needed)	Viscosity (% of gum needed)	Average (% of gum needed)	Rheological properties (Weight=4)	Sum	Overall Rank
Carageenan	1	23.20	6	12	2.2	2.2	2.5	2.4	2.1	2.2	16	35	4
Gellan	2	76.00	10	6	2.2	2.3	2.7	2.4	2.3	2.4	12	30	3
Guar	4	10.00	2	9	0.9	0.9	1.0	0.9	0.7	0.9	4	19	2
Locust bean	5	74.66	8	15	2.8	2.9	3.0	2.8	2.0	2.7	20	48	5
Xanthan	3	12.00	4	3	1.0	1.1	1.4	1.1	2.1	1.3	8	18	1

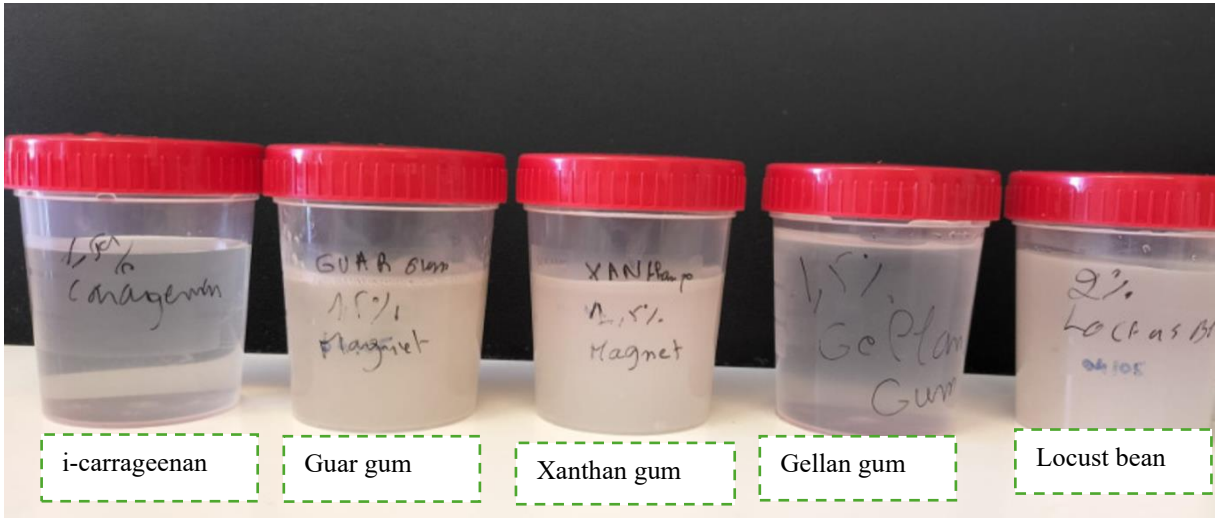

Figure 4.5. Visual appearance of gels produced with different gums.

The integrity of the gel properties over time was the second most weighted factor (3), assessed by monitoring rheological changes across four days. Xanthan gum performed best, showing minimal variation over time as mentioned in the last section. Locust bean gum received the worst score, as the gum precipitated during storage, significantly affecting consistency and appearance. *i*-Carrageenan ranked second worst in this category due to its tendency to form rigid gels over time, which reduces its suitability.

Commercial price was weighted less heavily (2), favoring economically viable options. Guar gum ranked best due to its low cost (€10 per kilogram), followed by xanthan gum. Gellan gum and locust bean gum were penalized for their higher costs. Finally, visual appeal, given the lowest weight (1), evaluated the clarity and texture of the gels. *i*-Carrageenan ranked highest for its appealing appearance, while locust bean gum scored lowest due to its unattractive precipitation and cloudy gel appearance, the low weight is due to the fact that the final product usually is in packed avoiding the visual aspect and the usual way to consume the product is direct from the package to the mouth, which avoids the visual appeal.

The total weighted scores were summed to rank the gelling agents. Xanthan gum emerged as the best candidate (total score of 14), followed by guar gum (23) and gellan gum (30). These three gelling agents demonstrated optimal performance across the evaluated criteria and were selected for the experimental mixture design phase to refine and optimize the sports gel formulation. *i*-Carrageenan and locust bean gum were excluded due to their less favorable performance, particularly in long-term stability and rheological properties.

4.4. Experimental design

Once the top three gelling agents were selected and their consistency with the control product's characteristics across the identified ranges was confirmed, experimental runs were prepared using the formulation intended for the final product. The gelling agents collectively accounted for 1% of the formulation, with the remaining 99% comprising the other ingredients described in the methodology. The decision to use 1% was based on the additional rheological contributions expected from honey and the other components in achieving the final product's desired characteristics.

4.4.1. Mixture design fitting

Therefore, after the selection, preparation of final formulation and execution of the experimental design described in the methodology section, the results were collected and presented in the **Supplementary Table S.1**, and after processing, the mixture design experiment

has proven successful in modeling the rheological properties of the system under study. This experimental approach allowed for the precise and reliable identification of the relationships between the proportions of the different gums (Xanthan, Gellan, and Guar) and the selected rheological responses (Firmness, Consistency, Cohesiveness, Index of Viscosity, and Viscosity).

The effectiveness of the design is evident in the ability of the developed models to accurately predict the rheological responses, as demonstrated by the high R^2 and adjusted R^2 values for most responses, indicating an excellent fit between observed and predicted values. Additionally, the high predicted R^2 values for nearly all responses confirm that the developed models have strong predictive power for new data. The adequate precision values, which measure the signal-to-noise ratio, also exceed the recommended threshold of 4 for all responses, further underscoring the reliability of the models.

The upper part of **Table 4.5** summarizes the key model fit statistics for each response. The R^2 values, which approach 1 for most responses—particularly for "Firmness," "Cohesiveness," "Index of Viscosity," and "Viscosity"—indicate optimal model fit. The adequate precision values above 30 for several responses, particularly for "Index of Viscosity," suggest a favorable signal-to-noise ratio, validating the model's ability to distinguish between significant effects and random variability. Furthermore, the low lack-of-fit values and the highly significant p-values for each model (all below 0.0001) confirm the statistical significance of the models.

While the lower part of **Table 4.5** presents the estimated coefficients for each gum's contribution to the various rheological properties, allowing for the assessment of each gum's primary effect and their interactions. These coefficients reveal the specific contribution of each gum to the system's rheological properties. For instance, Xanthan gum has the highest positive effect on Consistency and Viscosity, while showing a negative effect on Cohesiveness, suggesting that adjusting gum proportions can effectively tailor these properties.

The contour plots in **Figure 4.6 (a-e)** provide valuable insights into how the mixture components affect each rheological parameter across the experimental space. These visualizations reveal distinct patterns and optimal regions for each response variable.

Table 4. 5. Model fit statistics.

Model fit statistics						
Response	R^2	**Adjusted** R^2	**Predicted** R^2	**Adeq Precision**	**Model (*p*-value)**	**Lack of Fit**
Firmness	0.96	0.96	0.95	45.44	< 0.0001	0.464
Consistency	0.77	0.74	0.70	16.34	< 0.0001	0.613
Cohesiveness	0.94	0.93	0.91	34.11	< 0.0001	0.432
Index of viscosity	0.99	0.99	0.98	80.20	< 0.0001	0.412
Viscosity	0.95	0.95	0.94	33.78	< 0.0001	0.297

Gums Model coefficients estimates						
	A: Xanthan	**B: Gellan**	**C: Guar**	**AB**	**AC**	**BC**
Firmness	38.08	13.09	13.62	-26.47	-	-
Consistency	329.74	217.59	211.24	-241.73	-88.51	-
Cohesiveness	-13.61	-4.76	-5.78	8.15	5.03	-
Index of viscosity	-56.33	-3.75	-5.63	77.51	54.38	-
Viscosity	138.93	12.89	14.72	-124.84	48.27	-
Equation example:	Firmness = 38.08*Xanthan + 13.09*Gellan + 13.62*Guar - 26.47*Xanthane*Gellan					

Firmness (a) show a clear gradient pattern, with higher values (red region) concentrated in the Xanthan-rich region of the mixture space, while lower values (blue region) dominate in the Gellan-Guar axis. This pattern indicates that Xanthan gum is the primary contributor to product firmness, with values ranging approximately from 10 to 40 g. The interaction between Gellan and Guar gums produces relatively similar firmness values, suggesting interchangeability between these components when firmness is the primary concern.

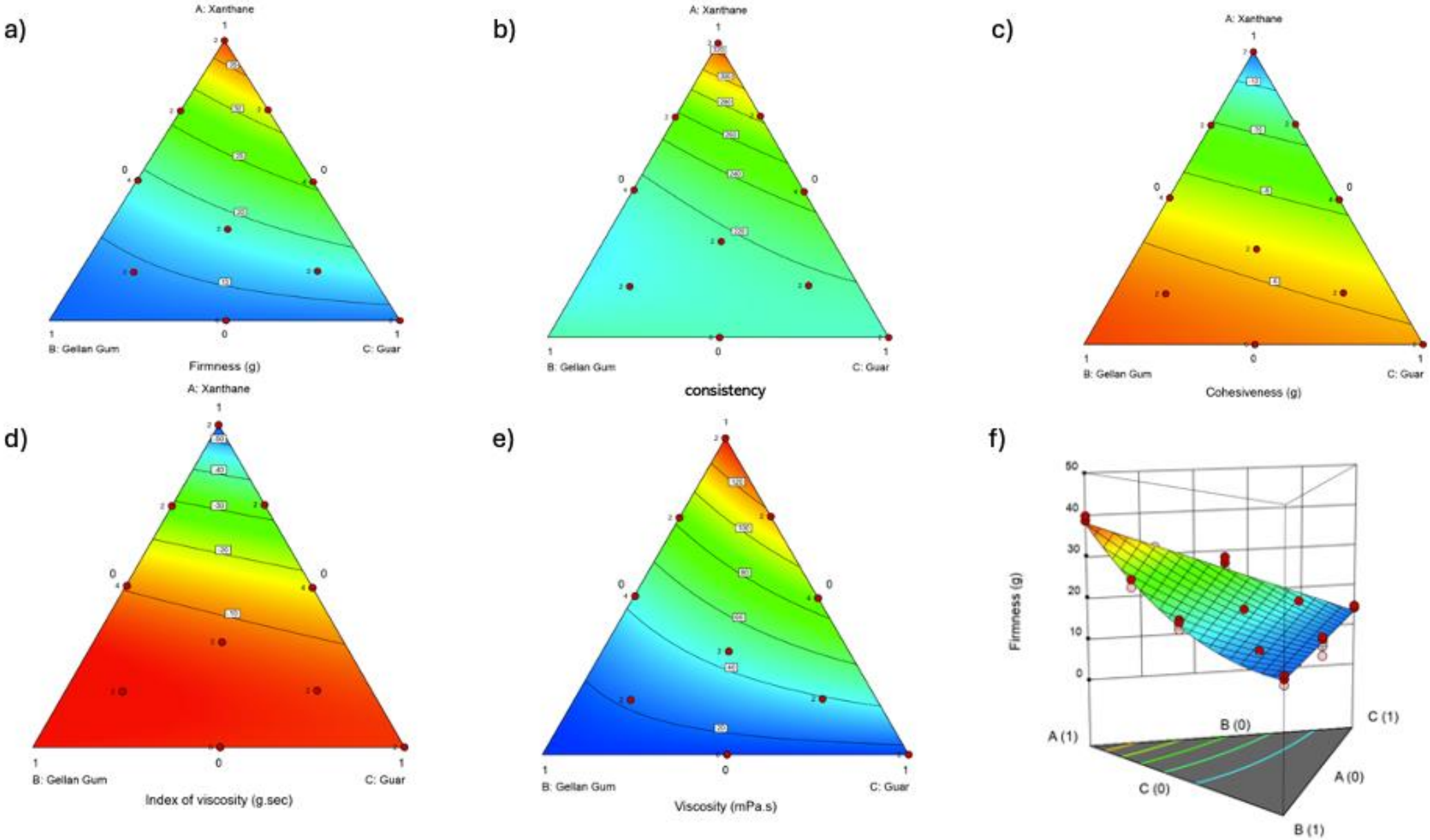

Figure 4. 6. Contour plots of the final formulation Reponses (a-e) and the response surface 3D plot of firmness (f).

Consistency (b) displays a more complex pattern, with moderate values (green region) occupying a substantial portion of the mixture space. The highest consistency values are observed in formulations with high Xanthan content, particularly when combined with moderate levels of either Gellan or Guar gum. This behavior aligns with the positive coefficients observed in the mathematical model and suggests that Xanthan gum could be used as the primary texture modifier when high consistency is desired.

Cohesiveness (c) exhibits an inverse relationship with Xanthan gum concentration, which it’s completely accurate due to the texturometer inverse response for Cohesiveness and Index of viscosity, this is evidenced by the lower values (blue region) in the Xanthan-rich area and higher values (orange-red region) towards the Gellan-Guar axis. This inverse relationship is particularly relevant for optimizing the product's mouthfeel and texture perception, as it suggests that a balance must be struck between achieving desired firmness and maintaining appropriate cohesiveness.

The Index of viscosity (d) response surface reveals a pronounced gradient from the Xanthan vertex (blue region) to the Gellan-Guar axis (red region), indicating that Xanthan gum significantly reduces this parameter. This pattern is particularly important for controlling the product's flow behavior and stability during storage and consumption.

Viscosity (e) measurements show a distinctive pattern with highest values (red region) concentrated in the Xanthan-rich area, particularly when combined with moderate levels of Guar gum. This synergistic effect between Xanthan and Guar gums is evidenced by the curved contour lines in this region, suggesting that optimal viscosity can be achieved with specific combinations of these two components while minimizing the use of Gellan gum.

The three-dimensional response surface for firmness (**Figure 4.6-F**) provides additional insights that complement the contour plot analysis. This 3D visualization clearly illustrates the pronounced curvature of the response surface, revealing the non-linear relationship between the gum components and firmness. The surface exhibits a distinct peak in the Xanthan-rich region, where firmness values reach approximately 40 g, and gradually descends towards the Gellan-Guar axis, where values decrease to around 10-15 g.

Of particular interest is the steep gradient observed along the Xanthan axis, which is not as immediately apparent in the contour plot representation. This 3D perspective emphasizes the dominant effect of Xanthan gum on firmness and reveals a critical formulation threshold where small changes in Xanthan concentration can lead to substantial variations in firmness. The

experimental points (shown as red and white dots above and below the surface, respectively) demonstrate the model's good fit to the observed data, with points closely distributed around the predicted surface.

The surface's asymmetric curvature, particularly evident in the 3D view, highlights the complex interaction between Xanthan and Gellan gums (AB interaction coefficient: -26.47). This interaction manifests as a deviation from planarity in the response surface, indicating that the effect of one gum on firmness is dependent on the concentration of the other. Such visualization is particularly valuable for formulation scientists, as it allows for more precise prediction of firmness values in regions where multiple gums are present in intermediate concentrations.

These visual representations of the response surfaces not only validate the mathematical models previously discussed but also provide practical guidance for formulation optimization.

4.4.2. Model optimization and validation

Based on the comprehensive understanding of the rheological behavior provided by the mathematical models, an optimization process was conducted to identify the optimal gum combinations that would match the target rheological properties of a commercial control product. The optimization criteria were established with different importance levels, prioritizing Cohesiveness, Index of Viscosity, and Viscosity (+++++), while maintaining adequate levels of Firmness and Consistency (+++).

The optimization process incorporated specific constraints for each gum component (**Table 4.6**), with Xanthan and Gellan gums allowed to vary within their full range (0-1), while Guar gum was set to zero to prevent syneresis issues in the final product. Target values were established based on the commercial control product's characteristics: Firmness (26.5 g) and consistency (247 g/sec). For cohesiveness and index of viscosity, minimization was pursued due to the observed higher values compared to the control product, while Viscosity was set to be maximized to ensure proper product stability and mouthfeel.

Table 4. 6. Constrains for optimization global responses

Constrains						
Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
A:Xanthane	is in range	0	1	1	1	3
B:Gellan Gum	is in range	0	1	1	1	3
C:Guar	is equal to 0	0	0	0	0	0
Firmness	is target = 26.5	10.09	39.8	1	1	3
Consistency	is target = 247	156.41	339.4	1	1	3
Cohesiveness	minimize	-14.7	-4.1	1	1	5
Index of viscosity	minimize	-60.1	-2.6	1	1	5
Viscosity	maximize	6.4	141	1	1	5

Among the eleven solutions generated by the statistical optimization, Solution 1 exhibited the highest desirability (0.616) with the following composition:

- Xanthan gum: 0.749
- Gellan gum: 0.251
- Guar gum: 0.000

Experimental validation of this optimal formulation was conducted in triplicate to verify the model's predictive capability. **Table 4.7** presents the comparison between predicted values and experimental results.

Table 4. 7. Comparison between predicted values and experimental results

	Predicted Mean	Std Dev	n	SE Pred	95% PI low	Data Mean	95% PI high
Firmness	30.20	1.56	3	1.05	28.06	28.43	32.35
Consistency	277.24	19.24	3	13.52	249.50	257.23	304.99
Cohesiveness	-10.99	0.66	3	0.46	-11.94	**-13.93**	-10.04
Index of viscosity	-36.71	1.69	3	1.19	-39.15	**-57.87**	-34.27
Viscosity	100.41	9.44	3	6.63	86.80	108.00	114.02

The experimental results demonstrated excellent agreement with the model predictions, with all responses falling within the 95% prediction intervals, except for Cohesiveness and Index of viscosity. Particularly noteworthy was the accuracy in predicting Firmness (predicted: 30.20 g, experimental: 28.43 g) and Consistency (predicted: 277.24 g/sec, experimental: 257.23 g/sec). The slight variations observed between predicted and experimental values for Cohesiveness (predicted: -10.99, experimental: -13.93) and Index of Viscosity (predicted: -36.71, experimental: -57.87) remain within acceptable ranges for practical applications.

Therefore, the validation results not only confirm the robustness of the developed models but also demonstrate the practical feasibility of achieving desired rheological properties using a binary gum system, which presents advantages in terms of both cost and processing complexity. This optimized formulation successfully achieves the target rheological properties while maintaining manufacturing efficiency through the elimination of Guar gum from the system.

4.5. Chemical characteristics of final products and SiS energy gel

Table 4.8 presents the final product (FP) physical and chemical characteristics compared to SiS Energy Sports Gel (SEG), which serves as the control. The parameters include texture parameters, firmness, consistency, cohesiveness, index of viscosity, and viscosity. Chemical characteristics include pH, moisture content, antioxidant activity, sugar content (glucose, fructose, maltodextrin, and total sugars), and aloin content.

Table 4. 8. Chemical characteristics of final product (FP) and SiS energy gel (SEG).

Parameter	**FP**	**SEG**
pH	4.1 ± 0.2	4.4 ± 0.2
Moisture content (%)	41.2 ± 1	66.5 ± 1
Antioxidant activity (µL Trolox equivalent)	10.42	Not Tested
Sugar content		
Glucose (g/100ml)	12.7 ± 0.1	36
Fructose (g/100ml)	15.98 ± 0.2	-
Maltodextrin (g/100ml)	*7.46 ± 0.1	-
Total sugars(g/100ml)	36.14 ± 0.2	36
Aloin content (mg/L)	0.83 ± 0.01	ND

ND=not detected. *= not quantified (manually added)

4.5.1. Sugar content

The final formulation of the energy sports gel (FP) delivers 36.14 g of total carbohydrates per serving, comprising glucose (12.7± 0.1 g), fructose (15.98±0.2 g), and maltodextrin (7.46± 0.1 g) (Table 1). Honey contributes primarily to fructose and glucose, while maltodextrin, a glucose oligosaccharide, further complements the glucose supply. This carbohydrate composition was strategically formulated to provide balanced and sustained energy, containing an optimal glucose-to-fructose ratio of approximately 1:0.8, which is considered ideal for optimal sugar absorption by the human body during extended periods (Rowlands et al. (2015). Glucose and fructose are absorbed at different rates—glucose provides immediate energy, while fructose offers a slower release (Fuchs, Gonzalez, & van Loon (2019). Furthermore, maltodextrin provides a further sustained supply of energy, as it needs to be hydrolyzed before being absorbed (Holesh et al., 2024). The carbohydrate composition of the developed energy sports gel offers enhanced absorption rates and improved carbohydrate availability during exercise. By combining glucose, fructose, and maltodextrin in specific proportions, the gel is optimized for efficient intestinal absorption and sustained energy release. The glucose-to-fructose ratio of approximately 1:0.8 aligns with recent research indicating that

this ratio allows for higher carbohydrate uptake compared to glucose alone like we observed that it's the only source of carbohydrates in the Sis Energy Sports gel.

The developed energy gel is designed to be consumed as one serving every 25 minutes, with each serving containing 36.14 g of total carbohydrates. This formulation results in an energy release rate of 1.44 g/min (36.14 g / 25 minutes), which falls within the optimal sugar absorption range of 1.3 to 2.4 g/min, as identified by Jeukendrup (2004). This carefully calculated dosage ensures that an athlete consuming the gel at the recommended interval can maintain a steady supply of carbohydrates that is efficiently absorbed by the body. The absorption rate of 1.44 g/min aligns well with the human body's capacity to maximize carbohydrate uptake, providing a reliable energy source for muscle activity and potentially enhancing athletic performance. By matching the body's optimal absorption capacity, the energy gel minimizes the risk of overloading the digestive system while still providing sufficient carbohydrates to sustain energy levels and support endurance during prolonged or high-intensity exercise.

The developed sports gel stands out from the control product and many commercial alternatives by offering an optimized sugar profile and energy delivery system. Unlike the control, which contains only glucose, the developed gel combines glucose, fructose, and maltodextrin in a strategic 1:0.8 glucose-to-fructose ratio, enhancing carbohydrate absorption through dual intestinal transport mechanisms (SGLT1 and GLUT5). This formulation not only increases total carbohydrate uptake by up to 65% compared to glucose alone (Fuchs et al., 2019) but also provides both immediate and sustained energy release, reducing the risk of gastrointestinal distress. By addressing the limitations of single-carbohydrate gels, the developed product delivers a more consistent energy supply, supporting endurance and performance during prolonged or high-intensity exercise. This innovative approach makes it superior to existing market options.

4.5.2. Moisture content and pH

The moisture content of the final formulation was 41.2%, which is lower than the 66.5% found in the control product. This decrease could be attributed to the high concentration of honey, which naturally has a low moisture content. This ultimately enhances the product's safety (van Boekel, 2023).

The pH value of 4.1 observed for the final formulation, as shown in **Table 1**, falls within the expected range for beverages, which is consistent with the findings of Bendaali and his team

(2024). The pH of 4 in the developed sports gel formulation is indeed comparable to many commercially available products in the market, as supported by scientific research. A study investigating the development of sports energy gels using chia seeds and different hydrocolloids found that the best formulation had a pH of 5.2 ± 0.38, which is slightly higher but still in a similar acidic range as the developed sports gel (Lestari et al., 2021). Another study focused on date-based sports energy gels and analyzed their physicochemical properties, including pH levels. These studies demonstrate that sports gels typically have slightly acidic pH values, confirming that the formulation with a pH of 4 is within the expected range for such products (Baroyi et al., 2023).

Hansson et al. (2001) confirm that maintaining a pH around 4 offers several benefits, including improved microbial stability, as the acidic environment inhibits the growth of both pathogenic and spoilage microorganisms. Additionally, this pH level could enhance the flavor profile, providing a refreshing taste by balancing sweetness and acidity.

4.5.3. Antioxidant activity

Recent studies have identified a rise in reactive oxygen species, free radicals, and reactive nitrogen species during intense physical activity, which can result in oxidative stress and negatively impact physical performance (Kruk et al., 2022). Consequently, using antioxidant supplements and sports foods enriched with antioxidants has become increasingly popular among athletes (Kruk et al., 2022; Cui et al., 2022). The developed product was formulated to be enriched with antioxidant compounds in this context. Both honey and Aloe vera are well-recognized as sources of phenolic compounds and polysaccharides, contributing to their antioxidant potential.

Figure 2 shows the antioxidant activity of the developed sports gel through the DPPH assay, which achieved a 10.42 µl Trolox Equivalent. This antioxidant activity likely originates from honey and aloe vera.

Although sports supplements have been developed with honey (Tomczyk et al., 2020), this is the first-time aloe vera has been introduced in sports gel formulations. Therefore, **Figure 2** also shows the effect of aloe vera incorporation on the antioxidant activity of the developed product. Samples without aloe vera showed a slightly lower anti-oxidant capacity than final product with aloe vera, but not statistically significant (p>0.05), antioxidant capacity (9.01 µl Trolox Equivalent). This suggests that at the incorporated concentration (1%), this ingredient

made a low contribution to the observed antioxidant activity. Therefore, the observed antioxidant activity is likely attributed primarily to honey.

On the other hand, the aloe vera gel alone (**Figure 4.7**) demonstrated a significantly (p<0.05) higher antioxidant capacity (47.33 Trolox equivalent) compared with the final product. This emphasizes the aloe vera's potential in enhancing the formulation's antioxidant properties.

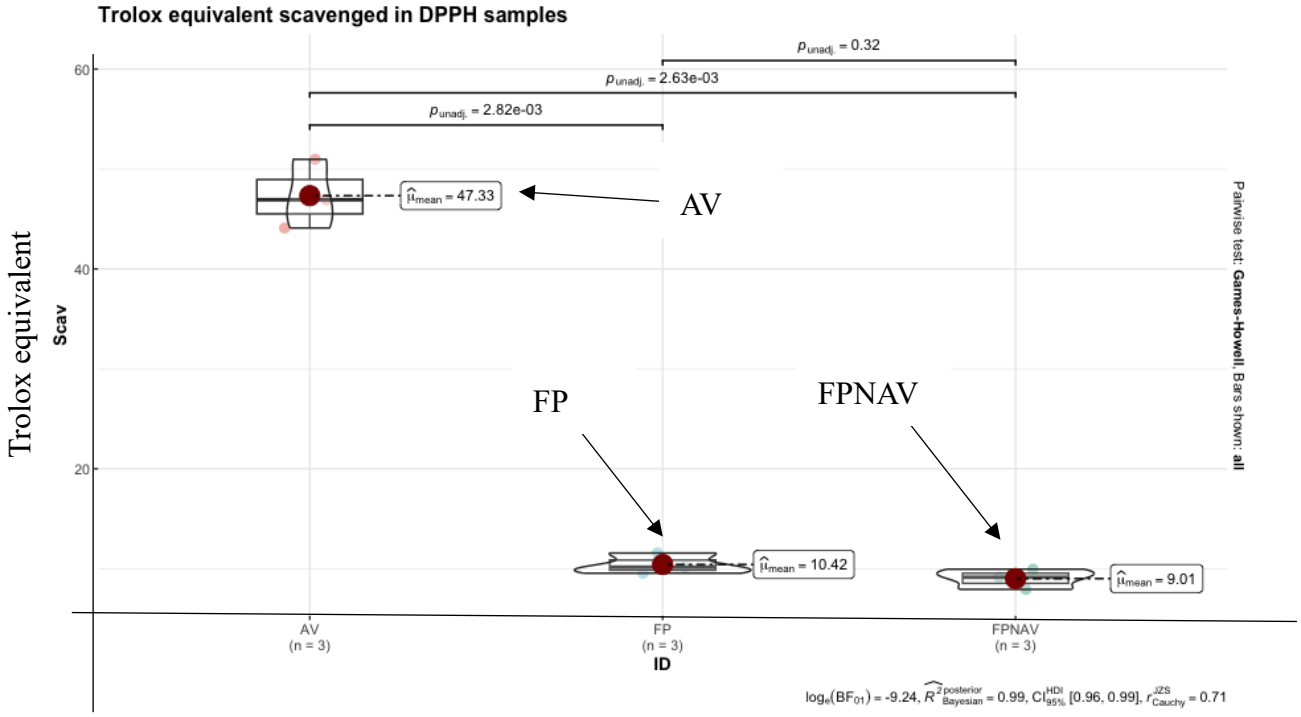

Figure 4. 7. Trolox scavenged in DPPH samples Aloe Vera, Final Product, Final Product No Aloe Vera.

These findings suggest that while aloe vera shows promising antioxidant activity, its integration into the formulation requires optimization to unlock its full potential. Further studies are needed to investigate the ideal concentration of aloe vera and its interactions within the formulation to achieve significant enhancements in antioxidant capacity.

In summary, the observed antioxidant activity offers preliminary evidence of the developed sports gel's potential to counteract free radicals. However, further studies are essential to confirm and validate its antioxidant efficacy.

4.5.4. Aloin content

The human consumption of Aloe vera extract in beverage form has substantially grown over the last several decades. However, incorporating aloe vera introduces a risk of aloin contamination, found in the latex of aloe vera and linked to adverse health effects, including digestive irritation, especially when consumed in large quantities over time. In beverages, aloin content is regulated at a maximum of 10 mg/L of aloin in beverages (Kim et al., 2023). Due to concerns related to the potential genotoxicity and carcinogenicity risk associated with the hydroxyanthraquinones in Aloe vera, specifically aloin, it is crucial to minimize their presence in food and beverages.

The results indicated that the pre-treatment utilized to eliminate Aloin efficiently reduced this compound from the aloe vera gel, as indicated in Table 1 and Figures 4.8b and 4.8c. After applying the pre-treatment, Aloin A reduced by 76% (from 7.07 mg/L to 1.70 mg/L), while Aloin B reduction achieved 93% (from 28.33 mg/L to 1.90 mg/L). Indeed, this pre-treatment has been well described as efficient to remove aloin from aloe vera gels (Brown et al., 2014b).

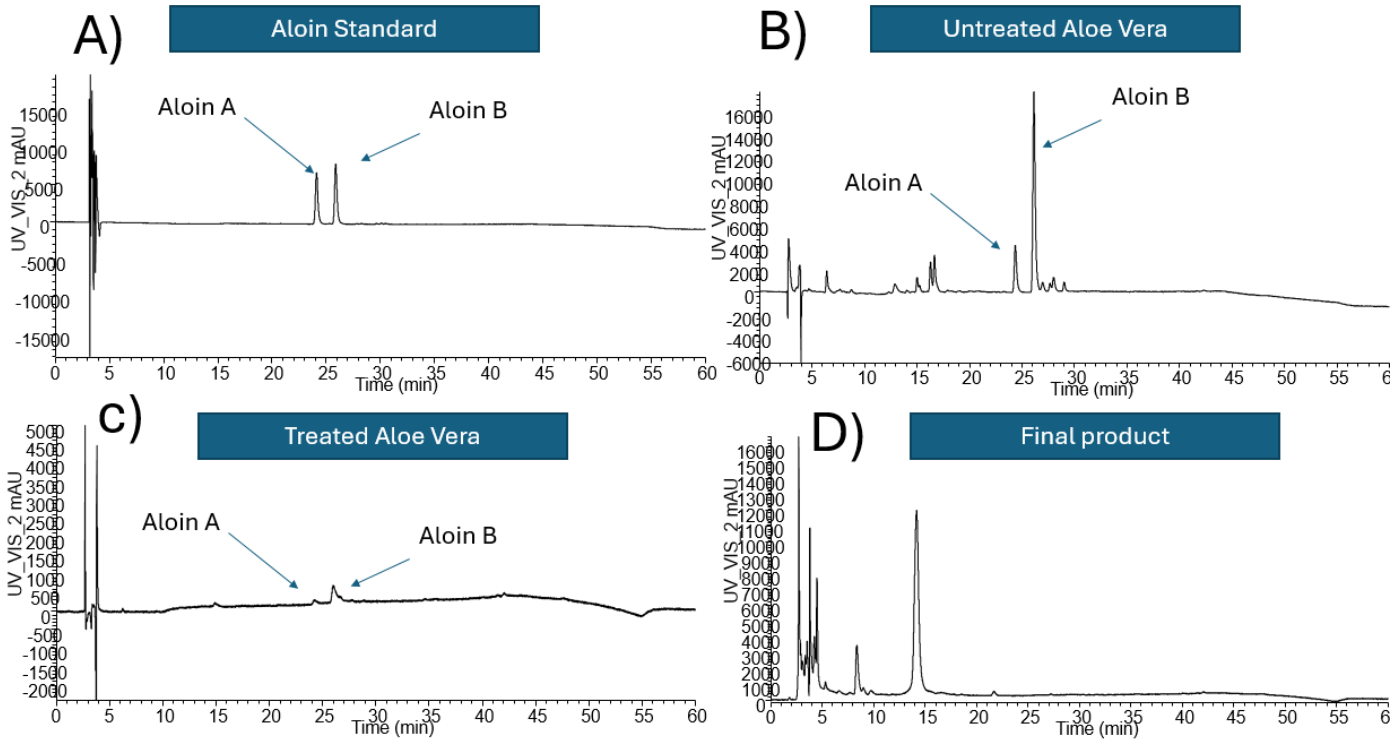

Figure 4. 8. Comparative HPLC Chromatograms of Aloin content in standard (A), untreated (B), treated *Aloe vera* (C), final product (D)

In the final product, Aloin B was found at a concentration of 0.83 mg/L, while Aloin A was undetectable (**Figure 4.8 D**). These concentrations are well below the allowed limit of aloin in beverages (10 mg/L) (Kim et al., 2023, International Aloe Science Council (IASC) (Brown et al., 2014b). In a recent study, Hayes et al. (2024) analyzed commercially available aloe vera gel beverages (Forever Living Products), and found on average 3.43 mg/L of aloin A and B in these products. The study also assessed the potential toxicity of the beverages by exposing male and female Sprague Dawley rats for 90 days with the drink as their sole liquid source. They found no adverse effect of the exposure regarding thyroid hormones, histological or histopathological changes and abnormal cell proliferation (Hayes et al., 2024). This data ensures the compliance of the developed sports gel with safety standards.

Table 4. 9. Aloin A and aloin B concentrations in different simples (mg/L) Final Product.

Samples	Aloin A	Aloin B
Untreated AV	7.07 ± 3.11	28.33 ± 13.75
Treated AV	1.70 ± 0.03	1.90 ± 0.21
FP	ND	0.83 ± 0.14

5. Conclusions

This study successfully developed mineral water, honey, and aloe vera-based sports gel with optimized rheological properties comparable to commercial gel (SIS energy sports gel). The successful implementation of a mixture design experiment has enabled the comprehensive exploration of the rheological properties of the aloe vera-based product as a function of the relative proportions of Xanthan, Gellan, and Guar gums. The statistically significant models developed demonstrate a strong predictive capability, accurately capturing the complex interactions between the gum components and their influence on the desired rheological responses.

The contour and 3D response surface plots offer critical insights into how varying proportions of gum components influence the system, with Xanthan gum emerging as a primary driver of Firmness, Consistency, and Viscosity. These visualizations highlight significant Xanthan-Gellan interactions, providing crucial information on how component balance affects the product's texture and stability. Notably, the 3D model analysis reveals that the optimal combination for desired properties is 0.75% Xanthan gum and 0.25% Gellan gum. This information is particularly valuable for guiding formulation adjustments to meet specific sensory and stability requirements, enabling formulators to make informed decisions when fine-tuning recipes. By leveraging these insights, manufacturers can precisely modify their formulations to achieve the desired texture, stability, and overall product characteristics, potentially leading to improved product quality and consumer satisfaction.

Through model optimization, an ideal gum combination was identified to closely match the target rheological properties of a commercial reference product. Experimental validation of this optimal solution confirmed the models' applicability, with predicted values aligning well with observed outcomes for all parameters except Cohesiveness and Index of Viscosity, where variations remained within practical limits. This optimized, binary gum system formulation achieves the desired rheological properties while simplifying manufacturing by omitting Guar gum, thus balancing efficacy and cost-efficiency. Overall, this study highlights the power of mixture design in optimizing product formulations and provides a solid foundation for further research and development in the field of aloe vera-based products.

This formulation offers several advantages compared to existing market formulations. By incorporating honey as a natural simple carbohydrate source, it provides a unique blend of glucose and fructose, allowing for both rapid and sustained energy release essential for athletic

endurance and recovery. The optimized fructose-glucose ratio enhances the energy release profile, ensuring a more balanced and sustained energy supply throughout physical activity. This dual-action carbohydrate profile offers athletes a more balanced energy source compared to traditional sports drinks or gels that often rely on refined sugars or single-source carbohydrates. Additionally, the use of honey introduces natural antioxidants and anti-inflammatory properties, which can aid in reducing muscle fatigue and supporting faster recovery post-exercise. This natural approach contrasts with many commercial products that may contain artificial additives or lack these additional beneficial compounds. The moderate glycemic index of honey makes it particularly suitable for prolonged sporting activities, offering a more stable energy supply than high-GI alternatives. Overall, this honey-based formulation presents a natural alternative to sports gels and drinks, while delivering comparable or potentially superior performance benefits, appealing to athletes seeking cleaner, less processed options for their sports nutrition needs.

This research culminated in the development of an innovative sports gel formulation, optimized for athletic performance and recovery. The final product comprises honey (47.4%) as the primary carbohydrate source, followed by pH-adjusted mineral water (43.14%) as the base. Maltodextrin (7.46%) complements the energy profile, while a carefully optimized combination of xanthan gum (0.75%) and gellan gum (0.25%) provides structure and stability. The formulation is enhanced with liquid aloe vera (1%) for its potential nutritional benefits. This unique blend offers athletes a natural, efficient energy source with optimized fructose-glucose ratios, providing both immediate and sustained energy release essential for endurance activities.

Future research will focus on exploring the bioactive properties of aloe vera within the gel and determining the optimal concentration to enhance hydration, antioxidant capacity, and potential health benefits.

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Supplement material

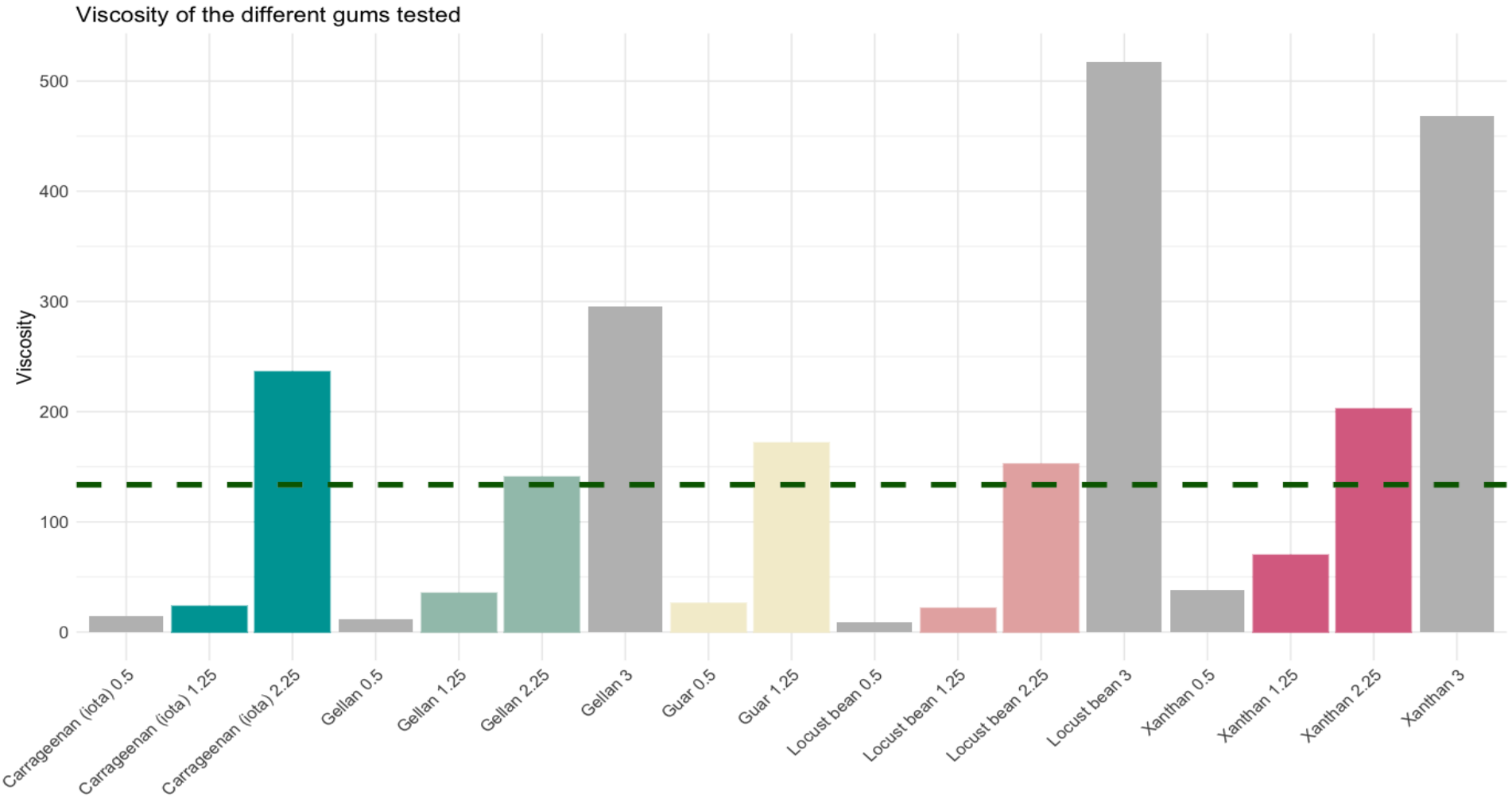

Figure S. 1. Visual representation of viscosity measured in 5 different gums

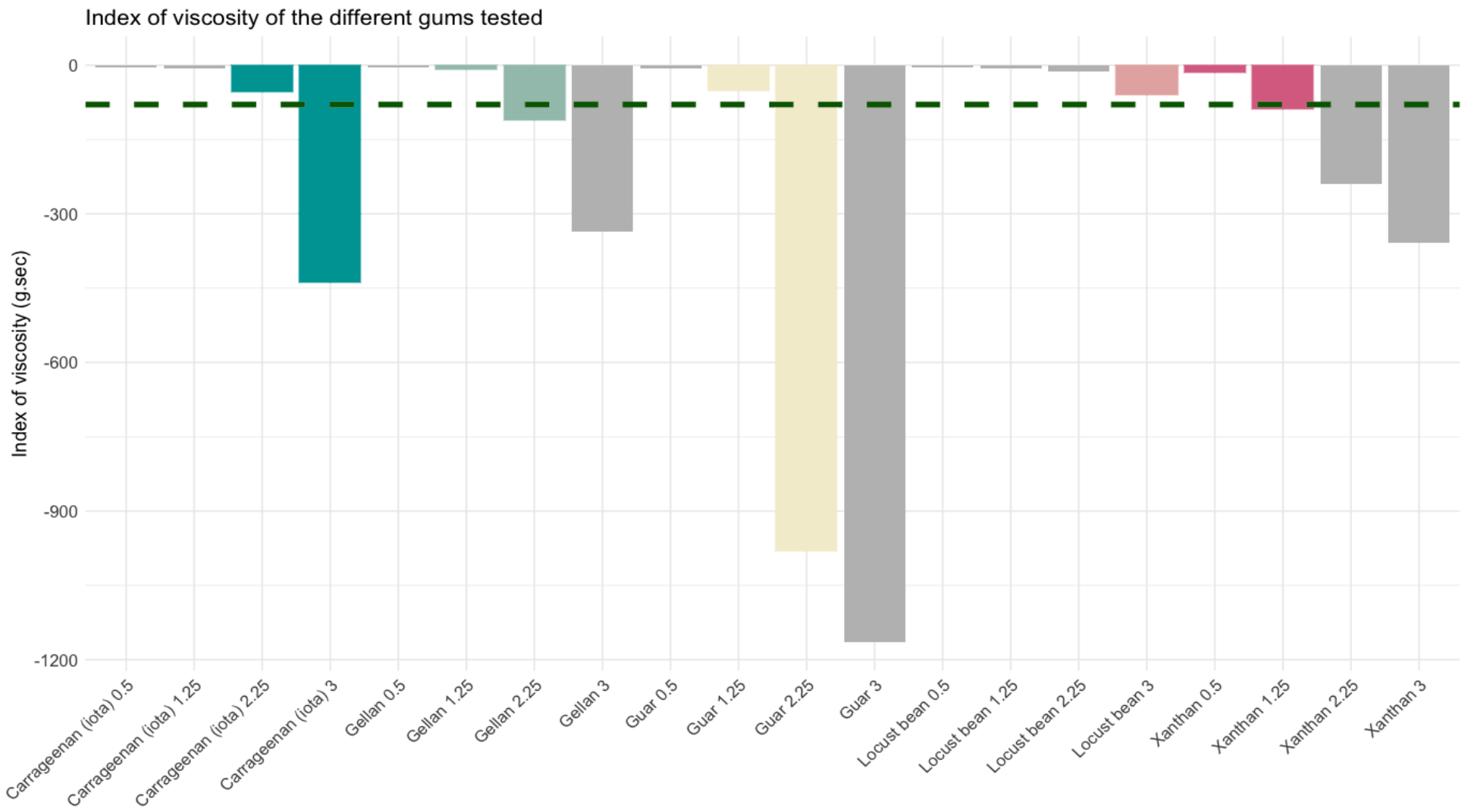

Figure S. 2. Visual representation of index of viscosity measured in 5 different gums

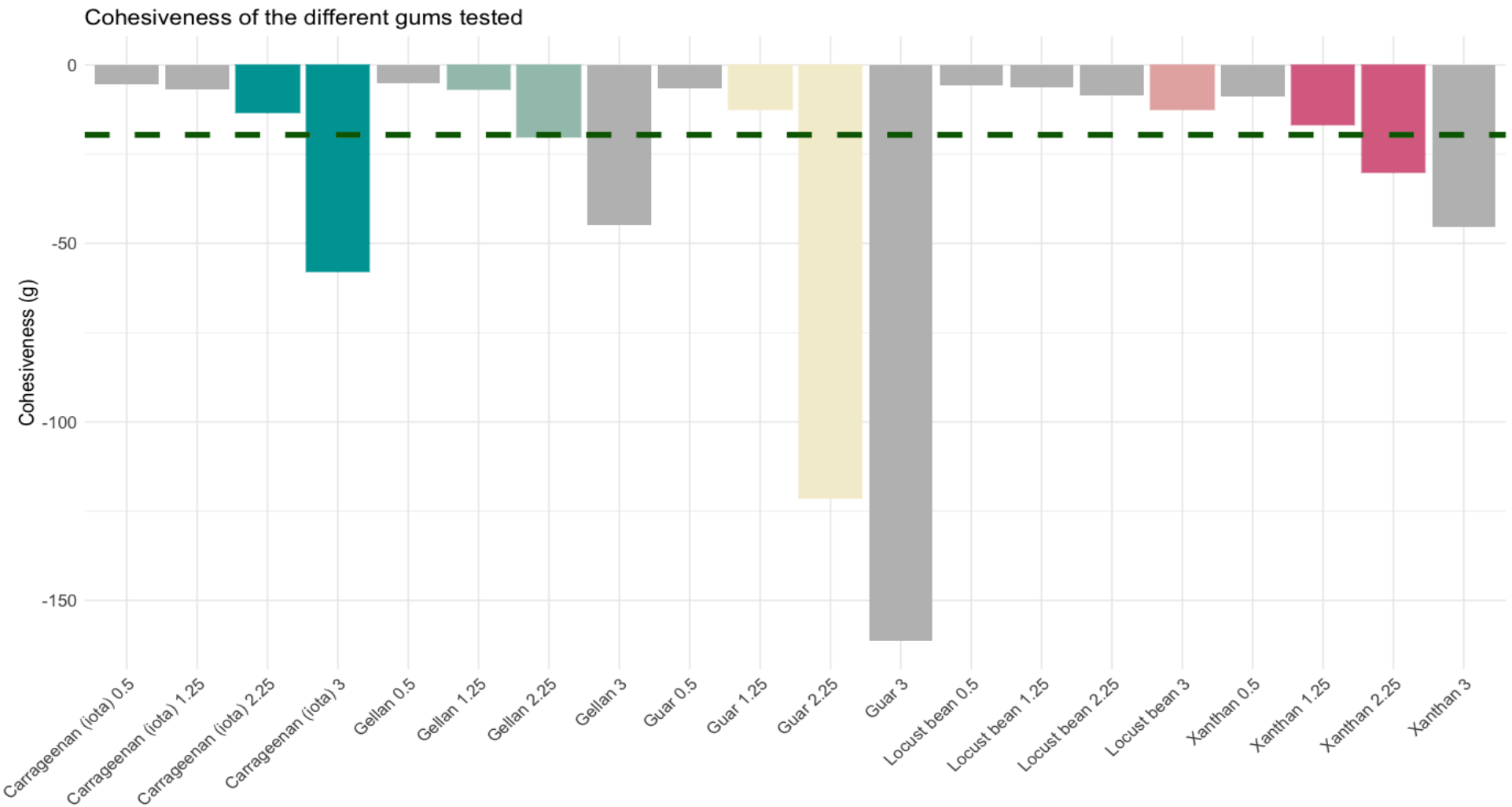

Figure S. 3. Visual representation of cohesiveness measured in 5 different gums

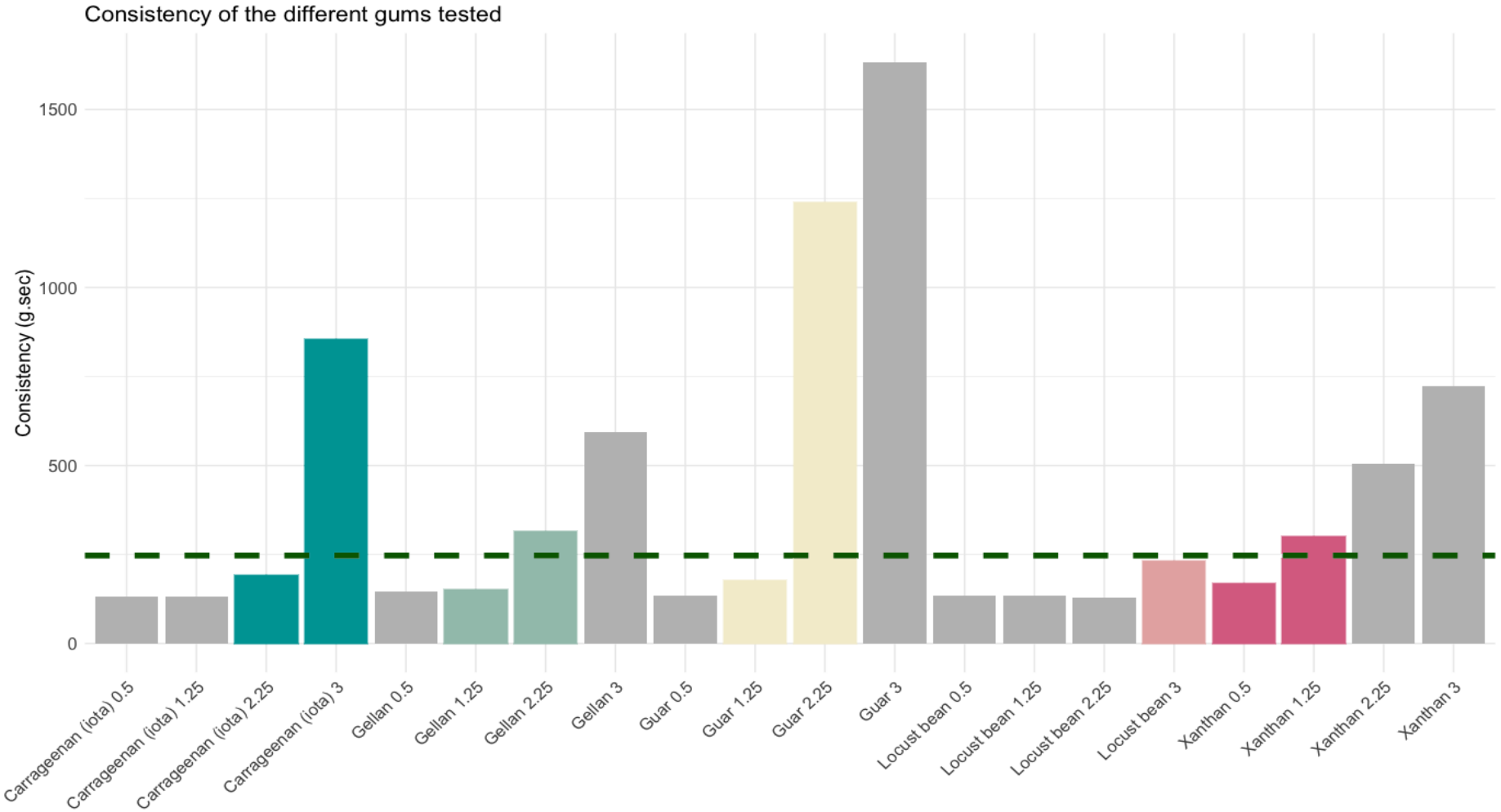

Figure S. 4. Visual representation of consistency measured in 5 different gums

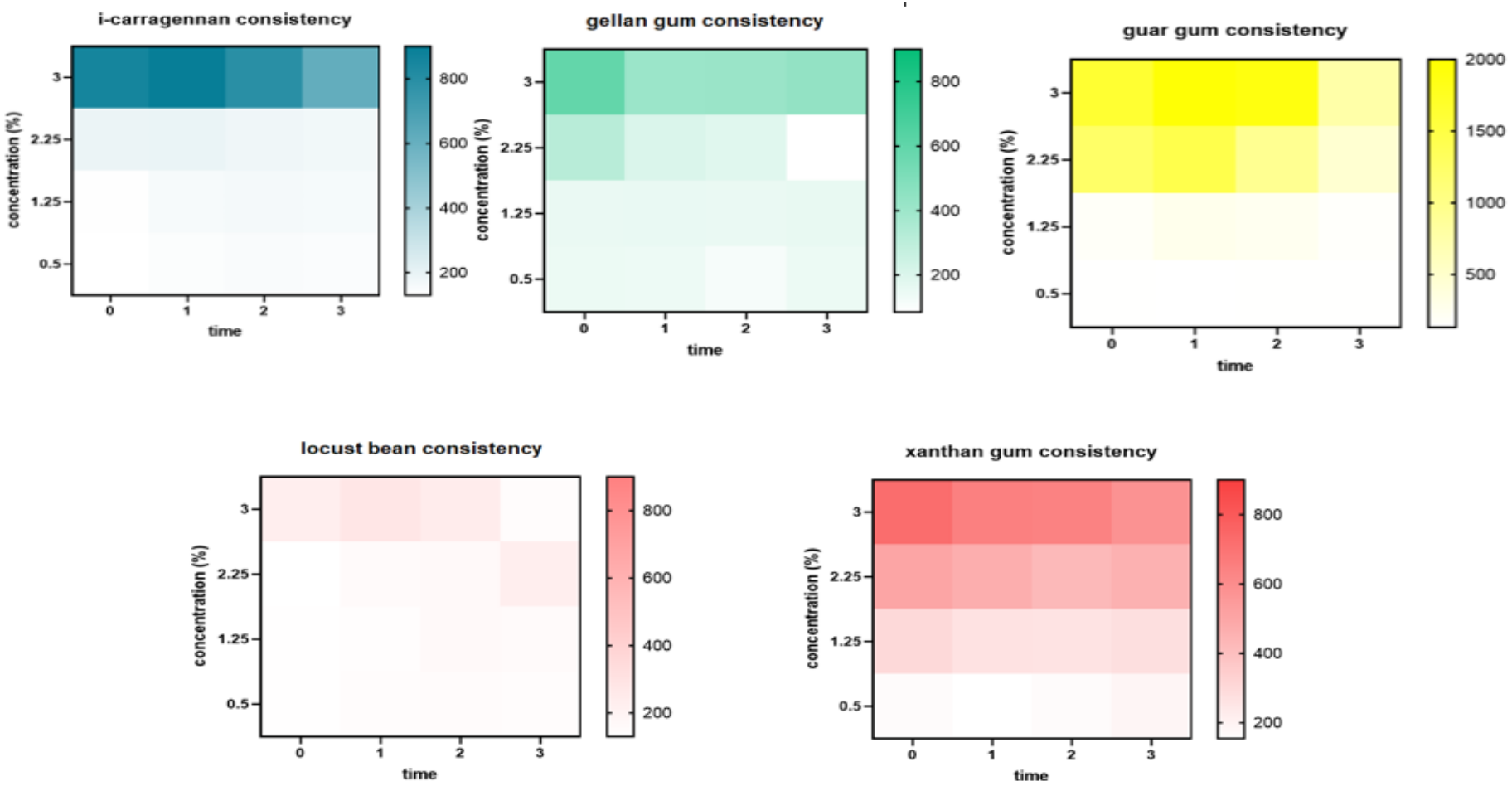

Figure S. 5. consistency of gelling agents along the time

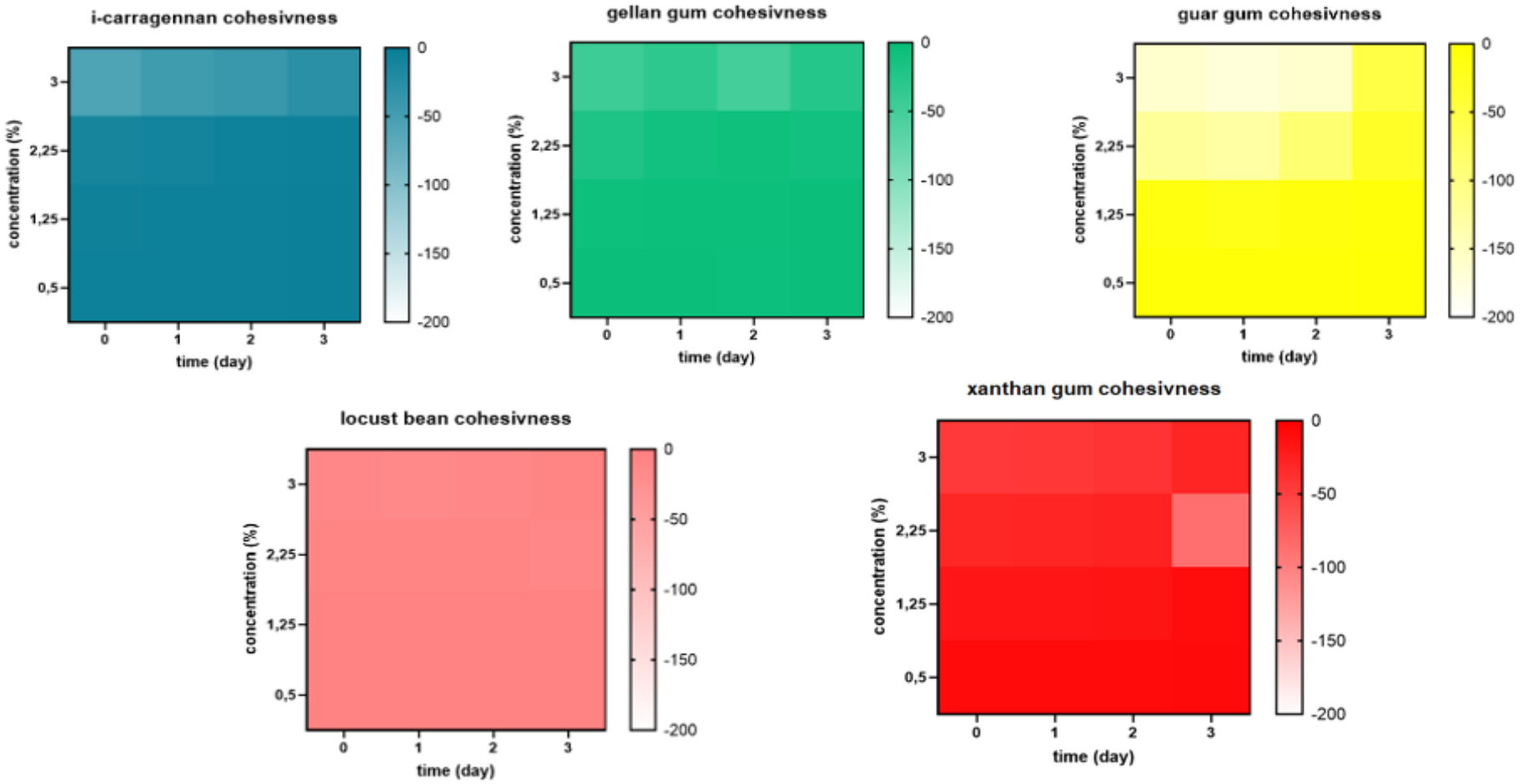

Figure S. 6. Cohesiveness of gelling agents along the time

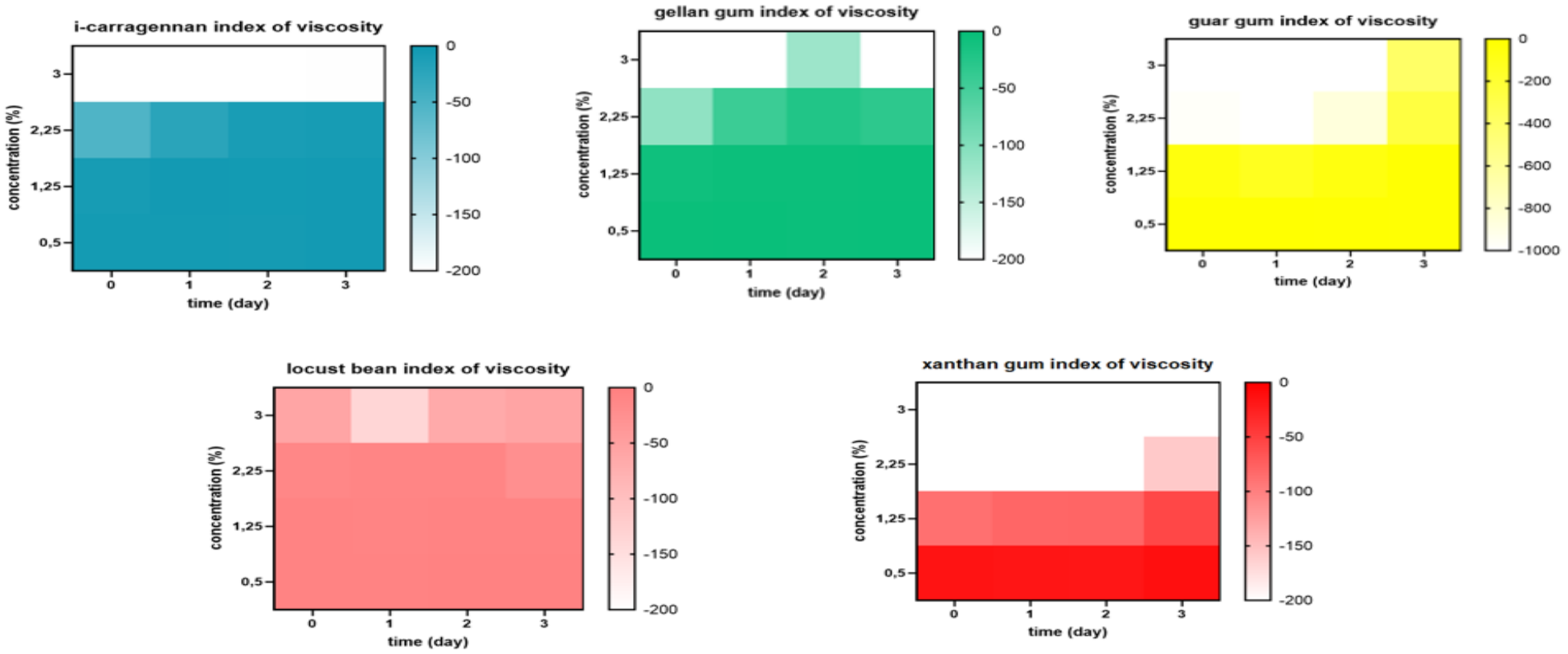

Figure S. 7. Index of viscosity of gelling agents along the time

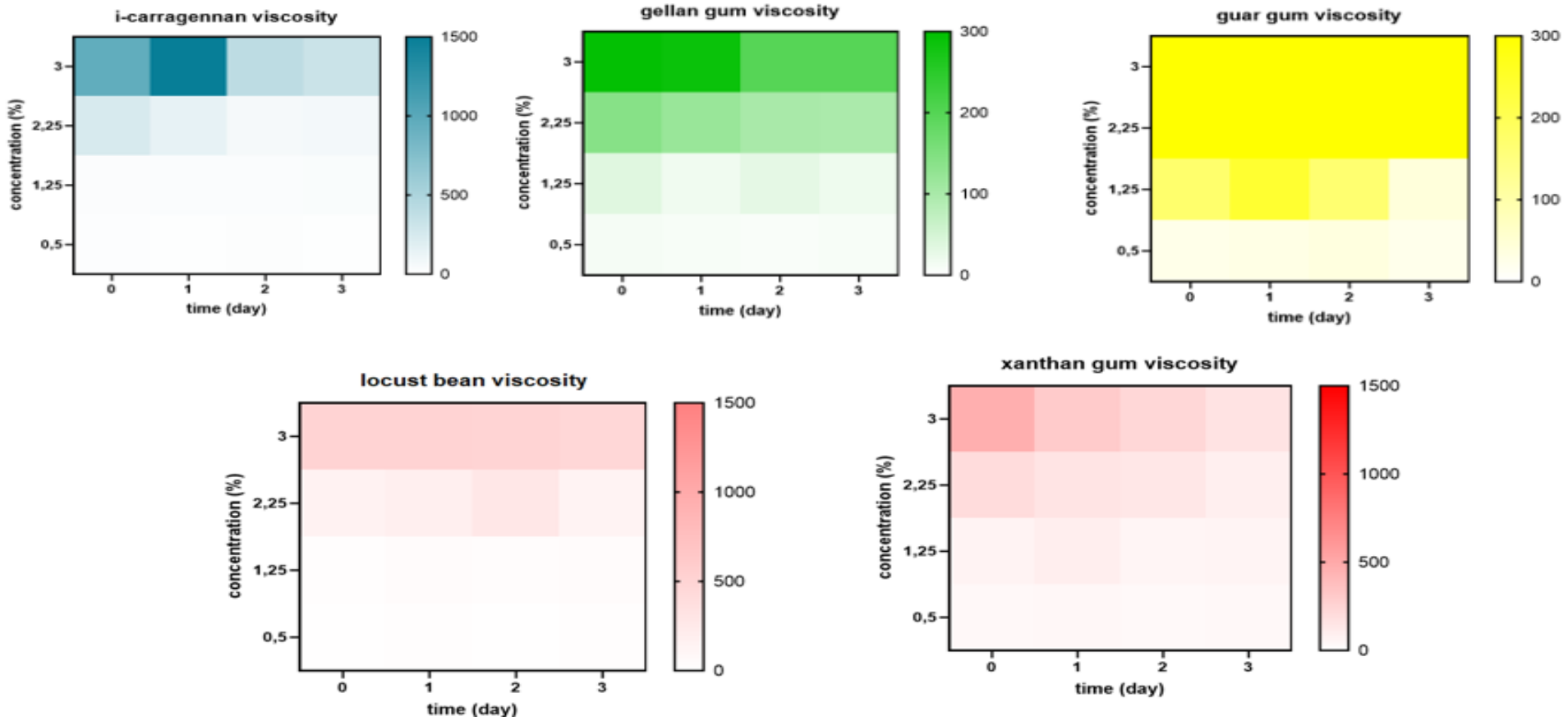

Figure S. 8. Viscosity of gelling agents along the time

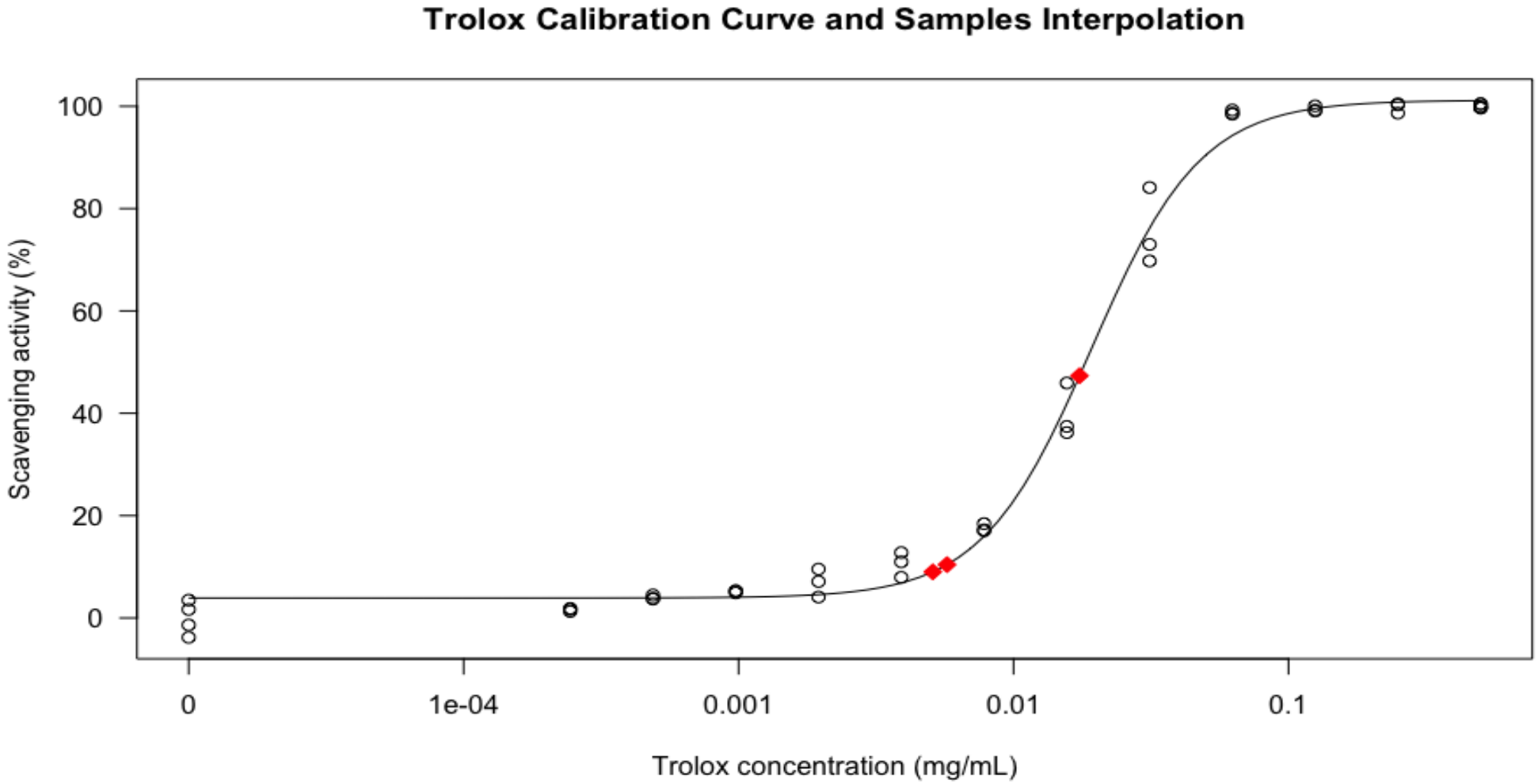

Figure S. 9. Trolox calibration curve and samples interpolation

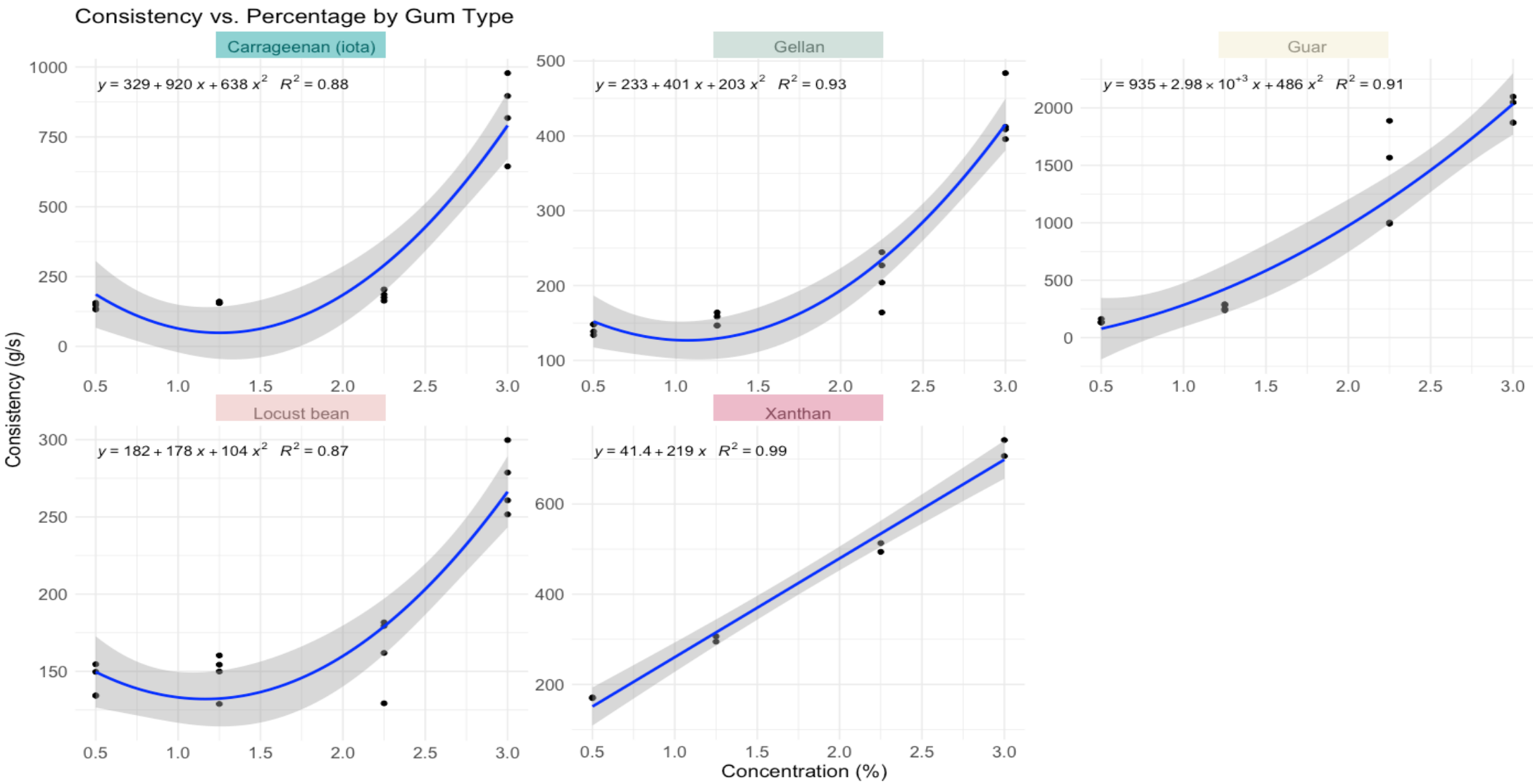

Figure S. 10. Regression equations for consistency across different GA concentration

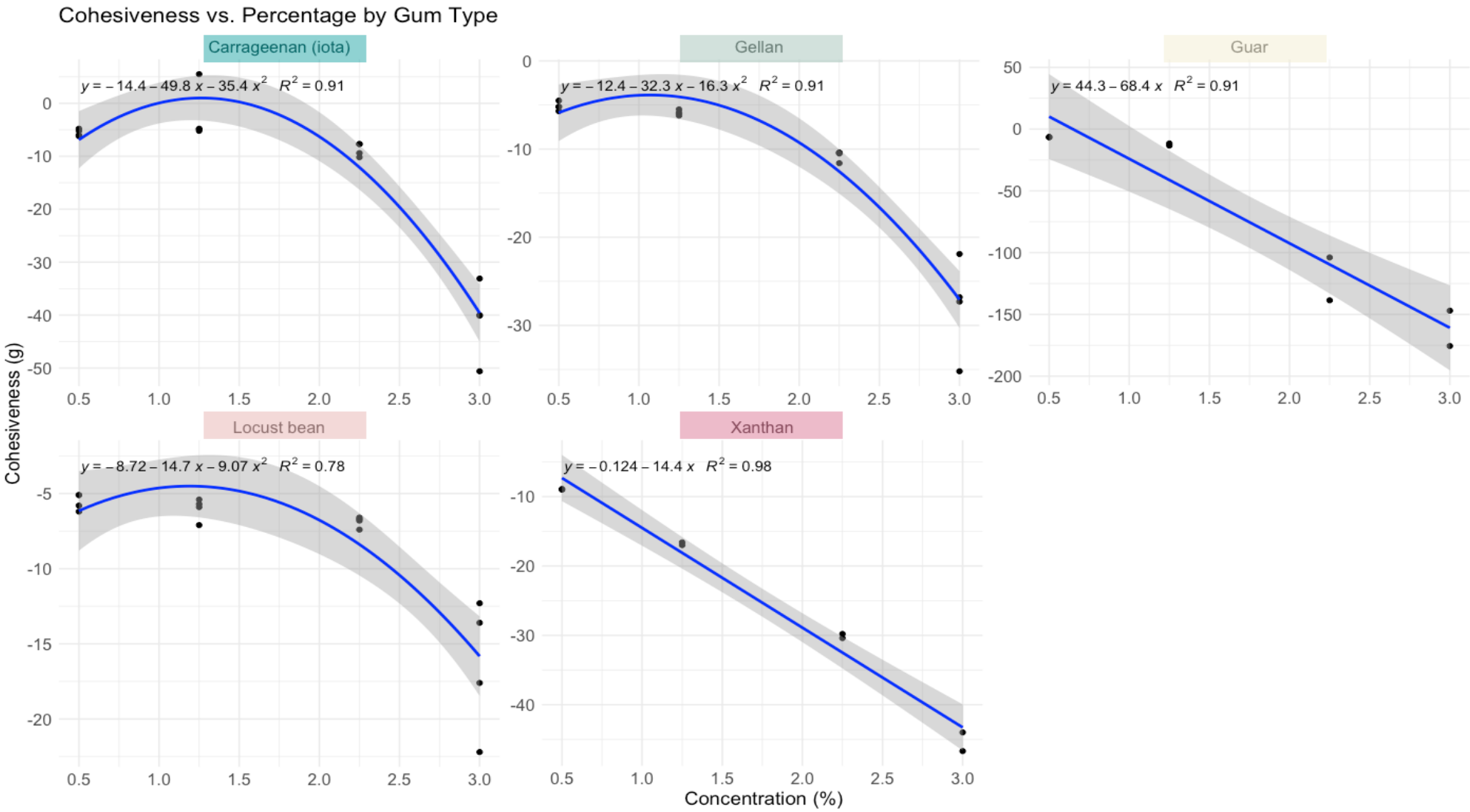

Figure S. 11. Regression equations for cohesiveness across different GA concentration

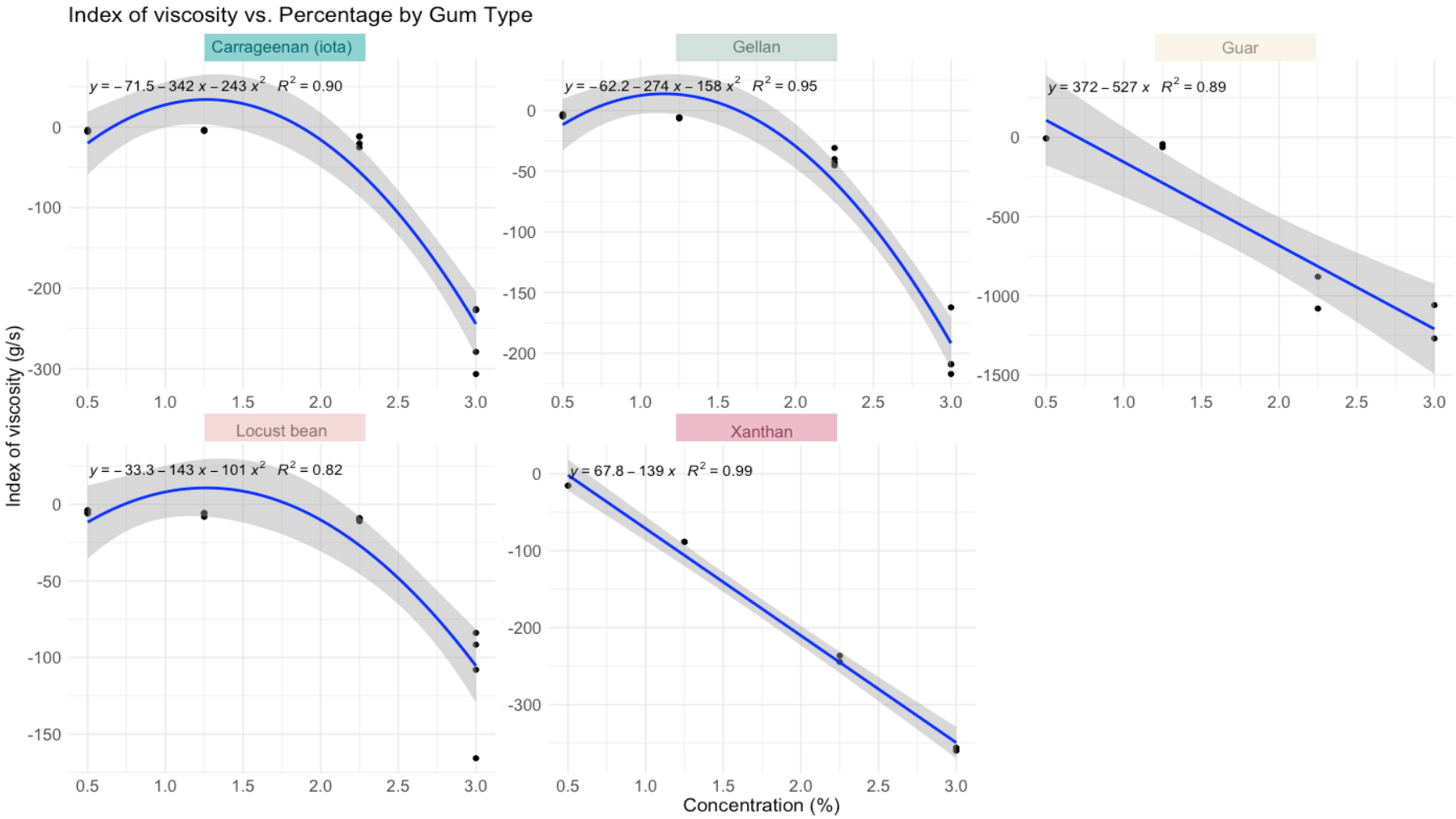

Figure S. 12. Regression equations for index of viscosity across different GA concentration

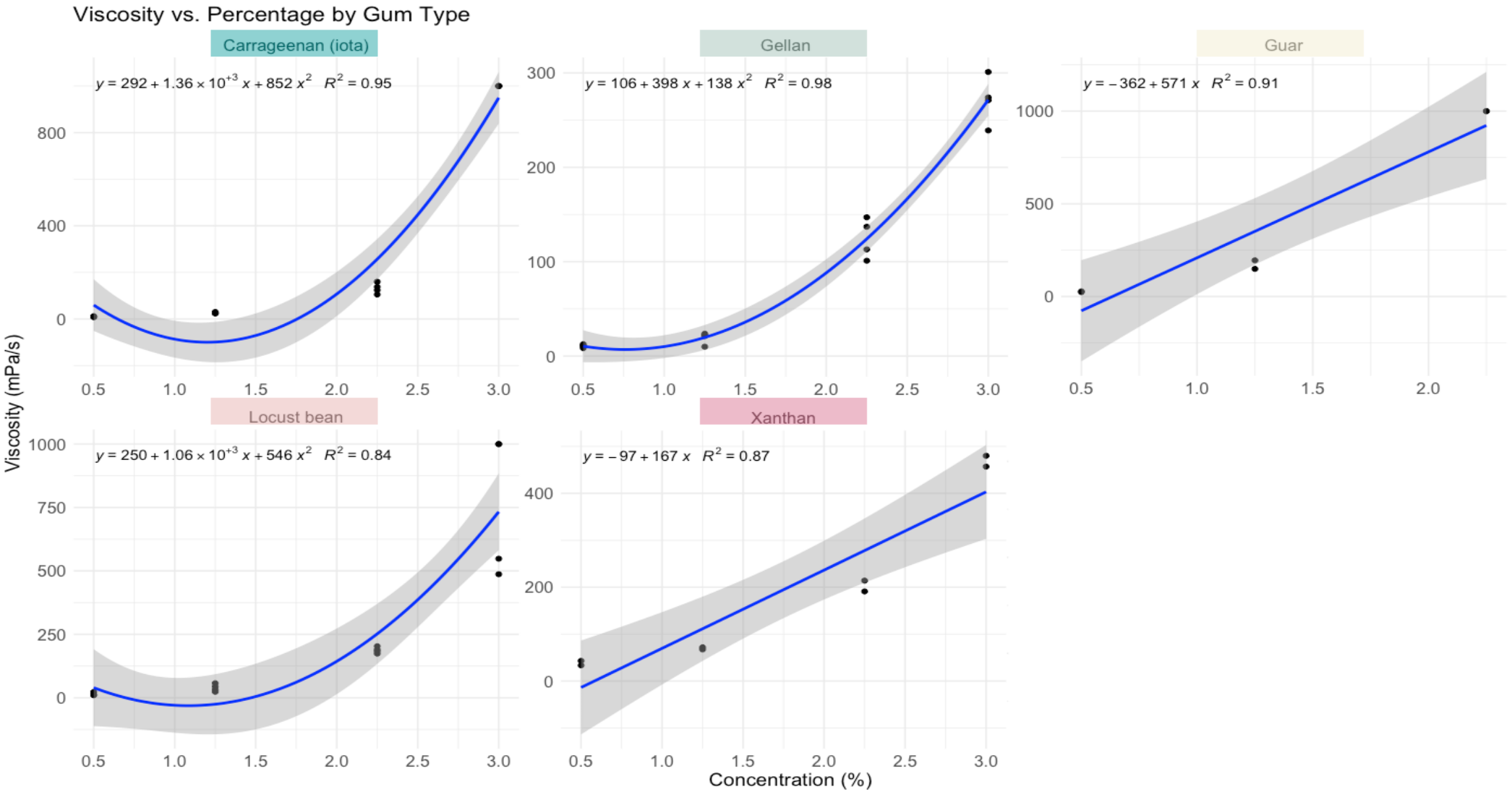

Figure S. 13. Regression equations for viscosity across different GA concentration

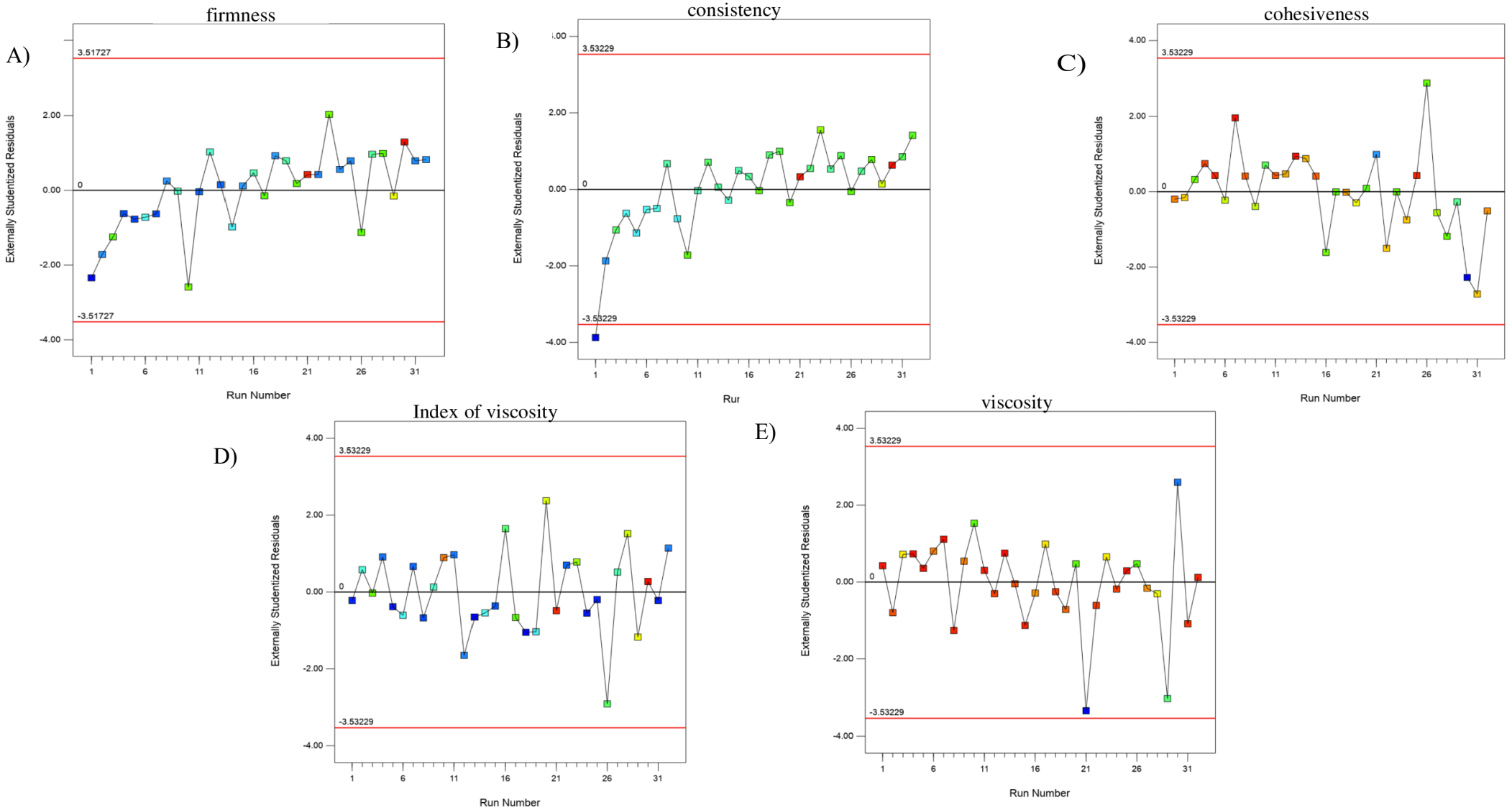

Figure S. 14. Residuals vs run of all the responses analyzed

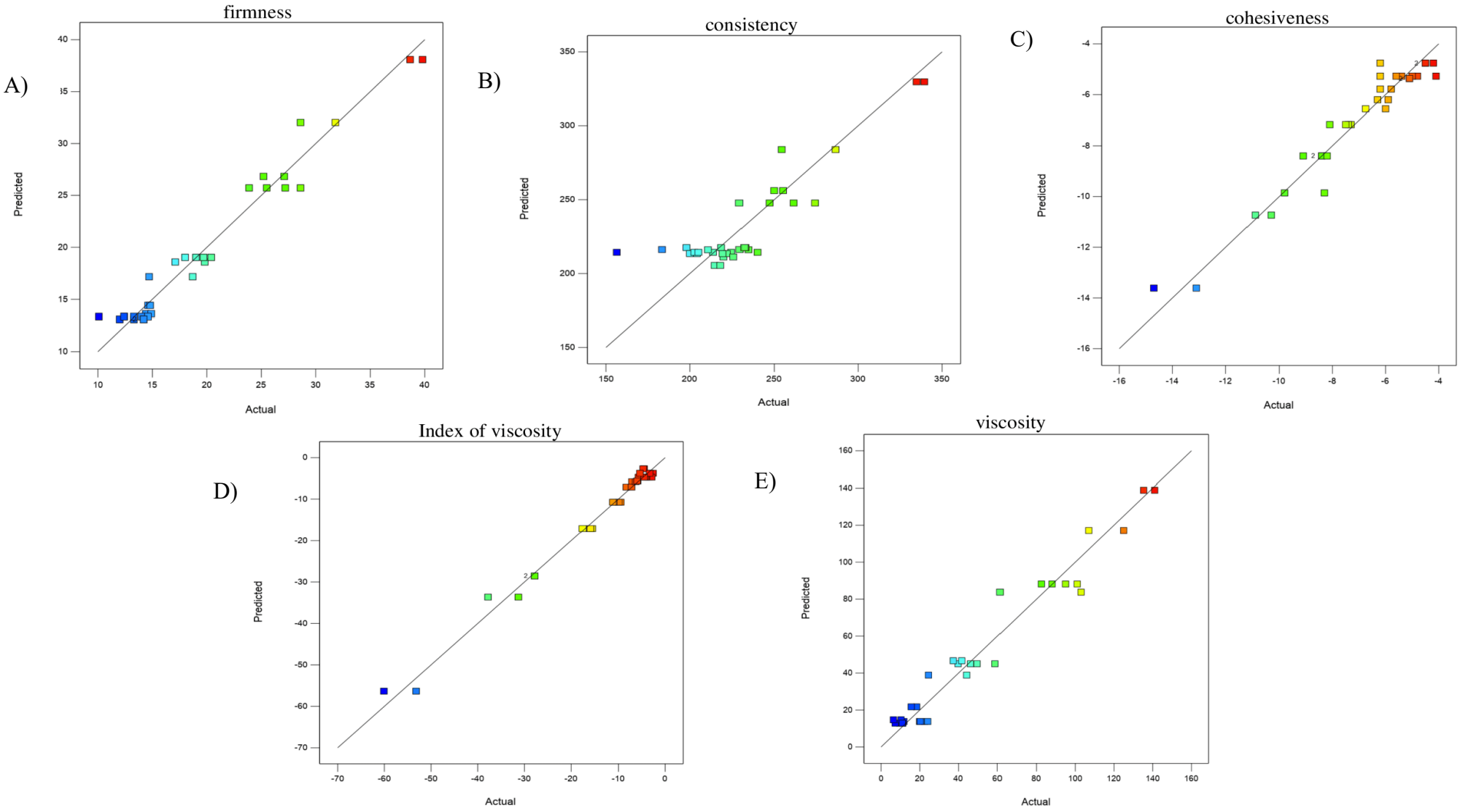

Figure S. 15. Predicted vs actual of all the responses analyzed

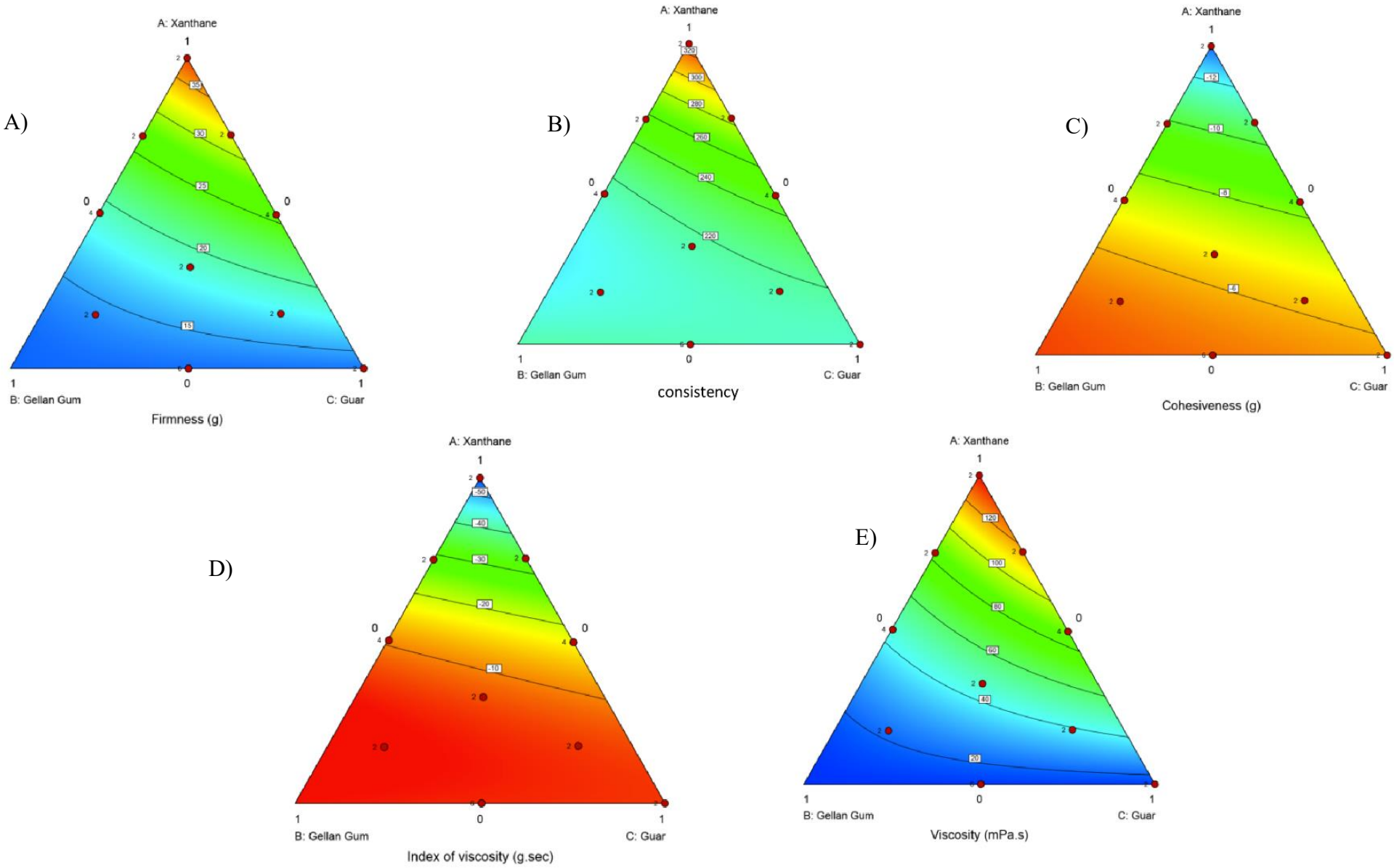

Figure S. 16. Contour plot of all the responses analyzed

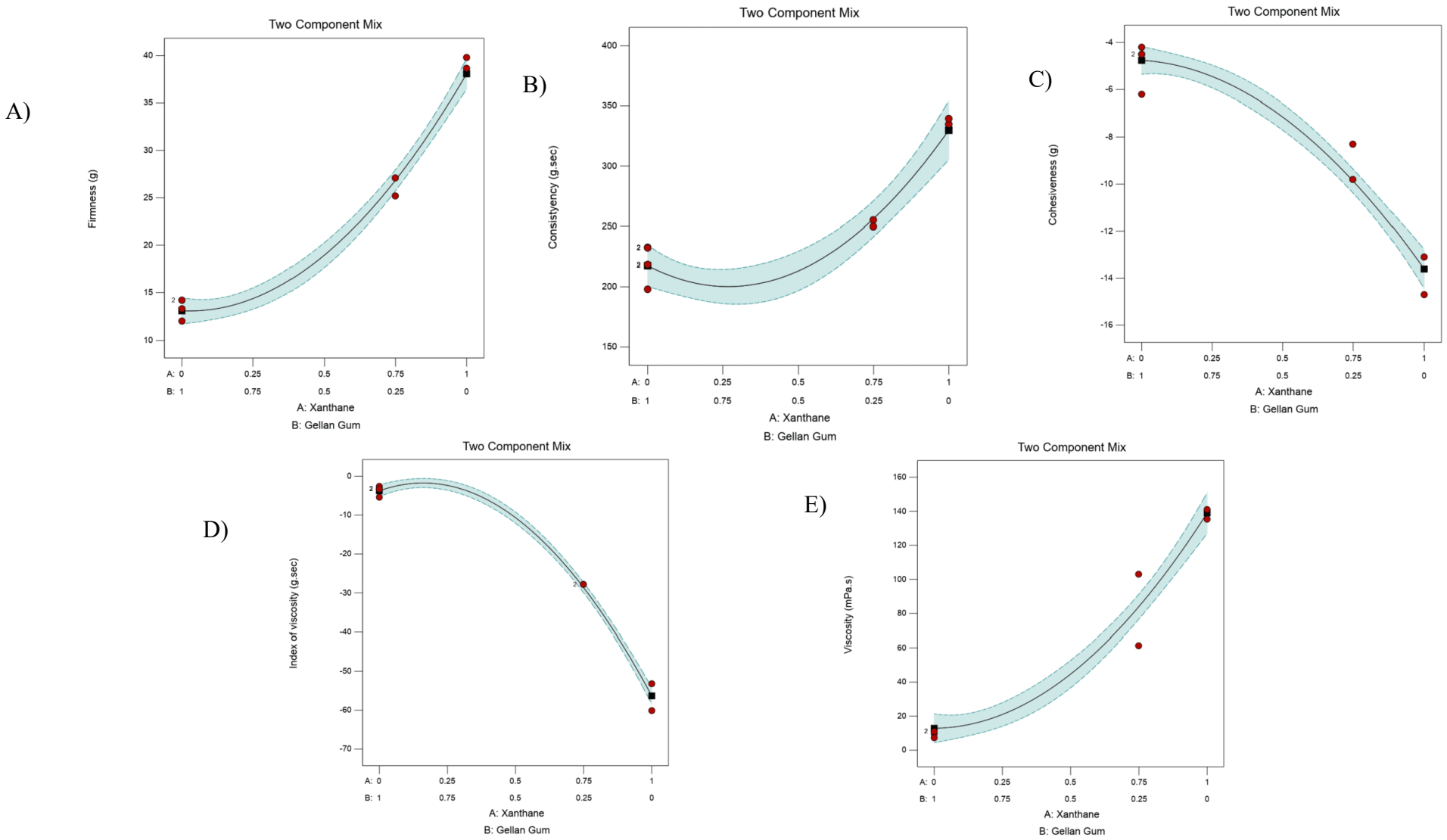

Figure S. 17. Two components mix of all the responses analyzed

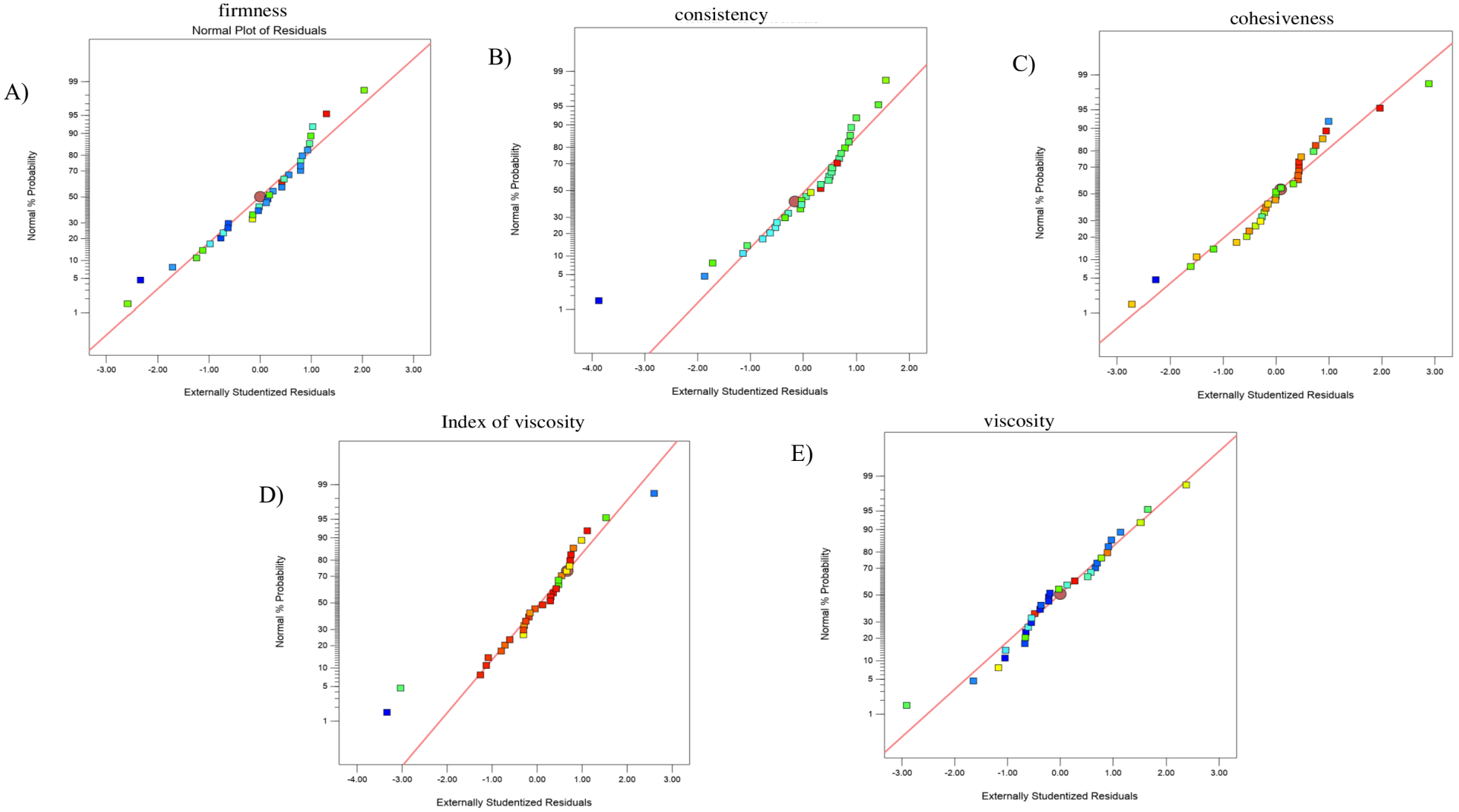

Figure S. 18. Normal plot of residuals of all the responses analyzed

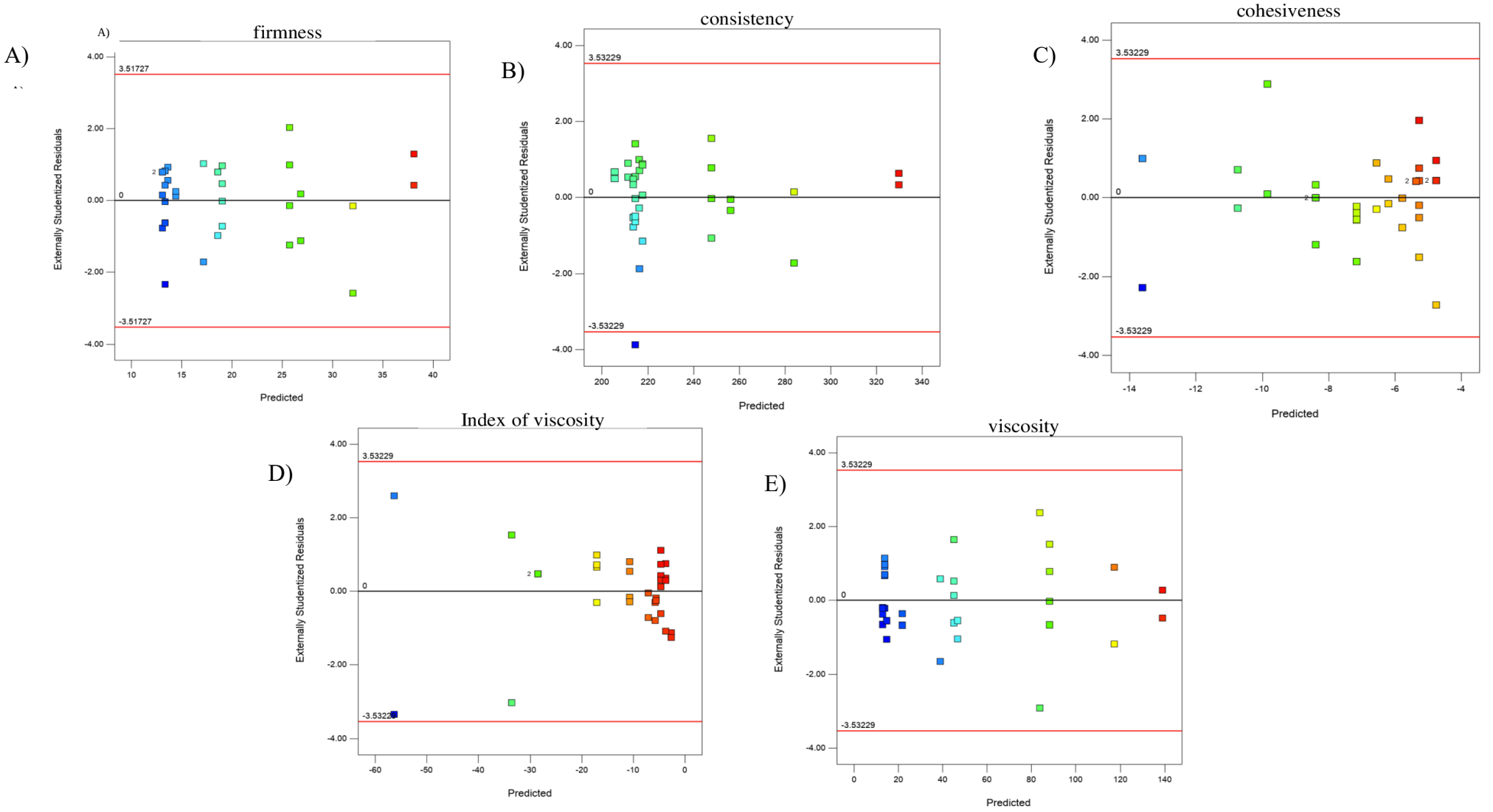

Figure S. 19. Residuals vs predicted of all the responses analyzed

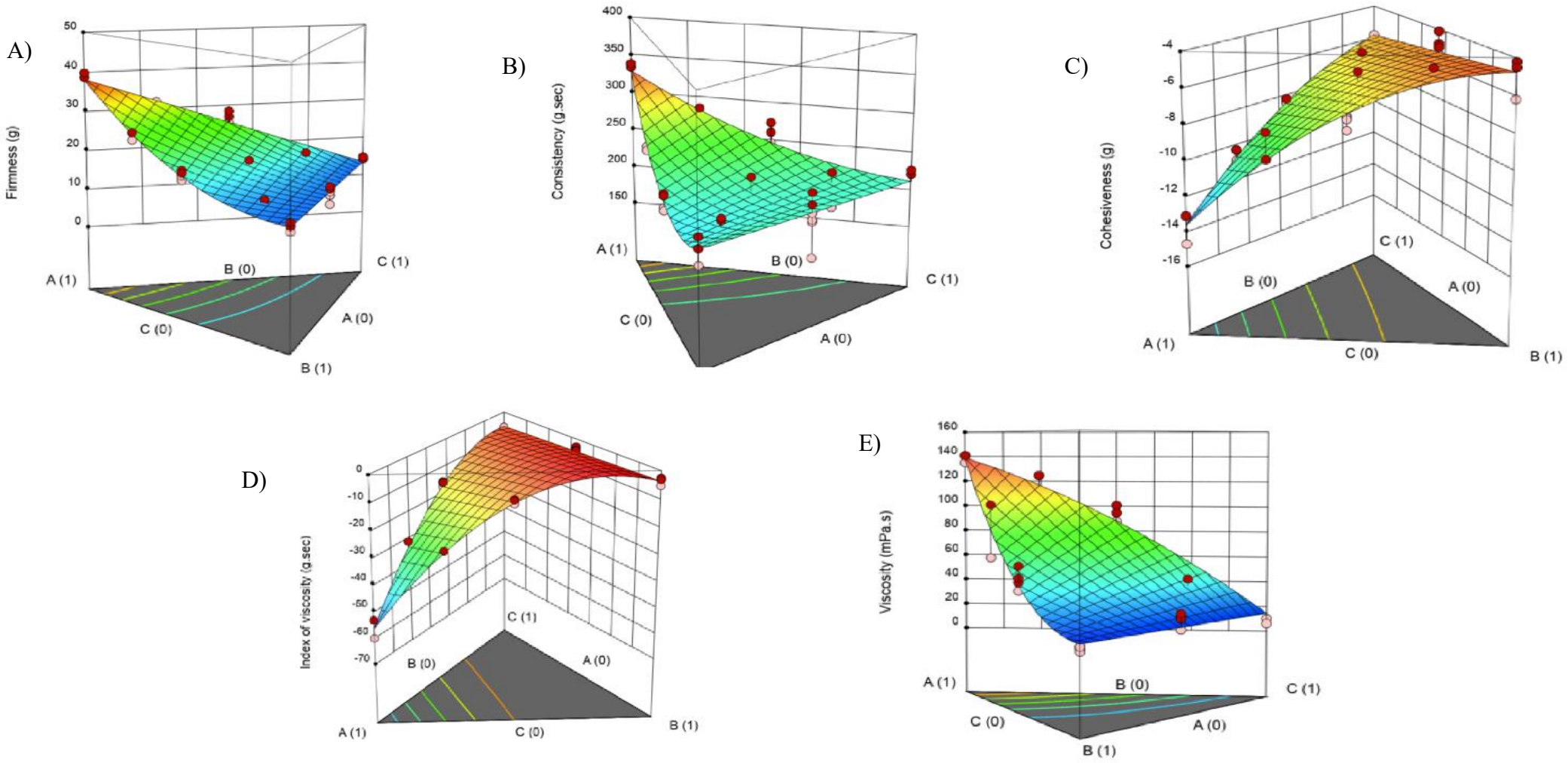

Figure S. 20. Response surface of all the responses analyzed

Table S. 1. Mixture of experimental conditions and responses collected during the experiments, responses data shown is the mean of three experimental measurements.

Run	Component 1 A:Xanthane	Component 2 B:Gellan Gum	Component 3 C:Guar	Component 4 D:Base	Response 1 Firmness g	Response 2 Consistency g/sec	Response 3 Cohesiveness g	Response 4 Index of viscosity g/sec	Response 5 Viscosity mPa.s
1	0	0.496	0.504	99	10.09	156.41	-5.4	-4	11.8
2	0.176	0.147	0.677	99	14.7	183.4	-6.3	-7.1	44.2
3	0.495	0	0.505	99	23.88	229.2	-8.2	-16	88
4	0	0.496	0.504	99	12.41	202.7	-4.8	-3.5	22.1
5	0	1	0	99	12	198	-4.5	-3.2	9.6
6	0.501	0.496	0.003	99	18	204.2	-7.3	-9.5	39.8
7	0	0.496	0.504	99	12.4	205.1	-4.1	-2.9	19.9
8	0.173	0.672	0.155	99	14.8	218.1	-5.1	-4.7	15.6
9	0.501	0.496	0.003	99	19	200	-7.4	-9.9	46.2
10	0.752	0	0.248	99	28.6	254.4	-10.3	-31.3	125
11	0	0.496	0.504	99	13.3	213.8	-5	-4.2	22.6
12	0.176	0.147	0.677	99	18.7	229.4	-5.9	-6.3	24.5
13	0	1	0	99	13.3	218.6	-4.2	-2.6	7.3
14	0.326	0.328	0.346	99	17.1	210.8	-6	-7.2	41.7

15	0.173	0.672	0.155	99	14.6	214.8	-5.1	-4.5	18.4
16	0.501	0.496	0.003	99	19.7	219.5	-8.1	-11.2	58.8
17	0.495	0	0.505	99	25.5	247.2	-8.4	-15.6	82.5
18	0	0	1	99	14.9	225.9	-5.79	-6	6.4
19	0.326	0.328	0.346	99	19.8	234.6	-6.75	-8.3	37.3
20	0.749	0.251	0	99	27.1	249.9	-9.8	-27.8	103
21	1	0	0	99	38.66	334.8	-13.1	-60.1	135.3
22	0	0.496	0.504	99	14	224.6	-6.2	-5.7	20.2
23	0.495	0	0.505	99	28.6	274.3	-8.4	-16.1	95
24	0	0	1	99	14.4	220	-6.2	-5.9	10.3
25	0	1	0	99	14.2	232.9	-4.5	-3.3	11.2
26	0.749	0.251	0	99	25.2	255.2	-8.3	-27.8	61.3
27	0.501	0.496	0.003	99	20.4	222	-7.5	-11	49.6
28	0.495	0	0.505	99	27.2	261.5	-9.1	-17.6	101
29	0.752	0	0.248	99	31.8	286.5	-10.9	-37.8	107
30	1	0	0	99	39.8	339.4	-14.7	-53.2	141
31	0	1	0	99	14.2	232.4	-6.2	-5.4	11
32	0	0.496	0.504	99	14.6	240	-5.6	-4.5	24.1