

HIGH-RESOLUTION MORPHOLOGICAL CHARACTERIZATION INVESTIGATION ON THE EFFECTS OF LOCAL MALAYSIAN CONDIMENTS ON HUMAN MINERALISED TISSUES.

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1. INTRODUCTION

There are a huge variety of locally produced Malaysian sauces available in the local market. In many instances they may formed the everyday condiments of the main menu especially during lunch and dinner to the local Malaysian citizen. The ingredients and taste of these products varies from state to state within the country and from fishery products (such as anchovies to fine shrimp) to soya bean ketchups. Among the local favourite condiments includes the Ketereh budu in Kelantan (anchovies sauce), cincaluk (fine shrimp sauce) in Malacca, maggi chilli sauce ketchup, Adabi soya sauce ketchup etc.

Mineralised tissues such as bones and teeth react to physical and chemical stimuli. These reaction is often observed quite differently to that of other tissues and organs of the body. The demineralisation of bone matrix and of fully calcified bone have been studied by Boothroyd

(1964), and Thorogood and Gray, (1975), where else on tooth tissue by Watson (1960) and Decker in 1973, but no relationship studies have yet to be carried out to observe the relationship between the consumption of local sauces with the natural process of demineralisation and loss of crystallites from the mineralised tissues that might be induced and attributed by regular consumption of this local condiments delights. Thus little is known of the histo morphological changes that might occurs and that might be related to high incidences of oral health issues such as white spots and fissures caries tooth formations that may have be the physical presentation of that relationship.

This study is undertaken with the knowledge that without knowing the nature and the possible consumptive effects of the local condiments available in the local market especially towards the human mineralised tissues, as such in their efforts in maintaining a healthy life style diet, it may be difficult to relate and provide reliable explanation to the causes and origin of such frequent ailment of the jaws and teeth

2. OBJECTIVE

- To register a national database on effects of local consumption of this sauces especially to the mineralised tissue of the human body.
- It is hope that insights gained from this study will further update knowledge of regular usage of the sauces.
- The aim of the present study is to evaluate and made an assessment of the progression of demineralisation reaction and changes to the tissue histomorphology as observed under stereoptical and high-resolution microscopy
- To investigate if there is any correlation with expiry dates of the condiments product to the strength, severity of demineralisation reactions towards the mineralised tissues of the body.

3. METHODOLOGY

Fish anchovies, tomato sauce ketchup and soya sauce ketchup will be randomly selected for the pilot study. Fresh varieties of the identified local condiments will be used. Each portion of the identified condiments will be placed into various labelled 100 ml bottle containers. These specimen containers will then be fixed to a variable speed rotator at room temperature; (this will help to improve the penetration of the condiments ingredients into the mineralised tissue). Ph and the expiry dates of each condiment will be noted. The mineralised tissue will be initially disinfected with Tymol gargle dan then directly immersed into each labelled condiment containers. Regular daily changes of

fresh portions of the various condiments will be subjected to the mineralised tissues. Each day the various morphological changes will be examined macroscopically using a stereomicroscope; each morphological change observed will be clerked and recorded. Under normal circumstance a complete demineralisation of a tooth structure will be achieved in the fourth to the fifth week, hence the morphological changes will be studied to the fifth week. On the fifth week, each mineralised tissues will be chemically fixed for duration of one day with full strength Karnovsky's (pH 7.0) following which they will then be rinse in Ringers solutions. (if needed, the chemically fixed specimens will then be x-rayed to check for radiolucency and radio-opacity. The specimen's will then be prepared as ground section where they will be-sectioned as unstained sections using a diamond saw microtome at selected 20µm specimen thickness increments. Each ground sections will then be grind and polished for optical microscope observations. The ground section will be observed under the stereomicroscope and variable pressure scanning electron microscope. The ground section will be initially washed with phosphate buffers solution and then osmificated with osmium tetraoxide. They will be examined under the VPSEM using its peltier cooling sub-stage at 44 to 48 Pascal pressure at an accelerating voltage of 15 kV and around 9mm to 15mm working distance. The tooth ground sections they will be examined from their occlusal surface to the pulpal border and clerked. Photomicrographs images of the sections will be taken and then transferred to Leica imaging workstation to enable accurate analysis of the

demineralised areas. The resultant image will be measured and colour-coded in accordance to various histogram classes.

4. OUTCOME, BENEFITS AND IMPORTANCE OF THE RESEARCH

Able to educate and be informed with better up-to-date knowledge of the local foodstuffs hazards especially of daily consumption of local condiments to the local community as such with the aim of improving the art of healthy eating and healthy life styles habits of the nation. The study will leads to the establishment of a national database on effects of daily consumption of local condiments especially on human mineralized tissue, as such will promote good general health awareness with regards to local almost ritual habits to condiments consumption.

5. RESULTS AND DISCUSSION

5.1 Ph and Immersion in Condiments

Experiments

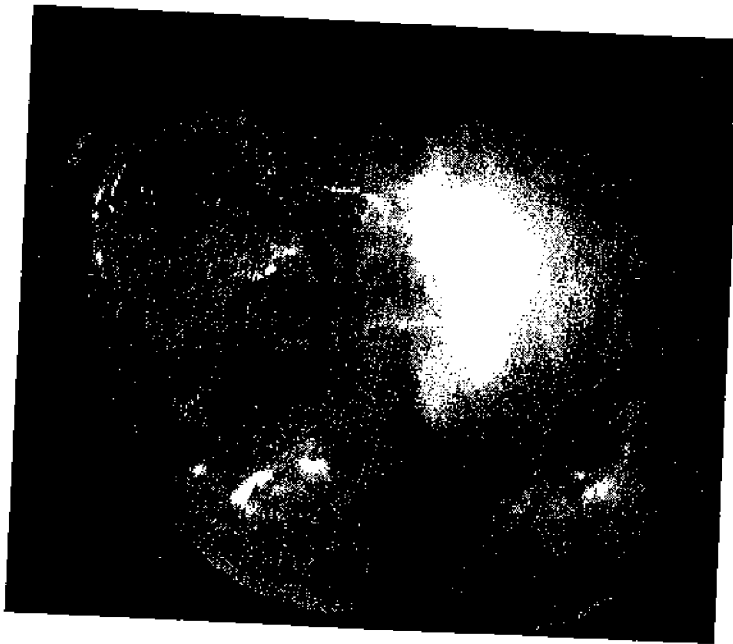
Ph of the condiment were consistent through the period of immersion experiments they as follows:

Tomato sauce Ph = 4.3. Anchovies sauce Ph = 5.97. Soya bean sauce Ph = 5.1

Tomato sauce specimens showed signs of demineralisation as early as week 4 in immersion. They were soft in texture and flabby like in nature, this phenomenon was not observed in the other condiment immersion samples.

5.2 Stereomicroscope imaging

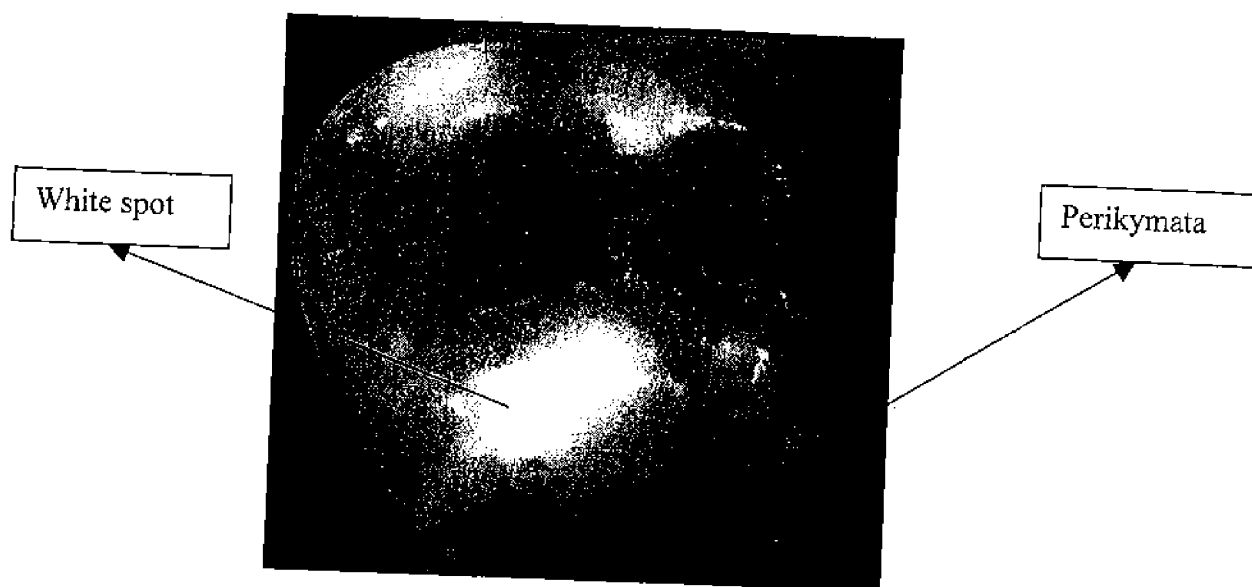
Observation was made at 16X. Surface occlusal morphology of the tooth crown was observed using the stereomicroscope. It was observed that white spots that is identified as a whitish chalky area or patches of islands was visible on the occlusal marginal ridges and cuspal tips of the tooth crown. At times the white spots appeared shiny and reflective. In anchovies specimens the peripheral outline of the white spots appeared more blurred as compared to a well defined margins observed in tomato sauce specimens



(Stereo-photomicrograph of tomato sauce molar specimen showing
present of white spot-1.25X magnification)

The perikymata of the enamel was well observed in tomato and anchovies specimens but blurred in some of the soya bean specimens. Perikymata are wave like patterns of concentric surface rings parallel to the tooth cementum enamel junction. Perikymata can be removed by attrition and abrasion. It is not cleared why the perikymata was blurred in majority of soya bean specimens. Perikymata on the enamel surface is continuously bombarded and linked to the adherence properties of the

local condiments consumed. Technically enamel is a hard non porous in soluble surface. These properties would suggest that it would resist the changes adopted by the adherence of the condiments. However this immersion experiments showed the present of white spots development and the lost of perikymata which only postulates the decalcifying effect brought about by the adherence of the condiment on the tooth surface. Clinically this is bad news for the oral cavity tissues.



5.3 Variable Pressure Scanning

Electron Microscope Imaging

The use of the saw microtome leads to the formation of smear layer impregnated on the cut surface of the tooth. The smear layers were thick and visible seems especially in the tomato sauce specimens. Observation of its surface topography was almost impossible, although attempt was made to remove the smear layer with the use of 35 phosphoric acid for 1 minute. The specimens were then gently fractured to avoid the cut surface. The fracturing technique was conducted gently with precision hammering with the use of a mallet. Observation was made using the VPSEM at 9mm working distance at 47 Pascal pressure with the (back scattering electron) BSED mode. The results can be summarised as below;

- a. there were no smear layer observed around the fractured surface in all specimens. Smear

layer was only observed on the cut surface and of the three groups of specimens-condiments samples; the least coated was the surface of specimens immersed with soya bean ketchup.

- b. the tomato sauce specimens were covered with a well-defined coating believed to be the adherence of the sauce and smear layer on the surface area of the specimens. This was interesting to be observed and noted as the phenomenon of coating was not observed to occur in other specimens with other condiments although all of them have been thoroughly washed under running tap water and the naked eye do not show any present of a coating or acquired pellicle like surface of what ever sort of colour to be present.

- c. The intertubular dentine in all dentin slabs used in the immersion experiments with various condiments showed various degree of shallow furrow like structures present. It is believed these structures are related to
- d. Intratubular or peritubular dentine is not seen in all these categories of samples as compared to control sample not immersed with condiments. The peritubular dentine is believed to be more mineralised than intertubular dentine. The lack of peritubular dentine is believed to be related to loss of octacalcium salts to demineralisation. Further investigation with EDAX will confirmed this.

6. CONCLUSION

The present conclusion of this pilot investigations suggest

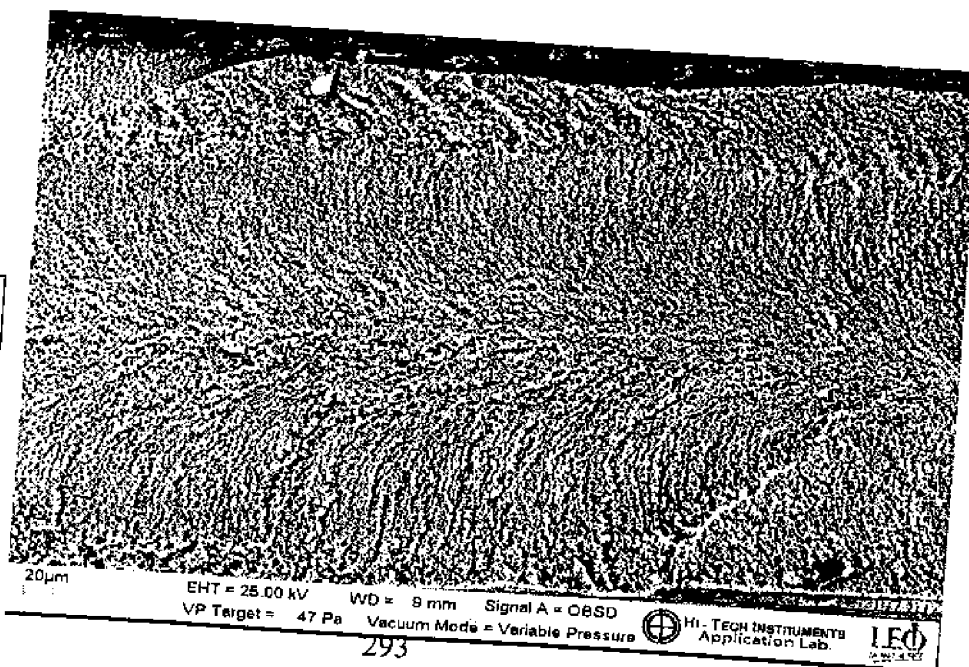
various ingoing process of shrinkage and lost of calcification linked to the process of demineralisation with prolong time of immersion with condiments.

- that adherence properties plays a major role in changes on tooth morphology especially with local condiment that strong acidic in preparation.
- elemental composition will be next step to further counter related to the morphological characterisation described here.

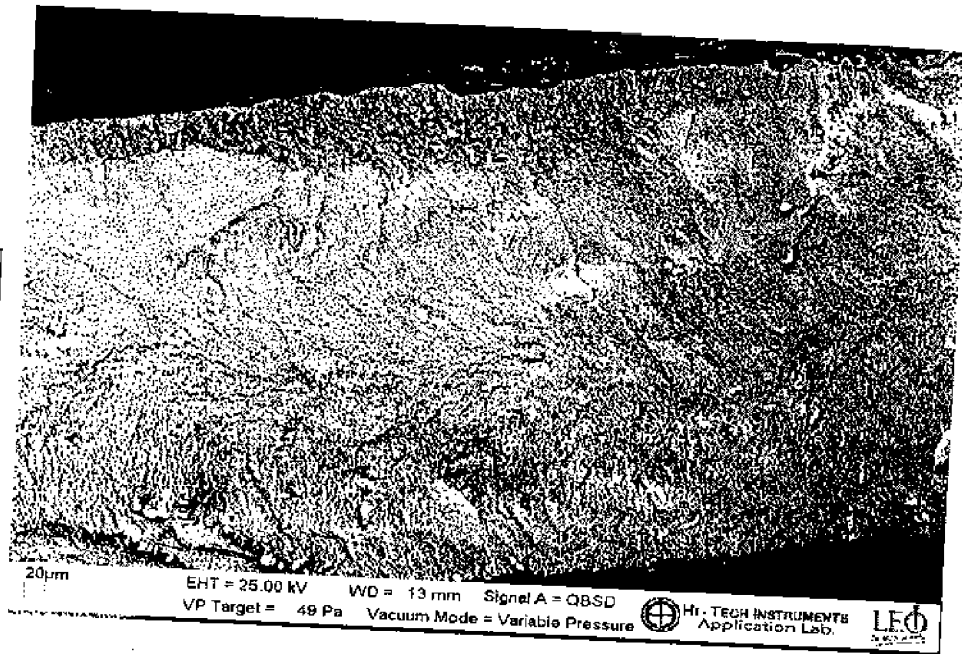
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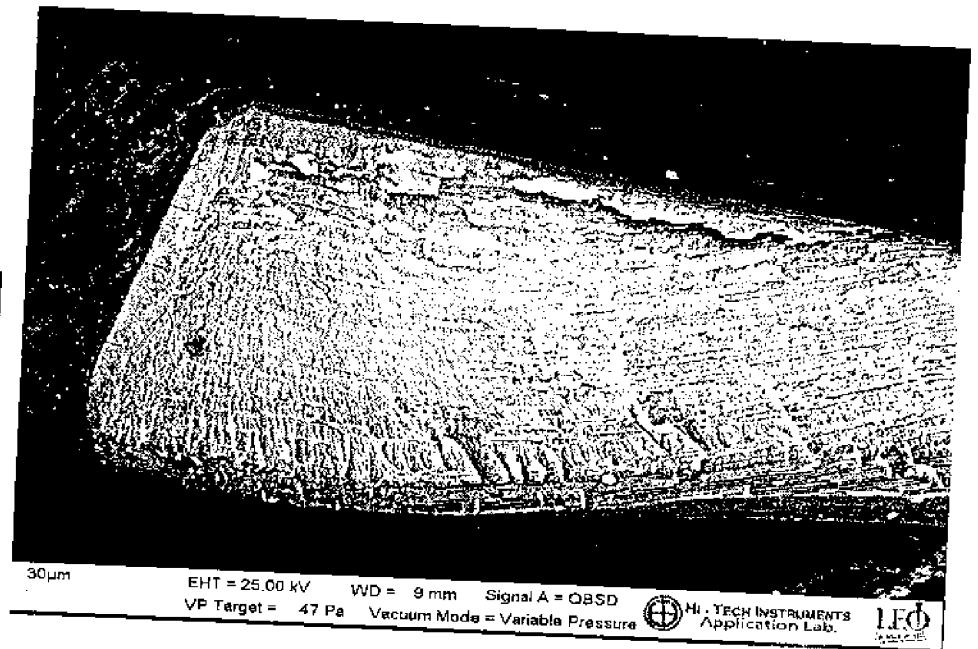
Anchovies



Soya bean



Tomato



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