

**DEVELOPMENT OF A NEW COLORIMETRIC SYSTEM
FOR MASTICATORY PERFORMANCE EVALUATION**

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**DEVELOPMENT OF A NEW COLORIMETRIC
SYSTEM FOR MASTICATORY PERFORMANCE
EVALUATION**

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DEVELOPMENT OF A NEW COLORIMETRIC SYSTEM FOR MASTICATORY PERFORMANCE EVALUATION

ABSTRACT

The two-color mixing ability test has been recently introduced for objective assessment of masticatory performance. However, the ideal bicolor specimens have not yet been identified, and the color analysis of digital images requires improvement. The purpose of this clinical study was to formulate a custom-made, two-color chewing gum and to develop a new image-processing method for color mixing analysis. Specimens of red-green (RG) chewing gum were prepared as a bicolor test food. A total of 300 gums were masticated by 20 healthy volunteers (mean age 21 ± 3.3 years) for 3, 6, 9, 15, and 25 mastication cycles. The boluses were retrieved, flattened to 1-mm-thick wafers, and scanned with a flatbed scanner. The digital images were analyzed using ImageJ software equipped with a custom-built plug-in Mastimeter[®]. Mastication Index (MI) was derived from the regression function using both spatial and value colorimetric parameters as predictors of the number of mastication cycles. Validity was assessed by the Pearson correlation between the Mastication Index and concurrent measurements using a previously well-established method, XYLITOL color-changeable gum, within a group of 10 dentate individuals and 10 denture wearers. Furthermore, the hardness and mass of RG chewing gum were measured before and after mastication. Hardness loss (%) and mass loss (%) were then calculated and compared with those of a commercially available chewing gum. Multiple regression analysis demonstrated a strong relationship between the color mixture and the number of mastication cycles ($r^2=0.81$, $P<.001$). Independent samples *t*-test showed a significant difference in MI between dentate individuals and denture wearers ($P<.001$). Significant correlation was observed between the MI and XYLITOL outcomes ($r=0.82$, $P<.001$). The hardness loss and mass loss of RG chewing gum were significantly lower than those of the commercial chewing gum ($P<.001$).

In conclusion, Mastimeter[®] seems valid and has the potential for evaluating masticatory performance in both the research and clinical settings.

Keywords: Mastication, digital assessment, two-color specimen, image processing, diagnostic technique.

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PEMBANGUNAN SISTEM KOLORIMETRI BARU UNTUK PENILAIAN KEBOLEHAN MENGUNYAH

ABSTRAK

Ujian keupayaan pencampuran 2-warna telah diperkenalkan sejak kebelakangan ini sebagai penilaian secara objektif kebolehan mengunyah. Walau bagaimanapun, spesimen dua warna yang ideal belum dapat dikenal pasti, dan analisis warna imej digital masih memerlukan penambahbaikan. Tujuan kajian klinikal ini adalah untuk merumuskan gula-gula getah dua-warna yang direka khas untuk menghasilkan kaedah pemprosesan imej bagi analisis pencampuran warna yang lebih baik. Spesimen gula-gula getah merah-hijau (RG) telah disediakan sebagai makanan ujian dua-warna. Sebanyak 300 gula-gula getah telah dikunyah oleh 20 subjek berusia muda bagi 3, 6, 9, 15, dan 25 jumlah kitaran. Bolus selepas kunyahan diambil, diratakan menjadi wafer setebal 1 mm dan diimbis dengan pengimbas rata. Imej digital dianalisa dengan menggunakan perisian ImageJ yang dilengkapi dengan plug-in yang dicipta khas Mastimeter[®]. Data pencampuran warna dianalisa dengan analisis regresi berganda menggunakan kedua-dua parameter ruang dan nilai warna sebagai peramal bilangan kitaran pengunyah. Kesahihan dinilai menggunakan korelasi Pearson diantara ujian Mastimeter[®] dan kaedah gula-gula getah berubah warna pada sekumpulan 10 subjek bergigi penuh dan 10 subjek yang memakai gigi palsu. Disamping itu, kekerasan dan jisim gula-gula getah RG, sebelum dan selepas pengunyah, juga diukur. Kehilangan kekerasan (%) dan kehilangan jisim (%) kemudiannya dikira dan dibandingkan dengan gula-gula getah yang boleh didapati di pasaran. Analisis regresi berganda menunjukkan hubungan yang kuat di antara parameter pencampuran warna dan bilangan kitaran pengunyah ($r^2=0.81$, $P<.001$). Ujian- t sampel bebas menunjukkan perbezaan ketara pada skor Mastimeter[®] di antara subjek bergigi penuh dan pemakai gigi palsu penuh ($P<.001$). Korelasi yang ketara telah ditemui di

antara keputusan Mastimeter[®] dan gula-gula getah berubah warna ($r=0.82$, $P<.001$). Kehilangan kekerasan dan kehilangan jisim gula-gula getah RG adalah jauh lebih rendah berbanding gula-gula getah yang berada dipasaran ($P<.001$). Sistem Mastimeter[®] mempunyai potensi untuk menilai kebolehan mengunyah samada bagi tujuan penyelidikan dan ketika sesi klinikal.

Kata kunci: Kebolehan mengunyah, keupayaan mencampurkan makanan, gula-gula getah dua warna, kolorimetri, teknik diagnostik.

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I wish to dedicate this work to my beloved mother Imane.

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LIST OF SYMBOLS AND ABBREVIATIONS

A	:	Area
ANOVA	:	Analysis of variance
ATP	:	Adenosine triphosphate
BFI	:	Bolus formation index
CIELab	:	International commission on illumination Lab color space
DMFT	:	Index of decayed, missing, and filled teeth
EMG	:	Electromyography
GD	:	Geometric dispersion
H_i	:	Initial hardness
H_f	:	Final hardness
HSI	:	Hue, saturation, and intensity color space
M_i	:	Initial mass
M_f	:	Final mass
MAI	:	Mixing ability index
MC	:	Mastication cycles
MI	:	Mastication index
MP	:	Masticatory performance
P	:	Perimeter
PVC	:	Polymerizing vinyl chloride
Q_a	:	Iso-areal quotient
R-group	:	Reference group of dentate volunteers
RG	:	Red-green chewing gum
RGB	:	Red, green, and blue color space
ROI	:	Region of interest

SA	:	Surface area
SA:Vol	:	Surface-area-to-volume ratio
SD	:	Standard deviation
SDHue	:	Standard deviation of Hue
SM	:	Wrigley's Spearmint chewing gum
T-group	:	Test group
TIF	:	Tagged image file format
TMJ	:	Temporomandibular joint
UF	:	Unmixed fraction
VAS	:	Visual analog scale
Vol	:	Volume
X ₅₀	:	Median particle size

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CHAPTER 1: INTRODUCTION

1.1 Background

Rehabilitation of masticatory function is a chief purpose of prosthodontics. Therefore, an objective method for quantifying masticatory function and monitoring prosthodontic treatment outcomes might be useful for dentists in both research and clinical domains. The process of measuring masticatory function “mastimetry” has been dominated by the use of either comminution tests or questionnaires. In the Glossary of Prosthodontic Terms, masticatory performance (MP) has been defined as “a measure of the comminution of food attainable under standardized testing conditions” (The Academy of Prosthodontics, 2017).

Various natural foods (peanuts, hazelnuts, carrots, apples, almonds, etc.) and artificial substances (silicone, gelatin, alginate, etc.) have been used for evaluating an individual’s ability to grind and pulverize test food specimens (Kaya, Guclu, Schimmel, & Akyuz, 2017). The particle size distribution of the resulting boluses has been measured using fractional sieving analysis and regarded as the gold standard for MP assessment (Silva, Nogueira, Rios, Schimmel, & Leles, 2018). However, as the procedures are relatively complicated and time-consuming, the comminution test is uncommon in clinical settings (Vaccaro, Pelaez, & Gil-Montoya, 2018).

Researchers have proposed simple and convenient alternatives on this matter, such as evaluating an individual’s ability to mix and knead a cohesive bolus of test food with two different colors (Liedberg, Spiechowicz, & Öwall, 1995; Liedberg, Stoltze, & Owall, 2005). The rationale for this method is that the higher the degree of two-color mixing, the higher the masticatory performance. The studies of concurrent validity have reported a significant correlation between the comminution ability and mixing ability tests, and

recommended the latter as especially suited for evaluating masticatory performance in pediatric and geriatric subjects (Kaya et al., 2017; van der Bilt, 2011; van der Bilt, Mojet, Tekamp, & Abbink, 2010).

Two-color chewing gum (Silva et al., 2018; van der Bilt et al., 2010; Weijenberg et al., 2013), and paraffin wax (Salleh, Fueki, Garrett, & Ohyama, 2007; van der Bilt, Speksnijder, de Liz Pocztaruk, & Abbink, 2012) have been used as test materials for assessing mixing ability. The level of color mixture has been assessed by either visual inspection or digital image analysis (Endo et al., 2014; Schimmel, Christou, Herrmann, & Muller, 2007); the digital method was recommended (Silva et al., 2018; van der Bilt et al., 2012).

The digital analysis involves computing unmixed area fraction of the specimen (Elsig et al., 2015; Schimmel et al., 2011), and variance of the color image histogram (Remijn, Vermaire, Nijhuis-van de Sanden, Groen, & Speksnijder, 2018), among other colorimetric parameters (Vaccaro, Pelaez, & Gil, 2016; Weijenberg et al., 2013). The measurements were performed using different software packages; such as image retouching software (Photoshop; Adobe Systems Inc, San Jose, CA, USA) (de Groot et al., 2019; Remijn et al., 2018), scientific image-analysis program (ImageJ; US National Institutes of Health, MD, USA) (Palomares, Montero, Rosel, Del-Castillo, & Rosales, 2018; Yousof, Salleh, & Yusof, 2019), computational software (Mathematica; Wolfram Research, Champaign, IL, USA) (Weijenberg et al., 2013), numerical analysis and programming language (MATLAB; MathWorks Inc, Natick, MA, USA) (Vaccaro et al., 2018; Vaccaro et al., 2016), specialized software (ViewGum; dHAL Software, Kifissia, Greece) (Kaya et al., 2017; Schimmel et al., 2015), and smartphone application (Hue-Check Gum App; ARTORG CENTER, University of Bern, Switzerland) (Buser et al., 2018).

1.2 Statement of Problem

Previous researchers used bicolor combinations of commercially available chewing gum to perform the mixing ability test and introduced computer-aided methods to quantify the level of color mixture of the bolus's digital images. Unfortunately, finding optimal bicolor specimens is a nontrivial task, in view of the fact that most manufacturers no longer add artificial colorings to their gums. Therefore, developing a custom-made, two-color chewing gum might be useful in this regard.

On the other hand, most proposed methods of image-analysis focused specifically on the heterogeneity of color values, regardless of the spatial distribution of the digital pixels (Halazonetis, Schimmel, Antonarakis, & Christou, 2013; Schimmel et al., 2015; Speksnijder, Abbink, van der Glas, Janssen, & van der Bilt, 2009). However, the spatial positioning of the pixels can be a fundamental parameter of the mixture characteristics (Fig. 1.1).

Moreover, none of the previous studies have combined both spatial and value parameters in the colorimetric analysis of the image, even though the combination of the two parameters in a comprehensive approach could produce a more in-depth analysis and more precise outcomes. Furthermore, to quantify the patients' masticatory handicap in relation to healthy individuals, a clinically interpretable mastication index needs to be identified.

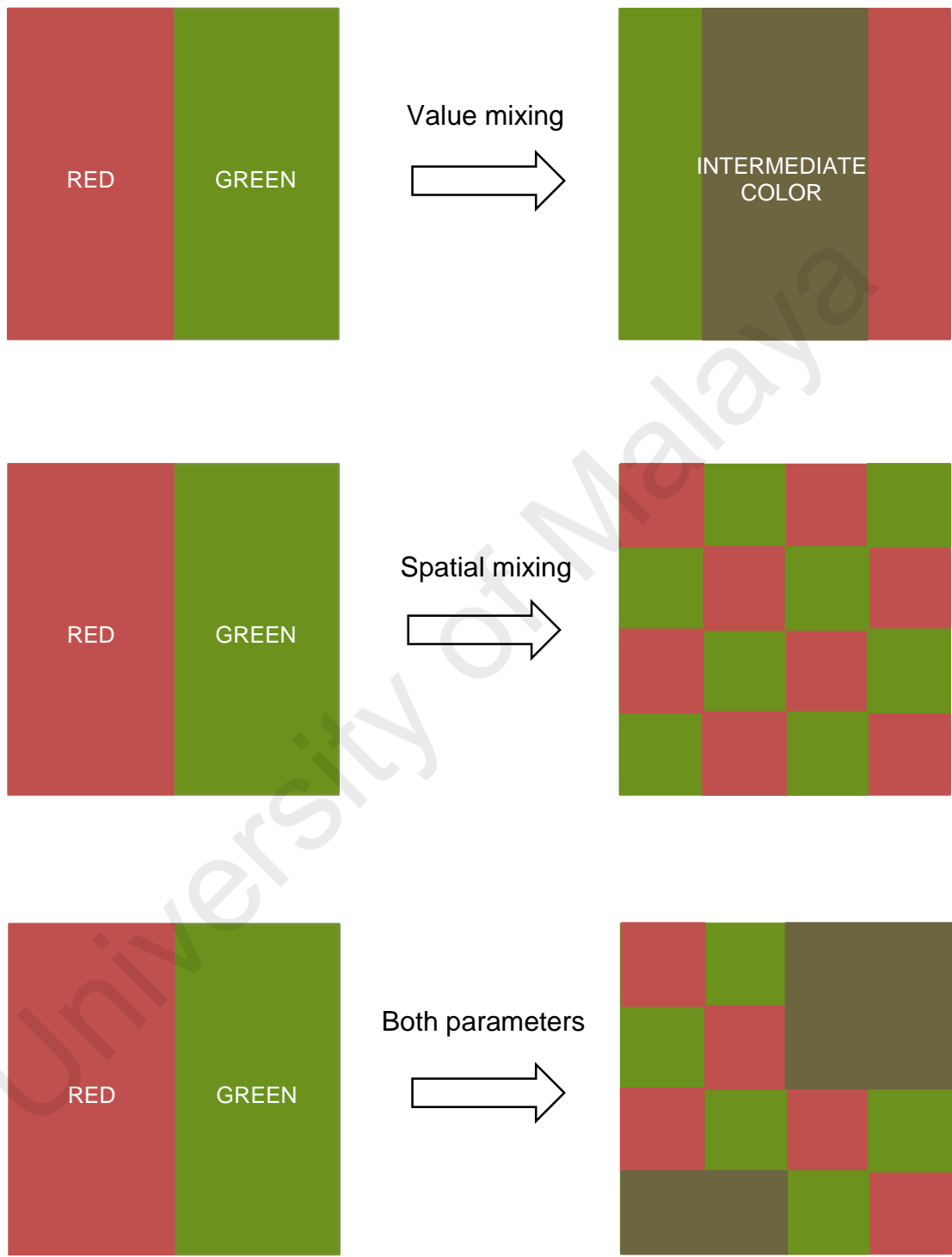


Figure 1.1: Diagram of color mixing parameters

1.3 Purpose of Study

The aim of this study was to develop a standardized system for masticatory performance assessment based on the holistic spatial-value analysis of two-color mixing ability.

The following five objectives were drawn:

1. To formulate a custom-made, two-color chewing gum for the mixing ability test.
2. To develop a new image-processing method for the spatial-value analysis of color mixing.
3. To combine both the spatial and value outcomes of color mixing in a one-dimensional mastication index, with clinical interpretability.
4. To investigate the validity of this new mastication index for masticatory performance assessment.
5. To evaluate textural characteristics of the new two-color chewing gum.

1.4 Research Hypotheses

The following five null hypotheses were tested:

1. The proposed colorimetric parameters (spatial and value) would not be able to discriminate among the groups of different numbers of mastication cycles.
2. There is no relationship between the degree of color mixing and the number of mastication cycles.
3. The new system Mastimeter[®] would not be able to discriminate among different dental conditions.
4. The results of the new system are not correlated with the measurements of a previously well-established method for evaluating masticatory performance.
5. The textural characteristics of the custom-made, two-color chewing gum are not significantly different from those of a commercially available chewing gum.

CHAPTER 2: LITERATURE REVIEW

2.1 Mastication Function

Mastication is defined as “the process of chewing food for swallowing and digestion” (The Academy of Prosthodontics, 2017). It represents the first step of the process of digestion and is meant to manipulate food substances and prepare it for deglutition and further digestive processes. During mastication, the solid or semisolid food is crushed and broken down into small particles and mixed with saliva in order to form a cohesive and slippery bolus ready to be swallowed (Posnick, 2014; van der Bilt, 2012).

Mastication is a complicated physiological function of the orofacial system, that requires the involvement of numerous structures like dentition, periodontium, tongue, lips, cheeks, palate, salivary glands, jaw muscles as well as temporomandibular joints. All of these different structures are functioning under close control and coordination by the central nervous system. If one component of the masticatory system is missing or is improperly working, the mastication capacity will be deteriorated (Laguna, Sarkar, & Chen, 2017).

Masticatory function has been related to the nutritional status, general well-being and quality of life, especially in elderly people (de Oliveira & Frigerio, 2004; Gil-Montoya, de Mello, Barrios, Gonzalez-Moles, & Bravo, 2015; Gonçalves, Campos, & Garcia, 2015). However, some controversies still exist regarding the strength of evidence and the type of relationship between the masticatory performance and the nutrient intake (Flores-Orozco et al., 2016; N'Gom & Woda, 2002; Wallace et al., 2018).

There are several factors that can potentially influence the masticatory performance, such as the number of natural teeth, malocclusion, periodontal status, salivary flow,

maximum bite force and prosthodontic rehabilitation (Ikebe et al., 2012; Ikebe et al., 2011; Kosaka et al., 2014; Magalhães, Pereira, Marques, & Gameiro, 2010; Wallace et al., 2018). Tooth loss is one of the major factors of the impairment of masticatory function (Hatch, Shinkai, Sakai, Rugh, & Paunovich, 2001; Ikebe et al., 2006). In order to restore this function, several strategies of prosthodontic treatment have been proposed to replace the missing teeth. However, edentulous people suffer from a significant masticatory handicap which may partially be compensated by dental prostheses (Moore & McKenna, 2016; Schimmel, Memedi, Parga, Katsoulis, & Müller, 2017; Wallace et al., 2018).

A wide variety of methods had been reported in the medical literature to evaluating the masticatory function (Elgestad Stjernfeldt, Sjögren, Wårdh, & Boström, 2019; Feine & Lund, 2006; Kimoto, Ogawa, Garrett, & Toyoda, 2004; Oliveira et al., 2014). For instance, measuring particle size distribution of comminuted food using a stack of graduated sieves (de Morais Tureli, de Souza Barbosa, & Gavião, 2010; Fontijn-Tekamp et al., 2000; van der Bilt et al., 2010), optical scanning and digital image analysis of masticated particles (Eberhard, Schneider, Eiffler, Kappel, & Giannakopoulos, 2015; van der Bilt, van Der Glas, Mowlana, & Heath, 1993), color change of chewing gum (Tarkowska, Katzer, & Ahlers, 2017; Wada, Kawate, & Mizuma, 2017), spectrophotometric determination of the dye released from a test food specimen (Käyser & van der Hoeven, 1977; Nokubi et al., 2010), and subjective assessment of masticatory performance using questionnaires of food preference or patient satisfaction (David & Finbarr, 2007; H Koshino et al., 2006; Hisashi Koshino et al., 2008).

This chapter reviews the most common methodologies for objectively evaluating masticatory performance, with special focus on elaborating the colorimetric methods (Fig. 2.1).

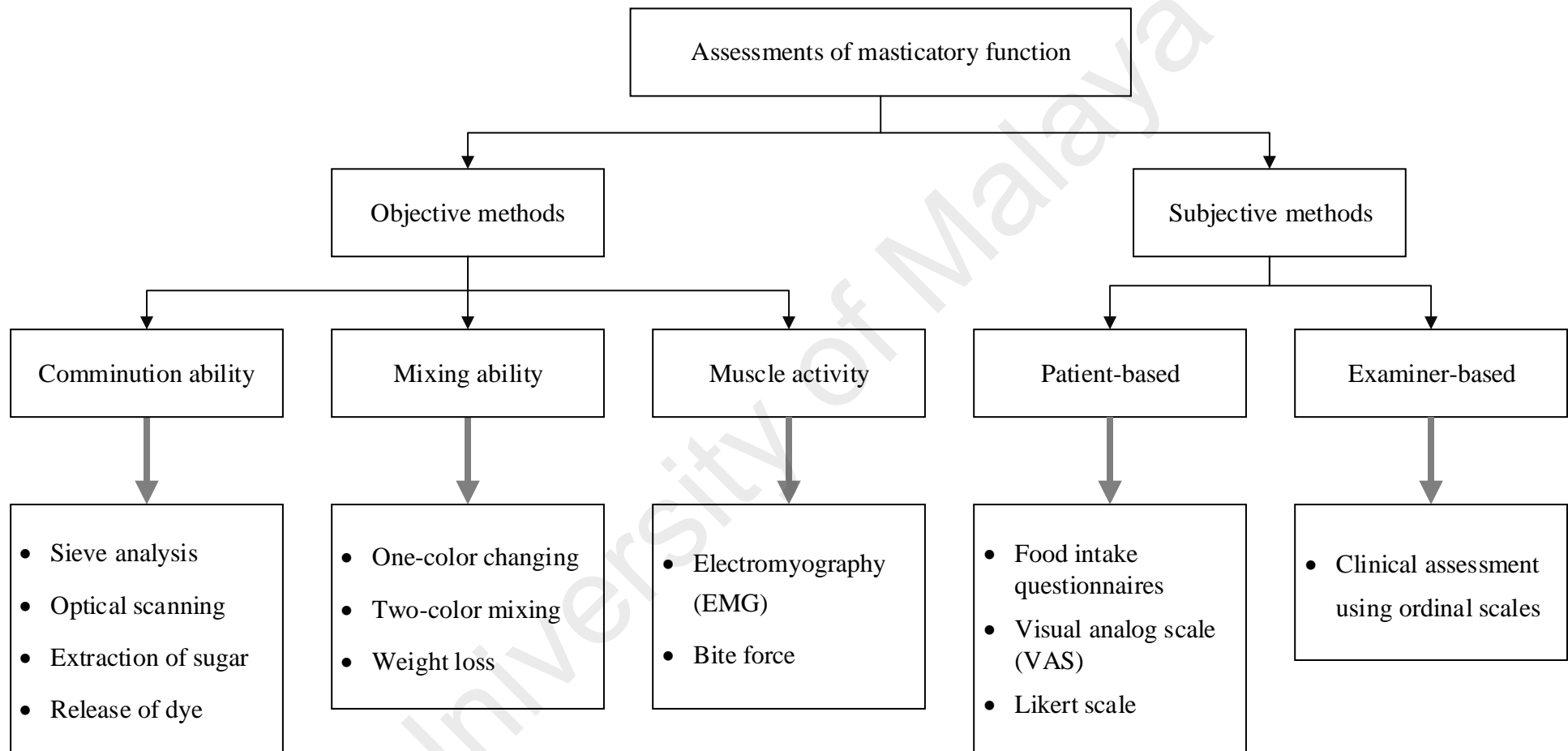


Figure 2.1: Categories of methods commonly used to evaluate masticatory function

2.2 Terminology of Masticatory Performance

The terms *masticatory performance*, *masticatory efficiency*, *masticatory ability*, *masticatory efficacy*, and *masticatory capacity* are frequently used in the literature to express the ability of person to masticate food (Kimoto et al., 2004). In general, masticatory performance (MP) refers to the objective assessment of masticatory function, whilst masticatory ability refers to the individual's self-assessment of his or her masticatory function (Boretti, Bickel, & Geering, 1995; van der Bilt, 2012). The Glossary of Prosthodontic Terms has defined the masticatory performance as "a measure of the comminution of food attainable under standardized testing conditions" (The Academy of Prosthodontics, 2017). In other words, masticatory performance refers to the individual's ability to grind or pulverize a specimen of test food with a predetermined number of mastication cycles (Oliveira et al., 2014). Some authors have included mixing of food within the definition of masticatory performance "a method that assesses an individual's ability to *mix* or *comminute* food/artificial food bolus" (Elgestad Stjernfeldt, Wårdh, Trulsson, Faxén Irving, & Boström, 2017).

The term masticatory efficiency has been used interchangeably with the term masticatory performance (Kapur & Soman, 2006). The Glossary of Prosthodontic Terms has defined the masticatory efficiency as "the effort required to achieve a standard degree of comminution of food" (The Academy of Prosthodontics, 2017). Hence, masticatory efficiency refers to the amount of mastication necessary to achieve a certain level of grinding and pulverizing of test food, independently of the number of mastication cycles (Oliveira et al., 2014). However, there is a lack of consensus among authors on the exact semantics of these terms (Kimoto et al., 2004; Oliveira et al., 2014).

2.3 Evaluation Methods of Masticatory Performance

Given the importance of masticatory function assessment, various methods have been described in the literature for quantifying the masticatory capacity (Fig. 2.1). In the majority of studies, individual's ability to grind or pulverize natural or artificial food specimens has been determined using the sieve analysis (van der Bilt, 2011; van der Bilt et al., 1993).

2.3.1 Fractional Sieving Analysis

The most common method for evaluating masticatory performance has been to measure particle size distribution of comminuted food using a stack of sieves with decreased apertures (Elgestad Stjernfeldt et al., 2017; Kimoto et al., 2004; Lucas & Luke, 1983; Olthoff, van der Bilt, Bosman, & Kleizen, 1984; Slagter, Bosman, & van der Bilt, 1993). Natural foods (almonds, peanuts, hazelnuts, carrots, apples, etc.) or artificial test foods (alginate-based, gelatin-based, or silicone-based materials such as Optocal and Optosil) have been masticated for a given number of mastication cycles or time period. Food bolus was then sieved, and particle size distribution was analyzed (Barbosa, Tureli, Nobre-dos-Santos, Puppini-Rontani, & Gavião, 2013; Marquezin, Kobayashi, Montes, Gavião, & Castelo, 2013; Marquezin et al., 2016; Sugiura, Fueki, & Igarashi, 2009; Woda et al., 2010).

The rationale of this technique is that the greater the proportions of chewed particles reaching the smaller mesh sieves, the better it is the masticatory performance. The amount of particles on each sieve and on the bottom plate are captured and quantified by weight. The weight of test food on individual sieves is expressed as a percentage of total weight, and the cumulative percentages are calculated, which can directly indicate to the degree of fragmentation of the food (Manly & Braley, 1950; Mishellany-Dutour, Renaud,

Peyron, Rimek, & Woda, 2008). The cumulative function may also be generated using a curve-fitting approach with a Rosin-Rammler equation to determine the median particle size (X_{50}), which is the aperture size of a theoretical sieve through which 50% of the weight of the comminuted material could pass. The value of X_{50} is inversely proportional to the masticatory performance (Aras, Hasanreisoglu, & Shinogaya, 2009; Barrera, Buschang, Throckmorton, & Roldan, 2011; Câmara-Souza, Figueredo, & Rodrigues Garcia, 2018; de Matos et al., 2010; Khoury-Ribas, Ayuso-Montero, Rovira-Lastra, Peraire, & Martinez-Gomis, 2018).

Although the general methodology of sieving analyses is similar (Table 2.1), the protocols may slightly differ in several points, such as:

- The natural/artificial test food.
- The given number of mastication cycles, or mastication period.
- The number of sieves and the aperture size.
- Washing of the test material while it passes through the sieves, or vibrating of the sieve-system containing dried particles.
- Quantification method and mathematical expression of the particle size distribution.

Table 2.1: Summary of studies investigating masticatory performance using comminution tests

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Umino et al., 2003	Adenosine triphosphate (ATP) enteric-coated granules; total 5 g	Post-maxillectomy patients with maxillary prostheses; N=43	50 mastication cycles	The amount of ATP eluted from the granules in a solution was determined with a spectrophotometer	Absorbance units
Shiga et al., 2006	Gummy jelly; Cylinder-shaped (10 mm diameter and 10 mm height); weight: 2 g	Natural dentate subjects (mean age 24.6 years); N=20	20 seconds	Glucose extraction from gummy jelly during mastication. Glucose concentration measured with blood glucose meter	Glucose concentration <i>mg/dl</i>
Fauzza & Lyons, 2008	Irreversible hydrocolloid impression material (alginate); column-shaped (12 mm diameter and 12 mm height)	Complete denture wearers (age 64–83 years); N=20	10 or 20 mastication cycles	Fractional sieving analysis; stack of 4 sieves	Percentage of particle weight for each sieve to the total particle weight (%)
Tureli et al., 2010	Optocal; 17 cubes (edge size 5.6 mm); total size: 3 cm ³	Children from 8 to 12 years old; N=97	20 mastication cycles	Fractional sieving analysis; stack of 10 sieves	Median particle size X ₅₀ ; Broadness of distribution
van der Bilt et al., 2010	Optocal and Optosil; 17 cubes (edge size 5.6 mm); total size: 3 cm ³	Elderly subjects (age 72 years); N=20 Young subjects (age 24 years); N=20	15 mastication cycles	Fractional sieving analysis; stack of 9 sieves	Median particle size X ₅₀

Continues

Table 2.1 Continued

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Eberhard et al., 2012	Optosil Comfort; 17 cubes (edge size 5.6 mm); total size: 3 cm ³	Natural dentate subjects (mean age 24 years); N=20	15 mastication cycles	Fractional sieving analysis; stack of 10 sieves Optical scanning and digital image processing; ImageJ software	Median particle size X ₅₀
Bessadet et al., 2013	Raw carrots; 4 g Peanuts; 4 g	Partially edentulous subjects with and without RPD; age from 24 to 79 years; N=19	Until swallowing	Optical scanning and digital image processing; PowderShape software	Median particle size X ₅₀ ; mastication time; number of mastication cycles; chewing frequency
Kosaka et al., 2014	Gummy jelly; 20×20×10 mm ³ ; weight: 5.5 g	Residents in Suita City in Japan (mean age 67 years); N=1623	30 mastication cycles	Concentration of extracted sugar is converted into surface area of particles using regression formula	Surface area mm ²
Meena et al., 2014	Raw carrots; 10 g	Bilateral missed lower molars before/after implant restoration; N=10 Complete natural dentate subjects; N=10	20 mastication cycles	Measuring the released dye from masticated raw carrots with a spectrophotometer	Optical density nm
Niwatcharoenchaiikul et al., 2014	Peanuts; 3 g	Complete denture wearers (age 67 years); N=10	20, 40 and 60 mastication cycles	Fractional sieving analysis; stack of 12 sieves	Median particle size X ₅₀

Continues

Table 2.1 Continued

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Sanchez-Ayala et al., 2016	Encapsulated fuchsine beads Optosil; 17 cubes (edge size 5.6 mm); total 3.4 g	Natural dentate subjects (mean age 23 years); N=20	20 mastication cycles	Fuchsine dye, released from mastication, was quantified with a spectrophotometer Fractional sieving analysis; stack of 10 sieves	Dye concentration $\mu\text{g/ml}$ Median particle size X_{50}
Tsuneoka et al., 2017	Chewing gum containing spherical resinous microparticles; Examastica Co.	Patients before and after orthognathic surgery (mean age 28 years); N=18	25 mastication cycles	Bolus is flattened and spherical particles per unit area are counted microscopically	Crushing ratio of microparticles %

For measuring particle size distribution of test food, single-sieve methods have been used to quantify the weight or volume percentage of masticated bolus that passes through a sieve with a specified aperture size (Kapur & Soman, 2006; Ow, Carlsson, & Karlsson, 1998). However, multiple-sieve systems have been recommended as it provides more detailed and precise estimation of the particle size distribution (van der Bilt & Fontijn-Tekamp, 2004).

Optical scanning of comminuted particles with a conventional flatbed scanner and digital image analysis has been used for estimating the median particle size in place of sieving method (Bessadet et al., 2013; Eberhard et al., 2012; Eberhard et al., 2015; Mowlana, Heath, & Auger, 1995; Mowlana, Heath, van der Bilt, & van der Glas, 1994; van der Bilt et al., 1993).

Fractional sieving analysis remains today the gold standard for masticatory performance assessment (Vaccaro et al., 2016; Wallace et al., 2018). However, as the procedures are time-consuming and necessitate a specialized laboratory setting, the use of sieving test is still limited only to research purposes (Schimmel et al., 2007; Schimmel et al., 2015).

2.3.2 Colorimetric Methods

A variety of colorimetric methods have been described in dental literature to evaluate masticatory performance (Fig. 2.2), such as the spectrophotometric measurement of dye release after masticating raw carrots (Awinashe & Nagda, 2010; Käyser & van der Hoeven, 1977; Meena et al., 2014) or dye-containing artificial test food (Nakasima, Higashi, & Ichinose, 1989; Nokubi et al., 2010; Shiratsuchi, Kouno, & Tashiro, 1991), the colorimetric assessment of a color-changing chewing gum (Hayakawa, Watanabe, Hirano, Nagao, & Seki, 1998; Matsui et al., 1996; Tarkowska et al., 2017), and determining the mixing degree of two-color chewing gum (Kaya et al., 2017; Liedberg & Owall, 1995; Schimmel et al., 2007).

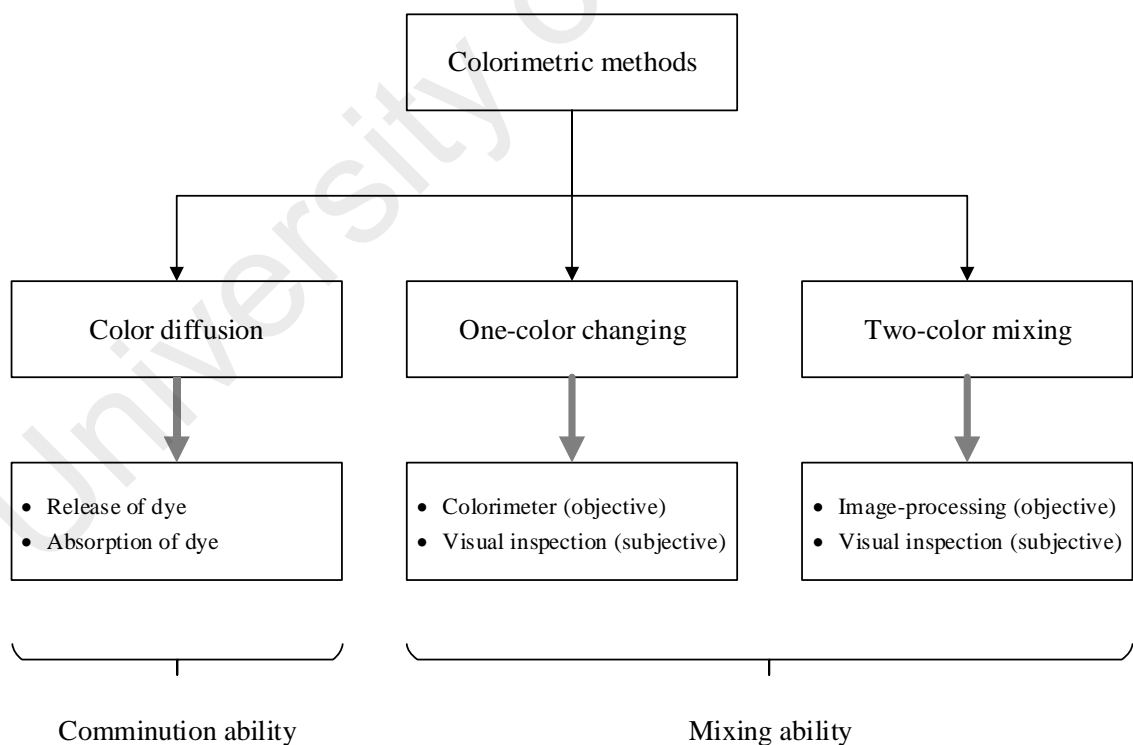


Figure 2.2: Categories of colorimetric methods used for evaluating masticatory performance

2.3.2.1 Color Diffusion

As a result of food mastication, the total surface area of food bolus increases substantially, i.e. comminution and fragmentation of food into small particles raises the surface-area-to-volume ratio (Fig. 2.3). It has been shown that the rate of substance diffusion into or out of an object is directly proportional to the surface-area-to-volume ratio (Taylor & Jones, 2009). The more surface area that substance can pass through, the more effective diffusion.

The concentration of dye in a solution of pigmented test food items is linearly correlated with the rate of color diffusion, which is in turn correlated with the overall surface area of test material. Such concentration corresponds to the surface area of food particles, i.e. the degree of comminution and break down of food determines the diffusion rate. The concentration of dye may be quantified with a spectrophotometer and used as a fragmentation indicator.

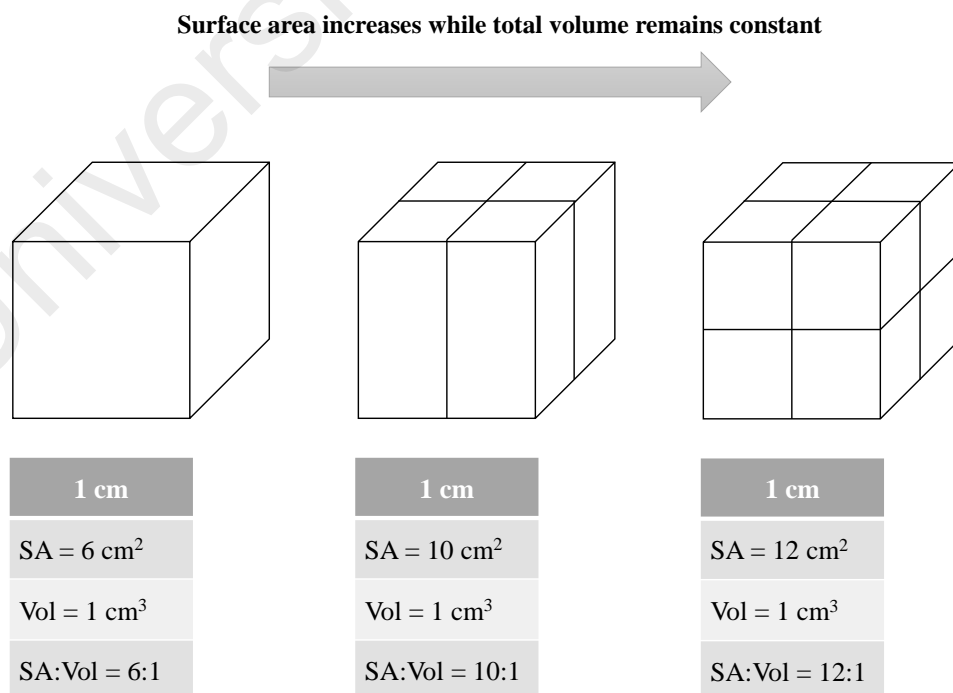


Figure 2.3: Surface-area-to-volume ratio

Raw carrots naturally contain beta-carotene which is responsible for the orange color of the carrots. When the vegetable is masticated, the dye is released from the carrot particles. The concentration of this dye can be determined using a spectrophotometer at 530 nm. The spectrophotometric analysis of masticated carrots to evaluate the masticatory performance has been described in the literature few times (Käyser & van der Hoeven, 1977; Meena et al., 2014).

The individual was asked to masticate a homogeneous piece of raw carrots for a given number of cycles without swallowing the particles of carrots or the saliva produced during the process of mastication. After mastication completion, the bolus and saliva were expectorated in a graduated cylinder. Water was added to 25 ml and the cylinder was stirred mechanically for 10 minutes. The contents were filtered and the solution was studied under a spectrophotometer at 530 nm. The masticatory performance was expressed in terms of optical density (Awinashe & Nagda, 2010; Käyser & van der Hoeven, 1977; Meena et al., 2014).

The samples of natural food are inconsistent in terms of hardness, texture and color. For instance, the concentration of carotene dye may vary between carrots and within carrots. Whereas, the artificial test items like gelatin are standardized regarding the texture and color. A measurement technique has been developed by a Japanese research group to evaluate the release of dye when masticating β -carotene-containing gummy jelly (Nokubi et al., 2010; Nokubi et al., 2013). However, the latter method requires a special measuring device, and the measuring procedures should be strictly controlled, such as water temperature and times for rinsing and dissolving gummy jelly.

Saliva and test food particles may be swallowed accidentally during mastication. To avoid swallowing any of the food, some researchers sealed the test items inside a rubber capsules (Borges et al., 2013; Nakasima et al., 1989; Sánchez-Ayala et al., 2016).

A capsule of fuchsine beads has been introduced as test material by a Brazilian research group (Escudeiro Santos, de Freitas, Spadaro, & Mestriner-Junior, 2006; Sánchez-Ayala et al., 2016). When the capsule is masticated, beads are smashed and fuchsine dye is released within the capsule according to masticatory efficacy. Capsule components are dissolved in water and the concentration of fuchsine in the solution is measured with a spectrophotometer at 546 nm.

Granules of approximately 1 mm in diameter were prepared from cellulose, corn starch, lactose in addition to fuchsine dye and other components. Each granule was coated with a layer of polymeric methacrylate. Then, about 250 mg of these beads were packed in polymerizing vinyl chloride (PVC) capsules (Fig. 2.4). The individual was instructed to freely masticate one capsule for a given number of cycles. After mastication completion, the capsule was cut, and its content was dissolved in 5 ml water with constant stirring for 30 seconds. The solution was filtered and the dye concentration was determined using a spectrophotometer at 546 nm (Borges et al., 2013; Casal, da Silva, Galo, Junior, & da Silva, 2016; Cunha et al., 2013; de Abreu et al., 2014; Felício, Couto, Ferreira, & Mestriner Junior, 2008).



Figure 2.4: Fuchsine capsules¹

¹ Reprinted from de Abreu et al. (2014) with permission from Elsevier.

In a study by Felício et al. (2008), the reliability of fuchsine beads to evaluate the masticatory performance and its correlation with the anterior temporal and masseter muscles' activity were investigated. High reliability was observed for the fuchsine beads test and a significant correlation with the electromyographic activity.

Capsules of erythrosine-containing granules (Nakasima et al., 1989) or adenosine triphosphate (ATP) granules (Masuda et al., 1981; Shiratsuchi et al., 1991; Umino et al., 2003) have been used by some Japanese investigators to determine the concentration of dye released from the crushed granules upon mastication.

The individual was asked to masticate 5 grams of ATP granules for 50 cycles without swallowing any of the test material. After mastication, the mouth was rinsed and the material was recovered in a beaker. Water was added to a total 2000 ml, the solution was filtered, and the amount of ATP in the solution was determined by measuring the absorbance with a spectrophotometer at 259 nm (Umino et al., 2003).

On the contrary of the above mentioned methods, if non-pigmented test food particles were immersed in a colored solution, the dye from the surrounding solution will diffuse into the particles. Consequently, the dye concentration in the colored solution will decrease in proportion to the total surface area of the food particles. The difference in dye concentration may be quantified with a spectrophotometer. Gelatin-hardened by formalin (Gunne, 1983) or tablets of a synthetic material incorporated with a color binder (Huggare & Skindhøj, 1997) have been used as test food items.

Spectrophotometric method seems to be easy to apply, and measure a considerable amount of specimens within a reasonable time period. However, it requires a special laboratory equipment and test materials, which may not be available in clinical settings (Kimoto et al., 2004).

2.3.2.2 One-Color Changing

Over the last two decades, researchers have widely used a convenient method (Table 2.2) which involves evaluating an individual's ability to mix and knead a cohesive food bolus (Hayakawa et al., 1998; Kubota, Kanazawa, Hama, Komagamine, & Minakuchi, 2017; Tarkowska et al., 2017). In clinical settings, it has been suggested that mixing ability is a simple, feasible test in comparison with the comminution techniques (H. Sato et al., 2003; Schimmel et al., 2015). Several colorimetric methods, that measure color change of a chewing gum upon mastication, were specially developed for the purpose of masticatory performance assessment (Hama, Kanazawa, Minakuchi, Uchida, & Sasaki, 2014a; Hayakawa et al., 1998; Kasahara et al., 1989; Matsui et al., 1996; Tarkowska et al., 2017).

In 1989, Kasahara et al. introduced the first color-changeable chewing gum which consisted of two portions. One included Phloxine ($C_{20}H_2Br_4Cl_4Na_2O_5$), which is a dye that can develop red color in alkaline conditions, and the other contained alkaline sodium bicarbonate ($NaHCO_3$). The degree of mixing upon mastication determines the chromatic value of Phloxine.

The individuals with xerostomia or the wearers of removable dentures may suffer undesirable gum adhesion to their teeth and dentures. Therefore, a low-adhesive color-changeable chewing gum has been developed and tested for its applicability in those conditions (Matsui et al., 1996). The results indicated that this type of low-adhesive gum is useful for masticatory performance assessment in both complete denture wearers and natural dentate individuals (Matsui et al., 1996).

One study has introduced a method using two-layer chewing gum, one layer contains purple-blue pigment of red cabbage and the other includes citric acid. As a result of the

acid reaction, the color changes from purple-blue to red according to the degree of mixing upon mastication (Hayakawa et al., 1998).

Another color-changeable chewing gum (Masticatory Performance Evaluating Gum XYLITOL;¹ Lotte Co., Ltd., Tokyo, Japan), which is now widely used, has recently been developed by researchers at Tokyo Medical and Dental University (Ishikawa, Watanabe, Hayakawa, Minakuchi, & Uchida, 2007; Kamiyama, Kanazawa, Fujinami, & Minakuchi, 2010). The gum base contains red, yellow, and blue dyes in addition to citric acid. Amongst these dyes, the red dye is pH-sensitive and remains invisible under the acidic conditions. Prior to mastication, the chewing gum looks yellowish-green because of the yellow and blue pigments (the low pH of the gum makes the red dye invisible). During the mastication process, the citric acid elution into saliva raises the pH inside the chewing gum, leading to the color of the chewing gum to turn gradually from yellowish-green to red (Fig. 2.5). Changes in color may be objectively measured using a colorimeter or visually assessed according to a graded color scale (Hama et al., 2014a; Hama, Kanazawa, Minakuchi, Uchida, & Sasaki, 2014b; Komagamine et al., 2012; Tarkowska et al., 2017; Wada et al., 2017).

The individual was instructed to masticate one piece of XYLITOL[®] Masticatory Performance Evaluating Gum for 100 mastication cycles. This task may be repeated three times consecutively with rest intervals of few minutes to avoid muscle fatigue. After mastication, the gum was picked up immediately from the mouth, placed between two polyethylene films, and compressed to a thickness of 1.5 mm with two glass plates. The color was measured through the polyethylene film with a colorimeter (Fig. 2.6). The International Commission on Illumination CIE Lab color space was used for the color

¹ XYLITOL color-changeable chewing gum is available at <https://www.oralcare.co.jp>

measurements, where a^* values express redness. The color readings were performed at several predefined points on the compressed gum, and the mean value was calculated (Ishikawa et al., 2007; Kamiyama et al., 2010; Wada et al., 2017). In the CIELab color space, L^* represents the lightness, a^* represents the color degree between red and green, and b^* represents the degree between yellow and blue opponent colors (Luo, 2015).

The CIELab measurements of XYLITOL gum before mastication revealed the following values L^* : 72.3, a^* : -14.9, and b^* : 33.0 (Hama et al., 2014b). The color change upon mastication may be simply expressed as Δa^* differences (Ishikawa et al., 2007; Kamiyama et al., 2010) or the total ΔE value might be calculated from the formula:

$$\Delta E = \sqrt{(L^* - 72.3)^2 + (a^* + 14.9)^2 + (b^* - 33.0)^2} \quad (2.1)$$

ΔE represents the mean difference between two colors in CIELab color space (Hama et al., 2017; Kubota et al., 2017; Tarkowska et al., 2017).



Figure 2.5: XYLITOL color-changeable chewing gum¹



Figure 2.6: XYLITOL gum and colorimeter²

¹ Reprinted from Wada et al. (2017) under Creative Commons Attribution License.

² Reprinted from Kamiyama et al. (2010) with permission from Elsevier.

Several research groups have developed specialized scales to easily evaluate the color of XYLITOL Masticatory Performance Evaluating Gum by mere visual inspection (Hama et al., 2014b; Kamiyama et al., 2010). A five-point color scale in addition to a 100-mm long visual analog scale (Fig. 2.7), and ten-point color scale (Fig. 2.8) have been used for the visual assessment of color. Validity and reliability of these scales have been tested and confirmed (Hama et al., 2014b; Kamiyama et al., 2010).



Figure 2.7: Five-point color scale with visual analog scale¹

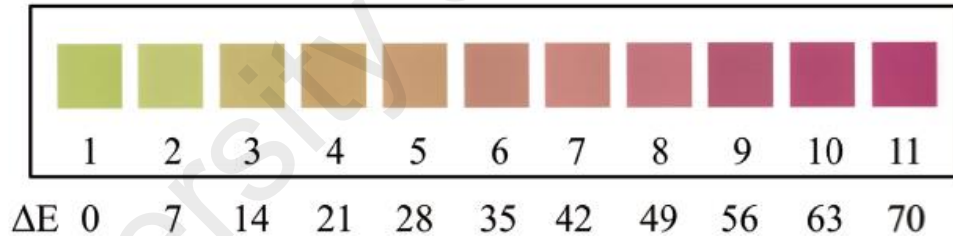


Figure 2.8: Ten-point color scale²

¹ Reprinted from Kamiyama et al. (2010) with permission from Elsevier.

² Reprinted from Hama et al. (2014b) under Creative Commons Attribution License.

Table 2.2: Summary of studies investigating masticatory performance using mixing ability tests

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Liedberg et al., 2005	Two-color chewing gum (red and blue)	Elderly men (Age 67–68 years); N=483	10 mastication cycles	Visual assessment of color mixing and bolus shaping using 5-point reference scales	Five grades
Sugiura et al., 2009	Two-color paraffin wax cube (red and green)	Healthy dentate adults (mean age: 25 years); N=32 Removable partial denture wearers (mean age: 65.5 years); N=40	10 mastication cycles	Assessment of color mixing and bolus shaping using digital image analyzer	Mixing ability index (MAI)
Abe et al., 2011	Two-color molded rice and rice cake (green and white)	Healthy dentate adults (mean age: 27.1 years); N=10	10, 15, 20, and 30 mastication cycles, and under instruction to “chew normally” and “chew well”	Two-color mixing was assessed with video endoscope in the oropharynx and digital image software (Adobe Photoshop). Proportion of non-white area to the entire food bolus on the image was defined as the bolus formation index (BFI)	Bolus formation index (BFI)
Schimmel et al., 2011	Two-color chewing gum (azure and pink)	Patients of stroke (mean age: 69 years); N=31	20 mastication cycles	Measurement of the ratio of unmixed baseline color in flattened specimen using Adobe Photoshop	Unmixed fraction (UF)

Continues

Table 2.2 Continued

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Komagamine et al., 2012	Color-changeable chewing gum XYLITOL	Complete denture wearers (age 75 years); N=93	100 mastication cycles	Color measurement using colorimeter	ΔE color difference in CIELab color space
Horie et al., 2014	Color-changeable chewing gum XYLITOL	Healthy dentate adults (age 28 years); N=44	60 mastication cycles	Color measurement using colorimeter	ΔE color difference in CIELab color space
Choi et al., 2015	Two-color paraffin wax cube (red and green)	Individuals with normal occlusion and malocclusion (mean age: 23 years); N=85	10 mastication cycles	Assessment of color mixing and bolus shaping using digital image analyzer	Mixing ability index (MAI)
Ishida et al., 2015	Color-changeable chewing gum XYLITOL	Patients underwent mandibulectomy; N=26	3 minutes	Visual inspection using 5-point color scale	Five grades
Weijenberg et al., 2015	Two-color chewing gum (pink and blue)	Elderly patients with dementia (mean age: 85 years); N=114	20 seconds	Calculating the difference in color intensity of each digital pixel and its neighbor using Mathematica software	DiffPix score between 0 and 1
Yamada et al., 2015	Color-changeable chewing gum XYLITOL	Healthy dentate adults (age 25 years); N=51	60 mastication cycles	Color measurement using colorimeter	ΔE color values and number of cycles (<i>N</i>)
Lin et al., 2016	Color-changeable chewing gum XYLITOL	Healthy elderly subjects (mean age: 64 years); N=25	3 minutes	Color measurement of digitized images using colorimetric software	ΔE color difference in CIELab color space

Continues

Table 2.2 Continued

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
Scudine et al., 2016	Color-changeable chewing gum XYLITOL	Healthy adolescents (age 14–17 years); N=91	60 seconds	Visual inspection using 10-point color scale	Ten grades
Kubota et al., 2017	Color-changeable chewing gum XYLITOL	Healthy dentate adults (age 27–43 years); N=10	60 mastication cycles	Color measurement using colorimeter	ΔE color difference in CIELab color space
Schimmel et al., 2017	Two-color chewing gum (pink and azure)	Patients with stroke (mean age: 64 years); N=27 Healthy subjects (mean age: 61 years); N=27	20 mastication cycles	Measurement of standard deviation of color intensities in hue channel of HSI color space using ViewGum software	Standard deviation of hue (SDHue)
Wada et al., 2017	Color-changeable chewing gum XYLITOL	Older adults (mean age: 82 years); N=30	2 minutes	Color measurement using colorimeter	a^* value in CIELab color space
Buser et al., 2018	Two-color chewing gum (pink and blue); Hue-Check Gum	Healthy dentate adults (mean age: 25 years); N=20 Edentulous subjects with implant overdentures (mean age: 73 years); N=20	5, 10, 20, 30, and 50 mastication cycle	Measurement of standard deviation of color intensities in hue channel of HSI color space using ViewGum software and Hue-Check Gum mobile application	Standard deviation of hue (SDHue)

Continues

Table 2.2 Continued

Study	Test food	Subjects	Mastication	Analysis technique	Outcome measure
De Groot et al., 2018	Two-color wax tablet (red and blue)	Patients with oral malignancies; N=123	10 or 20 mastication cycles	The sum of standard deviations of color intensities in red and blue channels of RGB color space using Adobe Photoshop	Mixing ability index (MAI)
Maeda et al., 2018	Color-changeable chewing gum XYLITOL	Patients underwent mandibulectomy; N=31	3 minutes	Visual inspection using 5-point color scale	Five grades
Shao et al., 2018	Two-color chewing gum (red and green)	Post-maxillectomy patients with maxillary prostheses; N=43	20 mastication cycles	Measurement of the ratio of unmixed baseline color in flattened specimen using Adobe Photoshop	Unmixed fraction (UF)

2.3.2.3 Two-Color Mixing Ability

Masticatory performance has been assessed in an objective method by determining an individual's ability to mix and knead food bolus before swallowing (Elgestad Stjernfeldt et al., 2017; Kaya et al., 2017; van der Bilt, 2011). Test materials for mixing upon mastication may be color-changeable chewing gum (Tarkowska et al., 2017), two-color chewing gum (Schimmel et al., 2015) or paraffin wax with different colors (Salleh et al., 2007; H. Sato et al., 2003). After a specified number of mastication cycles, masticated specimens may be analyzed by examiner's naked eye, colorimetric device or by computer analysis of digital images.

Validity and reliability studies have demonstrated a significant correlation between comminution ability and mixing ability methods (S. Sato et al., 2003; Sugiura et al., 2009; van der Bilt et al., 2010). The mixing ability was especially recommended for senior edentulous subjects, and patients with a masticatory handicap or mental disorders where traditional fragmentation tests are not applicable (Speksnijder et al., 2009; van der Bilt, 2011; van der Bilt et al., 2012). However, the comminution test was considered best in discriminating the masticatory performance in healthy adults with complete natural dentitions (Speksnijder et al., 2009; van der Bilt et al., 2010). In other words, mixing ability test has limited applicability in adults with high masticatory performance due to a ceiling effect with the results (Kaya et al., 2017).

The underlying rationale for this method is that the greater the degree of two-color mixing, the higher the masticatory performance. This approach provides a straightforward and convenient evaluation of masticatory function as compared with comminution tests described in previous sections, and it might have the potential for both clinical and research applications (H. Sato et al., 2003).

In 1995, a Scandinavian team (Liedberg & Owall, 1995; Liedberg et al., 2005) published the first study aimed at quantifying masticatory performance by evaluating the degree of color mixing and bolus formation with two-color chewing gum. The participants were asked to masticate pieces of red and blue chewing gum for 10 mastication cycles. After retrieving the boluses from the oral cavity, the color mixing and bolus shaping were subjectively scored by visual inspection into 5-point scale (Fig. 2.9).

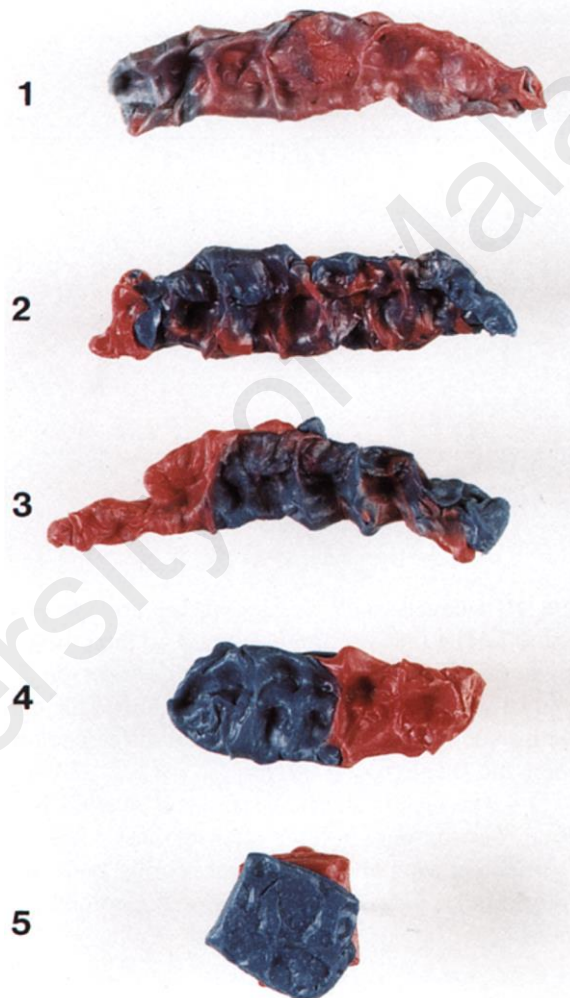


Figure 2.9: Five-point scale of two-color mixing ability¹

¹ Reprinted from Liedberg and Owall (1995) with permission from Springer Nature.

Japanese research group (H. Sato et al., 2003) developed a computer-aided system to estimate food mixing and kneading ability. This system used red and green semi-checked paraffin wax cube as a test food (Fig. 2.10). The individual was instructed to masticate paraffin cube for 10 cycles. After mastication, the specimen was picked up from the mouth, and photographed with a digital camera under standardized lighting and distance conditions. The images were assessed for color mixing and shape of the masticated cube using a specialized software (Luzex-FS; Nireco Co., Tokyo, Japan) or (Image-Pro Plus; Media Cybernetics Inc., MD, USA). Mixing Ability Index (MAI) was derived from the following parameters: the unmixed red area, the unmixed green area, the remaining mixed area, the maximum length and breadth of specimen, the total projection area, and the projection area above 50 μm in thickness (Asakawa, Fueki, & Ohyama, 2005; Bae, Jeong, Jeong, & Huh, 2015; Choi et al., 2015; H. Sato et al., 2003; Weijenberg et al., 2013).

The validity and reliability of this system for masticatory performance assessment have been confirmed (Fueki, Yoshida, Sugiura, & Igarashi, 2009; S. Sato et al., 2003), and the textural properties of the paraffin cube have been evaluated (Salleh et al., 2007). However, the required hardware and software are only available in Japan which poses practical difficulties in replication.

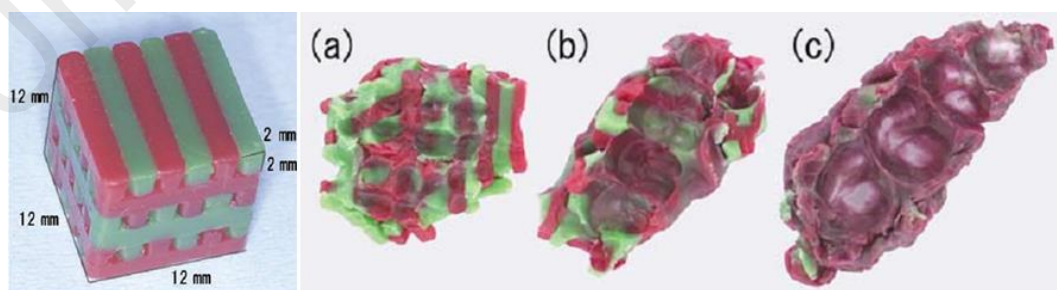


Figure 2.10: Paraffin cube before and after mastication¹

¹ Reprinted from H. Sato et al. (2003) with permission from John Wiley and Sons.

Prinz (1999) flattened the masticated bolus into wafer by pressing it between two glass plates with a 1 mm spacer. Tooth marks in the raw bolus and oblique illumination of the camera's or scanner's lamp may create high contrast shadows and confound color evaluation. Therefore, flattening the specimen helps to avoid the undesired shadows in the image, and provides a more reliable and accurate assessment of the color mixing (Prinz, 1999; Schimmel et al., 2007). However, the parameters of bolus shaping will be disregarded in the analysis of wafers' images.

One of the simplest methods for assessing the degree of two-color mixing is to calculate the unmixed fraction (UF) of baseline color (Endo et al., 2014; Schimmel et al., 2007). The individual was instructed to masticate a specimen of two-color chewing gum for 20 cycles. The masticated bolus was then retrieved from the oral cavity, flattened to a 1-mm-thick wafer, and both sides of the specimen were digitized with a flatbed scanner. The *magic wand* tool of Adobe Photoshop software was used to select the area of unmixed color, and the number of pixels was measured from the histogram panel for this area (Fig. 2.11). Thereafter, the unmixed fraction (UF) of the specimen was calculated semi-manually using Microsoft Excel spreadsheet with the following formula (Elsig et al., 2015; Elsyad & Khairallah, 2017; Elsyad & Shawky, 2017; Palomares et al., 2018; Schimmel et al., 2011; Shao et al., 2018):

$$UF (\%) = \frac{Unmixed\ Pixels_{1st\ side} + Unmixed\ Pixels_{2nd\ side}}{2 \times All\ Pixels_{one\ side}} \times 100 \quad (2.2)$$

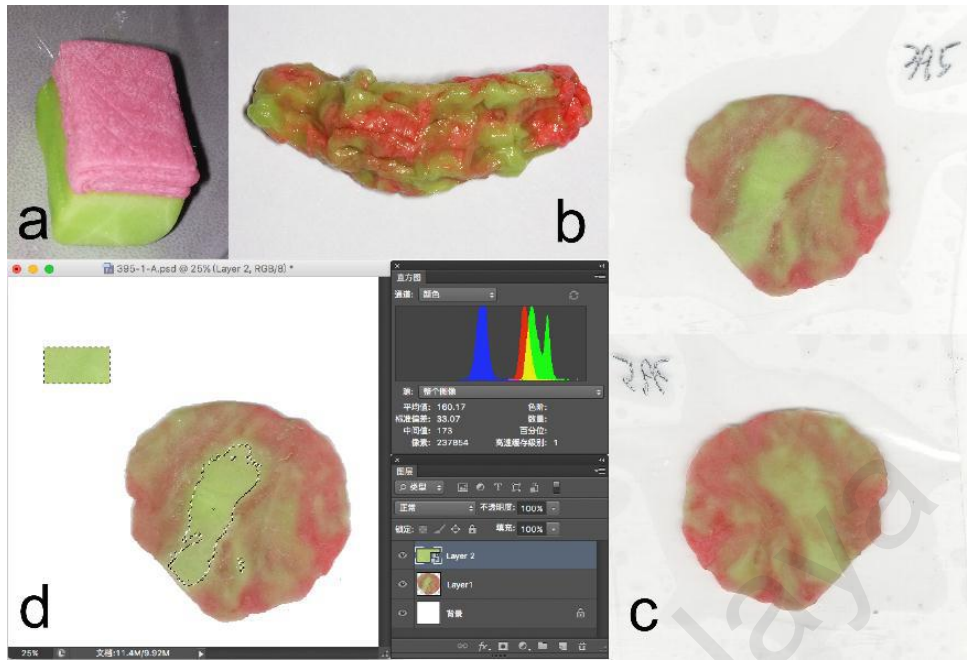


Figure 2.11: Unmixed fraction of green color, Adobe Photoshop¹

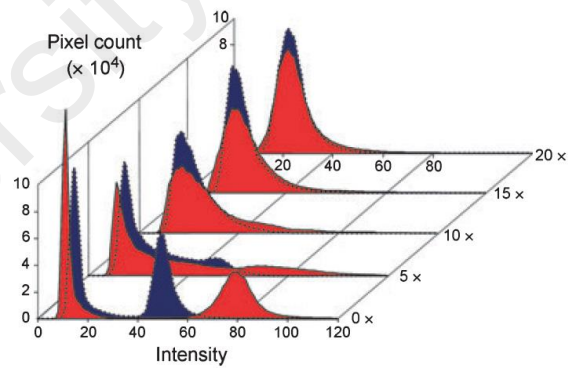


Figure 2.12: Image histograms of red and blue channels²

¹ Reprinted from Shao et al. (2018) with permission from Elsevier.

² Reprinted from van der Bilt et al. (2012) with permission from John Wiley and Sons.

Another approach that depends on the color image histogram has been presented by Speksnijder et al. (2009). The histogram of an image plots the number of pixels for each intensity value. Mastication mixes the two baseline colors and intermediate color intensities appear, thus the spreads of the intensities decrease. A lower spread of intensities implies a higher color mixing, and a better masticatory performance (Fig. 2.12) (Rozeboom et al., 2018; Speksnijder et al., 2009; van der Bilt et al., 2012).

The participant was asked to masticate a two-color wax tablet for 20 cycles. The tablet consisted of two layers (3 mm each) of red and blue soft wax, and had a diameter of 20 mm. After being masticated, the wax was fattened to a 2-mm-thick wafer and photographed on both sides using a flatbed scanner. The images of the two sides were combined and analyzed using Adobe Photoshop software. The sum of the standard deviations of the red and blue histograms was measured and used as indicator of the mixing ability (de Groot et al., 2019; Remijn et al., 2018; van der Bilt et al., 2010).

A specialized software (ViewGum; dHAL Software, Kifissia, Greece) was developed by Halazonetis et al. (2013) for the assessment of two-color mixing ability. This program measures the standard deviation of the Hue channel in the HSI color space (Fig. 2.13). The validity and reliability of this software for evaluating masticatory performance were confirmed through a series of studies (Buser et al., 2018; Halazonetis et al., 2013; Kaya et al., 2017; Schimmel et al., 2015; Silva et al., 2018).

The participant was instructed to masticate a specimen of two-color chewing gum for 20 cycles. The bolus was then retrieved from the mouth, flattened to a wafer of 1 mm thickness, and both sides were scanned with a flatbed scanner. The digital images were subjected to a colorimetric analysis using ViewGum[®]. The software transforms the images from RGB to HSI color space, calculates the circular variance of pixels in Hue channel, and displays the standard deviation of hue (SDHue). A lower SDHue indicates

higher color mixing and therefore better masticatory performance (Enkling, Saftig, Worni, Mericske-Stern, & Schimmel, 2017; Müller et al., 2013; Schimmel, Voegeli, et al., 2017).

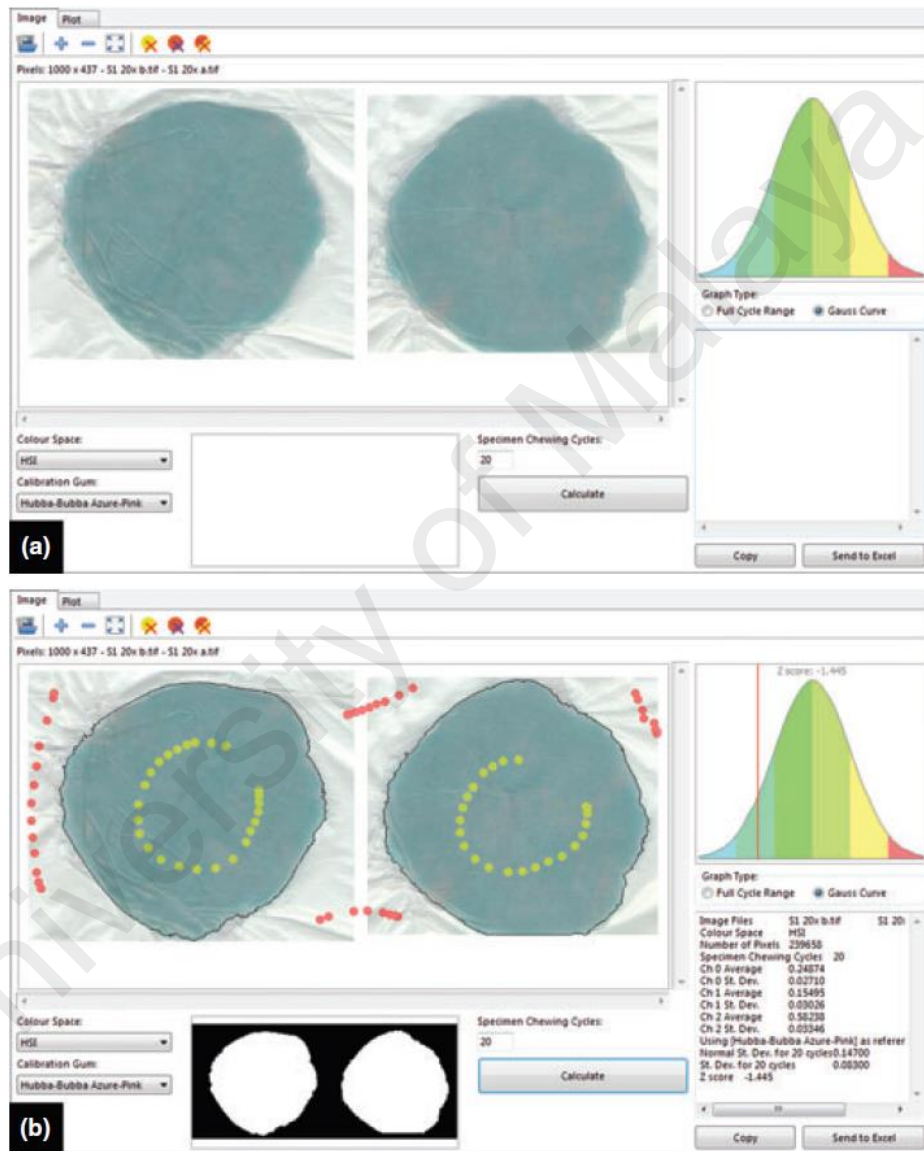


Figure 2.13: ViewGum[®] image analysis software¹

¹ Reprinted from Halazonetis et al. (2013) with permission from John Wiley and Sons.

Buser et al. (2018) introduced a custom-made, two-color chewing gum (Hue-Check Gum; Orophys GmbH, Bern, Switzerland) in blue and pink colors, and a custom-built smartphone application (Hue-Check Gum App; ARTORG CENTER, University of Bern, Bern, Switzerland) for the assessment of two-color mixing ability. After flattening the gums, each specimen was photographed from both sides with an iPhone 6 under standardized lighting and distance conditions. Hue-Check Gum App measured the standard deviation of pixels in Hue channel (SDHue) as an indicator of color mixing (Buser et al., 2018).

Weijenberg et al. (2013) used a computational software (Mathematica; Wolfram Research, Champaign, IL, USA) to calculate the difference in color intensity of each pixel and its neighbor, thus providing an indicator for spatial heterogeneity. The amount of mixing of the two-color specimen was expressed as a score between 0 and 1; a lower score indicates better mixing (Weijenberg et al., 2015; Weijenberg et al., 2013). However, the algorithm and computer script are not available for reproduction.

Finally, Vaccaro et al. presented a MATLAB-based software (MPAT v1.20, Perceptodent Project, University of Malaga, Spain, <http://perceptodent.lcc.uma.es>) and an expert system (MEPAT; <https://github.com/fabianvaccaro/perceptodent>) for objective assessment of masticatory performance using two-color mixing ability test (Vaccaro et al., 2018; Vaccaro et al., 2016). Nevertheless, the image processing methods used by Vaccaro et al. were similar to previous studies, which is the variance of color intensities in various color spaces.

2.3.3 Subjective Methods

Subjective methodology for masticatory performance evaluation might be either patient-based or examiner-based assessments. Questionnaires provide subjective information of patient's comfort, satisfaction, food preference, and self-perception of masticatory function. The degree of satisfaction is self-evaluated by the patients using category scales or visual analog scales (VAS). Feine and Lund (2006) suggested that patient-reported outcomes are the most appropriate measures of masticatory performance in edentulous populations. Slagter et al. (1992) recommended objective tests over questionnaires for the assessment of masticatory performance in complete denture wearers. However, the relationship between subjective and objective measures is still controversial, and it might be recommended to consider both aspects in the evaluation of masticatory function (Feine & Lund, 2006; Murakami et al., 2018; Pedroni-Pereira et al., 2018; Slagter, Olthoff, Bosman, & Steen, 1992; van der Bilt, Olthoff, Bosman, & Oosterhaven, 1994).

Examiner-based five-point or ten-point color scales have been also used to subjectively evaluate the masticated bolus by visual inspection (Endo et al., 2014; Hama et al., 2014b; Schimmel, Genton, & McKenna, 2019). However, the term "subjective" in the dental literature commonly refers to the patient-based outcomes.

CHAPTER 3: MATERIAL AND METHODS

3.1 Participants

A reference group (R) of 20 healthy volunteers were recruited and served as controls for generating a calibration dataset and establishing a reference regression model for the new system. The participants were 18 to 31 years old (average age of 20.9 ± 3.3 years), 10 men and 10 women, had complete natural dentition except third molars, showed normal occlusion (Angle class I), and scored less than 4 on the index of decayed, missing, and filled teeth (DMFT). Participants were either students or staff at the Faculty of Dentistry of the University of Malaya. Individuals with periodontal disease, severe tooth wear, or temporomandibular disorders were excluded.

A test group of 10 healthy volunteers (T1) and 10 denture wearers (T2) were recruited for testing the validity. The participants in T2-group were 54 to 75 years old, wearing clinically acceptable conventional complete dentures (Table 3.1). This clinical study was approved by the Medical Ethics Committee of the Faculty of Dentistry of the University of Malaya DF-RD1612/0045(P). Written informed consents were obtained from the participants before taking part in the study.

Table 3.1: Criteria for selecting participants

Group	Inclusion criteria	Exclusion criteria
<p>Natural dentitions (R & T1 groups)</p>	<ul style="list-style-type: none"> • Aged 18 to 35 years old. • Good general health. • Normal body mass index. • Complete natural dentition excepting third molars. • Normal occlusion; Angle class I canine-molar relationship. • DMFT score of 3 or less. 	<ul style="list-style-type: none"> • TMJ dysfunction symptoms. • Orofacial pain. • Bruxism. • Severe tooth wear. • Periodontal disease. • Tooth anomalies. • Presence of crowns or onlays. • Presence of fixed or removable orthodontic appliances.
<p>Complete dentures (T2-group)</p>	<ul style="list-style-type: none"> • No age limit. • Wearers of clinically acceptable complete dentures. 	<ul style="list-style-type: none"> • Orofacial pain. • Signs of severe TMJ dysfunction. • Neuro-muscular disorders. • Dementia.

3.2 Two-Color Chewing Gum

Sticks of custom-made, red-green (RG) chewing gum were prepared as test food specimens. The RG chewing gum consisted of four ingredients: gum base, softener, powder filler, and water-insoluble dyes. The gum base (Glee Gum; Verve Inc., Providence, RI, USA) was melted in a double-boiling water bath at 100°C for 20 minutes. Vegetable margarine (Daisy; Lam Soon Edible Oils Sdn. Bhd., Shah Alam, Selangor, Malaysia), food-grade talcum powder (Microtalc FC8; Mondo Minerals B.V., Amsterdam, Netherlands), and food-grade lake pigments (Idacol; ROHA Dyechem Pvt. Ltd., Mumbai, India) were then added and mixed thoroughly using a handheld electric mixer. Subsequently, the gum dough was flattened to a thickness of 2 mm using a rolling pin, and cut in dimensions of 25×10×2 mm. Two pieces of opponent colors were stuck together and wrapped in wax paper (Figs. 3.1–3.9). Ingredients of RG chewing gum are elaborated in Table 3.2.

Table 3.2: Ingredients of RG chewing gum and their ratios

Material	Manufacturer	Description	Weight (g)	wt.%
Glee Gum	Verve Inc.	Natural rubber gum base	100	71
Daisy margarine	Lam Soon Edible Oils Sdn. Bhd.	Palm-oil-based margarine	20	14
Microtalc FC8	Mondo Minerals B.V.	Food-grade talcum powder	20	14
Idacol L17 Lake Allura Red	ROHA Dyechem Pvt. Ltd.	Food-grade, water-insoluble pigment	1	<1
Idacol L13 Lake Pea Green	ROHA Dyechem Pvt. Ltd.	Food-grade, water-insoluble pigment	0.4	<1



Figure 3.1: Ingredients of RG chewing gum



Figure 3.2: Gum base in water bath

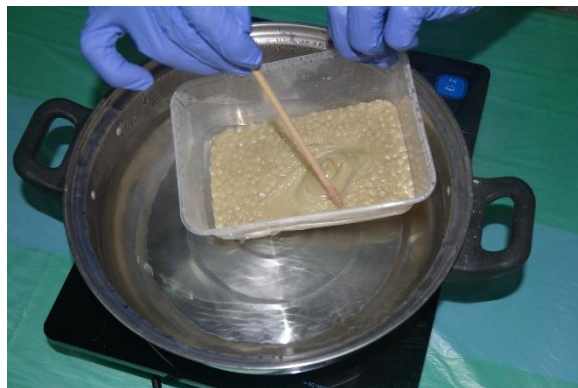


Figure 3.3: Gum base completely melted

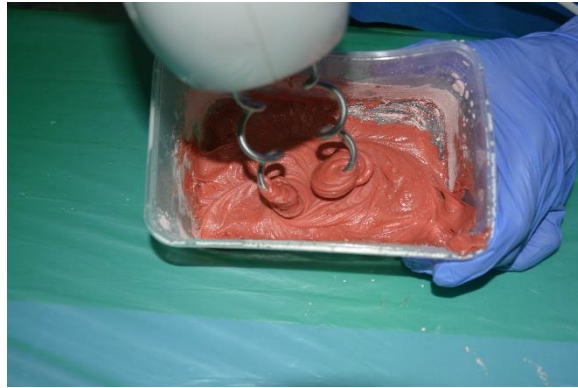


Figure 3.4: Mixing ingredients of RG chewing gum



Figure 3.5: Rolling of chewing gum. Arrows point to glass spacers (2-mm-thick)



Figure 3.6: Roller cutter used to cut strip of chewing gum (10-mm-width)



Figure 3.7: Cutting specimens of chewing gum (25-mm-length)



Figure 3.8: RG chewing gum specimens

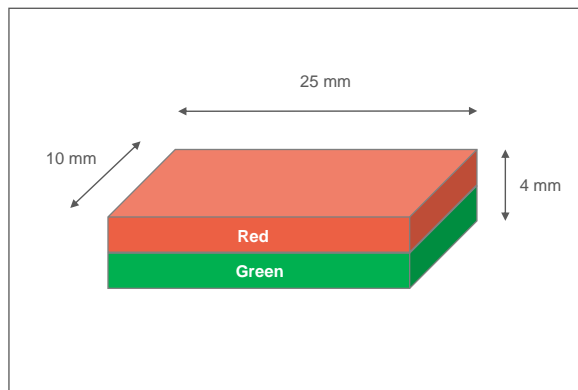


Figure 3.9: 3D-shaped diagram of RG chewing gum

3.3 Clinical Protocol

Each participant in R-group sat comfortably in an office chair with his or her head upright, and masticated five specimens of RG chewing gum freely on his or her preferred masticatory side for 3, 6, 9, 15 and 25 successive cycles. This sequence was repeated three times (total n=15 specimens for each participant). Rest intervals of 1 minute were imposed between the mastication sessions to avoid muscle fatigue. The boluses were then retrieved from the oral cavity, rinsed in running tap water, dried with air syringe, inserted between two clear polyethylene films, and flattened to 1-mm-thick wafers using a pair of glass plates. Both sides of the wafers were scanned with a flatbed scanner (CanoScan 4400F; Canon Inc., China) of 500 dots per inch (dpi) resolution, saved in tagged image file (TIF) format, and labelled with identification codes for the subsequent analysis (Fig. 3.10). A white cardboard was placed over the specimens during scan process to create a light scanning background. Figure 3.11 presents wafers of RG chewing gum subjected to different numbers of mastication cycles.

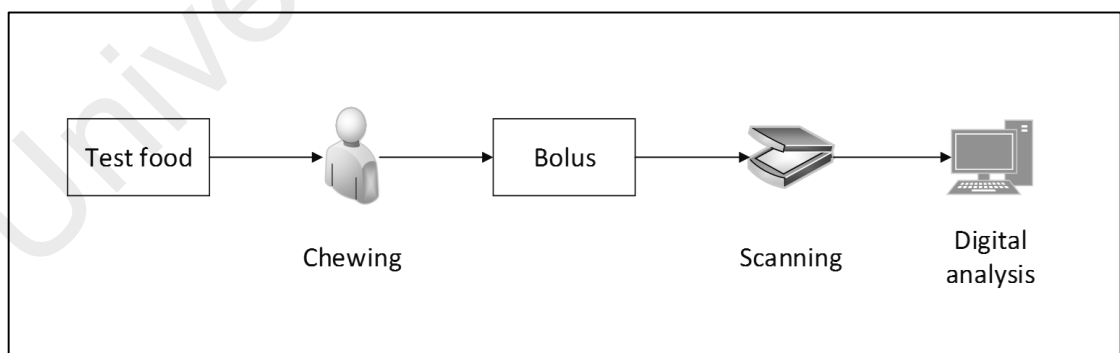


Figure 3.10: Workflow of mastication test



Figure 3.11: Specimens masticated (*from top*) for 3, 6, 9, 15 and 25 cycles

3.4 Image Analysis

A total of 600 digital images corresponding to both sides of the 300 specimens were analyzed using a scientific image-analysis program (ImageJ 1.51m; US National Institutes of Health) (Fig. 3.12). The International Commission on Illumination CIELab color space was used for the color measurements. In CIELab color space, the color is defined through three-dimensional components: L^* for the lightness, a^* represents the degree of red–green opposite colors, and b^* represents the degree of yellow–blue opposite colors. L^* runs in the range from $L^*=0$ for black to $L^*=100$ for white color. Each of the a^* and b^* components has the range $[-128$ to $+127]$ with red and yellow at positive values, and green and blue at negative values of a^* and b^* respectively.

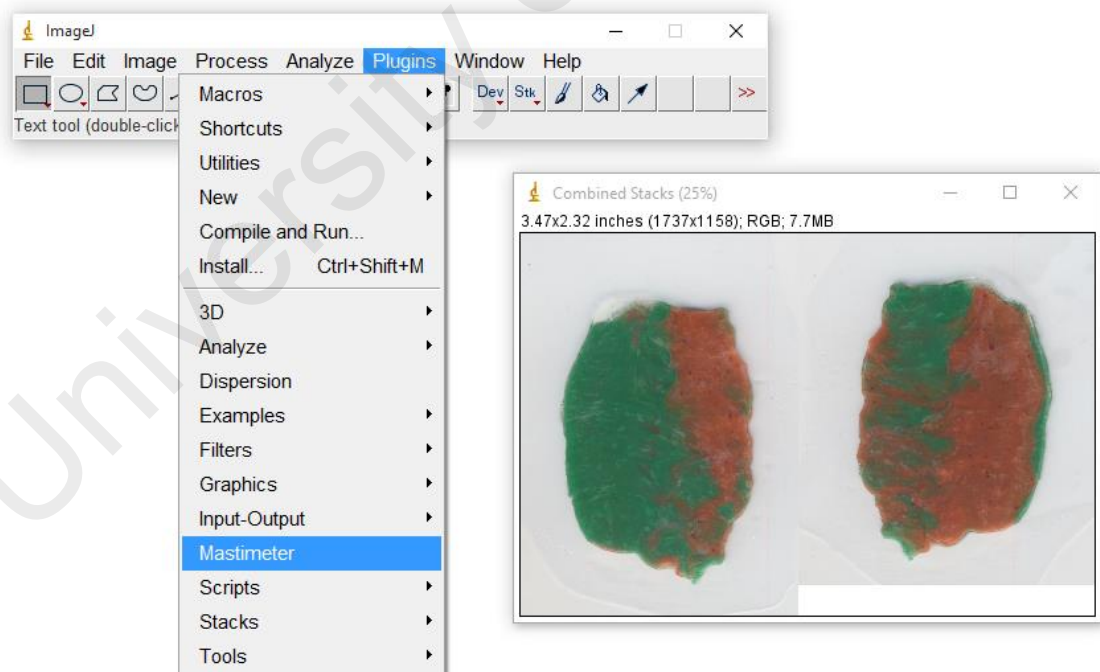


Figure 3.12: Screenshot of ImageJ software

The images of two opposite sides of the wafer were combined using the ImageJ combining tool; *Image>Stacks>Tools>Combine*.¹ Then, the combined image was segmented at the following threshold levels: $L^*=[0 \text{ to } 55]$, $a^*=[0 \text{ to } +127]$, $b^*=[-128 \text{ to } +127]$. As the images had light background with fairly dark wafer, the isolation of background was performed at the lightness level $L^*=55$. The segmentation of baseline colors (red versus green) into two mutually exclusive segments was done based on the cutoff point $a^*=0$. Thus, the red segment was our region of interest (ROI) (Fig. 3.13).



Figure 3.13: Threshold boundary (yellow line)

¹ Unused area in the combined image is filled with the background color. White color should be selected for the background from the drop-down menu: *Edit>Options>Colors*.

After segmentation, the perimeter (P) and the area (A) of the region of interest (ROI) were measured, and the geometric dispersion (GD)¹ of this two-dimensional object was calculated using the following formula:

$$GD = \frac{P}{2\sqrt{\pi A}} \quad (3.1)$$

where GD is geometric dispersion, P is perimeter, and A is area. GD is a dimensionless measure that relates the perimeter of an object to that of a circle with the same area (iso-areal quotient). The GD of a circle² is 1, and much more for scattered and irregular objects (Yusof et al., 2019). GD indicates to the spatial dispersion of the ROI (red segment in the present case).

Furthermore, the mean a^* values of the ROI was calculated and considered as a parameter of the red color value. We supposed that the degree of color mixing is directly proportional to GD , and inversely related to mean a^* value of our ROI.

¹ Custom-built Macro and JavaScript were developed for ImageJ to measure geometric dispersion of the 2D-object: *Help>Examples>Macro/JavaScript >Custom Measurement*. See appendices C, D, and E.

² Circle is the most compact 2D-object, whereas sphere is the most compact 3D-object. See appendices A and B.

Both spatial and value parameters (GD and a^*) of color mixing were combined as predictors of the number of mastication cycles by means of multiple regression analysis (inverse calibration) (Olivieri, 2018):

$$\text{Mastication cycles} = \text{function (color mixing)} \quad (3.2)$$

A new Mastication Index (MI) was defined as “the estimated number of mastication cycles that a healthy reference cohort would need to achieve a certain degree of color mixing.” Hence, the ratio of MI to the real number of cycles applied to the test food specimen represents the masticatory performance of a diagnosed individual against healthy reference people. The following formula was derived from the inverse multiple linear regression analysis of our calibration dataset (Blanco, Coello, Iturriaga, Maspoch, & Alaoui-Ismaili, 1999):

$$MI = b_0 + b_1x_1 + b_2x_2 \quad (3.3)$$

where MI denotes the Mastication Index, x_i the parameters of color mixing, and b_i the regression coefficients calculated from the reference dataset. By inserting the two parameters of color mixing of the specimen into the function, we obtain the Mastication Index. The latter formula was embedded into a custom-built plug-in Mastimeter[®] of ImageJ software for automated analysis of the digital images.¹

¹ <https://sites.imagej.net/RG.Mastimeter/plugins> and <https://www.youtube.com/watch?v=nJsGUcbiRwY>. Batch processing of the digital images; *Process>Batch>Macro* can be used to automatically analyze a batch of images, thus minimize human error and save time through process automation

The basic idea of our algorithm was as follows:

Step 1: Start

Step 2: Open color image

Step 3: Convert to CIELab color space

Step 4: Select region of interest (ROI)

if $L^* \leq 55$ and $a^* \geq 0 \rightarrow$ include pixel in ROI

if $L^* > 55 \rightarrow$ exclude pixel from ROI

if $a^* < 0 \rightarrow$ exclude pixel from ROI

whatever b^* value \rightarrow take no action

Step 5: Measure the area (A) of ROI

Step 6: Measure the perimeter (P) of ROI

Step 7: Measure the mean (a^*) of ROI

Step 8: Compute geometric dispersion (GD)

$$GD = 0.5(P/\sqrt{\pi A})$$

Step 9: Compute mastication index (MI)

$$MI = 17.6 + 0.2 \times GD - 12.4 \times \log_{10}(a^*)$$

Step 10: Display mastication index in results table

Step 11: Close image

Step 12: Finish



3.5 System Validation

To assess the validity of Mastication Index to discriminate between different dental states, each participant in T-group was asked to masticate a specimen of RG chewing gum for 15 cycles. This trial was repeated three times with 1-minute rest intervals. The boluses were then retrieved from the mouth, flattened and scanned by following the same preceding procedures. The digital images were automatically analyzed using Mastimeter[®] plug-in of ImageJ. The average of three trials was calculated for each participant.

Furthermore, the outcomes of Mastimeter[®] test were compared with those obtained from a well-established method using color-changeable chewing gum (Masticatory Performance Evaluating Gum XYLITOL; Lotte Co Ltd, Tokyo, Japan) (Fig. 2.5) (Wada et al., 2017). The color of XYLITOL chewing gum changes from yellowish-green to red upon mastication. The participants in T-group were instructed to masticate a specimen of XYLITOL chewing gum for 60 cycles. This trial was repeated three times with 1-minute rest intervals. The gum was retrieved immediately after mastication, and flattened to a thickness of 1 mm in polyethylene films using two glass plates. The color was then measured with a colorimeter (CM-5; Konica-Minolta, Tokyo, Japan) (Fig. 3.14) at three points; the center of the wafer, and approximately 5 mm above and below of the center. The CIELab color space was used for the color measurements. The average of a^* values represents each trial, and the average of three trials represents each participant (Fig. 3.15).



Figure 3.14: Colorimeter (CM-5; Konica-Minolta, Tokyo, Japan)

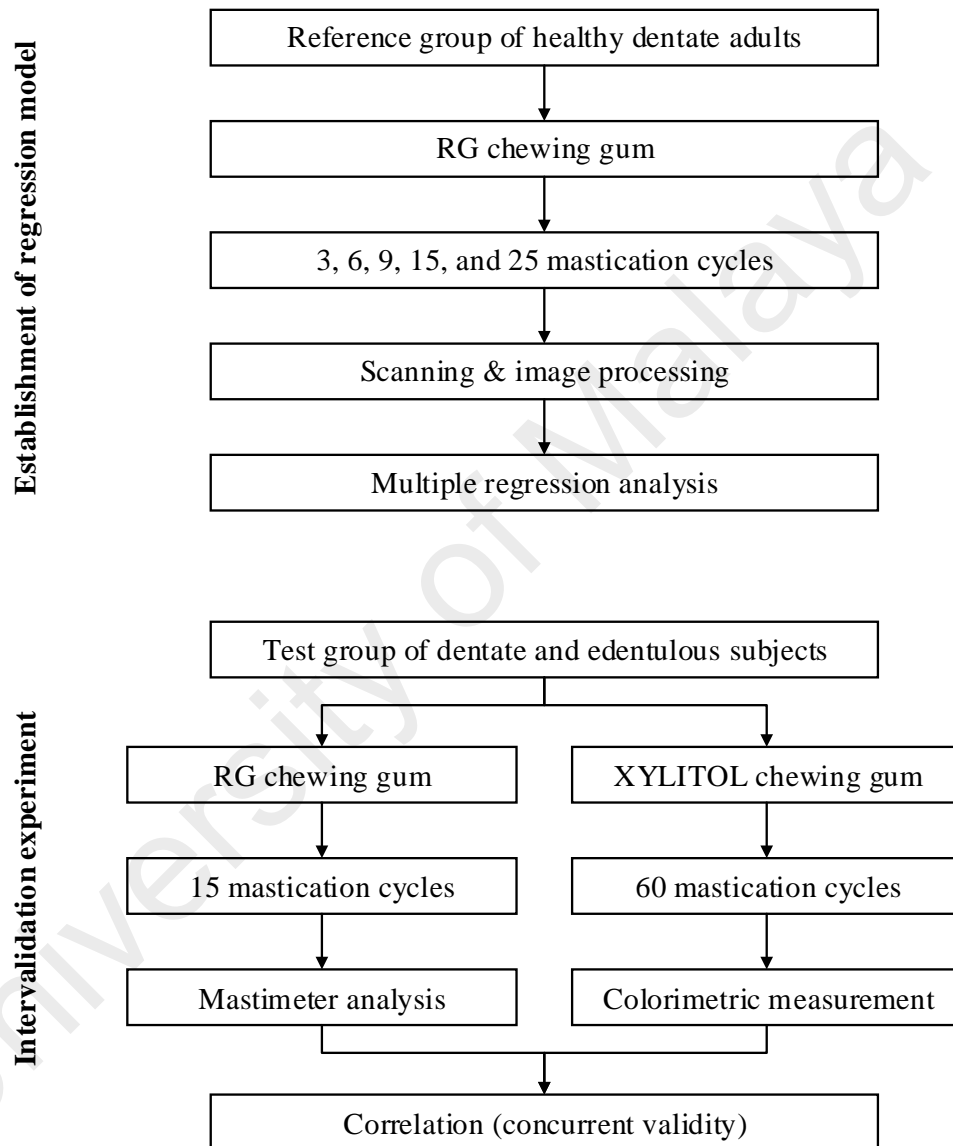


Figure 3.15: Flowchart of study design

3.6 Textural Characteristics

3.6.1 Hardness of Chewing Gum

The hardness of RG chewing gum was determined by penetration with a 6-mm-diameter cylinder probe using a texture analyzer device (TA-Xt Plus; Stable Micro Systems, Surrey, UK) (Fig. 3.16). The specimen was placed on the platform, and the probe moved downward with a pre-test speed of 1 mm/s, test speed of 10 mm/s, post-test speed of 10 mm/s, trigger force of 0.05 N, and distance of 3 mm (Table 3.3). The highest peak force (N) of the resulting force–time curve was considered the hardness value. Five specimens were measured at a room temperature of 23°C, and the average of hardness was calculated.

To evaluate the changes of hardness throughout mastication, one young participant masticated specimens of RG chewing gum for 15, 25 and 100 successive cycles. This sequence was repeated five times (total n=15 specimens). Hardness loss was calculated using the following formula:

$$\text{Hardness loss (\%)} = \frac{H_i - H_f}{H_i} \times 100 \quad (3.4)$$

where H_i is the initial hardness of unmasticated specimen, and H_f is the final hardness of masticated specimen. For comparison, sticks of a commercially available chewing gum (Wrigley's Spearmint [SM]; Wrigley Phil, Inc., Antipolo, Philippines) were folded into 4-mm-thick specimens, and the hardness was measured before and after 15, 25 and 100 mastication cycles by following the same preceding procedures.

Table 3.3: Parameters of texture analysis

Probe diameter	6 mm
Pre-test speed	1 mm/s
Test speed	10 mm/s
Post-test speed	10 mm/s
Trigger force	0.05 N
Distance	3 mm

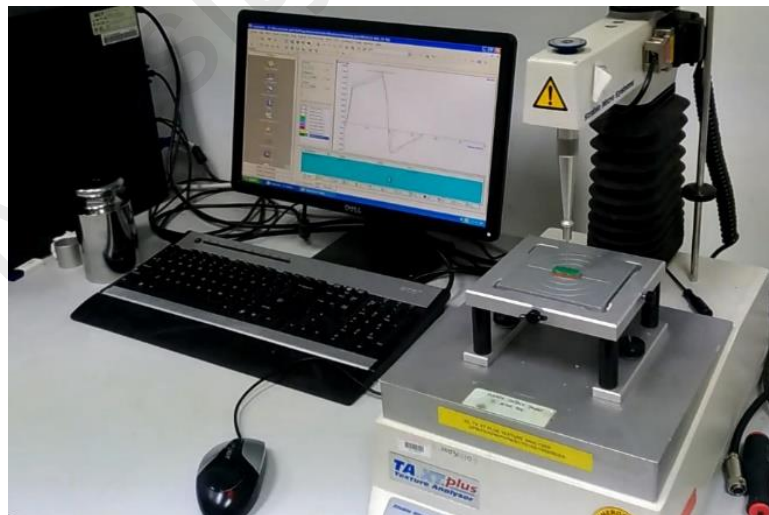


Figure 3.16: Texture analyzer (TA-XT Plus; Stable Micro Systems)

3.6.2 Mass Loss of Chewing Gum

One young participant masticated three preweighed specimens of RG chewing gum for 15, 25 and 100 successive cycles. This sequence was repeated five times (total n=15 specimens). One-minute interval was imposed between the mastication sessions to avoid muscle fatigue. The retrieved boluses were washed with running tap water to remove saliva from the surface of the specimens, dried with air syringe, desiccated for two weeks at 37°C, and weighed with an electronic scale of 0.1 mg accuracy (Sartorius BP221S; Sartorius AG, Gottingen, Germany). The percentage of mass loss of chewing gum after mastication was calculated using the following equation (Anastassiadou & Heath, 2001):

$$\text{Mass loss (\%)} = \left(\frac{M_i - M_f}{M_i} - \frac{m_i - m_f}{m_i} \right) \times 100 \quad (3.5)$$

where M_i is the initial mass of gum specimen, M_f is the final dried mass of masticated specimen, m_i is the initial mass of control unmasticated gum specimen, m_f is the final desiccated mass of control unmasticated specimen.

Similarly, the mass loss of a commercially available chewing gum (Wrigley's Spearmint [SM]; Wrigley Phil, Inc., Antipolo, Philippines) was measured by following the same procedures for the purpose of comparison.

3.7 Statistical Analysis

Data were explored for normality with the Shapiro-Wilk test. The ability of each parameter of the color mixing (GD and a^*) to discriminate between the specimens of different numbers of mastication cycles was investigated with the two-way¹ repeated measures ANOVA corrected with *post hoc* Bonferroni for pairwise multiple comparisons. The data of R-group were analyzed with multiple regression analysis using the two parameters of color mixing (GD and a^*) as predictors of the number of mastication cycles (dependent variable). Independent samples *t*-test was used for comparing the masticatory performance between T1 and T2 groups, and for comparing the hardness, hardness loss, and mass loss between RG and SM chewing gums. The relationship between the Mastication Index and XYLITOL outcomes was evaluated using Pearson correlation coefficient. All the analyses were performed using a statistical software (IBM SPSS Statistics v23; IBM Corp, Armonk, NY, USA) with a 5% significance level.

¹ Two-way ANOVA, one factor for the cycles number and second factor for the repetition.

CHAPTER 4: RESULTS

4.1 Reference Regression Model

From the reference group, a total of 600 images corresponding to both sides of the 300 wafers of RG chewing gum were recovered. Examples of RG wafers are presented in Figures 4.1 & 4.2, in which the region of interest (ROI) was determined according to predefined threshold levels in CIELab color space. The ROI (red in the present case) was used for the subsequent measurements.

The values of geometric dispersion (GD) for each step of the mastication cycles are given in Table 4.1 and Figure 4.3. The Shapiro-Wilk test detected signs of non-normality at 6 mastication cycles but the data of the remaining mastication cycles were normally distributed. The two-way repeated measures ANOVA with Bonferroni test confirmed the ability of GD to discriminate the groups of different numbers of mastication cycles. Pairwise multiple comparisons revealed significant differences between each pair of the groups (Table 4.2). The GD increased linearly with an increasing number of the mastication cycles (Fig. 4.3). Figure 4.4 shows results table of ImageJ software.



Figure 4.1: RG chewing gum masticated for 3 cycles (*top*) and 15 cycles (*bottom*)

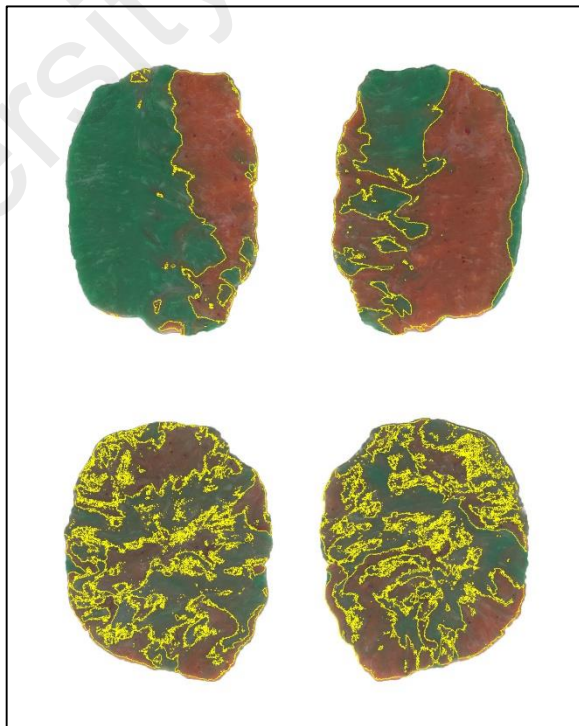


Figure 4.2: Region of interest (ROI)

Table 4.1: Descriptive statistics of geometric dispersion data

Cycles	N	Min	Max	Mean	Std. Dev.
0	5	2.4	3.0	2.9	0.5
3	60	5.1	12.3	8.8	1.6
6	60	10.5	27.5	16.0	3.8
9	60	12.7	35.8	23.8	5.3
15	60	19.7	56.1	37.2	8.6
25	60	29.0	75.9	51.9	12.6

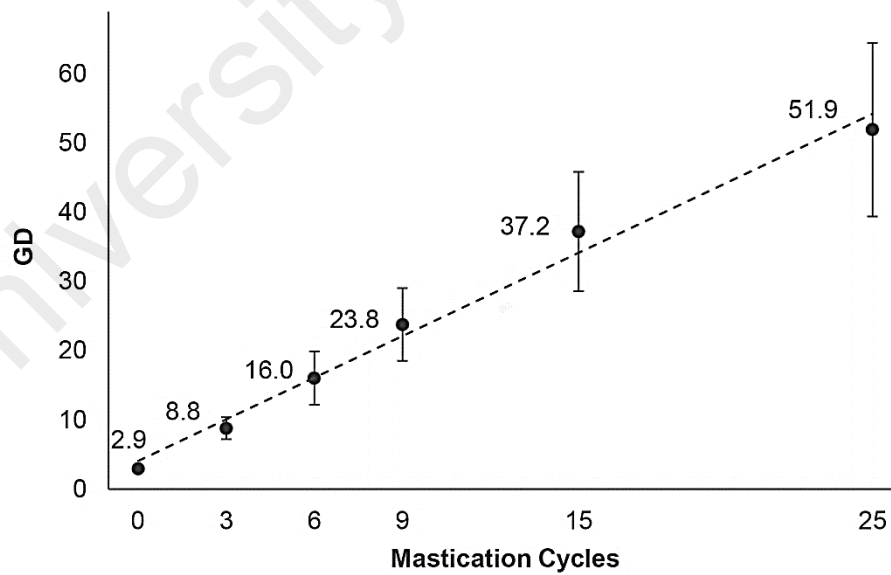


Figure 4.3: Geometric dispersion (mean \pm SD)

Table 4.2: Contrasts of GD values for different number of mastication cycles

Cycles		Mean Group Difference	Std. Error	P-value
3	6	-7.22	1.38	.000
	9	-14.99	1.41	.000
	15	-28.42	1.42	.000
	25	-43.15	1.55	.000
6	3	7.22	1.38	.000
	9	-7.77	1.33	.000
	15	-21.20	1.34	.000
	25	-35.93	1.47	.000
9	3	14.99	1.41	.000
	6	7.77	1.33	.000
	15	-13.43	1.36	.000
	25	-28.16	1.50	.000
15	3	28.42	1.42	.000
	6	21.20	1.34	.000
	9	13.43	1.36	.000
	25	-14.73	1.51	.000
25	3	43.15	1.55	.000
	6	35.93	1.47	.000
	9	28.16	1.50	.000
	15	14.73	1.51	.000

Bonferroni *post hoc* analysis. Sample size: 60. All contrasts were significant at the 0.05 level.

Table 4.3 and Figure 4.5 present the means and standard deviations of the color values of a^* channel for each step of the mastication cycles. The Shapiro-Wilk test showed that the data of a^* values were normally distributed. The two-way repeated measures ANOVA with Bonferroni test confirmed the ability of a^* to discriminate the groups of different numbers of mastication cycles. Pairwise multiple comparisons revealed significant differences between each pair of the groups (Table 4.4).

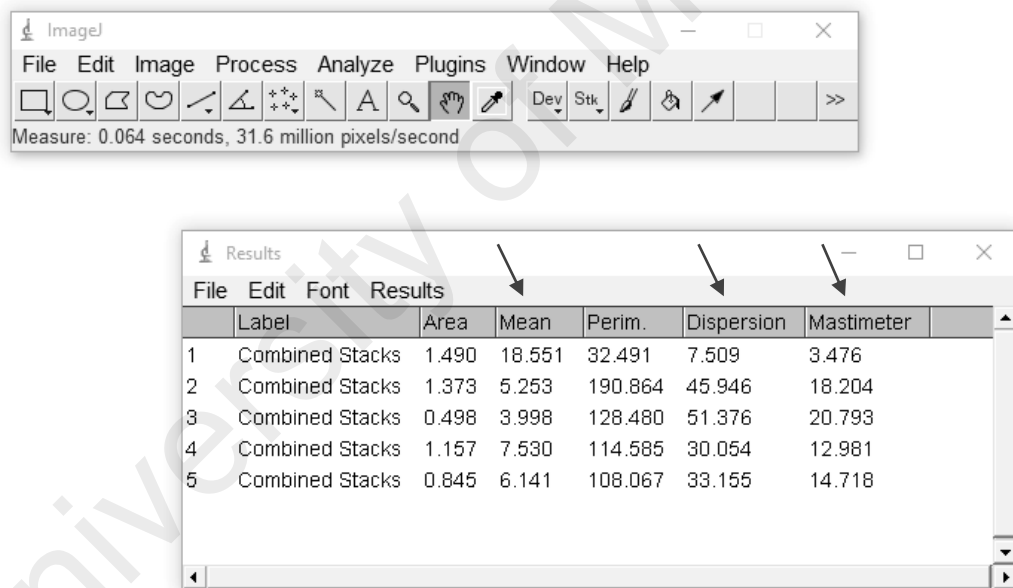


Figure 4.4: ImageJ results table. Arrows point to mean a^* , geometric dispersion and Mastication Index

Table 4.3: Descriptive statistics of a^* values

Cycles	N	Min	Max	Mean	Std. Dev.
0	5	27.5	29.3	28.5	1.3
3	60	16.4	26.4	21.2	2.5
6	60	7.9	19.4	13.9	2.8
9	60	6.0	15.4	10.4	2.2
15	60	3.1	10.2	6.2	1.5
25	60	2.3	7.4	4.2	1.5

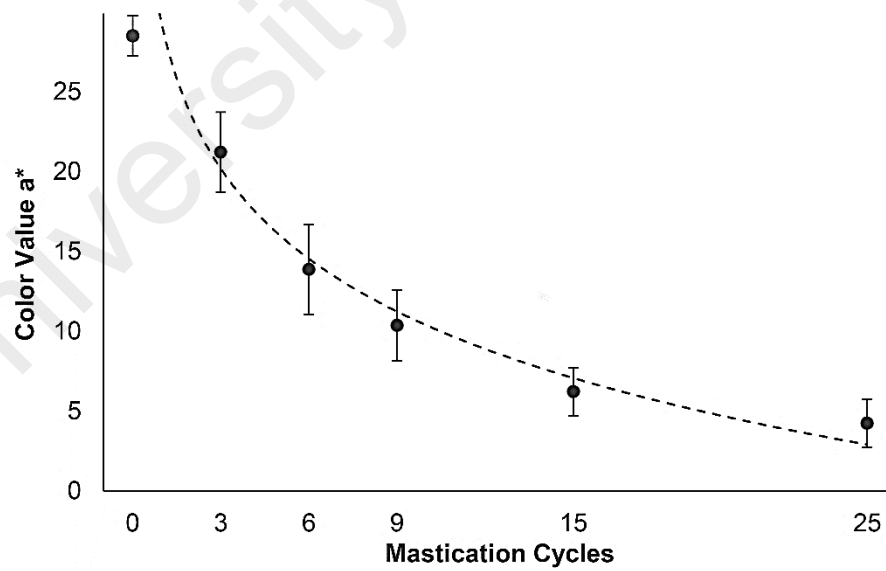


Figure 4.5: Color values of a^* channel (mean \pm SD)

Table 4.4: Contrasts of a^* values for different number of mastication cycles

Cycles		Mean Group Difference	Std. Error	P-value
3	6	7.36	0.44	.000
	9	10.87	0.44	.000
	15	15.01	0.45	.000
	25	16.99	0.49	.000
6	3	-7.36	0.44	.000
	9	3.51	0.42	.000
	15	7.65	0.42	.000
	25	9.63	0.47	.000
9	3	-10.87	0.44	.000
	6	-3.51	0.42	.000
	15	4.14	0.43	.000
	25	6.12	0.47	.000
15	3	-15.01	0.44	.000
	6	-7.65	0.42	.000
	9	-4.14	0.43	.000
	25	1.98	0.47	.000
25	3	-16.99	0.49	.000
	6	-9.63	0.47	.000
	9	-6.12	0.47	.000
	15	-1.98	0.48	.000

Bonferroni *post hoc* analysis. Sample size: 60. All contrasts were significant at the 0.05 level.

The regression analysis showed that with increasing number of mastication cycles, GD increased linearly ($r^2=0.80, P<.001$), while a^* decreased semi-logarithmically, indicating a higher level of color mixture. The a^* data were log-transformed to build a linear model ($r^2=0.79, P<.001$). Then, the two parameters were combined together as predictors of the number of mastication cycles (Mastication Index) by means of multiple regression analysis. As a result of the multiple regression analysis ($r^2=0.81, P<.001$), the following regression formula was obtained:

$$MI = 17.596 + 0.207 \times GD - 12.358 \times \log_{10}(a^*) \quad (4.1)$$

where MI is Mastication Index, GD is geometric dispersion, and a^* is the mean color value of a^* channel. The latter regression formula was embedded into a custom-built plug-in Mastimeter[©] of ImageJ software¹ for the automated analysis of the digital images.

¹ <https://sites.imagej.net/RG.Mastimeter/plugins> and <https://www.youtube.com/watch?v=nJsGUcbiRwY>.

See appendix F.

4.2 Intervalvalidation Experiment

In the data of test group, there were significant differences in masticatory performance between T1 and T2 groups for both Mastimeter[®] and XYLITOL tests (Figs. 4.6 & 4.7, Table 4.5).

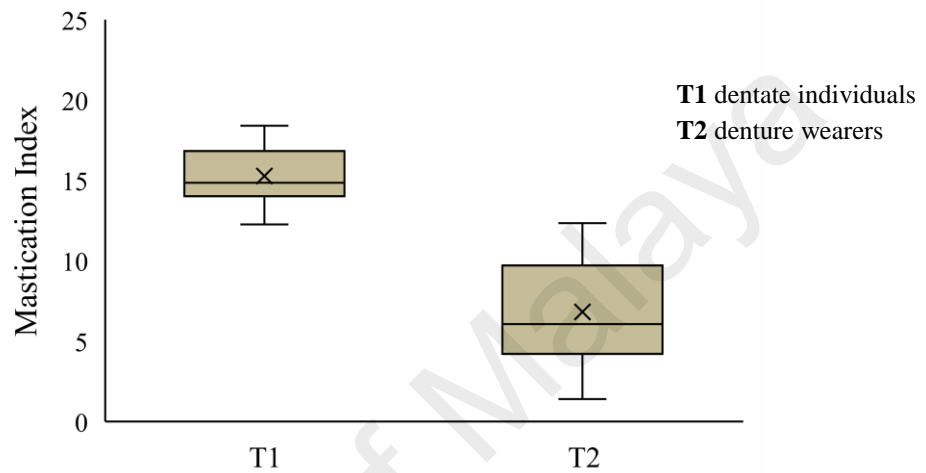


Figure 4.6: Mastication Index for both test groups

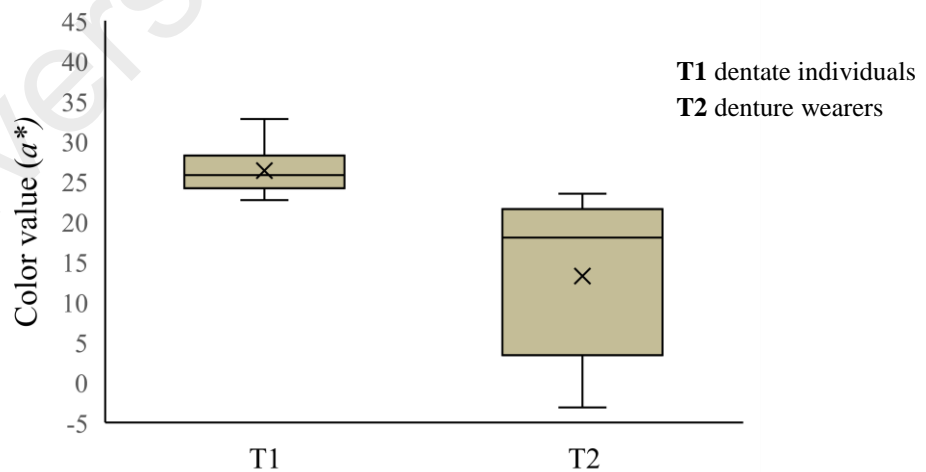


Figure 4.7: XYLITOL results for both test groups

Table 4.5: Masticatory performance (Mean \pm SD) for both groups

Group	Natural dentitions (T1) N=10	Complete dentures (T2) N=10	P-value
Mastimeter[®]	15.3 \pm 1.9	6.8 \pm 3.5	.000
XYLITOL	26.3 \pm 3	13.2 \pm 10.1	.002

Independent samples *t*-test

Table 4.6: Pearson correlation coefficients between Mastimeter[®] and XYLITOL masticatory performance tests

Group	N	Correlation coefficient	P-value
Natural dentitions (T1)	10	.40	.257
Complete dentures (T2)	10	.67	.036
All	20	.82	.000

Pearson correlation coefficients between the Mastication Index and the outcomes of XYLITOL color-changeable chewing gum were significant in T2-group and in the total test group (Table 4.6). In T1-group, no significant correlation was observed between the results of the two tests. Figure 4.8 illustrates a scatter plot of the results of masticatory performance obtained from both tests.

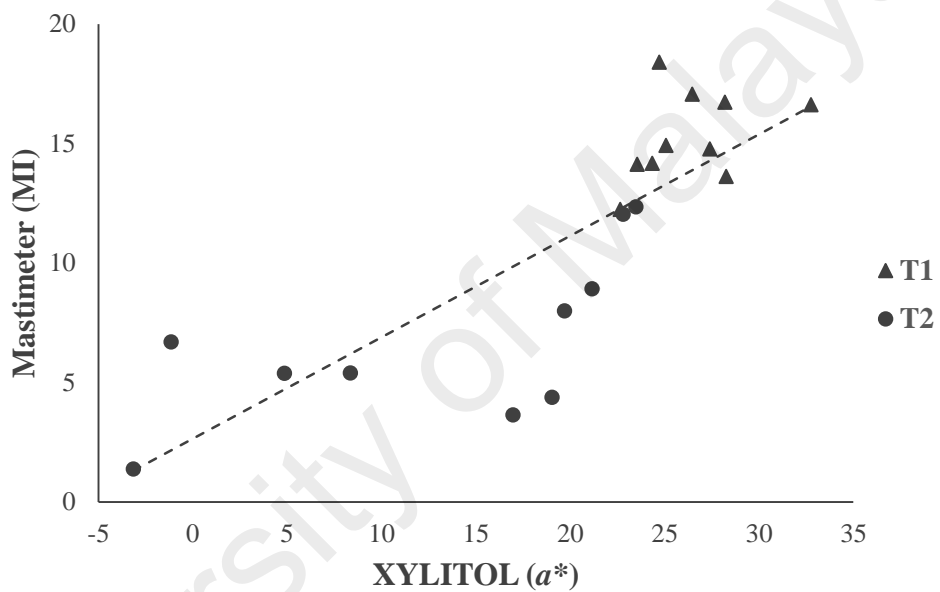


Figure 4.8: Scatterplot of Mastimeter[®] against XYLITOL

4.3 Characteristics of Chewing Gum

4.3.1 Hardness of Chewing Gum

The hardness of RG chewing gum (47.2 ± 2.5 newtons) was significantly lower than the hardness of Wrigley's Spearmint (SM) chewing gum (106.3 ± 1.7 newtons, $P < .001$). However, the latter showed a steep drop of hardness over the mastication process (97% hardness loss after 15 cycles) comparing with its RG counterpart (57% hardness loss after 15 cycles) (Fig. 4.9, Tables 4.7 & 4.8).

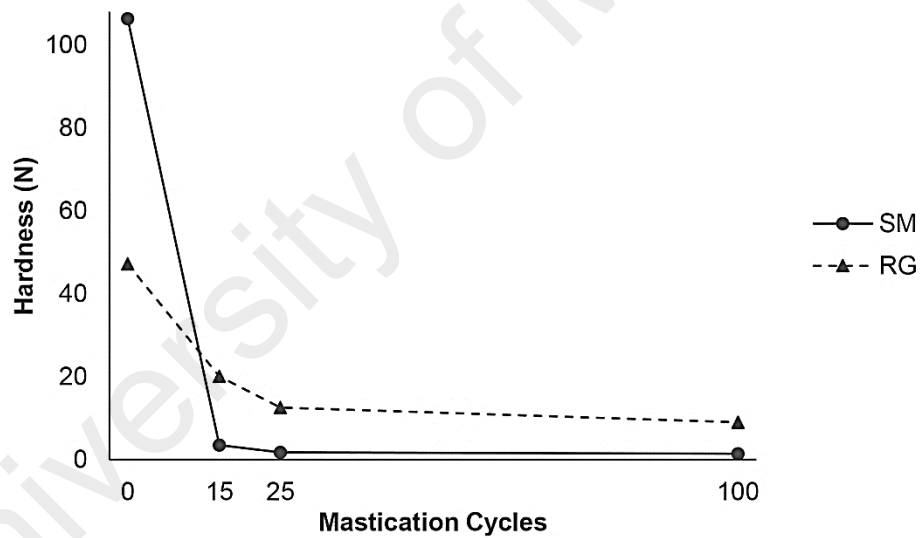


Figure 4.9: Hardness of chewing gum throughout different mastication cycles

Table 4.7: Hardness of chewing gum for different numbers of mastication cycles

Cycles	Force (N)		P-value
	RG	SM	
0	47.2 ±2.5	106.3 ±1.7	.000
15	20.1 ±2.3	3.5 ±0.7	.000
25	12.6 ±1.1	1.8 ±0.3	.000
100	9.0 ±1.0	1.5 ±0.3	.000

N: Newtons. RG: red-green gum. SM: Wrigley's Spearmint gum. Independent samples *t*-test. Sample size: 5.

Table 4.8: Hardness loss of chewing gum for different numbers of mastication cycles

Cycles	Hardness loss (%)		P-value
	RG	SM	
0	0	0	-
15	57.3 ±0.06	96.7 ±0.01	.000
25	73.4 ±0.02	98.3 ±0.01	.000
100	80.9 ±0.03	98.6 ±0.01	.000

RG: red-green chewing gum. SM: Wrigley's Spearmint gum. Independent samples *t*-test. Sample size: 5.

4.3.2 Mass Loss of Chewing Gum

Throughout the mastication process, RG chewing gum displayed a trivial mass loss (within 1%) under the presented testing conditions (Fig. 4.10). Whereas, a significant mass loss of Wrigley's Spearmint chewing gum was observed (48% mass loss after 100 cycles) (Fig. 4.10, Tables 4.9 & 4.10).

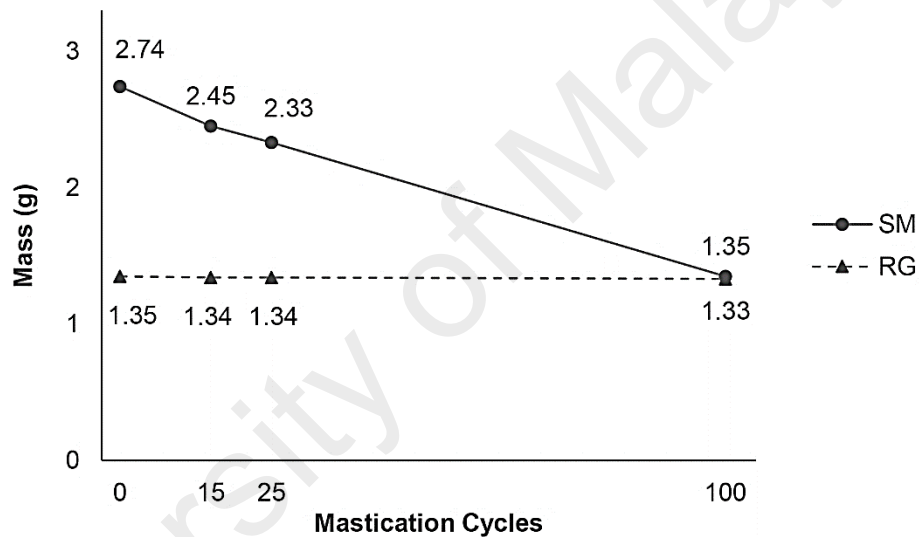


Figure 4.10: Mass of chewing gum throughout different mastication cycles

Table 4.9: Mass of chewing gum for different numbers of mastication cycles

Cycles	Mass (g)		P-value
	RG	SM	
0	1.35 ±0.10	2.74 ±0.02	.000
15	1.34 ±0.10	2.45 ±0.02	.000
25	1.34 ±0.11	2.33 ±0.04	.000
100	1.33 ±0.12	1.35 ±0.05	.783

RG: red-green chewing gum. SM: Wrigley's Spearmint gum.
Independent samples *t*-test. Sample size: 5.

Table 4.10: Mass loss of chewing gum for different numbers of mastication cycles

Cycles	Mass loss (%)		P-value
	RG	SM	
0	0	0	-
15	0.14 ±0.03	08.2 ±0.51	.000
25	0.14 ±0.05	12.6 ±1.25	.000
100	0.88 ±0.08	48.3 ±1.89	.000

RG: red-green chewing gum. SM: Wrigley's Spearmint gum.
Independent samples *t*-test. Sample size: 5.

CHAPTER 5: DISCUSSION

The present study demonstrated that measuring masticatory performance is feasible by the use of the new system Mastimeter[®]. Three sets of experiments were carried out. First, the two-color mixing ability was tested in 20 healthy participants (reference group) with a view to prove that the degree of color mixture depends on the number of mastication cycles applied, and to establish a reference regression model.

The data of the reference group support rejecting the first null hypothesis, as the colorimetric parameters (GD and a^*) were able to discriminate among the groups of different numbers of mastication cycles. Furthermore, the second hypothesis should also be rejected, as the regression analysis demonstrated significant relationship between the parameters of color mixing and the number of mastication cycles. The numbers of mastication cycles [0 to 25] were selected based on previous studies that investigated the mixing ability using two-color chewing gum (Palomares et al., 2018; Vaccaro et al., 2018; Vaccaro et al., 2016). From 10 to 20 mastication cycles, the two-color mixing ability test seems able to discriminate among different levels of masticatory performance. Beyond 25 cycles, the healthy dentate person almost attains the saturation level of color mixture, and thus, the test loses its ability to quantify the masticatory performance due to a ceiling effect with the results.

Based on the calibration data of our reference group, the two parameters of color mixing (GD and a^*) were combined as predictors of the number of mastication cycles (Mastication Index) by means of multiple regression analysis. Color parameters were previously converted into numbers of mastication cycles using specialized nomograms (Schimmel et al., 2011), machine learning techniques (Vaccaro et al., 2018), or regression models (Hama et al., 2014a). However, the regression analysis is a simple technique that

provides detailed outputs comparing to the nomograms or machine learning classifiers. The percentage of masticatory performance of a patient in relation to healthy individuals can then be calculated using the formula:

$$MP(\%) = \frac{MI}{MC} \times 100 \quad (5.1)$$

Where *MP* is masticatory performance, *MI* is Mastication Index, and *MC* is the number of mastication cycles applied. The $MP=100\%$ indicates an optimal masticatory performance, i.e. the diagnosed individual has a coequal level of mixing ability as the reference healthy persons. Conversely, the $MP=0\%$ denotes a total absence of mixing ability. For instance, when a complete denture wearer masticated a specimen of RG chewing gum for 15 cycles, the Mastication Index was 5.4, and the MP then is $5.4/15=36\%$.

In the second set of experiments, the outcomes of Mastimeter[®] test were compared with the results obtained from a color-changeable chewing gum (Masticatory Performance Evaluating Gum XYLITOL; Lotte Co Ltd, Tokyo, Japan) in a test group of young and elderly participants. The data of test group support rejecting the third null hypothesis, as the Mastimeter[®] test was able to identify a significant difference ($P<.001$) in masticatory performance between young and elderly individuals. Furthermore, the results of Mastimeter[®] presented in Table 4.5 are clinically interpretable as that our group of complete denture wearers attained almost 45% of masticatory performance as compared with healthy young individuals, whilst the current outcomes of XYLITOL chewing gum cannot be expressed as ratio scale due to the non-linear relationship between the color change and the number of mastication cycles (Hama et al., 2014a).

Significant correlations between the Mastimeter[®] and XYLITOL tests were observed for the group of complete denture wearers and for the total test group, but not for the

group of young dentate individuals. Therefore, the fourth null hypothesis is partly rejected. These findings are in accordance with results of other studies (Kaya et al., 2017; van der Bilt et al., 2010; Weijenberg et al., 2013). The Mastication Index of the young and elderly individuals are plotted against the outcomes of XYLITOL test in Figure 4.8. In this figure, it can be seen that the data points for T1-group are close to each other and concentrated in a limited region in comparison with those for T2-group. The T1-group consisted of 10 young participants with normal occlusion, complete natural dentition, and healthy masticatory system. Thus, we presumed that they have an optimal MP with a minimal variation (i.e. ideal chewers). The relatively small standard deviations of both Mastimeter[®] and XYLITOL results for T1-group in comparison with T2-group (Table 4.5) confirm the previous assumption. Apparently, the small amount of variability in the data of T1-group negatively affected the size and significance of the correlation ($r=0.40$, $P<.257$), whilst the more diverse data of the total test group led to the finding of a high correlation ($r=0.82$, $P<.001$).

In the third set of experiments, the hardness and mass of RG chewing gum were measured before and after mastication. Hardness loss (%) and mass loss (%) were then calculated and compared with those of a commercially available chewing gum. The data support rejecting the fifth null hypothesis, as the hardness loss and mass loss of RG chewing gum were significantly lower than those of commercial chewing gum ($P<.001$).

In the previous literature, various commercial brands of chewing gum have been used for the two-color mixing ability test (Schimmel et al., 2015; Vaccaro et al., 2016; Weijenberg et al., 2015). The appropriate specimens should comply with certain requirements (Table 5.1) (Schimmel et al., 2015). Unfortunately, finding specimens with optimal color combinations was not straightforward, as most manufacturers no longer add artificial colorings to their gums.

Table 5.1: Specifications for ideal specimens in two-color mixing ability test¹

<ol style="list-style-type: none">1. The specimen should have two colors, ideally pre-combined in one piece.2. The color combination should represent a large spread in hue values in the HIS color space (e.g. green/red or red/azure).3. The colors should not include white, which has an undefined hue value.4. The colors should both be visible in the unchewed gum, ideally one color per side (a colored “core” is unsuitable).5. The specimen should not stick to denture resin (PMMA).6. The specimen should not be too big or too hard, thus relatively easy to chew.7. The specimen should be storable and be widely available.8. The specimen should be individually packed for handling and hygienic reasons.9. The colors should be relatively stable over time, even once the specimen has been chewed.10. The taste should be enjoyable for most patients.11. The specimen should be sugar-free.

Some investigators used custom-made wax specimens (Salleh et al., 2007; H. Sato et al., 2003). However, people might prefer gum instead of wax because they are accustomed to its taste and texture. Therefore, RG bicolor chewing gum was formulated and validated.

According to color theory, red and green are a pair of opposite colors (Wuerger & Xiao, 2015). The high contrast of this combination enables color recognition both visually and digitally. Furthermore, mixing red and green pigments produces brown, which is an intermediate color located in mid-spectrum between the two baseline colors. Saliva might be a confounding factor in the color change, as the composition of saliva and its flow rate

¹ Reprinted from Schimmel et al. (2015) under Creative Commons Attribution License.

differs among people. Therefore, water-insoluble dyes were used to color the RG chewing gum.

The test food specimen should have a suitable texture and should be easy to masticate by individuals with compromised oral function. In food science, Texture Analyzer has been widely used to assess food texture by examining force-deformation/time curves (Salleh et al., 2007). Clearly, the mixing ability score depends on the texture of chewing gum. The hardness behavior of RG chewing gum demonstrates that it has much better hardness stability throughout the duration of mastication as compared with the SM commercial gum (Fig. 4.9).

The steep drop in the hardness of the SM chewing gum could be attributed to the gum base characteristics and the plasticizing effect of sugar-saliva content. The sugar might be harmful to patients with diabetes, and the release of sugar into saliva causes undesirable mass loss of the gum. Thus, a sugar-free chewing gum is recommended.

The commercially available chewing gum lost up to 13% of its weight after only 25 mastication cycles, which is consistent with the findings of a previous study (Anastassiadou & Heath, 2001). By contrast, RG chewing gum showed a minimal mass loss over 100 cycles (Fig. 4.10). The volume of the chewing gum is directly proportional to the surface area of the wafer. In other words, the longer the duration of mastication, the more lost pixels on the digital image. Therefore, mass stability is considered a favorable characteristic of RG chewing gum.

Some research groups have produced custom-made chewing gum (Buser et al., 2018; Endo et al., 2014), but the recipe and method of preparation remain exclusive to the manufacturing company. The ingredients of RG chewing gum (Table 3.2) are available by mail order, and its preparation is relatively straightforward.

In Euclidean geometry, the circularity (iso-perimetric quotient) has been frequently used to measure the compactness of two-dimensional objects (Schick, Fischer, & Stiefelhagen, 2014). However, the circularity outcomes of the data of our reference group were limited to a tiny span [0 to 0.05]. Therefore, a new measure of an object's anti-compactness "geometric dispersion" was derived from an iso-areal quotient formula and used in this study. The iso-areal quotient is a dimensionless measure that relates the perimeter of a shape P_s to that of a circle P_c with the same area ($A_s=A_c$). The iso-areal quotient for a circle is 1 and increases for more scattered and irregular objects.

The radius of a circle is $r = \sqrt{\frac{A_c}{\pi}}$

The perimeter of this circle is $P_c = 2\pi r$

The iso-areal quotient then is $Q_a = \frac{P_s}{P_c} = \frac{P_s}{2\pi\frac{\sqrt{A_c}}{\sqrt{\pi}}} = \frac{P_s}{2\sqrt{\pi A_s}}$

In CIELab color space, the pixel of a two-dimensional image is typically digitized by two sets of data: three color values (L^* , a^* , b^*), and two Cartesian coordinates (x , y) of spatial position. Both the contrast of color values and the spatial polarization of pixels can be considered as the main characteristics of color heterogeneity. The former feature has been extensively studied (Halazonetis et al., 2013; Speksnijder et al., 2009; Vaccaro et al., 2016). However, it might be useful to investigate the image from a spatial point of view and then evaluate the color mixture using a holistic spatial-value approach.

Color thresholding based on the cutoff point $a^*=0$ splits the baseline colors (red versus green) into two mutually exclusive segments. Each red and green segment has almost the same number of pixels (surface area), regardless of the degree of mixture, whereas spatial distribution of pixels defines the resulted shape perimeter. In the early stage of

mastication, reddish pixels—for instance—are concentrated and compacted in just a few wide areas. As kneading advances, the geometric dispersion increases due to the spreading and diffusion (spatial mixing), whereas the color intensity (a^*) decreases because of the value mixing.

Mastimeter[®] has many advantages as compared with previous methods of masticatory performance assessment. This system combines both spatial and value outcomes of color mixing in a one-dimensional score, which provides an immediate and useful clinical conclusion. The test is easy, and the whole procedure requires just few minutes to masticate, scan, and analyse the specimen. The ingredients of RG chewing gum are not costly, and the required software is open-source and online downloadable free of charge.

The limitations of this study include that the validity experiment enrolled a relatively small sample size, and Mastimeter[®] was not compared to any of the comminution tests, which are to date still considered the gold standard for evaluating masticatory performance. Furthermore, the Mastimeter's test-retest reliability and inter-examiner consistency are still to be proven.

Future studies are needed to determine the validity and reliability of Mastimeter[®] for evaluating masticatory performance in a larger sample of participants with different dental states, and with a comminution test as gold standard. A further comparative study is recommended to compare the trueness and precision of different digital techniques for quantifying color mixing ability.

CHAPTER 6: CONCLUSION

Based on the results of this study, the following conclusions were drawn:

1. The newly developed chewing gum has favorable structural and colorimetric characteristics for assessing food mixing ability.
2. The proposed colorimetric parameters (spatial and value) are able to discriminate among different degrees of color mixing.
3. Mastication Index combines both spatial and value colorimetric parameters in a one-dimensional score, with clinical interpretability.
4. Mastication Index is able to discriminate among different dental conditions.
5. Outcomes of Mastimeter[®] are significantly correlated with the measurements of a previously well-established method for evaluating mixing ability.
6. Mastimeter[®] proved to be valid and able to quantify the masticatory performance, and has the potential to be used in both research and clinical settings as a proxy to other traditional methods.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Yusof, Y., Salleh, N. M., & Yusof, F. (2019). Assessment of masticatory performance by geometric measurement of the mixing ability with 2-color chewing gum. *Journal of Prosthetic Dentistry*. 121(6), 916-921.

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