UNIVERSITI PUTRA MALAYSIA

ENZYME AIDED PEELING AND MEMBRANE REMOVAL OF LOCAL MANDARINS (CITRUS SUHUIENSIS)

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ENZYME AIDED PEELING AND MEMBRANE REMOVAL OF LOCAL MANDARINS (*Citrus suhuiensis*)

By

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Specially Dedicated

To

My Late Father Liu Shu Ping
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

ENZYME AIDED PEELING OF LOCAL MANDARIN (*CITRUS SUHUIENSIS*) AND LOCAL MANDARIN SEGMENTS

By

LIU FANNY

May 2004

Chairman: **Associate Professor Azizah binti Osman, Ph.D.**

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A combination of pectinases and cellulases are able to selectively alter the albedo and segment membrane structure of citrus fruits and, hence, aid the removal of the peel, adhering albedo layer and also the segment membrane. This study was carried out to determine the optimum conditions needed to peel local mandarins using pectinases (Peelzym® IV, Novozyme, Switzerland) and cellulases

(Celluclast® 1.5L, Novozyme, Switzerland). The experiment variables were enzyme concentration, vacuum pressure and vacuum infusion time. In the first part of the experiment, the local mandarins were first scored from stem end to the blossom end followed by immersion in 1000 ml of enzyme solution at a set vacuum pressure and ambient temperature (27 ± 1 °C). Only one parameter was varied in any one experiment. The latter part of the experiment was carried out using Response Surface Methodology (RSM) to determine the optimum combinations of enzyme concentration, vacuum pressure and vacuum infusion time to aid enzymatic segment membrane removal. Echip software was employed in the experimental design, calculate equations and statistical analysis. Peelzym® IV at 0.4 % v/w, 650 mm Hg vacuum and 16 minutes of vacuum time were found to be optimal for peel removal.

The enzyme-peeled fruits were judged by the panellists using three different sensory tests to ascertain its appeal to consumers. A significant ($P < 0.05$) difference between enzyme-peeled and hand-peeled segments was found, with the panelists preferring the enzyme-peeled segments. Celluclast® 1.5L at 4.52 % v/w, vacuum pressure at 370 mm Hg for 9 minutes was found to be optimal. After segment membrane removal, the membraneless local mandarin segments were then placed in different concentrations of sugar solutions to gauge consumer acceptance. Different sugar concentrations were used to emulate commercially available canned mandarin segments. Although varying concentrations of sugar solutions were used, the colour, odour, firmness, presence of adhering segment membrane and segment integrity were not affected as there was no significant ($P<0.05$) difference among the samples. It was also observed that local mandarins stored in 15 °Brix was the preferred sugar concentration. As an overview, enzyme-peeled segments were found to be much more appealing as it had a much more intense orange colour, was firm with no loss of segment integrity, hence, was very well accepted by the panelists. Thus, enzyme aided peeling has a great potential as an alternative method to replace conventional methods of peeling.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGUPASAN KULIT AND SELAPUT MEMBRAN LIMAU MADU (*CITRUS SUHUIENSIS*) DENGAN BANTUAN PENGUNAAN ENZIM

Oleh

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Pengerusi: **Profesor Madya Azizah binti Osman, PhD**

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Kombinasi pektinase dan sellulase telah didapati berupaya mengubah struktur "albedo" dan selaput membran segmen buah sitrus secara selektif dan seterusnya membantu dalam pengupasan kulit, lapisan "albedo" yang terlekat serta selaput membran segmen. Kajian ini dilaksanakan dengan tujuan mengenalpasti keadaan optimum yang diperlukan untuk mengupas kulit limau madu dengan menggunakan pektinase (Peelzym® IV, Novozyme, Switzerland) dan sellulase (Celluclast® 1.5L, Novozyme, Switzerland). Pembolehubah eksperimen adalah kepekatan enzim, tekanan vakum, serta tempoh infusi vakum. Pada mulanya, limau madu dikelar kulitnya, dari hujung tangkai ke hujung pangkal, tanpa menempuhi bahagian isi limau. Langkah ini diikuti dengan perendaman limau dalam 1000 ml larutan enzim pada tekanan enzim yang ditetapkan pada suhu bilik (27 ± 1 ^{o}C). Hanya satu parameter diubahsuai dalam setiap satu langkah eksperimen. Bahagian kedua eksperimen dilaksanakan dengan menggunakan Methodologi "Response Surface" (RSM) untuk menentukan titik optimum kombinasi kepekatan enzim, tekanan vakum serta tempoh infusi vakum dalam pengupasan selaput membran segmen limau madu. Perisian lembut Echip digunakan untuk menentukan rekabentuk eksperimen, mengira keseimbangan dan membuat penilaian statistik. Keputusan menunjukkan bahawa

Peelzym® IV pada kepekatan 0.4 % v/w, 650 mm Hg vakum dan tempoh infusi enzim selama 16 minit adalah keadaan optimum bagi pengupasan kulit limau madu. Limau madu yang telah dibuang kulitnya dinilai oleh para panel dengan menggunakan tiga jenis kaedah sensori bagi menentukan penerimaan dan daya tarikannya oleh pengguna. Perbezaan yang ketara ($P< 0.05$) diantara limau yang dikupas tangan berbanding dengan limau yang telah dikupas dengan bantuan enzim telah didapati di mana para panel telah menunjukkan yang limau yang telah dikupas dengan bantuan enzim menjadi pilihan mereka. Didapati juga bahawa kepekatan enzim Celluclast® 1.5L pada kepekatan 4.52 % v/w, tekanan vakum pada 370 mm Hg serta tempoh infusi vakum selama 9 minit adalah keadaan optimum bagi pengupasan selaput segmen limau madu. Selepas pengupasan selaput segmen, segmen limau madu telah direndam di dalam larutan gula pada kepekatan yang berlainan untuk mengenalpasti penerimaan para pengguna. Langkah ini dilakukan untuk menyamai produk limau yang boleh didapati secara komersil. Walaupun kepekatan larutan gula yang berlainan telah digunakan, ciri fisiko-kimia segmen limau madu tidak terjejas. Didapati tiada perbezaan yang ketara ($P < 0.05$) ke atas warna, bau, kekerasan, kehadiran selaput yang masih terlekat serta integriti segmen juga masih kekal. Didapati juga produk hasil rendaman dalam larutan gula pada 15 ^{o}Brix merupakan pilihan para panel. Secara amnya, boleh dikatakan bahawa limau madu yang telah dibuang kulit dengan bantuan enzim adalah lebih menarik memandangkan ia mempunyai warna yang lebih menarik serta ketara, tidak terlalu lembut teksturnya serta diterima dengan baik oleh para pengguna. Dengan itu boleh disimpulkan bahawa teknik pengupasan kulit dan selaput segmen limau madu dengan bantuan enzim berpotensi untuk mengambil alih cara pengupasan konvensional yang lain.

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CHAPTER 1

INTRODUCTION

Fruit and fruit products, in all its many varieties and variations, are major world commodities and part of the economic life-blood of many countries, particularly the developing world (Arthey and Ashurst, 1996). Fruits have long been valued as part of the staple diet of many living things. As a matter of fact, fruits have been inexorably linked to the welfare of life on our planet (Taylor, 1996). Fruits are a form or class of food that is not only affordable but delicious, easily obtainable and highly nutritious. Most fruits, are primarily water, but also contain over 400 other constituents and nutrients, i.e. carbohydrates, organic acids, amino acids, ascorbic acid, minerals, flavonoids, carotenoids, volatiles, lipids, etc.

Nutrients frequently consumed in sub-optimal concentrations by humans are proteins, iron, vitamin A, thiamin (Vitamin B_1), riboflavin (Vitamin B_2) and ascorbic acid (Fourie 1996). Members of this group are often known as the critical nutrients. Fruits also contain these critical nutrients, and sometimes some of these nutrients occur in higher concentrations than in other foods (Fourie, 1996). Frequent consumption of vegetables and fruits, especially green and yellow vegetables and citrus fruits, is associated with decreased susceptibility to some forms of cancer, although the mechanisms for their protective effects have not been fully determined. Therefore, fruits play an important role in balancing the human diet, mainly because the compositions of fruits differ markedly from other foods of plant and animal origins (Hugo, 1969). Fruits are often consumed fresh or processed, as a result of the many attributes of the many different varieties of fruits available. Therefore, it is inevitable that processing of fruits are important to maximize the utility of fruit and to encourage an increase in fruit consumption.

Peeling is one of the most important preparatory steps in the processing of some fruits and vegetables meant for canning, freezing and dehydration (Radhakrishnaiah-Setty *et al.*, 1993) and also as a fresh refrigerated item. In the beginning of the fruit processing industry, only hand peeling was practiced. Over the years, several other methods of peeling i.e. peeling by steam, mechanical peeling, cryogenic peeling, etc were introduced, improving the efficiency of peeling. With the advancement in technology, enzyme (pectinases and cellulases) -aided processing (peeling, segmentation and membrane removal) has been introduced. This new method of peeling has many advantages over the other conventional methods/ practices of peeling. Among the advantages are minimization of production losses, energy and chemical use, heat ring formation and pollution load, and in addition it peels to the extent dictated by the products (Radhakrishnaiah-Setty *et al.*, 1993).

The United States Department of Agriculture (USDA) patented (USDA Patent No. 4,284,651) enzyme infusion technology in 1981 (Bruemmer, 1981) but it is relatively new to Malaysia. It has not been thoroughly studied especially in the context of local citrus. Local fruits i.e. citruses have been found to be very high in nutrients and fiber. Moreover this is a good way of promoting local fruits, presented in a different form to local consumers.

Enzyme treatment offers a way to achieve very specific changes in whole foods or food ingredients. Moreover, the removal or inactivation of the enzyme requires no specific label reference to enzyme use making it very consumer friendly (McArdle and Culver, 1994). Interest in enzyme infusion has increased in proportion to the availability and purity of commercial enzymes (McArdle and Culver, 1994). Consumers are beginning to show an interest in less-processed, fresher tasting retail food products (especially by the catering and bakery industry) that are fresh, convenient, can be prepared and consumed in less time.

The availability and purity of commercial enzymes have also contributed to the interest in enzyme infusion. In addition to that, the load of pollution caused by lye peeling can be greatly reduced when using enzyme infusion. This will be of particular importance, as consumers are becoming more and more conscious of the importance of non-environmental polluting products.

In addition, "natural" alteration of food ingredients has been a major rationale for genetic manipulation of plants (Caransa *et al.*, 1998). Consumer concerns over genetically engineered food ingredients (Fraley, 1992) may produce a climate where alternative biological strategies such as enzyme infusion accomplish the same task as genetic engineering in a simpler scheme. Enzyme infusion may also lead to unique opportunities, such as improved processes for peeling and sectioning, changes that may be difficult or impossible through genetic manipulation (McArdle and Culver, 1994).

In recent years, consumers have become more health-conscious in their food choices but have less time to prepare healthful meals. As a result, the market demand for 'minimally processed' fruits and vegetables has rapidly increased (Jacxsens *et al.*, 1999). Enzyme infusion can be driven not only by the food industry's interest in responding to consumer needs but also the need to increase productivity.

Malaysia is rich in resources and has an abundant supply of local fruits that are mostly non-seasonal (easily available), cheap and highly nutritious. These resources are under-utilised. Therefore, it is important to realise the potential of using high technological methods of processing these fruits minimally. With this, Malaysia might be able to penetrate into the international food market as a major producer of processed and minimally processed fruits. There is great potential in texture-improved ready-to-eat citrus fruits with intact fruit sacs and also for separated fruit sacs that can be incorporated into fruit juice in Malaysia.

The local mandarin / "Limau Madu" (*Citrus suhuiensis*) is a local citrus that is easily obtainable and available. With the aid of enzymes, it is relatively easy to process citrus fruits to produce attractive, value added, peeled fruits. These minimally processed citrus would be in demand in the catering industry such as the airline, hotel and bakery industries as it is less time consuming and more efficient as compared to hand peeling (the conventional method used in Malaysia).

Therefore the objectives of this study were to:

1. determine and compare the physico-chemical characteristics of local mandarins with other citrus fruits.
2. determine the effects of enzyme concentration, vacuum pressure and vacuum time needed for optimum peel removal of local mandarins.
3. optimize enzyme aided membrane removal of local mandarins with RSM (Response Surface Methodology).
4. determine consumer acceptance of enzymatically peeled (removal of skin and segment membrane) local mandarins.

All the data obtained will be useful to determine the optimum conditions needed for peel and membrane removal in order to optimize processing and to reduce production cost. This is in the belief that this new technology has great potential as an alternative to conventional peeling.

CHAPTER 2

LITERATURE REVIEW

2.1 Citrus fruits

2.1.1 Citrus

Citrus is considered as one of the important fruit crops in tropical and sub-tropical regions. According to Davies and Albrigo (1994), the general area of origin of citrus is believed to be southeastern Asia, including that from eastern Arabia east to the Philippines and from the Himalayas south to Indonesia or Australia. Within this large region, northeastern India and northern Myanmar were believed to be the center of origin, but recent evidence suggests that Yunnan Province in south-central China may be as important due to the diversity of species found and the system of rivers that could have provided dispersal to the south (Gmitter and Hu, 1990). Extensive movement of the various types of citrus probably occurred within the general area of citrus origin from before recorded history. Currently, citrus is grown primarily between the latitudes 40°N to 40°S. More northern and southern locations of commercial production exist where temperatures are moderated by ocean winds (Davies and Albrigo, 1994).

Production of fresh and processed citrus surpasses that of bananas, apples, grapes, or mangoes (Davenport, 1990). Fresh citrus has been appreciated to the extent that it was carried from its area of origin and cultivated from before the fifth century BC