# Impact of Fibre Morphology on the Degradation of Salivary Amylase Using Starch-Iodine Indicators

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**Abstract**
Saliva is a frequently encountered biological fluid in forensic casework, with alpha-amylase serving as its primary biochemical marker. The starch-iodine test remains one of the simplest presumptive methods for saliva detection, yet its reliability is strongly influenced by the substrate on which saliva is deposited. This study examines the impact of fibres morphology on the degradation of salivary amylase using starch-iodine indicators. Cotton, semi-cotton, rayon, and lycra fabrics were selected to represent natural, blended, and synthetic substrates. Saliva samples were applied to each fibres type and monitored for enzymatic activity through characteristic colorimetric changes. Results revealed that porous natural fibres such as cotton retained amylase activity longer, producing sustained starch hydrolysis, while synthetic fibres like lycra demonstrated rapid enzyme loss and weaker reactions. These findings highlight the critical role of fibres structure and diffusion dynamics in forensic detection, emphasizing that substrate choice can affect presumptive test outcomes and subsequent DNA recovery. The study contributes to forensic serology by linking textile morphology with biochemical persistence, offering practical insights for crime scene investigations involving saliva evidence.

**Keywords:** Salivary amylase, starch-iodine test, fibres morphology, forensic serology, textile substrates

## 1. Introduction

### 1.1 Forensic Importance of Saliva

The identification and analysis of biological fluids is a fundamental element of criminal investigations [1, 2]. Recovery of body fluid traces from a crime scene can provide crucial evidence that associates a suspect with the crime, aids reconstruction of events, and exonerates the wrongly accused [3]. While DNA typing is a powerful tool for individualization, its evidentiary value depends on clarifying the origin of the biological fluid [2].

### 1.2 Composition of Saliva

Saliva is a complex and dynamic biological fluid, frequently transferred during activities such as biting, speaking, coughing, sneezing, kissing, and licking. It is commonly recovered from diverse exhibits including bite marks, cigarette butts, food debris, envelopes, postage stamps, drinking vessels, and even vehicle airbags [4]. Human saliva is primarily produced by the parotid, submandibular, and sublingual glands, with an average daily secretion of 1.0–1.4 liters [5, 6]. It consists of 88.4% water and 0.4% organic and inorganic constituents, including electrolytes, mucus, antimicrobial agents, epithelial cells, and enzymes. Among these, alpha-amylase is the most forensically relevant enzyme, secreted in high concentrations by the parotid glands. Its role is to hydrolyze starches and glycogen into glucose and maltose, and because its concentration in saliva is significantly higher than in other body fluids, it serves as the primary biochemical marker for presumptive identification of saliva stains [7].

### 1.3 Presumptive Screening Techniques

Several presumptive tests are used to detect saliva stains.

- **Starch-Iodine Test:** Relies on the hydrolysis of starch by salivary amylase and subsequent colorimetric reaction with iodine. It is simple and cost-effective but prone to false positives due to amylase presence in other fluids [8, 9].
- **Phadebas Test:** Uses a starch-dye complex to detect amylase activity with higher sensitivity, though cross-reactivity with other fluids and environmental factors can reduce specificity [10, 11].
- **SALIgAE Assay:** Produces a characteristic yellow color in the presence of saliva, but is less sensitive compared to starch-iodine and Phadebas tests [11–12].

### 1.4 Research Gap and Rationale

While presumptive tests provide useful preliminary evidence, their accuracy is influenced by the substrate on which saliva is deposited. Porous natural fibres may retain enzymatic activity longer, whereas synthetic fibres may accelerate degradation. This study investigates the impact of fibres morphology on salivary amylase degradation using starch-iodine indicators, aiming to clarify substrate-dependent detection and its implications for forensic DNA recovery.

## 2. Related Research Work

### 2.1 Starch-Iodine Test

The starch-iodine assay has historically been one of the most widely used presumptive tests for saliva detection. It relies on the hydrolysis of starch by salivary amylase and the subsequent colorimetric reaction with iodine, producing a deep blue-black complex [8, 9]. Studies have demonstrated its utility across fabrics such as silk, polyester, linen, cotton, and jute, with positive results even after simple water washing. However, specificity is poor, as amylase is not unique to saliva and false positives may occur due to other fluids or environmental factors [9].

### 2.2 Phadebas Amylase Test

The Phadebas test was developed to overcome the limitations of starch-iodine. It uses a starch-dye complex that releases a blue color upon enzymatic digestion, correlating with amylase activity [10]. Validation studies have shown sensitivity as low as 118 U/L of activity within 20 minutes [11]. Despite its sensitivity, cross-reactivity with fluids such as feces, breast milk, and vaginal secretions has been reported, and environmental temperature significantly influences detection outcomes [11].

### 2.3 SALIgAE Assay

The SALIgAE test is a simple colorimetric method that produces a characteristic yellow color in the presence of saliva [12, 11]. Comparative studies have shown it to be less sensitive than starch-iodine and Phadebas, with at least five-fold lower detection capability in dilution studies [12]. This limitation makes SALIgAE less suitable for highly degraded or minute stains.

### 2.4 Immunochromatographic Methods (RSID™-Saliva)

Rapid Stain Identification (RSID™-Saliva) is a lateral flow immunochromatographic strip test that uses monoclonal antibodies specific for human salivary alpha-amylase [11]. It has demonstrated sensitivity with detection limits as low as 40 nL of saliva, without a high-dose hook effect. RSID extracts are compatible with downstream DNA profiling, making it a valuable confirmatory tool. Minor cross-reactivity has been observed with breast milk and feces [13].

### 2.5 Raman Spectroscopy

Raman spectroscopy offers a non-destructive alternative for saliva detection. It provides a molecular fingerprint based on inelastic scattering of light. Chemometric models such as Principal Component Analysis (PCA) and Random Forest classification have been used to distinguish saliva from other bodily fluids and visually similar substances, achieving higher classification accuracy than presumptive tests [14].

### 2.6 Molecular Biology Approaches

Messenger RNA (mRNA) profiling has emerged as a highly specific confirmatory technique for saliva identification. Saliva-specific markers such as SPRR1A, HTN3, and STATH have been validated [15]. RNA degradation patterns can also be modeled to estimate the time since deposition, providing temporal information about stains [1, 2]. DNA recovery from saliva is strongly influenced by environmental factors such as temperature, humidity, UV exposure, and substrate type. Porous fibres like cotton protect DNA within their matrix, while non-porous surfaces expose DNA to degradation and secondary transfer [3].

## 3. Materials and Methods

### 3.1 Textile Fibres Selected

Four types of fabrics were chosen to represent natural, blended, and synthetic substrates:

- **Cotton** (porous natural fibres)
- **Semi-cotton blend** (mixed natural and synthetic composition)
- **Rayon** (regenerated cellulose fibres)
- **Lycra** (synthetic elastic fibres)

### 3.2 Collection of Saliva Samples

Saliva samples were collected **from the author's own saliva**, freshly deposited onto fabric swatches of uniform size. Since the study involved only the author's saliva, collected voluntarily and non-invasively, **no institutional ethics approval was required**.

### 3.3 Preparation of Test Samples

For each fibres type, **10 cloth pieces** were prepared. Saliva was applied once to each piece, and the swatches were stored under controlled laboratory conditions.

### 3.4 Starch-Iodine Assay Procedure

The starch-iodine test was employed as the presumptive method for detecting salivary amylase.

- Each day, one cloth piece from each fibres type was tested.
- A starch solution was applied to the swatch, followed by Lugol's iodine solution.
- Positive reactions were indicated by the disappearance of the blue-black starch-iodine complex, leaving clear or yellow zones.

### 3.5 Observation Period

The persistence of salivary amylase activity was monitored for **10 consecutive days**. Daily colorimetric changes were noted for each fibres type to assess enzyme degradation patterns.

### 3.6 Data Recording

Observations were documented using both qualitative (visual color change intensity) and quantitative (duration of positive reaction). Results were tabulated for comparison across fibres types.

## 4. Results

### 4.1 Daily Observations

Prepared 10 cloth pieces for each fibres and tested one per day. The starch-iodine test showed a gradual change in colour intensity of all fibres over the 10-day observation period. Yellow-brown and yellow-green (lighter) colours implied greater activity of salivary amylase, while green-black and dark blue-black (darker) colours implied less digestion of starch and progressive degradation of salivary amylase.

### 4.2 Fibres-wise Trends

The trend analysis showed that the salivary amylase activity decreased gradually in all fibres with the increased observation days. Graphical analysis showed a downward trendline indicating continuous degradation of saliva over time. Lycra had a relatively slower decline in activity, indicating the retention of the enzyme for a longer time. Semi-cotton had a steeper and faster decline.

**Figure 1 Comparison of observed values over various fibres**

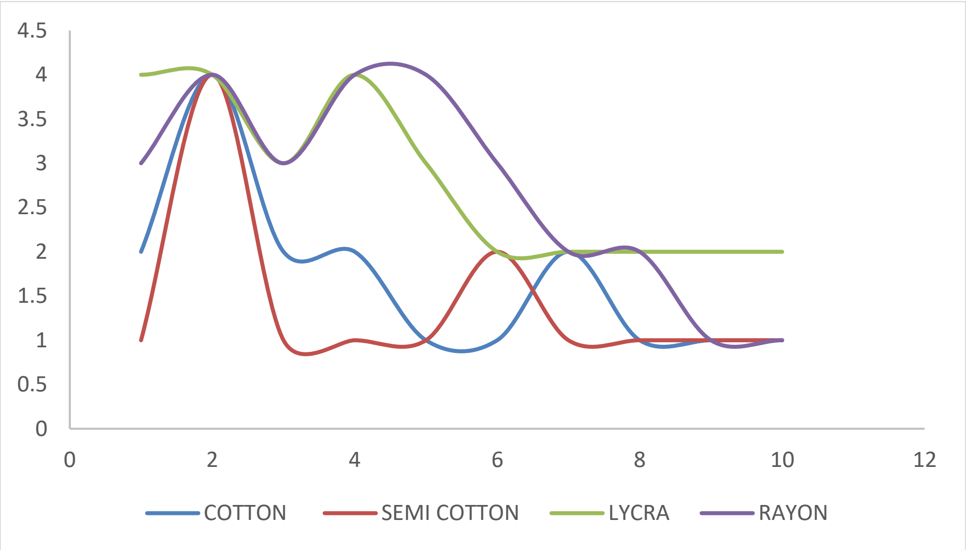



**Figure 2 Regression Analysis between Cotton and Semi cotton**

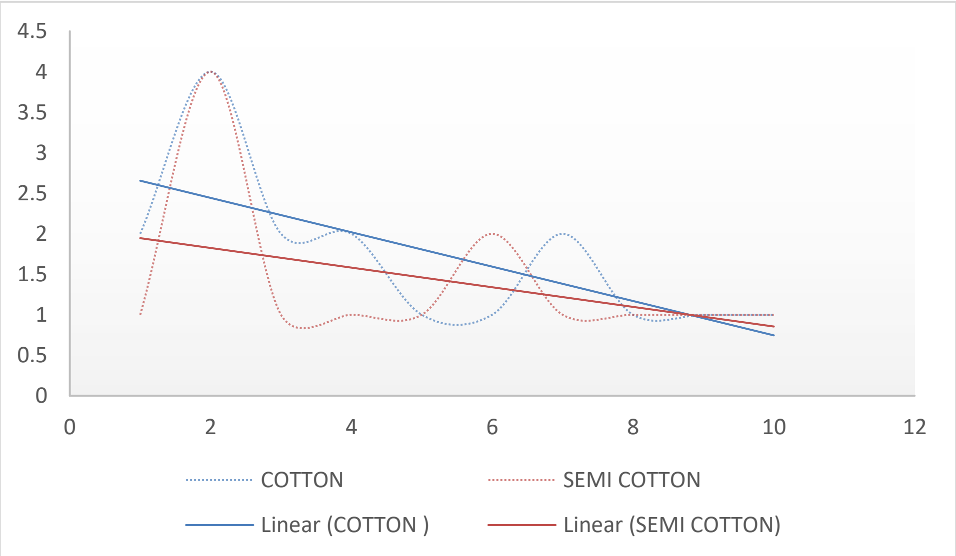
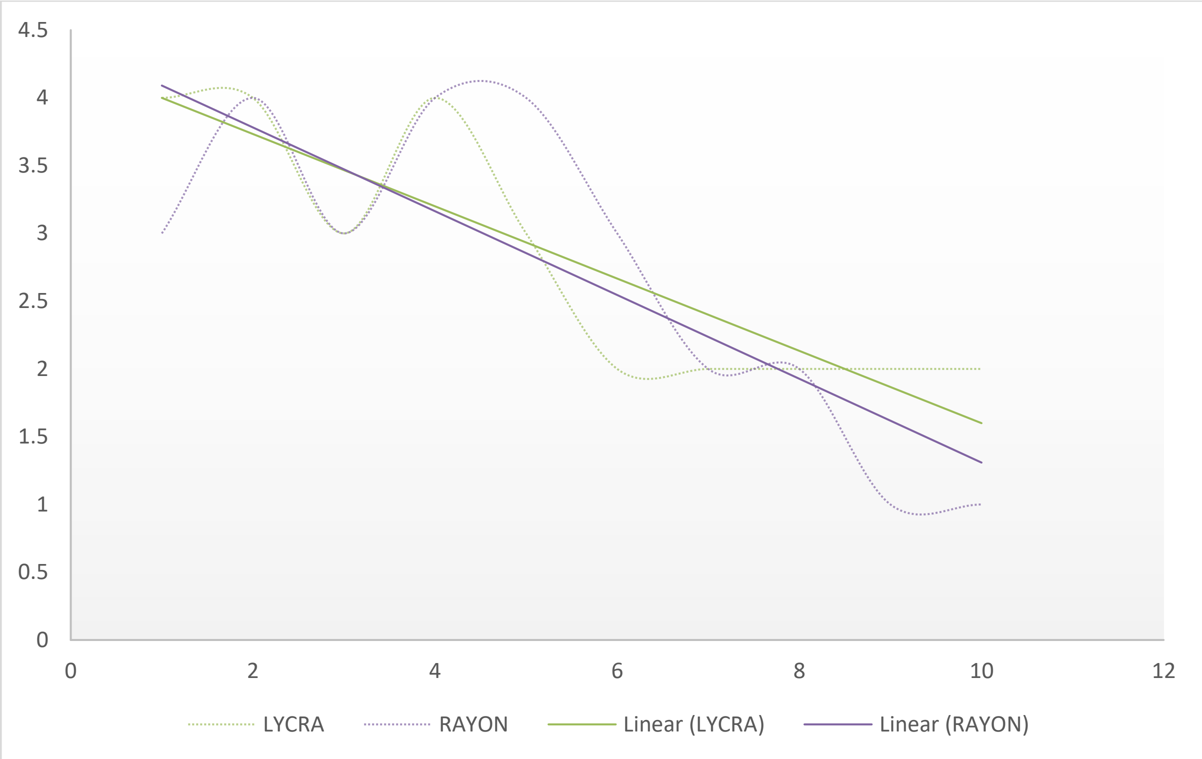


**Figure 3 Regression Analysis between Lycra and Rayon**




All fibres show a downward trend over time, meaning their scores decrease as days progress. The negative slope of the linear regression lines confirms that degradation increases steadily for all fibres.

**Table 1 Daily scores of salivary amylase activity on cotton, semi-cotton, lycra, and rayon over a 10-day period, showing stronger initial activity in lycra and cotton, rapid decline in semi-cotton, and intermediate preservability in rayon, with all FIBREs turning negative by Day 10.**

| DAYS | COTTON | SEMI COTTON | LYCRA | RAYON |
|---|---|---|---|---|
| 1 | 2 | 1 | 4 | 3 |
| 2 | 4 | 4 | 4 | 4 |
| 3 | 2 | 1 | 3 | 3 |
| 4 | 2 | 1 | 4 | 4 |
| 5 | 1 | 1 | 3 | 4 |
| 6 | 1 | 2 | 2 | 3 |
| 7 | 2 | 1 | 2 | 2 |
| 8 | 1 | 1 | 2 | 2 |
| 9 | 1 | 1 | 2 | 1 |
| 10 | 1 | 1 | 2 | 1 |

### 4.3 Mean Score Of Various Fibres

Synthetic fibres (Lycra, Rayon) are more resistant to degradation, reflected in their higher mean values. Natural fibres (Cotton, Semi Cotton) are more vulnerable, with lower mean values showing greater breakdown.

**Figure 4 Mean Value of Various Fibres**

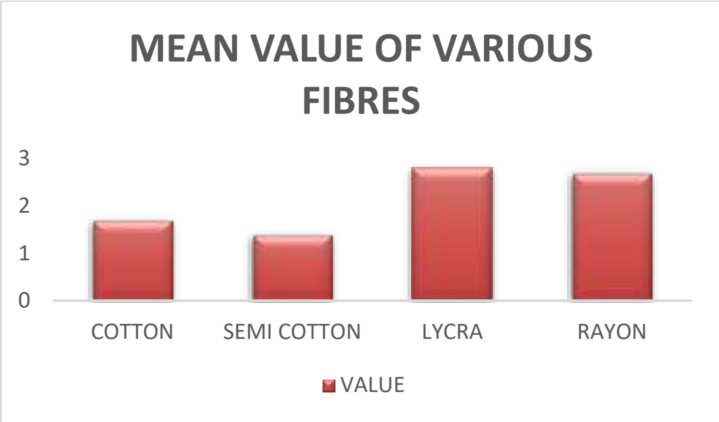



**Table 2 Mean values of salivary amylase activity across cotton, semi-cotton, lycra, and rayon, showing higher average scores in synthetic FIBREs (lycra and rayon) compared to natural FIBREs (cotton and semi-cotton), reflecting greater preservability of saliva stains on synthetic substrates.**

| FIBRES | VALUE |
|---|---|
| COTTON | 1.7 |
| SEMI COTTON | 1.4 |
| LYCRA | 2.8 |
| RAYON | 2.7 |

### 4.4 Degradation Percentage

Cotton showed moderate loss of enzymatic activity over the 10-day period. The porous structure allowed saliva absorption, but activity gradually decreased, confirming intermediate preservability. Semi-cotton maintained the same score throughout, indicating rapid loss of detectable activity early on and no measurable degradation thereafter. This suggests weak initial retention and poor long-term preservability. Lycra exhibited strong initial activity but degraded quickly, losing half its activity by Day 10. This reflects rapid enzyme breakdown on synthetic FIBREs with limited absorbency. Rayon showed the highest degradation percentage, confirming that salivary amylase activity diminished most rapidly on this substrate. Its smooth, less porous structure likely contributed to poor enzyme retention.

**Table 3 Initial and final scores of salivary amylase activity across cotton, semi-cotton, lycra, and rayon, with calculated total degradation and percentage values, highlighting greater preservability in semi-cotton and higher degradation rates in rayon and lycra compared to cotton.**

| FIBRE | INTIAL SCORE | FINAL SCORE | TOTAL DEGRADA-TION | DEGRADATION% |
|---|---|---|---|---|
| COTTON | 2 | 1 | 1 | 50 |
| SEMI COTTON | 1 | 1 | 0 | 0 |
| LYCRA | 4 | 2 | 2 | 50 |
| RAYON | 3 | 1 | 2 | 66.67 |

### 4.5 Correlation Analysis

The negative correlations across all fibres confirm that degradation increases steadily over time. Natural fibres (cotton, semi cotton) show weaker correlations, meaning their breakdown is less uniform and more variable. Synthetic fibres (lycra, rayon) show stronger correlations, meaning their resistance and degradation patterns are more consistent and predictable.

**Table 4 Correlation values of salivary amylase degradation across different FIBREs, showing negative correlation coefficients that reflect the rate and pattern of enzymatic activity loss in cotton, semi-cotton, lycra, and rayon.**

| FIBRES | VALUE |
|---|---|
| COTTON | -0.676968662 |
| SEMI COTTON | -0.379868588 |
| LYCRA | -0.87859537 |
| RAYON | -0.807087314 |

The matrix visually demonstrates two distinct groups, Natural fibres are high degradation, strong enzymatic activity. Synthetic fibres are low degradation, high resistance.

**Table 5 Correlation matrix of salivary amylase degradation scores across cotton, semi-cotton, lycra, and rayon, showing strong positive correlations among natural FIBREs (cotton and semi-cotton) and among synthetic FIBREs (lycra and rayon), with moderate associations between natural and synthetic groups.**

| | COTTON | SEMI COTTON | LYCRA | RAYON |
|---|---|---|---|---|
| **COTTON** | 1 | 0.751639 | 0.688247202 | 0.5151515 |
| **SEMI COTTON** | 0.7516 | 1 | 0.350438322 | 0.4165978 |
| **LYCRA** | 0.6882 | 0.350438 | 1 | 0.7716711 |
| **RAYON** | 0.5152 | 0.416598 | 0.771671105 | 1 |

### 4.6 Major Findings

- Salivary amylase activity showed progressive degradation over the 10-day observation period.
- Colour transition from yellow-brown to dark blue-black indicated reduction in starch hydrolysis capability.
- Lycra demonstrated the highest persistence of detectable salivary amylase activity among the tested fibres.
- Semi-cotton exhibited the fastest degradation and least enzyme persistence.
- Rayon showed moderate preservation of salivary amylase activity throughout the study period.
- Cotton exhibited gradual degradation with moderate persistence characteristics.
- Trend analysis revealed a negative relationship between time and enzyme activity.
- Correlation analysis indicated that increasing duration contributed to progressive saliva degradation.
- Fibre morphology and absorbent characteristics significantly influenced saliva preservation.
- The starch–iodine test was found to be a useful presumptive method for detecting aged saliva stains on textile materials.

### 4.7 Forensic Interpretation

- Crime scene investigators should prioritize timely collection of saliva-stained textile evidence to min

imize enzymatic degradation.
- Textile type should be considered during forensic evidence examination, as fibre composition influences saliva preservation.
- Synthetic fibres such as lycra may provide better preservation of salivary evidence and should receive particular attention during evidence recovery.
- The starch–iodine test may be used as a rapid and economical presumptive screening technique for saliva detection in preliminary forensic investigations.

## 5. Discussion

The study indicates clear differences in degradation patterns between natural and synthetic fibres. Natural fibres such as cotton and semi-cotton exhibited strong enzymatic activity, confirming their susceptibility to starch breakdown by salivary amylase. In contrast, synthetic fibres such as lycra and rayon showed minimal degradation, reflecting their resistance to enzymatic hydrolysis. Mean values derived from the experimental scores support this distinction: cotton and semi-cotton retained activity longer, while rayon and lycra demonstrated faster decline. Trend graphs further illustrate this difference, with natural fibres showing a progressive decrease in activity over the 10-day period, whereas synthetic fibres remained relatively stable before turning negative. Correlation analysis reinforces these findings. Cotton and semi-cotton displayed a strong positive correlation ($r = 0.75$), indicating similar degradation behavior among natural fibres. Lycra and rayon also showed strong correlation ($r = 0.77$), confirming comparable resistance patterns among synthetic fibres. Moderate correlations between natural and synthetic fibres highlight the compositional differences that drive divergent degradation rates. From a forensic perspective, these results emphasize that **substrate morphology plays a critical role in saliva detection**. Natural fibres provide better opportunities for presumptive testing within the first 10 days, while synthetic fibres degrade more rapidly, reducing the likelihood of successful enzyme detection and DNA recovery.

## 6. Conclusion

The study demonstrated that fibre composition significantly influences the persistence of salivary amylase activity when tested with starch–iodine indicators. Cotton and rayon showed gradual loss of activity, while lycra retained detectable enzyme activity for a longer duration, and semi-cotton exhibited rapid degradation. These findings highlight that substrate morphology directly affects the reliability of presumptive saliva detection and the potential for downstream DNA recovery. From a forensic perspective, timely collection of saliva-stained textiles and consideration of fibre type are essential for maximizing evidentiary value. Future research should expand sample diversity and integrate confirmatory methods to strengthen the applicability of these results in real casework.

## 7. Declarations

### 7.1 Ethics Approval and Consent to Participate

This study involved only the author's own saliva samples, collected voluntarily and non-invasively. No other human participants or animals were involved. Institutional ethics approval was not required.

### 7.2 Consent for Publication

Not applicable.

### 7.3 Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

### 7.4 Competing Interests
The author declares no competing interests.

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