A BRIEF OVERVIEW ON FACTORS AFFECTING THE LEVEL OF INOSINE 5′-MONOPHOSPHATE IN VARIOUS MEAT

Jutamart Kongkapan[1*], Apichaya Sakulthai[2], Nutdanai Boonoon[3], Tiranun Srikanchai[4]

[1,2,3,4]Faculty of Agro Industry, Department of Farm Technology of Management,
Panyapiwat Institute of Management, Thailand
[*]**Corresponding author, E-mail:** jutamartkon@pim.ac.th

Abstract

Due to the increasing of consumer's demand the quality of meat and caring obout healthiness, the processing of producing of good quality meat, particularly meat flavor is the most economically important. Umami is a highlight key for the flavor index. Inosine 5′-monophosphate (IMP), which is also call the umami compound, is a breakdown-nucleotide triphosphate after postmortem muscle which promotes to the taste in meat. There are several factors the influence IMP content in muscle including genetic, sex, age at slaughter, type of muscle, aging effect, cooking process, raising methods, nutrition factors, and season for harvesting. Therefore, if any change in the concentration of IMP in all meat products can potentially affect meat palatability. In this review, we conclude the potential factors influencing IMP level in various meat such as beef, pork, chicken, and aquatic meat.

Keywords: Inosine 5′-Monophosphate, Meat, Flavor

Introduction

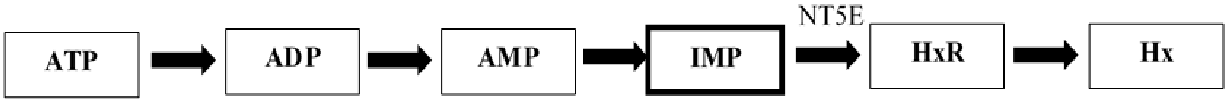
Nowadays, the trend of consumer demand for high-quality meat products, particularly in nutrition and sensory, has been valued over quantitative traits (Jung et al., 2013). The healthiness and a variety of sensory traits especially palatability and flavor are the major key decision of consumers. There are several factors which are generating the taste and flavor such as fatty acid, vitamins, amino acid, fat contents, and peptides. (Ramalingam, Song, & Hwang, 2019). The flavor of the meat is a combination of five basic tastes and the umami flavor, which its potency in the flavor and palatability of meat products, is a particularly important one. Umami flavor is a combination of savory and spicy and is the factor in broths and the fleshiness of cooked meats. The umami flavor is enhanced by a synergistic effect between free amino acids, the main ones being glutamic acid and free nucleotides such as inosine-5′-monophosphate (IMP), guanosine-5′-monophosphate (GMP), adenosine-5′-monophosphate (AMP) (Stapleton, Roper, & Delay, 1999; Mouritsen & Khandelia, 2012).

Inosine 5′-monophosphate (IMP) is a purine ribonucleotide in the muscle that most contributes to umami taste and it also developed the synergistic effect conjugated with monosodium glutamate (Kawai, Okiyama, & Ued, 2002; Koutsidis et al., 2008). IMP, is the main umami compound, has been found in livestock meat, and fish (Ramalingam, Song, & Hwang, 2019). The congeniality of fish meat has been confirmed by the close correlation with IMP (Resconi et al., 2013). Umami compounds can be found in other aquatic animals including sardine, crab, and lobster (Hong, Regenstein & Luo, 2017). IMP content is not only a key of flavor index but also an important determining of the economic value of chicken (Huang et al., 2020). In Addition, there have been reported that IMP is also plays a role as a dietary nucleotide that enhanced growth and immune response as the report of Pickering et al. (1998) confirmed that the humeral antibody response had increased after an infant fed milk supplement with nucleotides. Dietary nucleotides had shown improvement in the intestinal absorption of iron, nutritional effects on the liver and intestinal mucosa, and also decrease the rate of diarrhea (Schlimme, Martin, & Meisel, 2000). Therefore, the increasing IMP in meat not only develops the sensory quality of meat but also enhance the immune response.

IMP is a purine ribonucleotide which increases in skeletal muscle during muscle contractions as the hydrolysis rate of adenosine triphosphate (ATP) predominate the phosphorylation rate of adenosine diphosphate (Tullson & Terjung, 1999). In addition, IMP increasing in muscle after slaughter as the rapidly degraded of ATP to adenosine diphosphate (ADP) and adenosine monophosphate (AMP), after then degraded to IMP. IMP is further hydrolyzed to inosine (HxR) and hypoxanthine (Hx) (Howgate, 2006; Huang et al., 2020). Uemoto et al. (2017) reported that the reaction of hydrolyzed IMP into HxR is related with ecto-5′-nucleotidase (NT5E) enzyme in extracellular and then at which is degrading to HX (Picture 1). There is some differences in the degradation pathway of IMP among species. The most of ATP is transformed into AMP and further to IMP in death fish while harvesting procedure, and the pathway of ATP to IMP is normally complete in 2 days under storage in ice (Howgate, 2006). The degradation pathway of IMP to HxR and then converted to HX have also been reported in pork which might influence pork flavor (Tikk, 2006). The degradation of IMP progressed more rapidly at higher temperature, however there was slightly differences in the concentrations of HxR and Hx with heating temperature from 70 to 90 °C (Tikk, 2006)

The concentration of IMP in meat is an essential factor resulting to meat palatability. There are many factors influencing the IMP content in muscle such as genetic, feeding management, sex, age, nutritional level, and storage condition after slaughter (Huang et al., 2020). There are several reports that IMP content in muscle has shown higher level in the slow-growing chicken than fast-growing genotype-chicken. (Jianfan & Meinan, 1995; Jilan, Guiping, & Maiqing, 2002; Shiyou, Ying, & Qihua, 2007; Xiaojuan, Nianhua, & Rijun, 2010; Charoensin et al., 2021). The sex effect to IMP content in muscle have been reported that most significant higher level in female than male chicken (Jung et al., 2013) and, furthermore, age is the one factor effect on enhancing IMP content in muscle of chicken with the growing week of age (Huang et al., 2020). The IMP content in beef is affected by both genetic and also environmental factor particular postmortem condition and aging period. As the previous report, the sensory quality traits, particular umami intensity, tenderness and juiciness, in marbled beef had improved after aging over than 40 days (Iida et al., 2016). The content of IMP was rapidly significant increased during postmortem Japanese Black beef aging from day 0, day 1 and 14 into 78.4 ± 39.2 nmol/g,7,574.3 ± 1,402.1 nmol/g and 3,437.7 ± 205.6 nmol/g respectively (Muroya, Oe, Ojima, & Watanabe, 2019). For aquatic animal products, IMP is the most common umami compound found in most fish species after they have died (Hong, Regenstein, & Luo, 2017). The umami flavor of fish, on the other hand, increases after several hours of processing as the ATP breakdown begins (Komata 1990; Hong, Regenstein, & Luo, 2017).

The overall above-mentioned effect of crucial factors on the level of umami compound in meat products can potentially influence meat palatability. This review provided an updated overview on factors affected to umami compound, IMP, in various animal meat products such as chicken, pig, aquatic animals, and cattle.



Picture 1: Process of the IMP formation and degradation.
Source: Komatsu et al., 2019

Factors affecting the level of IMP in various meat

1. **Genetic**

There are several studies report that IMP contents are different between fast- and slow-growing genotype-chicken (Jilan, Guiping, & Maiqing, 2002). For example, IMP level in native chickens (4.7 mg/g) was higher than hybrid line (3.1 mg/g) (Jianfan & Meinan, 1995; Shiyou, Ying, & Qihua, 2007; Xiaojuan, Nianhua, & Rijun, 2010) as well as IMP level in Thai native chicken was higher than commercial breed (Charoensin et al., 2021). Moreover, the IMP level in muscle has shown related with gene control in beef. Uemo et al. (2017) report that SNPs in the exto-5'-nucleotidase (NT5E) gene were shown to effect the amounts of IMP and its breakdown products in beef frozen for 16 – 19 days after slaughter. The NT5E gene was encoded for the NT5 enzyme, which converted extracellular IMP to HxR. As well as the effect from species differences in aquatic animal on umami flavor, according to Sarower, Hasanuzzaman, Biswas, & Abe (2012) and Hong et al. (2017), the umami compounds can be influenced Umami flavor are lower in Loligo sp. than in Sepioteuthis lessoniana (Kani, Yoshikawa, Okada, & Abe, 2008). Umami flavor is slightly stronger in Todarodes pacificus than in S. lessoniana. (Kani, Yoshikawa, Okada, & Abe, 2008). As compared to other fish species, Lepidopus caudatus had the highest IMP concentrations at the start (Howgate, 2006).

2. **Sex**

In Korean native chicken, the influence of sex on IMP content was detected. The results indicated that female breast meat had a greater IMP concentration than male breast meat (p value < 0.05). (Jung et al., 2013). According to Jiang et al. (2003), gilts had a greater IMP concentration than barrows.

3. **Age at slaughter**

There have been many reports shown that the influence of age on the amount of IMP in chicken muscle was clear. Most of previous studies found that the IMP content in chicken muscle increased with increasing age, and the meat quality was better (Huang et al., 2020; Xiaojuan, Nianhua, & Rijun, Z, 2010). For example, the IMP content of 42-day-old chickens was significantly higher than that of 21-day-old chickens (Xiaojuan, Nianhua, & Rijun, 2010). The results showed that the IMP content together with the total IMP, HxR and Hx increased significantly with the increase in age (Jianjun & Jie, 2003). However, some studies had indicated that the amount of IMP decreased with increasing age. From 2 to 28 weeks of age, the IMP content of muscles in Taihe Silkies chickens decreased continuously with increasing age. (Haifeng & Qiulan, 2004).

The IMP content of cattle carcass was affected by the prolongation of the fattening period. According to Iwamoto, Oka, & Iwaki (2009), the IMP content in Japanese steer was greater (P 0.05) in the 24-month fattening period than in the 20-month fattening period. The results indicated that prolonging the fattening period might affect ATP content and AMP catabolism, increasing the IMP content of beef.

4. Type of muscle

IMP content in white muscle fiber was higher than in red muscle fiber (Huang et al., 2020). The previous study revealed that breast meat was greater than thigh or leg due to the difference composition of muscle fiber types in breast and thigh meats (Vani, Modi, Kavitha, Sachindra, & Mahendrakar; Jung et al., 2013). In rat skeletal muscle, type II muscle fiber accumulates more IMP than type I muscle fiber (Jaturasitha, Srikanchai, Kreuzer, & Wicke, 2008). Furthermore, in rat skeletal muscle, the activity of 5′-nucleotidase, which catalyzed the breakdown of IMP to inosine, was greater in type I muscle fiber than in type II muscle fiber (Tullson & Terjung, 1999).

5. Aging effect to IMP level in meat

Several studies confirm that when fresh meat is taken through the aging process, it will become tender, juicier and far tastier. This is due to the structure of the meat being digested by enzymes. There are two main methods of aging: dry-aging without vacuum bags and wet-aging with vacuum bags. The most common method is dry aging, as it can add a rich flavor to the meat. It has been reported that dry-aged meat is attractive to consumers when eaten due to its Umami flavor and rich taste. It is suitable for making premium meat in response to consumer demand (Kim et al.,2016).

After 14 days of postmortem aging in the Longissimus thoracis muscle of a Japanese Black (Wagyu) steer, researchers discovered that the accumulation of purine metabolism products, such as IMP, contributes to the improvement of aged beef quality traits (Muroya, Oe, Ojima, & Watanabe, A.2019). According to Dannert & Pearson (1967), the maximum level of IMP was discovered at 12 hr post-mortem on ageing beef for 28 days at 0.1-1.5°C (33-35°F), and then it stayed steady until the 4th day, when less than 15% of the peak IMP concentration remained after 28 days storage. (Table 1)

Table 1: IMP content of the Longissimus dorsi muscle in aging beef at 0.1 – 1.5oC.

Post-mortem time	IMP level (μg/ g)
0 hr	4.71
12 hr	5.44
24 hr	4.86
4 days	4.47
7 days	3.20
14 days	2.17
28 days	0.75

Furthermore, the umami intensity might occur under dry aging for a longer period of time, as a result of a synergistic action between umami compounds namely glutamic acid (Glu) and IMP. The finest dry aging time for highly marbled beef (Japanese black cow) was 40 days, resulting in the preservation of tenderness, juiciness, umami intensity, and flavor intensity (Iida et al., 2016). As well as aging pork meat, it was found that the longer the aging, the higher the free amino acids and nucleotides levels (Tikk et al., 2006; Hwang et al., 2019).

Lee et al. 2016 reported that aging pork tenderloin for 40 days increased the free amino acids by 73% (from 1159.55 mg per 100 g before aging to 2006.56 mg per 100 g. see Table 2). But in other cuts of meat concentrations of free amino acids may vary as each body part contains different quantities of enzymes, proteinase and peptidase that digest muscle fiber and muscle protein. For example, a study by Hwang et al. (2019), of aging the shoulder for 21 days, found that by dry-aging, free amino acids increased 120% and 112% respectively. When it comes to nucleotides and their digestible components, meat has lower GMP and IMP levels, which is the forerunner of meat flavoring, resulting in an Umami taste. GMP and IMP are digested as nucleosides by the enzymes, which are followed by purine, hypoxanthine, inosine, and ribose in meat, which may promote a stronger Umami flavor due to free amino acids (Hwang, Sabikun, Ismail, & Joo, 2019).

Table 2: Free amino acids from 40 days of dry-aging pig meat.

Free amino acids (mg/100g)	Control	Dry-aged pork	SEM[1]
Ala	7.14[b]	24.10[a]	0.537
Arg	802.84[b]	1336.22[a]	26.730
Asn	3.30[b]	16.79[a]	0.374
GABA	12.08[b]	23.09[a]	0.604
Gln	2.05[b]	5.53[a]	0.270
His	3.13[b]	13.54[a]	0.398
Ile	4.60[b]	18.90[a]	0.753
Leu	12.44[b]	44.64[a]	0.937
Met	5.0 [b]	17.56[a]	0.908
Phe	7.84[b]	34.20[a]	0.803
Pro	57.35[b]	106.33[a]	2.290
Ser	9.06[b]	34.74[a]	0.736
Thr	8.27[b]	29.89[a]	0.549
Try	0.15[b]	0.66[a]	0.122
Tyr	19.94[b]	79.70[a]	2.343
Val	3.98[b]	17.37[a]	0.374
Cys	9.54	9.83	0.494
Gly	172.51	178.40	4.710
Asp	18.10[a]	15.05[b]	0.472
Lys	0.20[a]	0.01[b]	0.023
Total	1159.55[b]	2006.56[a]	38.291
Guanosine 5'-monophosphate (GMP)	4.34[a]	2.55[b]	0.127
Inosine 5'-monophosphate (IMP)	238.92[a]	90.69[b]	4.313
Hypoxanthine	8.47[b]	27.40[a]	0.682
Inosine	66.24[b]	129.82[a]	1.599

[1]Standard errors of the least square means (n=10).
a,bThe superscript values with different letters within the same row indicate differ significantly ($p<0.05$).

Source: Lee et al.(2016)

6. Cooking process

The cooking process can also be a component to release the flavor, but when eating raw meat there may be a blood smell. There was a trial by Rotola-Pukkila, Pihlajaviita, Kaimainen, & Hopia (2015), to cook pork sirloin using the sous vide technique at 60 °C, 70 °C and 80 °C for 0, 60 and 120 minutes respectively. It was found that the juice from the meat in the group that was cooked at 80 oC had a higher amino acid concentration than the other two groups, but neither the temperature nor the time had any effect on IMP concentrations.

7. Raising method

The broilers were raised under free-range had shown significantly IMP level in muscle higher than that of caged broilers (Husak et al., 2008; Zang et al., 2018). The chicken also raised under flat-rearing and lower density had IMP contents in muscles significantly higher than that in high density and cage-rearing of chickens (Xiaojuan et al., 2010; Yuejiao et al., 2014). The reason of IMP in poultry muscle by different raising methods may be that different amount of exercise leads to different IMP content. The more intense the exercise, the higher the ATP content in poultry muscle, and the greater the metabolic activity of ATP, and the greater the ability to synthesize IMP (Huang et al.,2020).

8. Nutritional factors

The effect of dietary ingredients have an important influence on IMP content and chicken flavor. As several reports have shown that the addition of betaine in the diet can also significantly indirectly increase the IMP content in chicken muscle by enhancement of fatty acid oxidation (Poste, 1990) but the direct effect of betaine has not been report yet (Xianqing et al., 2015). In Addition, adding appropriate amount of Chinese herbal medicine, including licorice, eucommia, schisandra, codonopsis, cinnamon and allicin, to the feed can both improve survival rate of chicken and also increase IMP content with umami taste in chicken muscle (Jinge & Xiaoting, 2009; Yanci & Haihong, 2010). Moreover, natural bioactive compound has been reported which improve beef sensory quality. Addition level of 0.5, 1.0 or 1.5% w/w of mate extract to standard maize/soy cattle feed, resulted in improved inosine monophosphate, creatine and carnosine levels in fresh meat and also developed oxidative stability, nutritional value and sensory quality (Zawadzki et al., 2017).

9. Season for harvesting in fish

Season effect on IMP level has been reported in aquatic animal. Because of variations in environmental and nutritional conditions of geographical areas, IMP level in the muscle of puffer fish vary considerably since July to January, which is substantially higher than in the spawning season (March to May) (Hwang et al., 2000). This causes the muscle of puffer fish more palatable. (Hwang et al., 2000)

Conclusion

The meat quality particular umami flavor is highly indicator for consumer's purchasing habit as well as increasing meat economic value. The factors including genetic, sex, age at slaughter, type of muscle, aging effect, cooking process, raising methods, nutrition factors and season for harvesting are also influence the flavor and taste in all animal meat as well as significant correlate with umami level in muscle. The factors as above mentioned offers the guideline to maintain the good quality of meat. The most important is to served high quality of meat which meet to demand of consumers and also improvement of the data will provide a complete theoretical basis which will lead to the better quality of livestock, poultry products and fish (aquatic) meat.

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CLASSIFICATION OF OIL PALM FRUIT ON RIPENING STAGE USING UNIVERSAL TESTING MACHINE

Napat Kamthonsiriwimol[1*], Buntoon Chunnasit[2], Hideo Hasegawa[3], Siripong Luangvasuwat[4]

[1]Faculty of Innovative Agricultural Management, Panyapiwat Institute of Management
[2,4]Faculty of Agriculture at Kamphaengsaen, Kasetsart University
[3]Institute of Science and Technology, Niigata University
[*]**Corresponding author, E-mail:** napatkam@pim.ac.th

Abstract

This study was to the classification of oil palm fruit (*Elaeis guineensis* Jacq. var Tenera) by using frmness. In the experiment, the samples of oil palm fruits belonged to four ripening stages (unripe, underripe, ripe, and overripe) were harvested from Suphanburi Province. One hundred fruits per ripening stage were selected from five oil palm bunches. The firmness test of oil palm fruits was carried out by using a universal testing machine (LR 50K, Lloyd Instruments Ltd., UK). The firmness was measured under the compressive loading of a 6-mm cylindrical probe to determine the maximum compressive load of fruit in each ripening stage. Then ripening stage classification of oil palm fruit by the maximum compressive load with Euclidean distance was tested to investigate the technical feasibility. The results showed that the maximum compressive load of fruits in different ripening stages was significantly different. Moreover, the maximum compressive load of oil palm fruit had a strong relationship with the ripening stage. For the ripening stage classification, the correct classification in all ripening stages was around 96.0%. In addition, the ripening stage classification also indicated that unripe and overripe fruits were clearly distinguished from other stages while some misclassification took place between fruits that belonged to underripe and ripe stages. Since the ripening stage assessment of oil palm fruits in the mill has the requirement to separate the overripe and unripe fruits from ones of other ripening stages, the results of validation experiments may indicate that the developed classification method could provide acceptable accuracy and a possibility for practical use to assess the ripening stage of oil palm fruits.

Keywords: Classification, Firmness, Ripening stage, Oil palm fruit

Introduction

Oil palm (*Elaeis guineensis* Jacq.) is a crucial oil crop in the world. Initially, it was originated from West Africa and was introduced to Malaysia as an ornamental plant. Then it was brought to cultivate in Thailand in 1963 after successful growth in Malaysia (Wangrakdiskul & Yodpijit, 2015). Thailand is the third-largest oil palm producer in the world. In Thailand, the oil palm plantation significantly increased from 0.65 million Ha in 2011 to 0.98 million Ha on 2020. In contrary, the yield of oil palm dramatically decreased from 19.1 tons per Ha in 2011 to 18.1 tons per Ha in 2020 (Office of Agricultural Economics, 2021). Generally, palm oil is important raw material for cooking and a renewable source of fuel (Somnuek, Slingerland, & Grunbuhel, 2016). The palm-oil-based biodiesel project was launched in Thailand in 2005 and had been broadly utilized as an alternative fuel for running machines and vehicles (Wangrakdiskul & Yodpijit, 2015). Approximately, 5.8 million liters per day of biodiesel would be required to use in Thailand in 2021 (Krungsri research, 2021). To meet the demand for biodiesel, the Thai Cabinet approved the project to expand the oil palm plantation. Therefore, many farmers were decided to grow oil palm on their farm even if it located in unsuitable areas such as the northern and northeastern of Thailand. In these areas, the yields of oil palm production are low and not-cost effective according to the inappropriate climate conditions and insufficient rainfall.

Currently, the oil extraction rate (OER) in Thailand is around 16-17 % while the OER of neighboring countries such as Malaysia and Indonesia are around 20-24%. This low oil extraction rate stems partly from the harvesting without concerning of ripeness control. Some farmers may harvest the fresh fruit bunch (FFB) before the optimal ripening stage (Chucheep, Mahathaninwong, Limhengha, Petchi, &Templong, 2019; Junkwon et al., 2009a; Junkwon et al., 2009b). Therefore, the expansion of oil palm plantations is not the only one approach to increase the amount of palm oil in Thailand. Thus, the ripeness control for FFB or oil palm fruit may play an important role to increase palm oil.

In general, FFBs and oil palm fruits are oil palm products that farmers deliver to the oil palm mills. The mills may check the ripeness of the products by randomized sorting before purchasing as shown in Picture 1. The inspections are laborious work that requires many skilled specialists to inspect oil palm bunches and fruits.

(a) Inspection of FFBs and fruits

(b) Inspection of FFBs on the truck

(a) Inspection of FFB by using fruits' color

Picture 1: Ripeness inspection of oil palm bunches and fruits

Various techniques were developed to determine the ripening stage of oil palm bunches and fruits to seek a suitable approach that could be practically applied. Image processing technique by using various types of the camera such as CCD and the hyperspectral camera was intensively developed to classify the ripening stage of oil palm fruits and bunches (Junkwon et al., 2009a; Junkwon et al., 2009b; Fadilah, Mohamad-Saleh, Halim, Ibrahim, & Ali, 2012; Abdullah, Guan, Mohamed & Noor, 2002; Abdullah, Guan, & Mohd Azemi, 2001; Alfani, Shariff, Shafri, Saaed, & Eshanta, 2008) and it showed the promising results at laboratory scale. However, ripening stage determination by using image processing would require a suitable illumination unit and a sophisticate conveying system to carry the FFBs or the fruits through the camera. In this sense, the image processing technique may be not suitable to use at the mill or the oil palm plantation under unstable ambient conditions.

Beside image processing technique, the firmness of oil palm fruits was tested by Keshvadi, Endan, Harun, Ahmad, and Saleena, (2011) and the research 's results displayed that the firmness of oil palm fruits has strong relationships with the ripening stage of the fruits. Recently, the handheld firmness tester has been employed to measure the ripening stage of fruits and vegetables with a reasonable price and suitable functions. Therefore, the handheld firmness tester of oil palm fruits maybe another approach for users in terms of cost and functions. Unfortunately, the technical feasibility of ripening stage classification in oil palm fruits by using its firmness has not been investigated.

Research Objective

The objective of this research was to develop the technique for determining the ripening stage of oil palm fruits using fruits' firmness.

Literature Review

Oil palm is a perennial tree crop and the highest oil-producing plant in the world. It can produce 4 to 6 million tons of crude palm oil (CPO) and 0.4 to 0.6 tons of palm kernel oil (PKO) per hectare per annum (Sunilkumar & Sparjan Babu, 2013). It is a monocotyledon belonging to the species *Elaeis*. The genus *Elaeis* could be classified into two species, so-called *Elaeis guineesis* and *Elaeis oleifera* (Sambanthamurthi, Sundram, &Tan, 2000).

The oil palm fruit is a sessile drupe varying in shape from nearly spherical to ovoid shape. In general, oil palm fruits can be categorized into two types according to the pattern of color change that occurs before ripening. A fruit type that is purplish-black at the apex and pale greenish-yellow at the base before ripening is term *nigrecens* and the other fruit type that is green before ripening is called *virescens* (Corley & Tinker, 2003). *Nigrecens* is a dominant fruit type in Thailand (Junkwon et al., 2009b). In general, farmers will practically determine the ripening stage of oil palm bunches by noticing the detached fruits on the ground. The oil content of different ripening stages of fruits is a function of its degree of ripeness (Fadilah, Mohamad-Saleh, Halim, Ibrahim, & Ali, 2012). The presence of unripe FFBs could reduce the OER by 0.13% for every 1% of the total made up of unripe FFBs. Free Fatty Acid (FFA) content is also another important factor for oil quality. The recommended free fatty acid content is below 5%. The presence of overripe FFBs will increase the free fatty acid in the extracted oil (Abdullah, Guan, Mohamed & Noor, 2002). Therefore, it is very crucial that FFBs and oil palm fruits should be harvested and assessed to classify their ripening stage before extracting to maximize the oil extraction rate and quality of extracted oil.

Since the surface color of fruits is the simplest parameter to classify the ripening stage, many studies were done to determine the ripening stage of FFBs and fruits. Image processing becomes more and more popular for the ripening stage classification of FFBs and fruits due to the advantage of imaging technology that could be used to analyze the surface color.

For the ripening stage classification of fruits, Abdullah, Guan, and Mohd Azemi (2001) firstly studied the relationship between color perception and degree of ripeness. Next, a machine vision system by using a charge-couple-device (CCD) camera was applied to capture the images of oil palm fruits from four ripening stages as unripe, underripe, ripe, and overripe. Then HSI color space was selected to obtain the data of fruits' color. Finally, image analysis and discrimination were done to determine the ripening stage of oil palm fruits. The success rate of classification was greater than 90%.

To develop a more practical method, more complex classification methods were applied based on the image data from the CCD camera. RGB color space and fuzzy logic technique was employed by May and Amaran (2011) to automatically identify the oil palm fruits from three ripening stages as underripe, ripe, and overripe. The results demonstrated that the ability in distinguishing the three different ripening stages of oil palm fruits with an overall efficiency of 88.74%.

Since the data obtained from the CCD camera was just color image that consisted of grayscale images of red, green, and blue channels, Junkwon et al. (2009b) developed the classification for oil palm fruits in four ripening stage as unripe, underripe, ripe, and overripe by using the hyperspectral imaging that provided images by hyperspectral camera at 400-1000 nm. Ripening stage classification was done by calculating the ratio of colors in fruits. The correct ripening stage classification rate of 97.92% was gained by this method.

However, these studies were conducted under the control environment and involved a complex system for image acquisition. Therefore, some researchers tried to seek for other mechanical properties to classify the ripening stage of oil palm fruits such as firmness of mesocarp and hardness of exocarp.

Chucheep, Mahathaninwong, Limhengha, Petchi, and Templong (2019) examined the ripening stage of oil palm fruits by using the hardness by indenting with a steel ball on the exocarp of the fruits. The fruits were categorized into three ripening stages as unripe, underripe, and ripe. The results indicated that unripe oil palm fruit could be classified from other ripening stages but it was difficult to determine the fruits between stages of underripe and ripe.

Keshvadi, Endan, Harun, Ahmad, and Saleena (2011) studied the relationship between palm oil index development and firmness of oil palm fruits during the ripening process between 8,12,16, and 20 weeks after anthesis (WAA). The outer fruits from the region of the bunch expressed the significant difference of firmness of mesocarp and it indicated the possibility to apply in ripening stages classification of oil palm fruits.

Since the firmness of fruits generally related to their degree of ripeness and employed as a predictor of the ripening stages, many studies were done to assess the ripening stage of the fruits by their firmness.

Peleg, Ben-Hanan, and Hinga (1990) employed a sensor system for automatic non-destructive sensing of avocado fruit. The vibration technique was applied for classifying the firmness of avocado as soft, medium, and firm. The overall accuracy was about 90%.

Srisomboon, Boonmung, Pornchaloempong, and Pithuncharurnlap (2008) used the compression test to classify three different maturity stages of mango as 60%, 70%, and 80% of full ripeness by using a texture analyzer. The technique demonstrated the possibility of classification in two different stages between 60% and 80% of full ripeness but it could not detect the difference between 60% and 70% of fully ripeness or between 70% and 80% of full ripeness.

Methodology

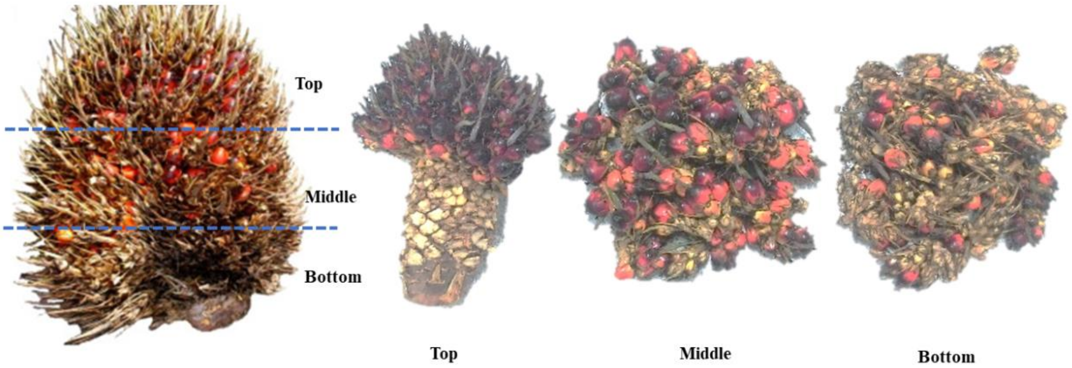
Initially, samples of oil palm fruits were categorized into four ripening stages by using colors that displayed on the surface of the fruits according to the standard from the Palm Oil Research Institute of Malaysia (PORIM) as unripe, underripe, ripe, and overripe as illustrated in Table 1. Ten years-old of Tenera variety oil palm trees of local plantation in Suphanburi Province were selected in this study. Five FFBs per ripening stage were harvested and then transported within one hour to the laboratory as shown in Pictures 2 and 3. Next, the fruits from each bunch were equally divided into three regions as top, middle, and bottom by their length and were separated from the bunch by an axe as shown in Picture 4. Then twenty fruits from the outer layer of the middle region that had good appearances such as no bruise or infection were randomly taken from each FFB. Totally, a hundred fruits per class were prepared.

Table 1: Classification of oil palm fruit (Abdullah, Guan, Mohamed & Noor, 2002)

Ripening stages	Description	
Unripe		The fruit exhibits a purplish black color covering more than 90% of the fruit's surface. The color at the base of the fruit appears colorless.
Underripe		The fruit displays the reddish black color over the upper part. The colors tend to lighten towards the base.
Ripe		The fruit displays the reddish orange color over the upper part. The colors tend to lighten towards the base.
Overripe		The fruit shows almost entirely reddish orange coloration.

Picture 2: Harvesting of oil palm bunches from local plantation in Suphanburi Province

Picture 3: Four ripening stages of oil palm bunches

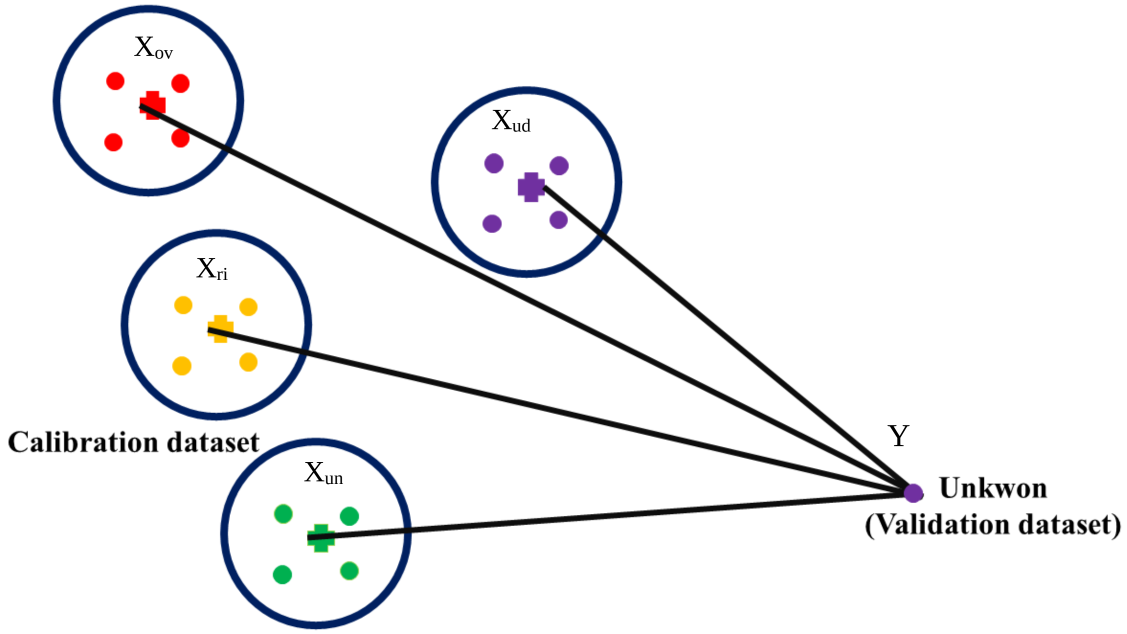


Picture 4: Sampling regions of oil palm bunches

To determine the firmness of oil palm fruits in each ripening stage, the firmness was measured by using universal testing machines (LR 50K, Lloyd Instruments Ltd., UK). The compressive loading of a 6-mm. diameter cylindrical probe was applied to the mesocarp until the deflection of the fruit was 3 mm to measure the firmness at the center of the fruits at 25 ˚C while the fruit was loaded in the horizontal position. Then the data of average maximum compressive load of fruits in each ripening stage were submitted to analyze the analysis of variance (ANOVA) by using SPSS version 24 (SPSS, INC., Chicago, IL). Duncan's Multiple Range Test was used for multiple comparisons and the significance level was $\alpha = 0.5$.

Euclidean distance was chosen and applied to develop the classification method by using the firmness of oil palm fruits to distinguish between ripening stage. The data of the average maximum compressive load of fruits in each the ripening stages were equally divided into two groups of data as calibration and verification datasets. Then, the centroids of the average maximum compressive load of fruits in each ripening stage were calculated from the calibration dataset for unripe, underripe, ripe, and overripes sample as X_{un},X_{ud}, X_{ri} and X_{ov}. Next, the Euclidean distance from the unknown sample to the centroid of each ripening stage was computed by using Equation 1. In the classification procedure, the distance from a sample to the centroid of each ripening stage was calculated. Finally, the unknown sample would be classified into the ripening stage for which the distance was the shortest as shown in Picture 5.

$$ED = \sqrt{(x-y)^2} \qquad \text{Equation 1}$$

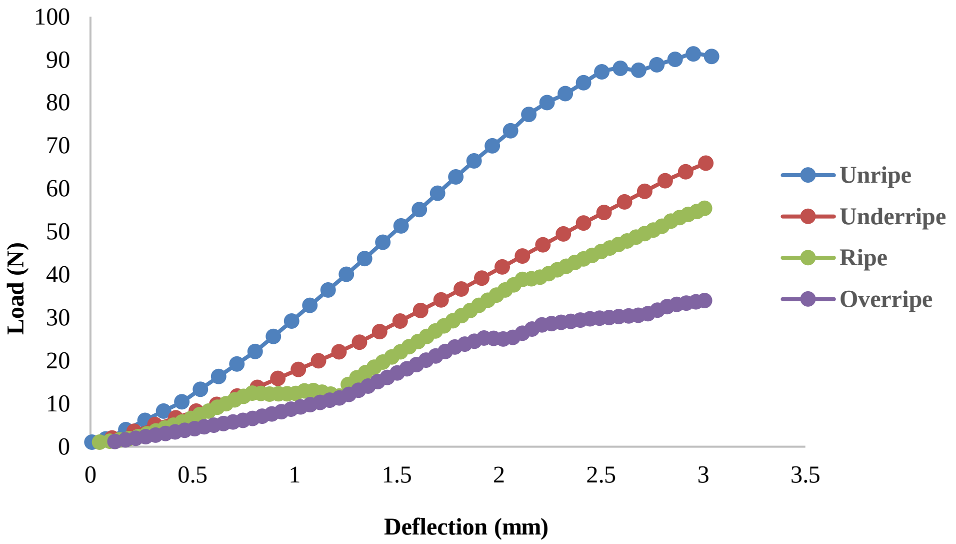


Picture 5: Concept of ripening stage determination by using Euclidean distance

Results

The results of the relationship between compressive load and deflection in the mesocarp of oil palm fruits in four ripening stages were illustrated as in Picture 6. The greater load expressed at the deeper level of deflection in all ripening stages. The greatest amount of compressive load was found in unripe fruit while the smallest one was overripe fruit. For the average maximum compressive load of oil palm fruit, the experimental results were shown as in Table 2. The average maximum compressive load was significantly different in all ripening stages.

Then, Euclidean distance was employed to classify the ripening stage of oil palm fruits. The classification results were displayed in Table 3. The overall success rate of the classification was around 96.0%. Moreover, the success rate of ripening stage classification also indicated that Euclidean distance could classify overripe and unripe fruits from the other samples. However, the classification between underripe and ripe fruits was more difficult than other ripening stages.



Picture 6: Relationship between compressive load and deflection of oil palm fruit with four ripening stages

Table 2: Maximum compressive load of oil palm fruit with four ripening stages

Ripening stage	Maximum compressive load (N)
Unripe	99.73a[1/] ±12.79
Underripe	74.88b ±6.25
Ripe	60.53c ±7.78
Overripe	40.82d ±13.25

[1/]Mean in the same column followed by a common letter are not significantly different at 5% level by DMRT.

Table 2: Confusion matrix for ripening stage classification of oil palm fruit by the maximum compressive load with Euclidean distance

Ripening stage	Unripe	Underripe	Ripe	Overripe	Total	%correct
Unripe	50	0	0	0	50	100
Underripe	0	46	4	0	50	92
Ripe	0	4	46	0	50	92
Overripe	0	0	0	50	50	100

Discussion

For the relationship between compressive load and deflection in the mesocarp, the lowest amount of maximum compressive load was detected in overripe fruits compared to ripe, underripe, and unripe fruits, respectively. This may cause by the cell wall modification during the development of the mesocarp of the oil palm fruit. In the ripening, the cells of the mesocarp continue to grow and expand for accumulating the lipid. Then, this lipid accumulation in the mesocarp makes the fruit become softer (Teh et al., 2014). Therefore, the maximum compressive load of oil palm fruit exhibits a tendency to decrease related to the progressive ripening stage. Generally, the oil deposition in mesocarp starts at approximately 15 weeks after anthesis and continues until fully ripening at 20 weeks after anthesis (Sambanthamurthi, Sundram, &Tan, 2000). Similar findings were reported by Keshvadi, Endan, Harun, Ahmad, and Saleena (2011).

As the classification results, the overall success rate of classification was satisfied in overripe and unripe fruits. This may affect by the clear differences between overripe and unripe fruits to other fruits in other ripening stages. For underripe and ripe fruits, it may require more samples to determine the centroids of the maximum compressive load of each ripening stage. The samples of fruits should be random sampling according to the ripening stages relate to weeks after anthesis.

Conclusion

In this study, oil palm fruits variety Tenera from four different ripening stages as unripe, underripe, ripe, and overripe were harvested from Suphanburi Province to develop the ripeness classification method. The conclusions are shown as follows:

(1) The technique to determine the ripening stage of oil palm fruits was successfully developed with 96% of the overall success rate of classification by using the differences of the maximum compressive load of oil palm fruits from four different ripening stages. This developed technique may be useful for the ripening stage classification of oil palm fruits and expressed the technical feasibility to apply in oil palm fruits assessment at the mill. However, the development of the maximum compressive load measuring device is required for a more practical ripening stage assessment.

(2) The unripe and overripe fruits were the fruits that belonged to two ripening stages were completely categorized from other ripening stages by using the maximum compressive load with Euclidean distance. However, misclassification still took place between underripe and ripe fruits. More numbers of oil palm fruits were suggested to improve the efficiency of classification in further research. In addition, the fruits from at different ripening stages before, during and after the major oil synthesis period should be taken into account for improving the efficiency of classification of the developed technique.

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