UNIVERSITEIT GENT

FACULTEIT BIO-INGENIEURSWETENSCHAPPEN

Wijitra Liaotrakoon

Characterization of dragon fruit (*Hylocereus* spp.) components with valorization potential

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

“to my dearest family”

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TABLE OF CONTENT

LIST OF NOTATIONS

List of abbreviations

AIR	Alcohol-insoluble residue
ANOVA	Analysis of variance
Ara	Arabinose
Araf	Arabinofuranosyl
CDTA	Cyclohexane-*trans*-1,2-diamine tetra-acetic acid
CFU	Colony forming unit
CSF	Chelator-soluble fraction
CT	Cold temperature
cyclo-Dopa	*cyclo*-Dihydroxyphenylalanine
Dha	3-Deoxy-D-lyxo-2-heptulosaric acid
DM	Degree of methoxylation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
ELSD	Evaporative light scattering detector
FAME	Fatty acid methyl ester
FOX	Ferrous oxidation-xylenol orange method
FRAP	Ferric reducing antioxidative power
Fuc	Fucose
GA	Gallic acid
Gal	Galactose
GalA	Galacturonic acid
Galp	Galactopyranosyl
GC	Gas chromatography
Glc	Glucose
H.	*Hylocereus*
HF	Hemicellulosic fraction
HG	Homogalacturonan
HME	High methyl-esterified pectin
HPAEC	High-performance anion-exchange chromatography
HPLC	High-performance liquid chromatography
HPSEC	High-performance size-exclusion chromatography

HTST	High-temperature short-time
Kdo	2-Keto-3-deoxy-D-manno-octulosonic acid
L	Linoleic acid (C18:2)
LME	Low methyl-esterified pectin
Man	Mannose
MM	Molar mass
MPBS	Phosphate-buffered saline containing milk powder
MUFA	Monounsaturated fatty acid
N	Number of measurements
NSF	Sodium carbonate-soluble fraction
O	Oleic acid (C18:1)
P	Palmitic acid (C16:0)
PAD	Pulsed amperometric detection
PBS	Phosphate-buffered saline
PG	Polygalacturonase
PL	Pectate lyase
PME	Pectin methylesterase
PUFA	Polyunsaturated fatty acid
RG-I	Rhamnogalacturonan-I
RG-II	Rhamnogalacturonan-II
Rha	Rhamnose
RT	Room temperature
S	Stearic acid (C18:0)
SFA	Saturated fatty acid
SD	Standard deviation
TAG	Triacylglycerol
TCC	Total colour change
UFA	Unsaturated fatty acid
UHT	Ultra-high temperature
WSF	Water-soluble fraction
XG	Xylogalacturonan
Xyl	Xylose

List of symbols

γ	Shear rate (s^{-1})
φ	Volume fraction (dimensionless)
η_s	Apparent viscosity of supernatant phase (mPa.s)
η_p	Apparent viscosity of puree (mPa.s)
ΔC^*	Difference of a* colour parameter (dimensionless)
ΔH^*	Difference of b* colour parameter (dimensionless)
ΔL^*	Difference of L* colour parameter (dimensionless)
ΔH	Enthalpy (watt/g)
ε	Molar extinction coefficient (l/mol.cm)
τ	Shear stress (mPa)
A	Properties measured value of the heated puree
A_0	Properties measured value of the unheated sample
Abs	Absorbance value (dimensionless)
C	Crystallization temperature (°C)
CDG•	cyclo-Dopa-5-O-β-glucoside radical
DF	Dilution factor (times)
E_a	Activation energy (kJ/mol)
K	Consistency coefficient ($mPa.s^n$)
k	Rate constant (min^{-1})
k_0	Frequency or pre-exponential factor (min^{-1})
K_C	Parametric factor of chroma component (dimensionless)
K_H	Parametric factor of hue component (dimensionless)
K_L	Parametric factor of lightness component (dimensionless)
LC	Path length of cuvette (cm)
M	Melting temperature (°C)
MW	Molecular weight (g/mol)
n	Flow behaviour index (dimensionless)
PV	Peroxide value (mequiv O_2/kg)
R	Universal gas constant (8.314 J/mol.K)
R•	Lipid alkyl radical
RH	Fatty acid
ROO•	Peroxy free radical
ROOH	Hydroperoxide

R_T	Interactive term between chroma and hue differences (dimensionless)
S_C	Weighting function of chroma component (dimensionless)
S_H	Weighting function of hue component (dimensionless)
S_L	Weighting function of lightness component (dimensionless)
T	Absolute temperature (K)
t	Heating time (min)
T_g	Glass transition temperature (°C)
T_{ge}	Endpoint glass transition temperature (°C)
T_{gm}	Midpoint glass transition temperature (°C)
T_{go}	Onset glass transition temperature (°C)
Toc	Tocopherol
Toc•	Tocopheroxy radical
Toc-OOR	Tocopherol-lipid peroxy complex

SUMMARY

Dragon fruit (*Hylocereus* spp.), also known as pitaya or pitahaya, is increasingly gaining interest in many countries, including Thailand which is a country with a climate ideal for breeding different varieties of tropical and subtropical fruits in general, and dragon fruit more specifically. The benefits of dragon fruit for human health can be explained by its essential nutrients such as vitamins, minerals, complex carbohydrates, dietary fibres and antioxidants. Dragon fruit is also an essential source of betacyanin which serves as a red/purple pigment with antioxidative properties. In Thailand, most of the fresh dragon fruits are consumed domestically, while some are traded globally as fresh fruit and processed products as juice or puree. As it is, dragon fruit peel and seeds are often considered as waste or by-products from fruit processing and have been less successful at adding value to the fruit. Currently, there is limited literature on dragon fruit properties, its processed products and constituents as well as potential utilization. Therefore, the main objective of this doctoral research was to gain deeper insight in the characteristics of dragon fruit and its derived products and components, specifically of two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). The characteristics of these two species of dragon fruit and their possible application are extensively discussed in the doctoral thesis/research.

Chapter 1 provides a state-of-the-art of current and relevant research on dragon fruit with respect to botanical classification, cultivation, economic aspects and chemical composition (particularly nutrients, pigment and antioxidative components) as well as an overview of techniques to process fruits (processing), i.e. freeze-drying and thermal processing. The impact of these processes on quality attributes (e.g. physicochemical and rheological properties) of different fruits is critically reviewed. The review also aims at giving a summarized overview of the most important aspects of seed oil (e.g. chemical composition and lipid oxidation) and cell wall polysaccharides (e.g. structure of pectic and hemicellulosic substances).

In **Chapter 2**, the properties/characteristics of the whole fruit, peel, pulp (seed-free flesh) and puree (flesh containing seeds) of the two species of dragon fruit were performed. Results demonstrated that the peel of both dragon fruit species as well as the pulp and puree of the red-flesh species contained large amounts of betacyanin. As it is, they have the potential to be utilized as a natural colouring agent. Dragon fruit pulp and puree also exhibited significant antioxidative activities. This was even more pronounced for the red-flesh species due to the presence of betacyanin. The pulp and puree showed shear-thinning behaviour due to the presence of mucilaginous components. Throughout this doctoral

research, these characteristics of dragon fruit, for example, pigment, antioxidative activities and rheological properties, provide a deeper insight with regard to further valorization of the fruit components and optimization of the fruit processing.

Chapter 3 describes the characterization of the freeze-dried pulp and peel of the two species of dragon fruit to be further utilized of dragon fruit's pigment (betacyanin) as a food additive. It was found that freeze-drying could satisfactorily preserve colour and pigment concentration. The freeze-dried red-flesh pulp and the freeze-dried dragon fruit peel from both species contained high contents of betacyanin. The freeze-dried dragon fruit pulp was well-soluble in water, whereas this was not the case for the freeze-dried dragon fruit peel. Additionally, due to the pH-sensitivity of betacyanin, the influence of pH (1-11) on the colour shift of the freeze-dried dragon fruit peel and pulp was monitored. The colour of the freeze-dried peel was stable within a pH range of 3-7, whereas the freeze-dried red-flesh pulp had high colour stability over a pH range of 1-11 and is available in a convenient form making it suitable for the use as a food colourant.

In order to gain insight into the effects of thermal treatment on the characteristics of the white-flesh and red-flesh dragon fruit purees, the antioxidative, rheological, physicochemical and microbiological properties of the purees were investigated at different process conditions (between 50 and 90 °C for 60 min). The results are presented and discussed in **Chapter 4**. It is interesting to see that the antioxidative properties of the heated dragon fruit puree increased during heating treatment probably because of the superior antioxidative attributes of the dragon fruit seeds present in the puree and the formation of Maillard reaction products. During thermal processing, the L* value (lightness) and b* value (yellowness) of the processed dragon fruit puree can be used to control the quality online. Total colour change (TCC), which is an indicator of different colour between the unheated and heated puree samples, of the red-flesh dragon fruit puree showed a strong negative correlation with betacyanin content. The kinetics of colour changes and betacyanin degradation in the heated dragon fruit puree followed a second-order models. The apparent viscosity of the heated dragon fruit puree increased with heating time and temperature. The rheological data fitted very well with the power-law model, showing shear-thinning behaviour. Thus, the heated dragon fruit puree, particularly the red-flesh puree, offer possibilities to be applied in foodstuffs due to their interesting attributes after thermal treatment.

The dragon fruit seeds are considered to have a high antioxidative potential. As it is, oil was extracted from the white-flesh and red-flesh dragon fruit seeds with petroleum ether as cold extraction. The characterization of the seed oil is described in **Chapter 5**. Dragon fruit

seeds contained significant amounts of oil, which accounted for about 32-34% of the dried seed weight. The predominant fatty acids of both dragon fruit seed oils were linoleic acid (C18:2, 45-55%), oleic acid (C18:1, 19-24%), palmitic acid (C16:0, 15-18%) and stearic acid (C18:0, 7-8%), respectively. Dragon fruit seed oil is interesting from a nutritional point of view as it contains a high amount of essential fatty acids, amounting to ~56%. The triacylglycerol (TAG) composition in the seed oil was also analyzed. It was shown to contain mainly triunsaturated and diunsaturated TAGs. A significant amount of tocopherols in the dragon fruit seed oil was clearly observed in which α-tocopherol was the most abundant tocopherol in the oil (~72% of total tocopherol content). In addition, a storage assessment of 3 months was performed, monitoring the oxidative and tocopherol stabilities in the dragon fruit seed oil. A low oxidation rate of both dragon fruit seed oils was obtained, while tocopherol content decreased on storage. However, the concentration of tocopherols in the dragon fruit seed oil after 12 weeks remained high content compared to the initial state. Thus, the dragon fruit seed oil could be considered as a good source of essential fatty acids and tocopherols, with a high oxidative stability.

In **Chapter 6**, the activity of pectic enzymes, i.e. pectin methylesterase (PME) and polygalacturonase (PG), of the pulp and peel of white-flesh and red-flesh dragon fruits as well as inactivation of pectic enzymes by thermal treatments (30-90 °C for 10 min) were examined. The untreated white-flesh dragon fruit had a higher PME activity compared to the red-flesh dragon fruit, whereas PG activity was absent in all dragon fruit samples. Results also demonstrated that no significant effects of PME activity at temperature below 70 °C for 10 min in either the pulp or the peel of dragon fruit were observed, while a moderate heat treatment (80 and 90 °C for 10 min) could efficiently inactivate PME.

In the last part of this doctoral study, the polysaccharides (pectic and hemicellulosic substances) from cell wall materials of the pulp and peel of white-flesh and red-flesh dragon fruits were isolated and structurally compared, as described in **Chapter 7**. Prior to cell wall polysaccharide isolation, blanching at 80 °C for 10 min allowed PME inactivation. Subsequently, cell wall material was extracted and sequentially fractionated using various solutions to obtain three different pectic fractions, a hemicellulosic fraction and a remaining residue fraction which could not be solubilized by the procedure used. These polysaccharide fractions were chemically characterized in terms of galacturonic acid (GalA) content, degree of methoxylation (DM), neutral sugar composition, molar mass distribution and affinity towards some specific anti-pectin antibodies. Results showed that the cell wall polysaccharides in the pulp and, to an even greater extent, in the peel of both white-flesh and red-flesh species contained significant amounts of pectic substances. The pectic

substances in the dragon fruit peel as well as in the dragon fruit pulp were shown to be lowly methyl-esterified pectin (DM $\leq$ 39%) and highly water-soluble pectin (38-47%). A higher contribution of pectic homogalacturonan (HG) region in the peel samples was observed compared to the pulp samples, whereas there were no large differences between the pectic substances of both dragon fruit species. Despite the low average DM value of the pulp and peel pectins, pectic substances in both pulp and peel samples showed an affinity for anti-HG antibodies with different specificities, indicating that a wide range of epitopes (including long blocks of unesterified GalA residues as well as a few consecutive esterified GalA residues) was present.

In this doctoral research, the key findings of the experimental work are clearly shown and summarized in **Chapter 8**. Dragon fruit is an excellent source of bioactive compounds such as betacyanin and tocopherol which both have high antioxidative properties and health-promoting functions. Dragon fruit also provides many valuable products and components that could have high potential of being applied in food industry. For example, the freeze-dried dragon fruit pulp could be used as a red/purple colouring agent (betacyanin) as well as an instant juice powder. The heated dragon fruit puree with significant antioxidative activity could serve as a semi-processed product, the dragon fruit seed oil, on the other hand, was defined as a high-value oil. The extracted cell wall polysaccharide from dragon fruit peel demonstrated to be a low methyl-esterified pectin. Thus, the dissemination of the research findings may significantly increase the value of dragon fruit, resulting in an increased revenue for growers and producers.

SAMENVATTING

Pitaya's of drakenvruchten (*Hylocereus* spp.) winnen aan belang in verschillende landen, bijvoorbeeld Thailand, waar het klimaat geschikt is voor de groei van tropische en substropische vruchten. Pitaya's hebben een positief imago daar ze een bron zijn van essentiële nutriënten zoals vitaminen, mineralen, complexe koolhydraten, voedingsvezels en antioxidantia. Meer specifiek bevatten pitaya's betacyanine, een antioxidant dat ook dienst doet als pigment. In Thailand wordt de meerderheid van deze vruchten thuis geconsumeerd terwijl een beperkt deel wordt verhandeld onder de vorm van sap of puree. De schil en de zaadjes in het vruchtvlees worden beschouwd als afvalstromen en tot op heden werden deze met weinig succes gevaloriseerd. Daarenboven is er in de literatuur weinig informatie te vinden over de eigenschappen, samenstelling en verwerking van deze vruchten. Dit doctoraatsonderzoek had dan ook als doel een beter inzicht te verwerven in de samenstelling, eigenschappen en verwerkbaarheid van twee soorten pitaya's, nl. deze met wit (*H. undatus*) en rozerood (*H. polyrhizus*) vruchtvlees.

Het literatuuronderzoek in **hoofdstuk 1** omvat drie delen. In het eerste deel wordt een algemeen overzicht gegeven van de eigenschappen van pitaya's. Er wordt dieper ingegaan op de origine, de classificatie, het cultiveren, de marktwaarde en chemische samenstelling van deze vruchten. Een tweede deel bespreekt de processen die belangrijk zijn voor de verwerking van fruit, nl. warmtebehandelingen en vriesdrogen. De invloed van deze processen op de kwaliteit van verschillende fruitsoorten wordt in detail besproken. De samenstelling (minor- en majorcomponenten) en oxidatie van oliën geëxtraheerd uit zaden van verschillende origine worden in een volgend deel in detail beschreven. Een laatste deel bespreekt de structuur en modificatie van polysachariden aanwezig in de celwand, nl. pectine en hemicellulose.

In **hoofdstuk 2** worden de eigenschappen van de volledige drakenvrucht, de schil, het vruchtvlees (met en zonder zaadjes) van *H. undatus* en *H. polyrhizus* in detail besproken. Er kon besloten worden dat zowel in de schil van beide varianten als in het vruchtvlees van de rozerode pitaya's grote hoeveelheden betacyanines aanwezig waren. Deze componenten kunnen mogelijks gebruikt worden als natuurlijke kleurstoffen. Zowel het vruchtvlees als de puree vertoonden antioxidatieve eigenschappen. Bij *H. polyrhizus* was dit meer uitgesproken door de aanwezigheid van betacyanines. Het reologisch gedrag kon geclassificeerd worden als "shear-thinning" door de aanwezigheid van slijmerig plantenmateriaal. De resultaten van de karakterisatie zorgden voor een beter inzicht in de mogelijke valorisatie en verwerking van de pitaya's.

Om de pitayapigmenten te gebruiken als additief, werden in **hoofdstuk 3** het gevriesdroogde vruchtvlees en de schil van zowel *H. undatus* en *H. polyrhizus* gekarakteriseerd. Tijdens het vriesdrogen werden de pigmenten goed bewaard. Zowel het gevriesdroogde rozerode vruchtvlees als de schil van beide soorten bevatten hoge concentraties betacyanines. Het gevriesdroogde vruchtvlees was goed oplosbaar in water terwijl dit niet het geval was voor de gevriesdroogde schil. Aangezien betacyanines pH gevoelig zijn, werd de invloed van de pH (1-11) op de kleurverandering onderzocht. De kleur van de gevriesdroogde schil was stabiel in pH gebeid van 3-7. Het gevriesdroogde rozerode vruchtvlees bevatte een hoge concentratie betacyanines met een goede kleurstabiliteit in een breed pH-gebied (pH 1-11). Daarenboven is het gevriesdroogde vruchtvlees goed oplosbaar in water waardoor het mogelijks gebruikt kan worden als een natuurlijke kleurstof.

In **hoofdstuk 4** werd het effect van verschillende warmtebehandelingen (temperatuur 50-90 °C, 60 min) op de fysicochemische en antioxidatieve eigenschappen van het vruchtvlees van *H. undatus* en *H. polyrhizus* onderzocht. Er kon vastgesteld worden dat de antioxidatieve eigenschappen van het vruchtvlees verhoogden tijdens de warmtebehandelingen. Dit was te verklaren door de antioxidatieve eigenschappen van de zaadjes aanwezig in het vruchtvlees en de vorming van Maillard producten. De kleurparameters L* (helderheid) en b* (geel/blauw) kunnen gebruikt worden als een online kwaliteitsparameter tijdens de warmtebehandeling van een puree. De parameter “TCC” (globale kleurwijziging) van de rozerode pitaya vertoonde een sterk negatieve correlatie met het gehalte betacyanines. De kinetiek van de kleurverandering en de betacyaninedegradatie van de verhitte puree vertoonde een tweede orde verloop. De schijnbare viscositeit van de verhitte puree steeg in functie van de verhittingstijd en -temperatuur. De reologische data vertoonden een goede fit met het power-law model wat dus duidt op shear-thinning gedrag. Er kon besloten worden dat de verhitte puree van de pitaya, en voornamelijk deze van de rozerode soort, door zijn interessante nutritionele eigenschappen toegepast kan worden in levensmiddelen.

Aangezien de zaadjes uit het vruchtvlees van *H. undatus* en *H. polyrhizus* interessante antioxidatieve eigenschappen vertoonden, werd de olie uit deze zaadjes geëxtraheerd met een koude extractiemethode op basis van petroleumether. **Hoofdstuk 5** beschrijft de karakterisatie van deze oile. De zaadjes bevatten een significante hoeveelheid olie, namelijk 32-34% van het gewicht van de gedroogde zaden. De belangrijkste vetzuren in beide oliën waren linolzuur (C18:2, 45-55%), oliezuur (C18:1, 19-24%), palmitinezuur (C16:0, 15-18%) en stearinezuur (C18:0, 7-8%). De olie uit de zaadjes heeft een interessant nutritioneel profiel

door de hoge concentratie essentiële vetzuren (tot 56%). Uit de analyse van de triglyceridensamenstelling bleek dat er voornamelijk tri-onverzadigde en di-onverzadigde triglyceriden aanwezig waren. Er werd ook een significante hoeveelheid tocoferolen gedetecteerd, met α-tocoferol als belangrijkste (~72% van de totale tocoferolconcentratie). In een bewaarexperiment van drie maanden bij kamer- en koelkasttemperatuur werden de oxidatieve stabiliteit en de concentratie tocoferolen opgevolgd. De oxidatiegraad was laag terwijl de concentratie tocoferolen daalde tijdens de bewaring. Deze daling was echter beperkt in vergelijking met de startconcentratie. Er kon dus besloten worden dat naast de aanvaardbare oxidatieve stabiliteit, de olie ook een interessante bron is van essentiële vetzuren.

In **hoofdstuk 6** werd de activiteit van de pectine enzymes (nl. pectine methylesterase, PME en polygalacturonase, PG) uit het vruchtvlees en de schil van *H. undatus* en *H. polyrhizus* bestudeerd. Daarnaast werd ook de inactivatie van deze enzymes onderzocht bij verschillende warmtebehandelingen (30-90 °C voor 10 min). De onbehandelde pitaya's met het witte vruchtvlees vertoonden een hogere PME activiteit in vergelijking met de pitaya's met het rozerode vruchtvlees. In geen enkel van de stalen werd er PG activiteit waargenomen. Temperaturen van 70 °C of lager gedurende 10 min hadden in het vruchtvlees noch in de schil een effect op PME activiteit terwijl bij 80 en 90 °C PME wel geïnactiveerd kon worden.

In een laatste deel van dit doctoraatsonderzoek werden de polysachariden, nl. pectine en hemicellulose gebaseerde componenten, uit het celwandmateriaal van het vruchtvlees en de schil van zowel *H. undatus* en *H. polyrhizus* bestudeerd. Deze werden eerst geïsoleerd en dan verder gekarakteriseerd zoals beschreven in **hoofdstuk 7**. Het celwandmateriaal werd voor de isolatiestap eerst geblancheerd bij 80 °C gedurende 10 min zodat PME geïnactiveerd werd. In een volgende stap werd het materiaal geëxtraheerd en vervolgens gefractioneerd met verschillende oplossingen om drie verschillende pectinefracties, een hemicellulose en een onoplosbare residuele fractie te bekomen. Deze fracties werden daarna chemisch gekarakteriseerd. Het gehalte galacturonzuur, de graad van methoxylatie, de neutrale suikersamenstelling, de distributie van de molaire gewichten en de affiniteit voor specifieke anti-pectine antilichamen werden bepaald. Er kon besloten worden dat zowel het vruchtvlees als de schil van beide soorten drakenvruchten significante hoeveelheden pectine-achtige componenten bevatten. Voor zowel het vruchtvlees als de schil waren dit pectines met een lage graad van methoxylatie en een hoge wateroplosbaarheid (38-47%). In de schil werd een groter aandeel van de homogalacturonaan regio teruggevonden in vergelijking met het vruchtvlees. De verschillen in pectine samenstelling tussen de twee

soorten pitaya's waren beperkt. Ondanks de lage graad van methoxylatie van de pectines in het vruchtvlees en de schil, vertoonden deze toch een grote affiniteit voor de anti-homogalacturonaan antilichamen. Dit duidt aan dat er een brede range aan epitopen (zowel grote blokken van niet veresterde gehalte galacturonzuur residuen als een aantal opeenvolgende veresterde gehalte galacturonzuur residuen) aanwezig waren.

In **hoofdstuk 8** worden de belangrijkste resultaten van het doctoraal onderzoek duidelijk samengevat. Pitaya's of drakenvruchten zijn een interessante bron van bioactieve componenten, zoals betacyanines en tocoferolen. Ze kunnen ook waardevolle producten aanleveren. Het gevriesdroogde vruchtvlees kan bijvoorbeeld als natuurlijke kleurstof of instant vruchtenpoeder gebruikt worden. De verhitte puree met interessante antioxidatie eigenschappen kan dienst doen als half-afgewerkt product. De olie uit de zaden heeft door zijn samenstelling een hoge toegevoegde waarde en de polysachariden uit de celwand zijn een bron van pectines met een lage graad van methoxylatie. Deze resultaten illustreren dat er duidelijk een toegevoegde waarde gecreëerd kan worden voor zowel telers als producenten. De verspreiding van de resultaten van dit onderzoek kunnen de waarde van pitaya's verhogen, zowel voor de teler als de producent.

OUTLINE OF THE RESEARCH

Dragon fruit (*Hylocereus* spp.) is cultivated in many tropical and subtropical areas, including Thailand which is today one of the main producers and exporters of dragon fruit in the world. Many tropical fruits produced in Thailand are, therefore, considered of great economic importance for local and national markets. Due to its nutritional and functional potentials as well as its appearance, there is an interest in dragon fruit among consumers on the world market. Although dragon fruit contains a variety of components, the characteristics of dragon fruit have not yet been evaluated in detail. There are also no comprehensive data available in literature related to the properties of dragon fruit during thermal processing, oxidative stability of dragon fruit seed oil and the structure of cell wall polysaccharides of dragon fruit. Therefore, the overall strategic objective of this doctoral research was to provide a better insight into the characteristics of two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). The outcome of this research may use to add value to the fruit and its by-products.

Figure 1 schematically summarizes the outline of this research. All chapters are connected to some extent. The outline of this dissertation consists of three main parts: an introduction and a literature review in **Chapter 1**, an experimental research, including research strategies and report research findings in **Chapter 2-7**, and a general conclusion in **Chapter 8**.

A review of the current literary: research and critical review relating to the botanical classification, cultivation, economic aspects, chemical composition (e.g. nutrients, pigment and antioxidative components) and functional properties of dragon fruit as well as the particular results from relevant works, is given in **Chapter 1**. The general principles of freeze-drying and thermal processing are also described, followed by a critical review on the impact of these processes on the characteristics of different fruits. A general introduction of seed oil (e.g. oil composition, oxidation mechanism and relevant factors influencing oil oxidative stability) is given. Focusing mainly on pectic substances and their conversions, this chapter provides a concise review on the structure of plant cell-wall polysaccharides.

An investigation into the physicochemical, antioxidative, microbiological and rheological properties of different fractions of the white-flesh and red-flesh dragon fruits takes place in **Chapter 2**. These fractions include the whole fruit, peel, pulp (seedless) and puree (with the presence of the seeds). They were investigated as they provide an ideal base for following experiments. **Chapter 3** discusses the quality attributes of the freeze-dried pulp and peel of the two species of dragon fruit. It is primarily pointing at colour parameters and concentration of betacyanin. The influence of pH on colour changes of the rehydrated

freeze-dried dragon fruit was also studied in order to specify the utilization of dragon fruit's pigment as a colouring agent. **Chapter 4** exhibits the antioxidative, rheological, physicochemical and microbiological properties of the dragon fruit puree as affected by various thermal treatments (at 50-90 °C for maximum 60 min). The derived results make it possible to predict and control the desired quality of the dragon fruit puree during thermal processing. Hence, the kinetic parameters such as rate constant and activation energy, of colour and pigment degradation as well as changes of antioxidative properties of the thermally processed dragon fruit puree were analyzed.

Focusing on the dragon fruit seeds as a potential source of antioxidants, they can be further explored as valuable components of the dragon fruit. Therefore, oil was extracted from the dragon fruit seeds and the seed oil characteristics such as the fatty acid profile, TAG composition, tocopherol content, and physicochemical and thermal properties are discussed in **Chapter 5**. The oxidative stability and the present tocopherols in the dragon fruit seed oil were also weekly monitored at two different storage conditions, cold and room temperatures, over a 3-month storage period. **Chapter 6** shows the activity of pectic enzymes (i.e. PME and PG) in the untreated pulp and peel of dragon fruit as well as the residue activity of pectic enzymes in the heated dragon fruit at different thermal treatments (at 30-90 °C for 10 min). The knowledge related to the thermal inactivation of pectic enzymes is of interest for dragon fruit processing. The blanching condition can be also applied as a pre-treatment prior to the cell wall polysaccharide isolation, as described in **Chapter 7**. In this chapter, the fractionation of cell wall polysaccharides of the pulp and peel of white-flesh and red-flesh dragon fruits obtained different pectin and hemicellulose fractions by using different solvents. The structural characterization (i.e. GalA content, DM, neutral sugar composition, molar mass distribution and some specific pectin structures by using anti-pectin antibodies) of the cell wall polysaccharide fractions from dragon fruit is accordingly discussed here.

In the final part of the manuscript, a general conclusion of the work is shown in **Chapter 8**. The most important findings in the doctoral research are formulated and discussed. A realistic view on the utilization of dragon fruit and by-products mainly with respect to the yield is given. Additionally, the limitations and recommendations for further research are suggested.

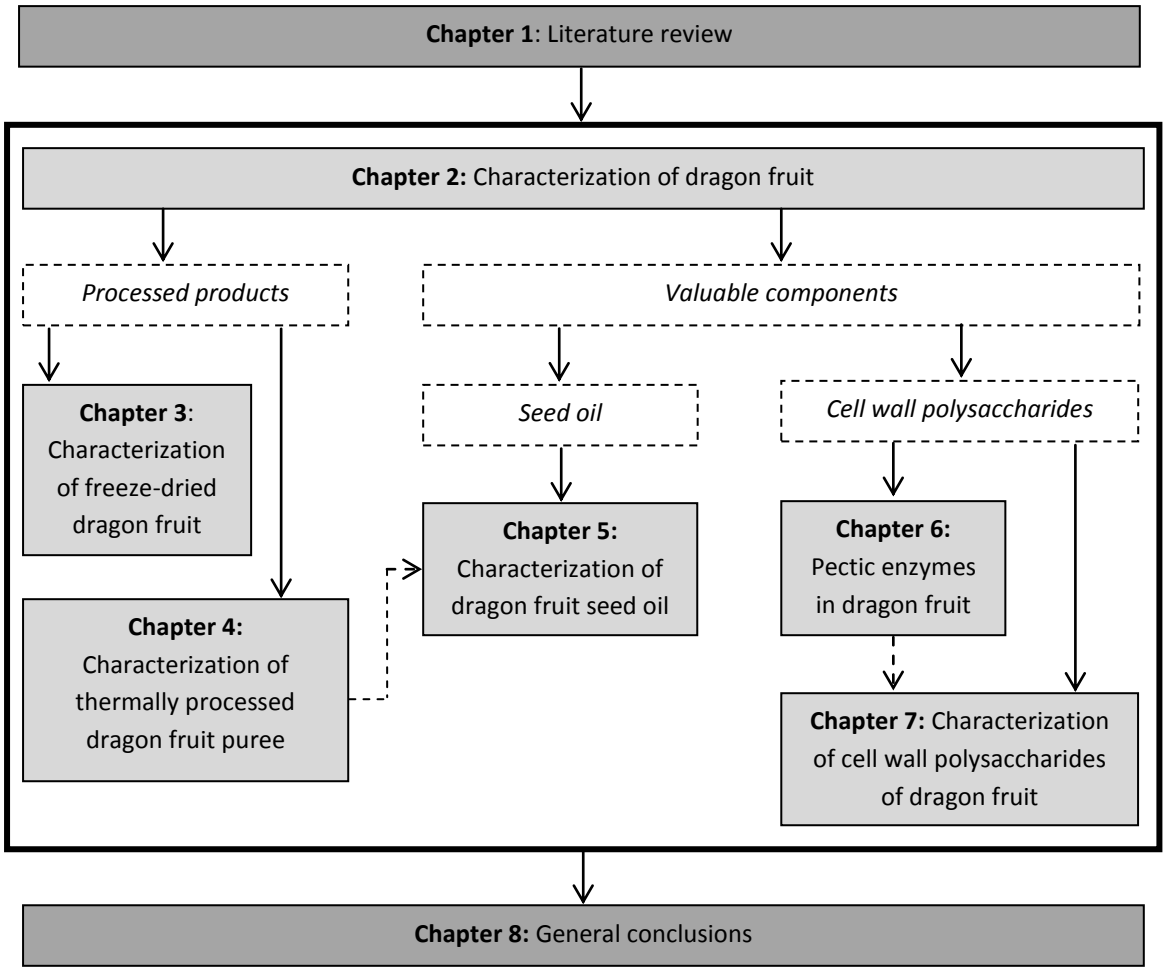


Figure 1 *Schematic outline of the research strategy*

CHAPTER 1

Literature Review

1. Literature review

1.1 Introduction

Dragon fruit (*Hylocereus* spp.) is a promising tropical fruit which can be cultivated in different tropical and subtropical parts of the world such as Southeast Asia, and Central and South America. The demand for dragon fruit extensively increases and the fruit today can be found on almost all exotic fruit markets around the world. This success can be partly explained by the fruit qualities, e.g. appearance, nutritional values and health benefits, but also by the commercial policies of producing and exporting countries such as Thailand and Vietnam, which are the main producing countries of dragon fruit. Tropical fruit crops in Thailand are steadily becoming an important aspect of agricultural production. The exports of fresh and processed tropical fruits from Thailand have shown upward trends since 1980 (Salakpetch, 2000).

Besides being consumed fresh, dragon fruit can also be processed into, for example, juice and puree. Dragon fruit and its products may be used as ingredients for innovative food products that respond to consumers' interest (Le Bellec *et al.*, 2006; Sabbe *et al.*, 2009). They are widely used in various food products such as sweets, yogurts, ice creams, pastries, jams, jellies and wines. This is due to its special colour (especially red/purple pigments in red-flesh dragon fruit), high nutritional values and antioxidative properties of the fruit (Mohd, 2010). A high increase in dragon fruit processing results in high amounts of waste materials such as peels and seeds. Since food waste management is a serious environmental issue, converting these wastes into value-added components must be given more attention. Therefore, the knowledge related to the characterization of dragon fruit is necessary to improve quality of dragon fruit and its co-products.

This literature review is intended to give a complete background of dragon fruit. It involves key information about classification, cultivation, harvesting, production and marketing as well as fruit composition (e.g. chemical, pigment and antioxidative components) of the fruit. A concise overview of available research relating to dragon fruit is also given. The knowledge of freeze-drying technology and thermal processing, and a critical review of research dealing with the impact of these processing technologies on important attributes of various fruits are included. The chemical properties (e.g. fatty acid composition and nutrients) and oxidative stability of the seed oils are described. This review ends with an

outline of structure of plant cell-wall polysaccharides (pectic and hemicellulosic substances) and their modification either by non-enzymatic reactions or by cell wall-related enzymes.

1.2 Dragon fruit

Dragon fruit (*Hylocereus* spp.) is a tropical climbing cactus. It is also known as pitaya or pitahaya in Latin America (Le Bellec *et al.*, 2006), *päniniokapunahou* or *päpipi pua* in Hawaii, and night-blooming cereus or strawberry pear in Southeast Asia (Zee *et al.*, 2004). The fruit crop is grown in the subtropical and tropical countries, and is normally available all year round. Dragon fruit is very attractive because of its exotic appearance. The pulp is juicy and contains numerous small black seeds. It is also considered as a potential source of micronutrients and antioxidants (Ariffin *et al.*, 2009: Jaafar *et al.*, 2009: Lim *et al.*, 2010a: Lim *et al.*, 2007: Mahattanatawee *et al.*, 2006: To *et al.*, 1999: Wu *et al.*, 2006). Dragon fruit is classified as a non-climacteric fruit (Enciso *et al.*, 2011; Zee *et al.*, 2004). The ripening mechanism is, therefore, based on the absence of ethylene burst during fruit ripening which corresponds to the inability to synthesize ethylene autocatalytically (Périn *et al.*, 2002).

1.2.1 Origin and classification

Genus *Hylocereus* is a group of tropical epiphytic cacti and believed to originate from the rainforests of Latin (Le Bellec *et al.*, 2006) and/or Central America (Barbeau, 1993). The genus *Hylocereus* is a member of the Cactaceae family, a dicotyledonous flowering plant family, in the Caryophyllales order. Within this family, there are 120-200 genera which group 1500-2000 species, naturally present in the semi-desert and tropical regions of Latin America (Spichiger *et al.*, 2000). Cactaceae are mainly appreciated for their ornamental qualities, but also include nearly 250 cultivated species of fruit-bearing crops. Only a few species are of economic value such as *Opuntia* spp. and *Hylocereus* spp. (Le Bellec *et al.*, 2006).

Up-to-date, there are 16 species of *Hylocereus* (Le Bellec *et al.*, 2006). The generic and vernacular names of *Hylocereus* species render their botanical classification difficult (Mizrahi *et al.*, 1997). There are many contradictions related to the botanical classification of the species that are probably explained by similar morphological characteristics. However, this doctoral dissertation focuses on two *Hylocereus* species, i.e. *H. undatus* (white-flesh dragon fruit) and *H. polyrhizus* (red-flesh dragon fruit). These two dragon fruit species are diploid

(2*n* = 22) (Dios, 2004; Lichtenzveig *et al.*, 2000) and their botanical classification is based on the main components, including stem, flower and fruit, as illustrated in **Figure 1.1**.

Figure 1.1 *Botanical classification of (A) Hylocereus undatus and (B) Hylocereus polyrhizus (adapted from Mohd, 2010)*

Morphologically, both *Hylocereus* species (i.e. *H. undatus* and *H. polyrhizus*) are cactus species with greenish long stems having spines at a margin. The stems are typically 3-sided or triangular stems with multiple branches. The flower of both *Hylocereus* species is known as queen of the night because it perfectly blooms at night. It is generally a very long flower, measuring up to 25-30 cm. The fruit of the two *Hylocereus* species is oblong with a typically intense dark pink or reddish-purple peel with green leaf-like scales. The small edible blackish seeds are usually embedded in its flesh with a sticky gelatinous carbohydrate layer. The seeds of the two *Hylocereus* species are similar to kiwi seeds in terms of their appearance (i.e. colour and size) and texture (Barbeau, 1993; Britton and Rose, 1963; Le Bellec *et al.*, 2006).

It can be derived from **Figure 1.1** that the stem of *H. undatus* has a margin with a whitish layer, whereas the stem of *H. polyrhizus* is occasionally 4- or 5-sided with more spines at the

margin compared to *H. undatus*. The large flower of *H. undatus* has outer greenish and inner whitish perianth segments, whilst the outer perianth segments of *H. polyrhizus* flower are reddish. The fruit of *H. polyrhizus* is a scarlet fruit with rather wider and shorter leaf-like scales compared to *H. undatus* (Barbeau, 1993; Britton and Rose, 1963; Le Bellec *et al.*, 2006). The fruit of *H. undatus* and *H. polyrhizus* is easily recognized by white flesh and red flesh, respectively. The well-ripe fruit of *H. undatus* is soft and sweet, whereas the pulp of *H. polyrhizus* is usually very sweet and mild acidic (Gunasena *et al.*, 2007; Le Bellec *et al.*, 2006). The size of fruit is variable. The fruit of *H. polyrhizus* (length: 10-12 cm, weight: 130-350 g) is generally smaller than *H. undatus* (length: 15-22 cm, weight: 300-800 g) (Mohd, 2010).

1.2.2 Cultivation and harvesting

Dragon fruit is a semi-epiphytic plant which prefers a dry tropical or subtropical climate with an average temperature of 21-29 °C, but can withstand temperatures of 38-40 °C and freezing temperature (as low as 0 °C) for short periods. This crop requires sunshine and rainfall of 600-1300 mm with alternating wet and dry seasons (McMahon, 2003). Plant growth is rapid and continuous, though possibly with a vegetative rest period when the climatic conditions are unfavourable. Dragon fruit can be multiplied easily by cutting off the stem or by sowing the seeds. Dragon fruit climbs and attaches naturally to the supports either in vertical or horizontal directions. Southeast Asia, including Thailand and Vietnam, is today the main producing region of dragon fruit. **Figure 1.2** shows a dragon fruit plantation in these countries. The fruit is normally planted with vertical support of wood or cement and its stem must be attached to the support with a clip.

Figure 1.2 *Plantation of dragon fruit in (A) Thailand and (B) Vietnam*

When vertical support is used, a 2-3 m distance of planting lines is required between 2000 and 3750 cuttings per ha at a rate of three cuttings per support. The height of the support should be between 1.4 and 1.6 m to facilitate management of the crop. Pruning is also important (Le Bellec *et al.*, 2006). During the first year after planting, the major pruning is carried out and the crop needs to be watered regularly (about once a week). The first harvest may begin after the first year of planting (FAO, 2004).

In maturation stage, dragon fruit peel changes from green to red or rosy-pink colour after 25-27 days of fruit set. Four or five days later, dragon fruit reaches its maximum coloration, but it is better to delay harvest (as much as 50 days after fruit set) to allow more sweetness and to grow in size (Zee *et al.*, 2004). The fruiting stage is reached more rapidly with the cuttings, about 1 year after planting, as opposed to about 3 years for plants grown from seed. After this stage, dragon fruit can be harvested by simply twisting the fruit that probably very often injures fruit peel. Therefore, fruit harvesting by secateurs should be applied to obtain high productivity and to ensure a good quality of the fruit (Le Bellec *et al.*, 2006). Dragon fruit can be stored for 25-30 days at 4 °C, but it can only less 10 days at room temperature (Zee *et al.*, 2004).

1.2.3 Production and economic aspects

Dragon fruit has drawn much attention of growers worldwide including Thailand. It was first introduced to Thailand in 1991 and successfully produced in 1994 with good quality and high production. The suitability of tropical climate, rainfall requirements, light intensity and soil types may attribute to the successful cultivation of this exotic fruit in this country (Jantrachu, 2013). Dragon fruit is productive all year round in Thailand, but the normal bearing period of dragon fruit is basically from April to October. The fruit is a high yielder and fast-return fruit crop with production in the second year after planting and full production in 5 years. However, it can be harvested continuously for 10-15 years (ThaiSME, 2013). Dragon fruit plantation in Thailand can be about 44 to 65 tons per ha per year (Duangtaweesub *et al.*, 2011). This is comparable with the yield of a well-managed plantation in Malaysia (70 tons per ha per year) (Mohd, 2010). On the other hand, yield of dragon fruit productivity in Vietnam could reach about 50 to 80 tons per ha per year with adaptation of good cultural practice (FAO, 2004). However, dragon fruit plantation can also be found in Taiwan, but production is quite low (16-27 tons per ha per year) (Zee *et al.*, 2004).

Dragon fruit is classified as a group of minor tropical fruits together with, for example, durian, litchi, longan, starfruit and rambutan, whilst pineapple (the highest production in Thailand), banana, mango, mangosteen and guava are classified as the major tropical fruits offering high production and income. The minor and major tropical fruits are defined depending on market availability, production, planted acreage and economic considerations (ESCAP, 2006; FAO, 2012). Asia and the Pacific regions are the biggest producing regions of tropical fruits (FAO, 2003). The regions produced about 68% of the share of minor tropical fruits in the world market during 1986-1988 (Singh, 1993). **Figure 1.3** shows production of tropical fruits in Thailand between the years of 1997 and 2010. Production of pineapple in Thailand increased from 1.3 to 2.6 million tons between 1981 and 1999, whereas production of minor tropical fruits was stable at 0.7 million tons with about 15% increase in total planted acreage. In the years of 2000 to 2010, pineapple production remained stable at average of 2.2 million tons, while the minor tropical fruits did not. The production of minor tropical fruits jumped from 0.7 million tons in 1999 to 2.5 million tons in 2000 with an increase of the production area of tropical fruits (up to 46%) (FAOSTAT, 2013).

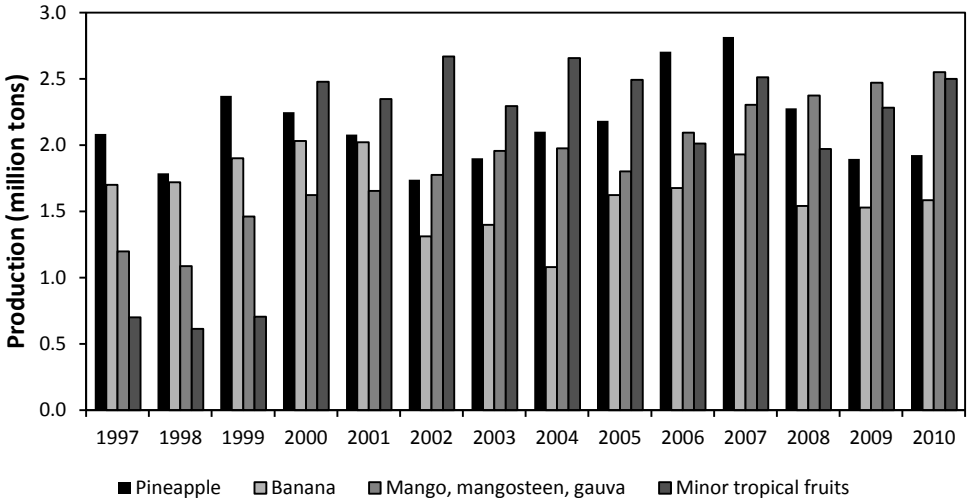


Figure 1.3 *Production of tropical fruits in Thailand (FAOSTAT, 2013)*

Southeast Asia is a big exporter of tropical fruits. The key countries include Thailand (the highest export value), Philippines, Indonesia, Malaysia and Singapore (ESCAP, 2006; FAOSTAT, 2013). Fruit production is one of the most important businesses in Thailand contributing greatly to the country's national income. Thailand also has the capacity to export fresh tropical fruits globally all year round. **Figure 1.4** illustrates the export value and

volume of tropical fruits in Thailand between the years of 1997 and 2010. From 2000 onwards, the export income of minor tropical fruits from Thailand to the world increased up to US$ 116 million (~89% of total export value from Southeast Asia), whereas fruit production in Thailand was only 30% compared to total production in Southeast Asia. However, about 90% of tropical fruits were consumed domestically, while the rest of the fruits was exported globally (FAOSTAT, 2013).

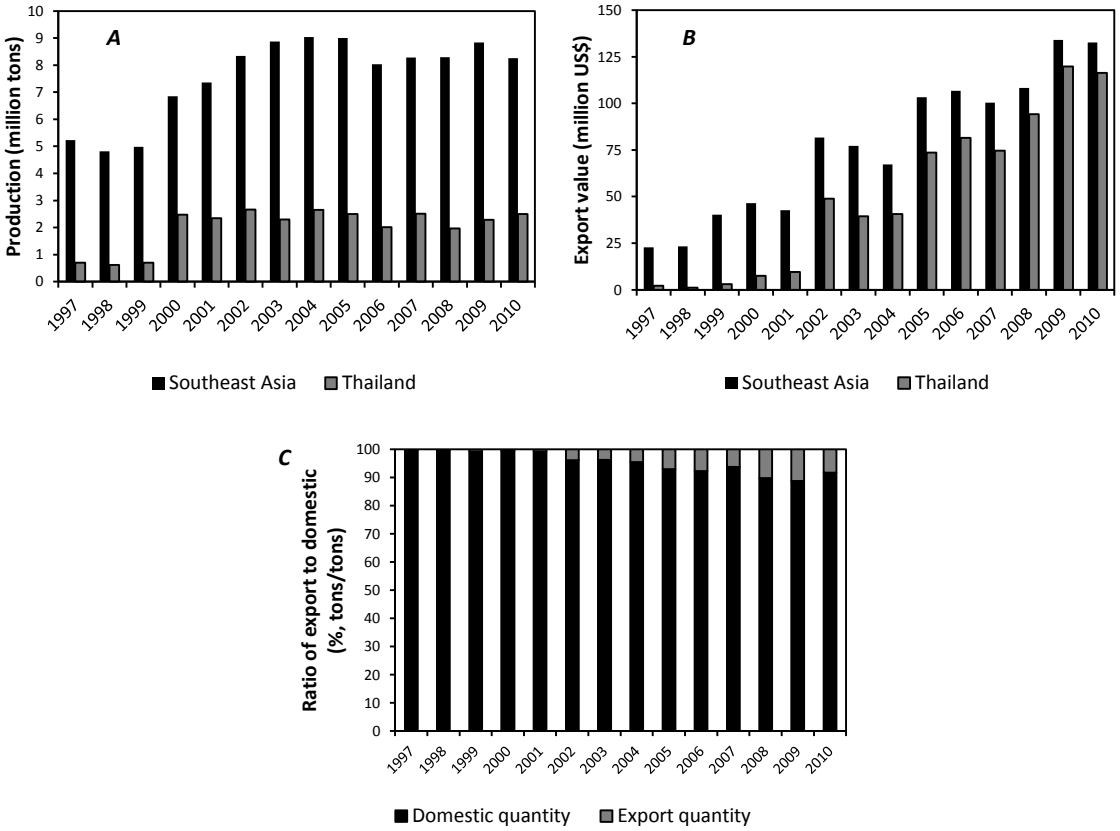


Figure 1.4 *Production and export of minor tropical fruits: (A) production in Thailand compared to Southeast Asia, (B) export value of Thailand and Southeast Asia, and (C) quantity ratio of export to domestic volume in Thailand (FAOSTAT, 2013)*

Dragon fruit has been cultivated almost in all states of Thailand where East part is the main production area. The domestic market price of dragon fruit greatly depends on the fruit quality, e.g. variability in size, taste and colour. Dragon fruit is currently sold at an average of US$ 1 per kg in Thai local market. Thus, a hectare of dragon fruit can be sold at estimated of US$ 44000-65000 per year, whilst the investment costs of dragon fruit cultivation are

estimated at US$ 5670-8400 per ha per year (Jantrachu, 2013; ThaiSME, 2013). The value of the exotic fruits in global market can be negotiable due to consumer demand, supplier, available transportation, and policies of producing and exporting countries. Dragon fruit is exported mainly to European countries with Vietnam being the first country to ship the fruit to Europe. The market in Thailand emerged in 2003 with the market share increasing from below 10% in 2001 to 17% in 2004. Thailand has an advantage over Vietnam in the cost of airfreight, a key factor in competitiveness (Le Bellec *et al.*, 2006). Thus, dragon fruit has a great potential in Thai fruit industry with good economic aspects and can be a profitable crop to venture.

1.2.4 Chemical composition

Dragon fruit has interesting nutritional and functional components. **Table 1.1** shows the chemical composition per 100 g of dragon fruit compared to some other tropical fruits. Dragon fruit contains significant amounts of minerals such as potassium, phosphorus, sodium and magnesium. The amount of these minerals in dragon fruit is clearly higher than those of mangosteen, mango and pineapple (Gunasena *et al.*, 2007; Stintzing *et al.*, 2003; To *et al.*, 1999). Vitamins such as vitamin C (33 mg/100 g) (Choo and Yong, 2011) and vitamin B3 (0.2-2.8 mg/100 g), are also found in dragon fruit at high concentrations, whereas small amounts of vitamins B1, B2 and A are present ($\leq$ 0.05 mg/100 g) (Stintzing *et al.*, 2003; To *et al.*, 1999). The estimated energy value of white-flesh dragon fruit is relatively low compared to red-flesh dragon fruit (130 kJ/100 g compared to 283 kJ/100 g) and other tropical fruits. However, the energy value of red-flesh dragon fruit is lower than jack fruit and banana (410 and 356 kJ/100 g, respectively), but it is comparable value to mangosteen, mango and pineapple (238-283 kJ/100 g) (To *et al.*, 1999). The chemical composition of dragon fruit can, however, vary considerably depending on the species, origin and harvesting conditions.

The predominant sugars in dragon fruit are glucose (Glc), followed by fructose. These sugars account for 2-6 g/100 g (Nomura *et al.*, 2005; Stintzing *et al.*, 2003). A lower amount of sorbitol is also present (0.3 g/100 g) (To *et al.*, 1999), whereas no measurable amounts of sucrose and maltose are observed in dragon fruit (Stintzing *et al.*, 2003; To *et al.*, 1999). Dragon fruit is a low acid food. Its pH values vary between 4.4 and 5.1 (Gunasena *et al.*, 2007; Stintzing *et al.*, 2003) with malic acid being the main acid in the fruit (Nomura *et al.*, 2005). Similar to most fruits, the moisture content of dragon fruit is relatively high amount (83-89 g/100 g fresh weight), accounting for the juicy attribute of the fruit (Mahattanatawee *et al.*, 2006; To *et al.*, 1999).

Table 1.1 *Chemical composition (per 100 g edible portion, fresh weight) of dragon fruit, mangosteen, mango and pineapple (Gunasena et al., 2007; Kansci et al., 2008; Stintzing et al., 2003; To et al., 1999)*

Composition	White-flesh dragon fruit	Red-flesh dragon fruit	Mangosteen	Mango	Pineapple
Energy (kJ)	130	283	238	276	243
Protein (g)	0.5	0.2-1.1	0.5	0.7	0.3
Fat (g)	0.1	0.6-0.9	0.3	0.4	0.2
Carbohydrates (g)	9.5	11.2	14.7	16.8	13.7
Glucose (g)	5.5	4.7-5.7	-	0.8-1.5	-
Fructose (g)	1.9	1.8-3.2	-	6.4-9.4	-
Crude fibre (g)	0.3	0.7-1.3	5	0.9	0.4
Calcium (mg)	3.1-6	2.3-10.2	10	10	17
Magnesium (mg)	26.6	31.3-38.9	Not detected	8.8	13
Sodium (mg)	3.3	7.3-8.9	1	7	1
Potassium (mg)	399.5	272-328.4	135	189	146
Iron (mg)	0.4	0.6-3.4	0.5	0.4	0.5
Phosphorus (mg)	19	27.5-36.1	10	13	8

A promising source of pectin is observed in dragon fruit pulp (Mahattanatawee *et al.*, 2006) and even in dragon fruit peel (Jamilah *et al.*, 2011). The peel is also a good source of dietary fibre, accounting to 69 g/100 g of dried peel (Jamilah *et al.*, 2011). The mucilaginous material in its flesh tissue which envelopes surrounding the seeds consist of polysaccharide substances (Wichienchot *et al.*, 2010). Dragon fruit contains oligosaccharides (~90 g/kg) which are suggested to consist of some fructooligosaccharides, i.e. 1-kestose, 6-kestose and neokestose (1 Glc unit and 2 fructose units), or nystose, bifurcose and neobifurcose (1 Glc unit and 3 fructose units), or stachyose (3 Glc units and 1 fructose unit). These fructooligosaccharides have prebiotic properties and are beneficial to the gastrointestinal system which include resistance to acid conditions in human stomach, partial resistance to human salivary α-amylase, and can enhance the capability to stimulate the growth of lactobacilli and bifidobacteria (Wichienchot *et al.*, 2010). Fructooligosaccharides may be partly hydrolyzed by gastric acid, but they are thought to be completely digested in the human upper intestine and be fermented by the colonic microflora (Cummings and Englyst, 1995).

The nutritional value of dragon fruit is not only limited to its pulp and peel, but also present in dragon fruit seeds. The edible seeds of dragon fruit contain a high amount of oil having health beneficial properties (Ariffin *et al.*, 2009). This is due to a significant amount of vitamin E (tocopherol) and essential fatty acids (Chemah *et al.*, 2010; Lim *et al.*, 2010a).

Besides tocopherol and fatty acids in dragon fruit seeds, the seeds may consist of insoluble dietary fibre (e.g. cellulose, hemicellulose and lignin) which probably originates from cell wall materials and seed coats like found in most seed plants such as flaxseed (Tarpila *et al.*, 2005). The consumption of dragon fruit has been linked to positive health effects due to chemical components present in its flesh and seed, and their bioavailability.

1.2.5 Pigment component

Pigment components in plant-based food naturally occur in diverse groups, resulting in various attractive colours. Chlorophyll appears green, carotenoid exhibits brilliant red, orange or yellow colours, anthocyanin has a colour range from orange/red to blue, and betalain appears yellow and red/purple (Delgado-Vargas *et al.*, 2000). The most important pigments in cactus fruits (e.g. *Hylocereus* spp. and *Opuntia* spp.) are betalain which can be divided into 2 groups: betaxanthin (orange/yellow pigment) and betacyanin (red/purple pigment) (Gibson and Nobel, 1986).

Betacyanin, a pigment group of water-soluble phytochemical components, is the most abundant pigment in the pulp of red-flesh dragon fruit and also can be found in the peel of both red-flesh and white-flesh dragon fruits (Harivaindaran *et al.*, 2008; Moreno *et al.*, 2008; Stintzing and Carle, 2004; Stintzing and Carle, 2007; Stintzing *et al.*, 2002; Wybraniec *et al.*, 2001). The chemical structure of betacyanin is shown in **Figure 1.5.** It comprises a betalamic acid (a central intermediate in the formation of all betalains) and a derivative of *cyclo*-dihydroxyphenylalanine (*cyclo*-Dopa) with ammonium conjugates (Moreno *et al.*, 2008). The pigment consists of non-acylated betacyanins (e.g. betanin which is a simple betacyanin component and displays as the most stable structure of betacyanins), acylated betacyanins (e.g. phyllocactin and hylocerenin), and their isomers and derivatives (Herbach *et al.*, 2006a; Herbach *et al.*, 2006b; Stintzing *et al.*, 2002; Wybraniec *et al.*, 2001).

Since dragon fruit is a rich source of betacyanin, it has been widely studied in terms of qualitative and quantitative properties. Dragon fruit mainly contains betanin (betanidin-5-*O*-β-glucoside), phyllocactin, hylocerenin and their isomer forms with a minority group of unidentified betacyanin compounds (Kim *et al.*, 2011; Stintzing *et al.*, 2002; Wybraniec *et al.*, 2001). During dragon fruit plantation, betacyanin content increases with the maturity level of the fruit (Phebe *et al.*, 2009). Total betacyanin content in the mature red-flesh dragon fruit varies from 32 to 47 mg/100 g (Herbach *et al.*, 2004a; Vaillant *et al.*, 2005). These values are comparable with the results found in red beetroot, a commercial betacyanin source (Azeredo *et al.*, 2007). Currently, the red-flesh dragon fruit has been

proposed as a promising new source of betacyanin pigments for red/violet colouring foodstuff (Harivaindaran *et al.*, 2008; Moreno *et al.*, 2008; Stintzing and Carle, 2004; Stintzing and Carle, 2007; Stintzing *et al.*, 2002; Vaillant *et al.*, 2005; Wybraniec *et al.*, 2001).

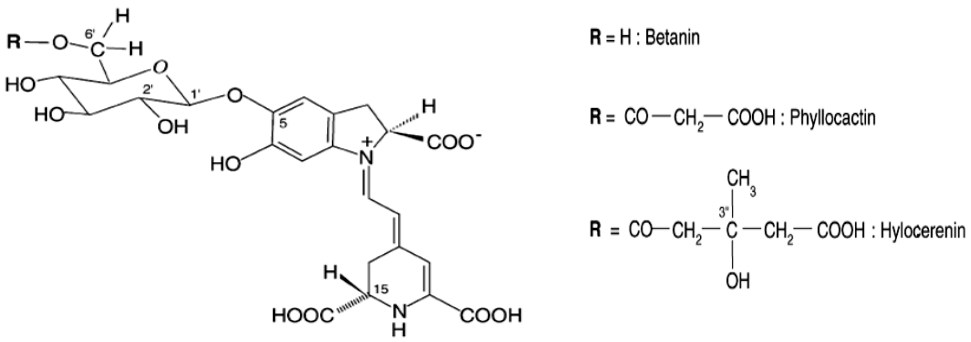


Figure 1.5 *Chemical structure of betacyanin (Stintzing et al., 2003)*

In general, betacyanin pigments can be degraded and/or formed to many derivative compounds during fruit harvesting and processing. This is due to their instability as affected by various specific and external factors, e.g. pigment concentration, degree of glucosylation or acylation, matrix constituent, chelating agents, antioxidative components, temperature, pH, oxygen, light, water activity and nitrogen atmosphere. In order to ensure the optimum pigment concentration and the colour retention in processed foods, process conditions (particularly pH and heating conditions) during manufacture need to be considered and controlled. The stability of betacyanin basically refers to degradation and deformation of betanin (Herbach *et al.*, 2006a; Moreno *et al.*, 2008). **Figure 1.6** illustrates various mechanisms of degradation and formation of betanin. Herbach *et al.* (2004b) explained that the isomerization of betanin to its isomer probably occurs. At alkaline conditions, betanin brings about aldimine-bond hydrolysis, resulting in the generation of the bright yellow betalamic acid and the colourless *cyclo*-Dopa-5-*O*-β-glucoside. In contrast to high alkalinity, the recondensation of betalamic acid and *cyclo*-Dopa-5-*O*-β-glucoside, and the C15 isomerization of betanin and betanidin into isobetanin and isobetanidin are induced at an acid treatment. This pathway probably causes the formation of the yellow 14,15-dehydrobetanin or neobetanin (Herbach *et al.*, 2006a).

Despite a strong effect of pH on the colour shift of betacyanin, a constant colour appearance, however, can be expected at pH between 3 and 7 (Herbach *et al.*, 2006a;

Stintzing and Carle, 2004). In addition, Stintzing *et al.* (2003) stated that the pigments of the genera *Opuntia* and *Hylocereus* are presumed to be stable over pH between 1 and 8 with a slight darkness at pH lower than 1.5 and pH higher than 7. Since betacyanin pigments retain their appearance in this pH range, application of the red-flesh dragon fruit as a natural food colourant appears to be promising. Hence, the addition of 1% ascorbic acid at pH 4 is suggested to maximize the pigment retention and to prevent betacyanin degradation upon a thermal treatment (Herbach *et al.*, 2006b).

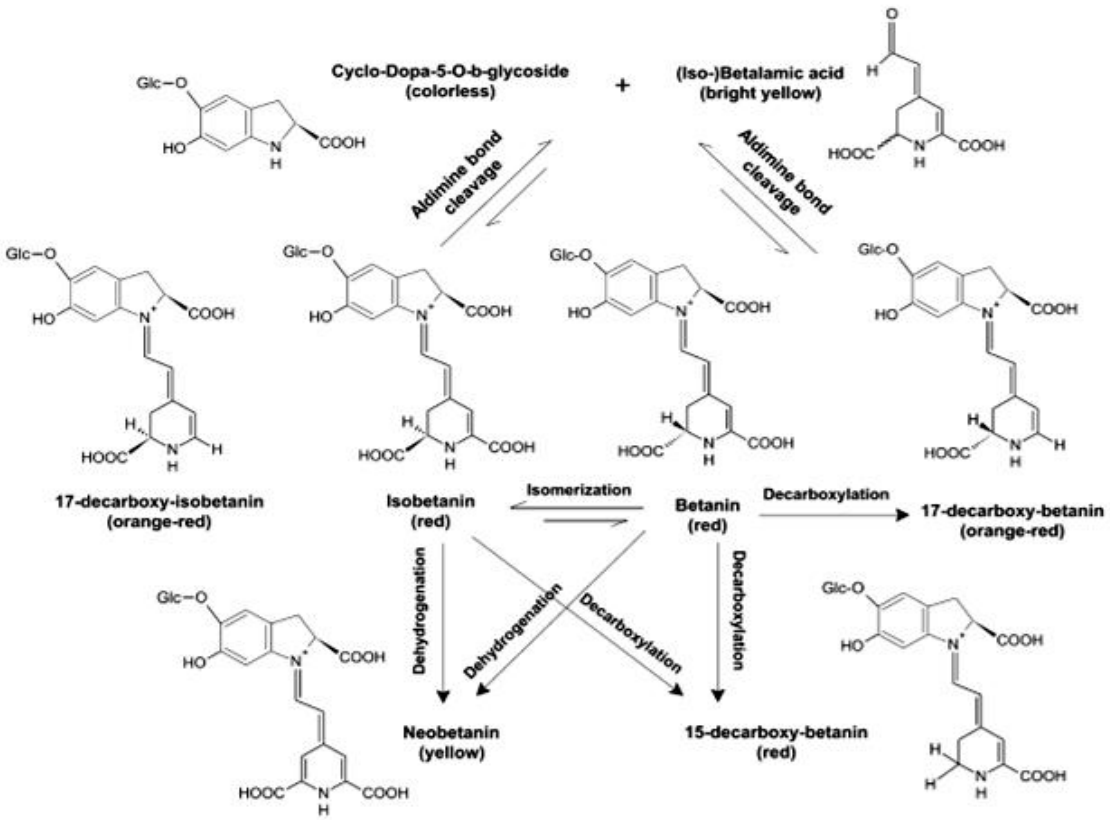


Figure 1.6 *Degradation and formation pathways of betanin under acidic and basic conditions (Azeredo, 2009; Herbach et al., 2004b)*

Thermal processing greatly affects the decomposition and formation of betacyanin. Heating time and temperature must be controlled to ensure the optimum pigment and the colour retention of the processed product. The formation and degradation of betacyanin (and their derivatives) in the red-flesh dragon fruit upon a thermal treatment result in colour changes of the fruit (Herbach *et al.*, 2006b). **Figure 1.7** shows the degradation and formation of betanin, a representative component of betacyanin, as affected by a thermal treatment.

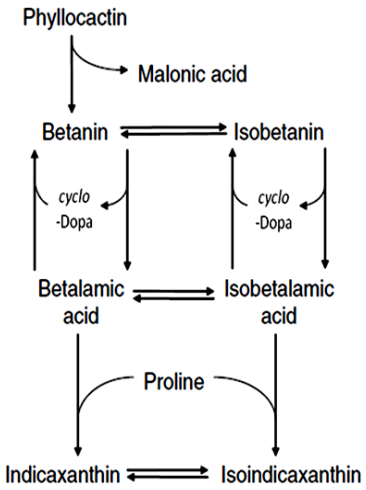


Figure 1.7 *Degradation and formation of betanin undergoing thermal treatment (Herbach et al., 2006b)*

The deacylation of phyllocactin to form betanin during thermal treatment is assumed to proceed until equilibrium between betanin and phyllocactin is reached. Afterwards, C15-isomerization of betanin also occurs. Since malonic acid is removed, only *cyclo*-Dopa derivatives (colourless components) are available as condensation partners for betalamic acid. Indicaxanthin and its C11-epimer isoindicaxanthin may be formed in the thermally treated product, especially at the elevated pH values. The association of proline and betalamic or isobetalamic acid is generated by hydrolytic cleavage of betacyanin upon a thermal process. Since a thermal treatment can lead to an increase in the inverse betanin/isobetanin isomerization in the red-flesh dragon fruit (Herbach *et al.*, 2006a), it is possible that betanin and isobetanin can be further formed and degraded by decarboxylation and/or dehydrogenation which leads to neobetanin formation and brings about a yellow tint during thermal processing (Herbach *et al.*, 2004a; Herbach *et al.*, 2004b).

1.2.6 Antioxidative component

The dietary intake of antioxidative components plays a major role in protecting cell membranes in humans against oxidation (lipid peroxidation) by eliminating reactive oxygen species. Oxidation may contribute to human ageing and disease (Tesoriere *et al.*, 2009). In general, phenolic compounds, which consist of a hydroxyl group directly attached to an aromatic ring, are the most abundant antioxidative components in most plant-based foods. They have a wide variety of structures: simple phenolic (a phenolic ring which contains a

hydroxyl group bonded to an aromatic ring), phenolic acid (carboxyl group attached to a phenolic ring) and polyphenolic (two or more phenolic rings). In that way, they can exhibit different functions, e.g. colouring, sensory functions and biological functions as bioactive components having health benefits (Ignat *et al.*, 2011; Jaiswal *et al.*, 2011; Maestri *et al.*, 2006; Nagasaka *et al.*, 2007; Parr and Bolwell, 2000).

Phenolic compounds in dragon fruit consist mainly of gallic acid (GA) (Kim *et al.*, 2011) and ferulic acid with minor amounts of other hydroxycinnamic acids (Mahattanatawee *et al.*, 2006). **Figure 1.8** shows the chemical structure of GA, which is the main phenolic acid, and other common phenolic acids. Most of the hydroxycinnamic acids such as caffeic, ferulic, *p*-coumaric and sinapic acids, are commonly present in higher plants (Ignat *et al.*, 2011), for example, in purple grape, pomegranate and apple (Mullen *et al.*, 2007). Besides phenolic acids, dragon fruit also contains some flavonoid compounds (polyphenolic compounds), i.e. phloretin-2-*O*-glucoside and myricetin-3-*O*-galactopyranoside, which are much higher in concentration in the peel than in the pulp (Kim *et al.*, 2011).

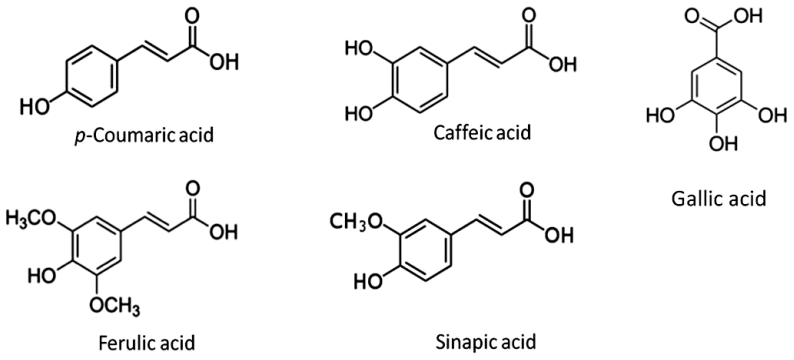


Figure 1.8 *Structure of phenolic acids*
(adapted from Ignat et al., 2011; Kawsar et al., 2008)

The antioxidative activity of phenolic acids is mainly due to their reduction potential. This allows them to act as reducing agents, hydrogen donators (to free radicals which are atoms or molecules with at least one unpaired electron) and singlet oxygen quenchers. They also have a metal chelation potential which is similar in function to polyphenolic compounds (Karadag *et al.*, 2009; Maestri *et al.*, 2006; Stevenson and Hurst, 2007). This explains why dragon fruit exhibits a high level of antioxidative activity (Mahattanatawee *et al.*, 2006; Vaillant *et al.*, 2005; Wu *et al.*, 2006). The antioxidative activity of dragon fruit is even higher than some other tropical fruits such as mango, lychee, longan and papaya. These fruits mostly contain polyphenolic compounds such as flavone glycosides, gallotannins and

catechin conjugates (Mahattanatawee *et al.*, 2006). The phenolic acids can be directly absorbed via the colon and have a relatively high bioavailability (Stevenson and Hurst, 2007). On the other hand, flavonoids are poorly absorbed by the human body, and therefore, they likely have little antioxidative value in human (Suzuki, 2009).

In addition, the antioxidative properties of red-flesh dragon fruit are high (Moreno *et al.*, 2008; Vaillant *et al.*, 2005; Wu *et al.*, 2006) and even higher compared to white-flesh species (Kim *et al.*, 2011; Mahattanatawee *et al.*, 2006). The peel of both dragon fruit species also shows remarkable antioxidative potential (Choo and Yong, 2011; Kim *et al.*, 2011). It is suggested that the antioxidative properties can be contributed to betacyanin (dragon fruit's pigment). Besides its colour function, betacyanin has other health benefits (Delgado-Vargas *et al.*, 2000). This can be explained by its chemical structure. Betacyanin contains a phenol moiety and a cyclic amine group (**Figure 1.5**), and has been considered to be a reducing compound having antioxidative and anti-radical activities. Moreover, the pigment is an excellent electron donor making it possible to stabilize free radicals, thereby exhibiting its antioxidant role (Kanner *et al.*, 2001). **Figure 1.9** illustrates the antioxidative reaction mechanism of betanin. Due to the presence of the phenol moiety, the intermediate betanin radical is generated when betanin (glucose-substituted monophenol pigment) comes into contact with a peroxyl radical. Subsequently, betalamic acid and the *cyclo*-Dopa-5-*O*-β-glucoside radical are released by cleavage of the aldimine bond (Tesoriere *et al.*, 2009).

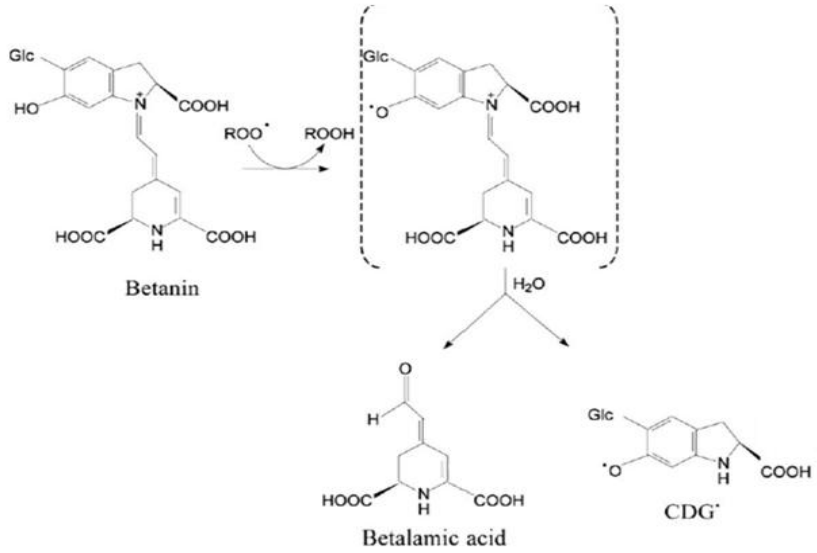


Figure 1.9 *The antioxidative reaction mechanism of betanin: intermediate betanin radical (in bracket), peroxyl radical (ROO•), lipid hydroperoxide (ROOH) and cyclo-Dopa-5-O-β-glucoside radical (CDG•) (adapted from Tesoriere et al., 2009)*

Besides the presence of phenolic acids, flavonoids and betacyanin, dragon fruit is also rich in other promising natural antioxidative components such as tocopherol (Chemah *et al.*, 2010; Lim *et al.*, 2010a) and ascorbic acid (vitamin C) (Choo and Yong, 2011). Tocopherol reacts with organic peroxyl radicals accounting for its antioxidative activity. This reaction is related to its major biochemical function. Tocopherol can protect tissue lipids from free radical attack. These free radicals can originate from polyunsaturated fatty acids (PUFA) in membrane phospholipids or in lipoproteins after hydrogen removal during the initiation process of the oxidation. On the other hand, free radicals can also originate from the addition of an oxygen molecule (Choe and Min, 2009; Sarin *et al.*, 2007; Sies *et al.*, 1992). Ascorbic acid is considered to be the most important cellular antioxidant. It can protect biomembranes against peroxidative damage by scavenging superoxide, hydroperoxide, hypochlorite, hydroxyl radical, peroxyl radical and singlet oxygen. It is more effective in inhibiting lipid peroxidation initiated by a peroxyl radical initiator compared to other plasma components. This vitamin can also protect membranes against peroxidation by enhancing the activity of tocopherol, the main lipid-soluble chain-breaking antioxidant (Sies *et al.*, 1992).

Next to its antioxidative properties, dragon fruit also has antiproliferative activity (Kim *et al.*, 2011; Wu *et al.*, 2006). The peel has been shown to have stronger activity against cancer cells than the flesh (Kim *et al.*, 2011). The phytochemical components can have cancer chemopreventive, antiproliferation, antimicrobial and anti-inflammatory actions on the living cells (Nagasaka *et al.*, 2007; Parr and Bolwell, 2000). This possibly reduces the risk of cardiovascular disease and cancer (Stevenson and Hurst, 2007), neurodegenerative disease (Joseph *et al.*, 2009), inflammation (Suzuki, 2009) and liver damage (Morisco *et al.*, 2008).

1.2.6.1 Assessment of antioxidative activity

Antioxidative components are molecules capable of retarding and/or preventing the oxidation of other molecules, especially free radicals (Moon and Shibamoto, 2009). The mechanisms to inactivate oxidation have generally been divided into two types: hydrogen and electron transfer reactions. Both mechanisms nearly always occur together (Prior *et al.*, 2005). In order to assess the antioxidative activity of molecules, different techniques can be used. Each method may differ in terms of reaction mechanism, oxidant and target/probe species, reaction conditions, analytical instruments and expression of results. There are two types of antioxidant tests: a straight chemical method, for example, by spectrophotometry,

gas chromatography (GC) or high-performance liquid chromatography (HPLC), and a biological assay such as enzyme-linked immunosorbent assay (Moon and Shibamoto, 2009).

a. Total phenolic content

The Folin-Ciocalteu method is an electron transfer-based assay. It has been widely used for the quantitative determination of the total phenolic content in plant-based foods due to its convenience, simplicity and reproducibility (Prior *et al.*, 2005). The method is a colorimetric assay and is based on the reaction of Folin-Ciocalteu reagent with the functional hydroxyl groups of phenolic compounds in alkaline medium (in the presence of sodium carbonate). The reaction results in the formation of blue complexes that can be monitored spectrophotometrically at wavelengths between 750 and 765 nm. A pure phenolic compound including GA may be used to perform the calibration. The total phenolic content is normally expressed in GA equivalents (mg GA/100 g sample) (Karadag *et al.*, 2009; Lim *et al.*, 2007). This colorimetric assay provides results of the total phenolic content that are sufficiently reliable and that are comparable with the HPLC results. Therefore, the method is suitable for routine work with reduced cost, solvent use and analysis time (Hrncirik and Fritsche, 2004).

b. Ferric reducing antioxidative power (FRAP)

The FRAP determination is based on the ability of phenolic compounds to reduce the ferric complex (Fe^{3+}) into the ferrous complex (Fe^{2+}). In acidic conditions, this reduction reaction is performed by electron-donating antioxidants in order to maintain iron solubility. The method is more specific for hydrophilic antioxidants. The ferrous complex can be measured spectrophotometrically. A strong reduction of Fe^{3+} indicates a high reducing power of the antioxidant (Giada and Mancini-Filho, 2009; Karadag *et al.*, 2009). The advantage of the FRAP assay is that the method is simple, rapid, reproducible and inexpensive as it requires no specialized equipment (Moon and Shibamoto, 2009; Prior *et al.*, 2005).

c. DPPH radical scavenging activity

The antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) in organic media has currently received a lot of attention in natural antioxidant studies because the method is simple and highly sensitive (Karadag *et al.*, 2009; Moon and Shibamoto, 2009). The DPPH

radical is a long-lived organic nitrogen radical and is relatively stable. Antioxidants can donate an electron or proton to the DPPH radical. Thereby, the purple chromogen radical is reduced to the corresponding yellow-coloured diphenylpicryl hydrazyl (Ebada *et al.*, 2008; Gressler *et al.*, 2010; Molyneux, 2004; Moon and Shibamoto, 2009).

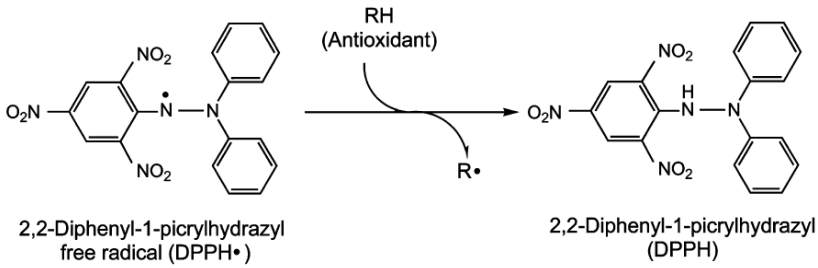


Figure 1.10 *Reaction between DPPH• and an antioxidant to form DPPH (Moon and Shibamoto, 2009)*

The DPPH radical scavenging capacity assay is based on an electron transfer mechanism and is more specific for lipophilic antioxidants. The reducing ability of antioxidants towards DPPH can be evaluated by electron spin resonance or by monitoring the decreased absorbance at wavelengths between 515 and 528 nm (Giada and Mancini-Filho, 2009; Karadag *et al.*, 2009; Wu *et al.*, 2006).

1.3 Freeze-drying technology

Food preservation allows a time span between production and consumption by reducing biological and chemical activities. Drying is the oldest food preservation method aimed at removing moisture from the food material. It basically results in inferior product quality as degradation of colour and nutrients takes place. This is a major concern for dried products (Que *et al.*, 2008). Therefore, advanced dehydration methods have been applied to optimize the quality of dried products. Freeze-drying or lyophilization is a drying technique which provides dried products with superior quality due to low temperature processing (Ratti, 2001). It may preserve colour and nutrients of food material. Freeze-dried products have an appealing physical appearance with a high specific surface area making it rapidly reconstitutable by the addition of water (Oetjen and Haseley, 2004). Although it is an expensive method because of its equipment and operation costs, the freeze-drying process

could be considered as a valuable alternative to protect the quality of food items (Ratti, 2001).

1.3.1 Principle of freeze-drying

During freeze-drying of foods, water is eliminated at low temperature under vacuum conditions by sublimation from a solid phase into a vapour phase (Khalloufi and Ratti, 2003; Marques *et al.*, 2007). Freeze-drying is mostly done with water (in foods) as a solvent. The phase diagram of pure water shows the area in which the transfer from solid to vapour is possible (**Figure 1.11**), but the situation may become more complicated in solutions containing more than one component (Oetjen and Haseley, 2004).

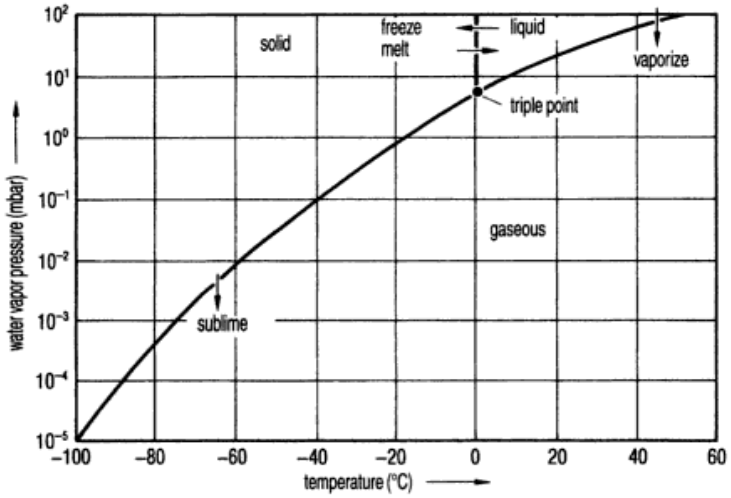


Figure 1.11 *Phase diagram of water (Oetjen and Haseley, 2004)*

Freeze-drying basically involves three stages: the freezing stage, the primary drying stage and the secondary drying stage. In the freezing stage all the material matrices might be in a completely frozen state if the temperature is below the solidification temperature of the material. Since water usually exists in a combined state in a food material system, the material must be cooled below 0 °C to keep the water in the frozen state. In the case of a eutectic solution (a uniform mixture of two or more phases), the freezing temperature has to be set below the eutectic point (the lowest temperature at which a liquid can exist in the solution). At the end of the freezing stage, 65-90% of the initial water content is generally in

the frozen state, whilst the remaining part (10-35%) is in the non-frozen state (Liapis and Bruttini, 2007; Oetjen and Haseley, 2004).

Subsequently, the primary freeze-drying stage (sublimation drying or main drying) is performed. At low temperature and under vacuum conditions, the frozen solvent is removed by means of sublimation. The pressure must be less than or near the equilibrium vapour pressure of the frozen solvent to dry the materials. The maximum temperature at which the frozen layer remains frozen also depends on the freezing behaviour of the material. If the material contains a eutectic form, the temperature should not exceed the lowest eutectic point to prevent melting in the frozen layer. Melting at the sublimation surface or any melting that would occur in the frozen layer can cause marked material faults, e.g. puffing, shrinking and structural topologies filled with liquid solution. It is possible that melting occurs at some point in the frozen layer and the solvent at that point cannot be removed by sublimation. This is a process failure in the drying of the frozen material because the solvent (water) can no longer be removed from the frozen layer by sublimation alone. There is also, at least, loss in structural stability. If the material has a glass form and the minimum freezing temperature exceeds the glass transition temperature (T_g), the phenomenon of collapse can occur. During the primary drying stage, a porous network is formed in the dried layer due to the sublimation of the frozen water. The pore shape, pore size distribution and pore connectivity of the porous network depend on the ice crystals that are formed during the freezing stage. This dependence is of extreme importance because the parameters that characterize mass and heat transfer rates in the dried layer are influenced significantly by the porous structure (Liapis and Bruttini, 2007; Oetjen and Haseley, 2004).

Eventually, the secondary drying stage involves the removal of sorbed or bound water that did not freeze. When the sublimation of the frozen material (ice phase) has been completed, the heating plate temperature is raised (e.g. to 20-40 °C for heat-sensitive products, to 50 °C or more for less heat-sensitive products) and the chamber pressure is further decreased to allow occurring of the bound water desorption. The desorbed water vapour is transported through the pores of the dried material. In this way, the dried product obtains the target moisture content (Pisano *et al.*, 2011). The bound moisture present is attributable to physical and mechanical adsorptions and water crystallization. It has a significant effect on the drying rate and overall drying time. The bound water is removed by heating the product under vacuum. However, the amount of heat supplied to the product cannot be increased freely as there are certain constraints that have to be satisfied during the secondary drying stage concerning the moisture content and the temperature of the

product. These two variables influence the structural stability as well as the product stability during and after freeze-drying (Liapis and Bruttini, 2007; Oetjen and Haseley, 2004).

1.3.2 Quality changes as influenced by freeze-drying

In general, a freeze-dried product has an amorphous form (a non-equilibrium metastable state) and is very sensitive to changes in temperature and moisture content. The amorphous matrix may exist either as a very viscous glass or as a liquid rubber structure during dehydration (**Figure 1.12**). The reversible transition from a glassy state into a rubbery molten state can be defined as a glass transition. The temperature at which this glass transition occurs is specific for each material. Products in the rubbery state have an elasticity and toughness comparable to rubber and have a decreased viscosity. This allows molecular movement needed for crystallization (Bhandari and Howes, 1999; Bhandari *et al.*, 1997).

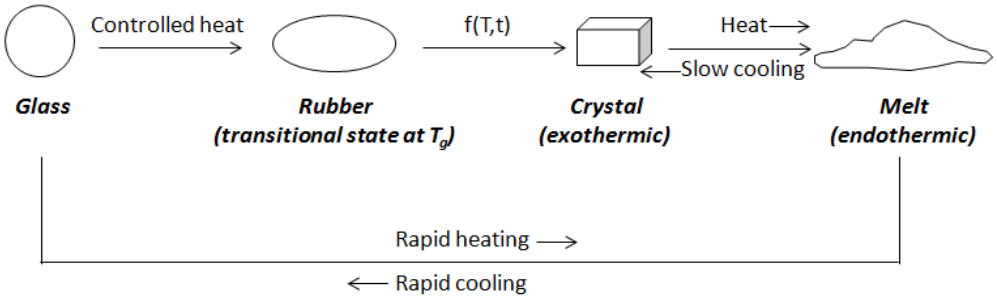


Figure 1.12 *Changes of the physical state of an amorphous material as a function of temperature (T) and time (t) (adapted from Bhandari and Howes, 1999)*

T_g is a useful index to assess the physical stability of amorphous materials during processing and storage (Bhandari *et al.*, 1997; Rao, 2007). It can be estimated by differential scanning calorimetry (DSC). **Figure 1.13** displays a DSC thermogram which shows the glass transition as a function of temperature. The glass transition region basically consists of three T_g values, i.e. onset, midpoint and endset values. The midpoint value, which is the value at one-half of the specific heat change ($\triangle$H), shall be used as a reported T_g value (Rao, 2007).

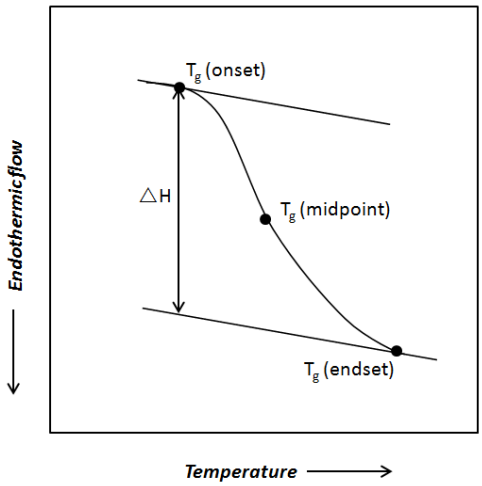


Figure 1.13 *DSC thermogram used to estimate the glass transition temperature (adapted from Rao, 2007)*

The freeze-dried product from fruit juice normally has a low T_g value because raw fruit juice consists mainly of water and is naturally rich in mono- and disaccharide sugars and acids. **Table 1.2** shows T_g values of water, and common sugars and acids present in fruit juice. It can be seen that these components have relatively low T_g values. The low T_g value of the dried product might be associated with the sticky behaviour during processing and storage. Stickiness is the tendency of powder to adhere to a contact surface (Bhandari and Howes, 1999). Also, the shrinkage of fruit pieces during freeze-drying is related to T_g and the internal structure of the material (Khalloufi and Ratti, 2003; Marques *et al.*, 2007).

Table 1.2 *Glass transition temperatures of various food components*

Food material	T_g (°C)	Reference
Water	-135	Roos (1995)
Sorbitol	-4	Slade *et al.* (1993)
Fructose	5	Bhandari and Howes (1999)
Glucose	31	
Sucrose	62	
Lactic acid	-60	Bhandari *et al.* (1993)
Malic acid	-21	
Citric acid	6	
Tartaric acid	18	

1.3.2.1 Nutritional value

Due to the low temperature processing, preservation of quality and functional value is expected for freeze-dried products. In general, vitamin C is sensitive to heat, oxygen and light. This implies that if the vitamin C content is well retained, other nutrients are also likely to be preserved. Therefore, the vitamin C preservation can be used as a quality index of processed products (Awuah *et al.*, 2007). Small reductions of vitamin C and phosphorus contents are found (~16 and 2%, respectively) in some freeze-dried tropical fruits, i.e. pineapple, cherry, guava, papaya and mango. On the other hand, calcium content of these freeze-dried tropical fruits is not significantly affected by freeze-drying. Thus, these freeze-dried tropical fruits could be promoted for consumption due to their high nutritional values (Marques *et al.*, 2006). Freeze-drying does not exert any considerable effect on vitamin C content of tropical fruits, i.e. starfruit, mango, papaya, muskmelon and watermelon. In contrast, the β-carotene concentration in freeze-dried mango and watermelon reduces up to ~26 and 43%, respectively, compared to their initial content (Shofian *et al.*, 2011). No significant losses of vitamin C in carrot are found upon freeze-drying, whereas small losses of α- and β-carotenes are observed, attributable to vitamin A activity during freeze-drying (Lin *et al.*, 1998). Freeze-drying can preserve vitamin C better than conventional drying methods. Freeze-dried chilli, for example, contains about 2 and 4 times higher vitamin C content than hot air and sun-dried chillies, respectively (Toontom *et al.*, 2012).

1.3.2.2 Colour and pigment degradation

Colour is an important attribute of food that affects consumer perception. Freeze-drying seems to be the most efficient method compared to conventional (hot air), vacuum, microwave and osmotic drying methods. It can improve colour of plant-based foods after drying (Krokida *et al.*, 2001a). The colour changes of freeze-dried strawberry and apple are not noticeable compared to the fresh material, while the colour of pear clearly shifts after freeze-drying (Khalloufi and Ratti, 2003). Freeze-dried chilli has a more bright-red colour compared to sun dried chilli at 37 °C and hot air dried chilli at 60 °C (Toontom *et al.*, 2012). Similarly, the browning process of freeze-dried pumpkin significantly reduces after processing, so the freeze-drying can preserve the redness of the product (Que *et al.*, 2008). The yellow and red colours of carrot are attributed to the presence of carotenes. The decrease in a* value (redness) in carrot dried by different methods (i.e. air drying, vacuum microwave drying and freeze-drying) correlates with the loss of α- and β-carotenes. However, this loss of pigment is not correlated with the recorded b* value (yellowness). The

degradation of carotenoids and structural changes in the carrot tissue may occur during processing (Lin *et al.*, 1998).

1.3.2.3 Antioxidative property

Antioxidative activities of mango, papaya and watermelon do not significantly reduce upon freeze-drying, whereas a marked decrease occurs in starfruit. Total phenolic content of these tropical fruits, which is perfectly related to the antioxidative properties, decreases after freeze-drying. This is possibly due to the fruit preparation and the slow freezing (at -20 °C for 24 h) prior to freeze-drying. During size reduction and processing, the fruit is normally susceptible to cut-edge browning caused by enzyme activity such as polyphenoloxidase. The enzyme acts as a catalyst for the hydroxylation of monophenols to diphenols and the oxidation of diphenols to quinones (Shofian *et al.*, 2011). High antioxidative capacity is also found in freeze-dried pulp and peel of acai (*Euterpe oleraceae*) (Schauss *et al.*, 2006). Freeze-drying may enhance flavonol content in onion. This might be attributed to the liberation of phenolic compounds from the plant matrix due to freeze-drying. During 6 months of storage at room temperature, no considerable effect on the flavonol content in freeze-dried onions is observed (Pérez-Gregorio *et al.*, 2011). It is also interesting to note that pumpkin dried by the hot air method shows stronger antioxidative activities compared to freeze-dried pumpkin. This can possibly be attributed to the production of Maillard products or intermediates with potential antioxidative activity (Que *et al.*, 2008).

1.3.2.4 Rehydration behaviour

Some advantages of freeze-dried foods are that they are light in weight, can be reconstituted quickly and will have the appearance of fresh foods after rehydration. This is because the process can eliminate almost all moisture (water) in raw food and leave microscopic pores in the dried product. Rehydration behaviour may be considered as an important parameter of freeze-dried products as it is a measure of the induced damage in the material (structural changes) during freeze-drying. This damage can include integrity loss and a reduction of the hydrophilic properties which decreases the rehydration ability. Marques *et al.* (2009) reported that the water absorption capacity, porosity and T_g value have a great influence on the rehydration behaviour of some freeze-dried tropical fruits, e.g. pineapple, mango, guava, acerola and papaya.

The process conditions can induce structural and compositional changes in the food tissue. Therefore, some authors have focused on the optimization of the freeze-drying conditions to improve the quality of freeze-dried products. To produce freeze-dried strawberry with a high quality, e.g. appearance, colour, texture and rehydration ratio, the optimum pressure and heating plate temperature of the secondary drying stage are programmed at 30 Pa and 50 °C, respectively, with a total freeze-drying time of 60-65 h to remove about 99% of the water content of the fruit (Hammami and Rene, 1997). An increase in the heating plate temperature of the secondary drying stage from 30 to 70 °C allows a considerable increase in the drying rate of freeze-dried strawberry. It, thus, results in process time reduction, whereas there are no significant effects on colour and volume. However, the strawberry dry layer temperature is higher compared to the estimated T_g of the dried fruit. A high heating plate temperature, therefore, increases the risk of collapse after freeze-drying (Shishehgarha *et al.*, 2002).

1.4 Thermal processing

Thermal processing is the most commonly used method for the preservation of foodstuffs. It is based on the inactivation of micro-organisms and enzymes, but it normally results in nutritional and sensory quality losses. Although this negative effect of the thermal process cannot be avoided, it can be minimized with regard to aesthetic and nutritional qualities (Manvell, 1997). Fruits are an important target for thermal processing due to their limited life span. Besides being consumed as fresh, they can be processed and can be applied in a number of food products. However, the consumption tendency is focused on fresh-like products having a high nutritional value and an extended shelf-life (Argaiz *et al.*, 2004). This demand has stimulated the food scientist to conduct more research on improving the quality of processed fruits by optimization of the fruit processing.

1.4.1 Principle of thermal processing

Nowadays, thermal processing under aseptic conditions is used to produce a wide range of high quality products (Awuah *et al.*, 2007). For example, fruits can be processed into juice and puree using thermal processing, e.g. pasteurization and sterilization (Younis *et al.*, 2011). These fruit products may be further applied in many kinds of food products such as jams, sweets and beverages. Pasteurization aims to destroy pathogenic micro-organisms (disease-causing microbes) and may reduce the level of non-pathogenic organisms.

Pasteurized products have a rather limited shelf-life and may need to be stored under certain conditions (e.g. at refrigeration temperature and low pH) in order to minimize microbial growth and prevent spore germination during storage. On the other hand, an effective sterilization process eliminates or destructs almost all vegetative non-pathogenic and pathogenic organisms, including spores and viruses. In this way, it results in shelf stable foods (Ramaswamy, 2005; Ramaswamy and Marcotte, 2006). High-temperature short-time (HTST) and ultra-high temperature (UHT) processes promote a better quality retention of the product while ensuring commercial sterility. These processes involve the sterilization of the food product (using a direct or indirect heat exchanger), followed by a holding period to achieve required lethality and a rapid cooling to minimize the impact of heat on the nutrients (Awuah *et al.*, 2007; Silva, 1996). Currently, the most favourable techniques for fruit juice processing are the HTST system using a tubular heat exchanger (Herbach *et al.*, 2007). Commercial sterility in HTST operations is obtained in the holding tube at a constant temperature within seconds, whereas in-container sterilization (e.g. canning) is most lethal at the end of the heating stage and beginning of the cooling phase. HTST application is challenged by the apparent difficulty in destroying heat-resistant enzymes and its limitation to pumpable fluids with low viscosity (Awuah *et al.*, 2007). Since economic aspects are important in industry, thermal processing conditions can be optimized to minimize the energy consumption and to maximize the productivity. In this situation, a compromise must be attained in terms of the final product quality (Silva, 1996).

Thermal processing might be used commercially to inactivate the pectic enzymes in fruit juice, resulting in the quality attribute (e.g. cloudiness) of the juice. A cloudy appearance of fruit juice results from particles in suspension which can be found in, for example, orange juice (Molinari and Silva, 1997) and dragon fruit juice (Herbach *et al.*, 2007). The loss of cloudiness is possibly due to the activity of the pectic enzymes. This affects the appearance and decreases the juice commercial value. However, the relatively high temperatures necessary for enzyme inactivation may produce an undesirable cooked off-flavour and a degradation of the juice aroma (Molinari and Silva, 1997).

1.4.2 Quality changes as influenced by thermal processing

Thermal processing has proven to be effective in terms of product safety. The concern with thermally processed foods is the fact that heating leads to either minor or major changes in physicochemical properties (e.g. appearance, texture, flavour and colour), nutritional and sensory characteristics. The challenge of maintaining both quality and safety of foods after

thermal processing is, thus, of great interest. In this respect, process optimization is needed to minimize damage of the sensory and nutritional qualities and to maximize the safety and shelf-life of the thermally processed products. This is accompanied by the relevant reaction kinetic constants and mathematical models to predict quality variations in the product during thermal processing. The influence of a thermal treatment on the physicochemical properties, which are fundamental in analyzing the unit operations in food industry, has been investigated for various fruit sources (Argaiz *et al.*, 2004; Chutintrasri and Noomhorm, 2007; Garza *et al.*, 1999; Herbach *et al.*, 2004b; Kim *et al.*, 2009; Nindo *et al.*, 2007; Vasquez-Caicedo *et al.*, 2007).

1.4.2.1 Nutritional value

Among a number of food nutrients, vitamins are the most sensitive to heat processing, particularly water-soluble components (Awuah *et al.*, 2007). Vitamin C (ascorbic acid) content of mango puree and juice decreases as the temperature increases from 75 to 88 °C (Argaiz *et al.*, 2004). The amount of β-carotene, total carotene, thiamin, riboflavin, ascorbic acid and free amino acids of carrot purees slightly decreases after thermal treatment, although the sugar content increases (Yim and Sohn, 2004). In thermally processed tomato puree, the content of furosine, a marker for the Maillard reaction, increases, whereas the ascorbic acid content, as expected, decreases. There are no significant differences in lycopene content of tomato puree before and after thermal treatment (Zanoni *et al.*, 2003). Since vitamin E or tocopherol (fat-soluble compound) is also found in some plant-based foods, the effect of thermal processing on tocopherol content has also been studied. Heat processing (i.e. blanching and boiling) appeared to have little effect on total tocopherol content of carrot and potato, whereas boiling increased the total tocopherol content of broccoli and spinach compared to their raw products (from 1.8 to 2.4 mg/100 g edible weight and from 2.1 to 3.7 mg/100 g edible weight, respectively) (Chun *et al.*, 2006). Dietary fibre, a common component in fruit, is becoming crucial for health-conscious consumers. Hence, the need exists to study changes in the dietary fibre content of foods during heat treatments. Azizah and Zainon (1997) found that both insoluble and soluble dietary fibres of some legumes and cereals increased after thermal processing at 100 °C for 15 min. This was especially the case for the high protein samples and may be attributed to the production of Maillard reaction products.

1.4.2.2 Colour and pigment degradation

The natural pigments in fruits are normally susceptible to degradation during heating. This results in an undesirable colour of the thermally heated product which strongly influences consumer acceptability. Researches have been reported that investigate colour degradation in different plant-based foods as affected by a thermal treatment. Betacyanin in red-flesh dragon fruit decreases with heating time. After heating at 85 °C for 5 h, about 25% of the initial pigments are retained and a slight change in background colour from blue to yellow (according to hue angle data) is observed. Upon heating, betanin decomposes into *cyclo*-Dopa-5-*O*-β-glucoside and betalamic acid, leading to a loss in red colour. The decomposition products are generally regarded as unstable components at the elevated temperatures (Herbach *et al.*, 2004a). The addition of ascorbic, isoascorbic and citric acids to dragon fruit juice positively affects the betacyanin stability. They also cause an increase in lightness value upon thermal treatment. It was reported that heated dragon fruit juice at pH 6 exhibits considerably lower betacyanin retention than at pH 4. Addition of 1% ascorbic acid at pH 4 provides the high betacyanin retention (91%) after heating at 85 °C for 1 h (Herbach *et al.*, 2006b). The colour shift of red beetroot (*Beta vulgaris* L. ssp. *vulgaris*) occurs after heating at 85 °C for 8 h, suggesting the formation of yellow products at the expense of red genuine pigments (Herbach *et al.*, 2004b).

The thermal stability of betacyanin in clarified dragon fruit juice was investigated by Herbach *et al.* (2007). The dragon fruit juice was pasteurized at 92 °C using three different heat treatments: an HTST system (preheating 7 sec and holding time 26 sec), a tubular heat exchanger system (preheating 53 sec and holding time 26 sec) and a vessel (preheating 85 min and no holding time). The HTST system and the tubular heat exchanger resulted in overall pigment retentions of 77%, whereas heating in a vessel retained ~63% of the initial pigment content. Phyllocactin, an acylated betacyanin, is the least stable throughout these processes due to the deacylation. This results in betanin (a non-acylated betacyanin) generation, which also accounts for the apparently higher betanin stability. The high mucilage content of dragon fruit is disadvantageous for juice clarification by filtration. It is because the process requires high enzyme dosages and further concentration of pigments is hindered. The mucilage material in dragon fruit may contribute to the minimization of pigment degradation upon heating and storage. This makes it possible to apply red-flesh dragon fruit as a colouring agent for cloudy products which contain mucilage substances.

Some authors have stressed the relationship between TCC and natural pigment degradation during thermal treatment. The linear correlation between thermal degradation of anthocyanin and TCC of plum puree during heating at 50-90 °C for 20 min is reported

(Ahmed *et al.*, 2004). The colour changes in pumpkin puree are found to be a direct manifestation of the changes in β-carotene content during heating at 60-100 °C for 2 h (Dutta *et al.*, 2006).

Colour degradation kinetics of food products are a complex phenomenon. However, the kinetic models of thermal destruction should be studied in order to have a better understanding and to be able to use them in process control to obtain safe and high quality products. The kinetic of colour degradation of fruit purees during thermal processing has been studied. The studies may also provide the activation energy (E_a) which indicates the sensitivity of the reaction rate to temperature. Some significant colour parameters can be used as a potential indicator for processed product quality. Ahmed *et al.* (2002) found that the yellow colour degradation of mango puree follows a first-order reaction kinetic. TCC and b* (yellowness) values may be used to describe the colour changes during thermal processing between 50 and 90 °C for 0-20 min. Similar results have also been reported in peach puree. The b* value significantly reduced with heating time and temperature (Garza *et al.*, 1999). On the other hand, TCC and L* (lightness) values in pineapple puree are the most sensitive colour parameters during heating between 70 and 110 °C. They can be recommended as an on-line quality control parameter. This temperature range covers the temperatures used during preheating and sterilization of commercial aseptic pineapple puree. At high temperature regions (95-110 °C for 100 min), TCC and L* values provide higher E_a values compared to low temperature regions (70-90 °C for 500 min) (94 and 129 kJ/mol compared to 84 and 69 kJ/mol, respectively) (Chutintrasri and Noomhorm, 2007). Similarly, TCC and L* values can be used to estimate the heat-sensitivity of concentrated apple, peach and plum pulps upon heating at 56-94 °C for 700 min. It has been shown that these parameters follow a first-order kinetic (Lozano and Ibarz, 1997).

1.4.2.3 Antioxidative property

The antioxidative potential of a wide variety of fruit sources as affected by a thermal treatment has been analyzed. The natural antioxidants in plant-based foods are basically lost during thermal processing and storage due to the instability of antioxidative components (Murcia *et al.*, 2009). A decrease of the antioxidative activity in carrot puree after heating at 90 °C for 30 and 60 sec (Quitao-Teixeira *et al.*, 2009) has been reported. This is also the case when tomato puree is heated at 90 °C for 160 min, at 95 °C for 51 min and at 100 °C for 15 min (Zanoni *et al.*, 2003). Nevertheless, it has been demonstrated that processed plant-based foods may retain or even have an improved antioxidative activity

after heating. This suggests that some components can be extracted and released by heating. The phenolic content and overall antioxidative activity in Shiitake mushrooms increase as a function of heating temperature and time (100 and 121 °C for 15-30 min). This indicates that the heat treatment significantly enhances the overall antioxidative activity of Shiitake mushrooms (Choi *et al.*, 2006). Some researchers are convinced that thermal treatments can induce the formation of Maillard reaction components. This results in the presence of antioxidative potential effects. Nicoli *et al.* (1997b) demonstrated that the overall antioxidative properties of tomato are maintained or even enhanced by the development of Maillard reaction products after thermal treatment. Nevertheless, the concentration of natural antioxidants was significantly reduced. The results were confirmed by Nicoli *et al.* (1997a) who demonstrated that coffee brew showed strong overall antioxidative properties after roasting which may mainly be attributed to the Maillard reaction products formed during processing. It can be hypothesized that polyphenolic components, which are present in crude coffee, are partially lost during thermal treatment, while the Maillard reaction products probably become the prevailing contributors to the antioxidative activity. Similarly, Kusznierewicz *et al.* (2008) reported that the antioxidative properties of heated cabbage juice can be attributed to the formation of both phenolic reactive components and Maillard reaction products (represented by the formation of a brown pigment value) during heating.

1.4.2.4 Rheological behaviour

The rheological behaviour of food depends on many factors such as temperature, food composition, total soluble solid content and structural behaviour. Knowledge of the rheological properties of food is essential for product development, quality control, sensory evaluation and process engineering calculations. **Figure 1.14** illustrates the correlation between shear rate and shear stress for different types of food rheological behaviour. The flow behaviour of most fluid foods is non-Newtonian, whereas some foods can be Newtonian fluids. Shear-thinning and shear-thickening are time-independent flow behaviour types. They occur in most food items due to the complex food matrix. Some fluid foods may also have a yield stress that must be exceeded for flow to occur. This is expressed in the Bingham and Herschel-Bulkley models (Rao, 2007). Most fruit purees show shear-thinning or pseudoplastic behaviour, where the viscosity decreases with an increasing rate of shear stress. This rheological behaviour can be found, for example, in raspberry, strawberry, peach and prune purees (Maceiras *et al.*, 2007), blueberry puree (Nindo *et al.*, 2007) and white guava puree (Sanchez *et al.*, 2009). Dragon fruit puree also shows shear-thinning

behaviour which is probably due to the mucilage material present in the fruit (Chuah *et al.*, 2008). The power-law model, also known as Ostwald-de Waele model, is an empirical model used for fitting rheological data and is ideal to predict the behaviour of shear-thinning fluids as well as Newtonian fluids (Whittingstall, 2001).

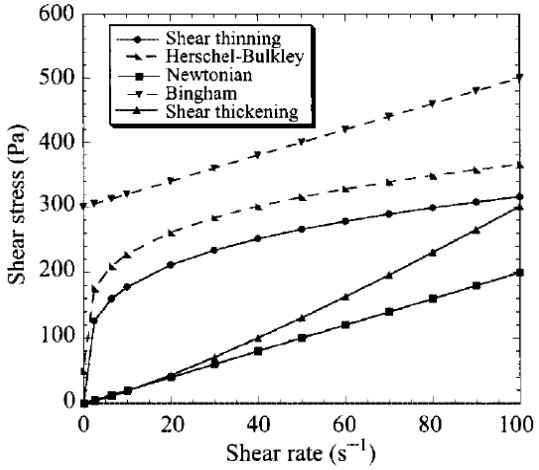


Figure 1.14 *Shear diagram showing the correlation between shear rate and shear stress (Rao, 2007)*

The viscosity of fruit puree can be affected by thermal processing as the physical and/or chemical attributes may be altered. It is essential to understand the rheological behaviour of heated fruit puree to better design and control the processing conditions and handling applications involving pumping and mixing. Therefore, the effect of heating temperature on flow behaviour parameters has been widely studied for various fruit purees. Nindo *et al.* (2007) found that the apparent viscosity of blueberry (*Vaccinium corymbosum* L.) puree decreases with temperature increase (25-60 °C). The Sisko model, which is a combination of Newtonian and power-law models, fits the data very well. Maceiras *et al.* (2007) reported that the apparent viscosity of raspberry, strawberry, peach and prune purees is influenced by heating at 20-40 °C. The yield stress and consistency coefficient decreased with increasing temperature, while the flow behaviour index increased. The power-law and Herschel-Bulkley models fitted reasonably well with the experimental data. Sanchez *et al.* (2009) concluded that the rheological properties of white guava (*Psidium guajava* L.) puree depend on the temperature and fit to the power-law model. The consistency coefficient decreased, while the flow behaviour index increased with an increase in temperature. Similarly, Shamsudin *et al.* (2005) clearly observed a decrease of the apparent viscosity in

guava juice upon heating from 30 to 80 °C. Dutta *et al.* (2006) noted that the rheological characteristics of pumpkin puree in the heating temperature range of 60-100 °C fit adequately with the Herschel-Bulkley model and that the yield stress decreases exponentially with temperature.

1.5 Seed oil

The world production and consumption of seed oil are growing steadily over the last two decades. Apart from its nutritional value, seed oil (e.g. sesame seed, rapeseed, linseed and sunflower seed oils) is of particular economic importance for developing countries, contributing considerably to export earnings (Wiemer and Altes, 1989). Plant seed oil consists of different chemical components that are nutritionally grouped as macronutrients (e.g. fatty acids), micronutrients (e.g. vitamins and minerals) and other components such as phenolic, antioxidative and pigment components. Some chemical components have been demonstrated for potential health benefits related to oxidative stability. Many seeds of fruit crop, e.g. grape seed and berries seed, can be obtained from the primary fruit juice and puree processing with the seed being discarded as waste or by-products. Fruit seed oil can be conventionally extracted from ground seed using organic solvents, followed by solvent evaporation. After extraction of the seed oil, the remaining residue is the flour. However, beneficial components with added value might be retained in cold pressed seed oil (Van Hoed *et al.*, 2011). They could possibly be lost by evaporation or chemical modification when the conventional solvent extraction method is used (Parker *et al.*, 2003b).

1.5.1 Major component

The fatty acid composition greatly affects the oil quality. Three fatty acids are bound to one glycerol molecule, which is called a TAG. TAG units may lose one, two or all fatty acids to become, respectively, a diacylglycerol and free fatty acid, a monoacylglycerol and two free fatty acids, and a glycerol molecule and three free fatty acids. The carbon chains may differ in length. In seed oil, lengths between C8 and C24 are commonly present (Dyer *et al.*, 2008). The fatty acids can be classified into two groups: a straight chain structure without double bonds, i.e. saturated fatty acid (SFA), and a group with the presence of one or more double bonds, i.e. with one double bond (monounsaturated fatty acid: MUFA) or with more than one double bond (polyunsaturated fatty acid: PUFA). These fatty acid structures have a significant effect on the physicochemical properties and the oxidative stability of the oil.

Table 1.3 shows the composition of some seed oils regarding the major fatty acids present. Seed oils are generally composed of fatty acids with different carbon chain length, mainly between C16 and C18. The major type of fatty acids present in seed oils is PUFA, followed by MUFA and SFA, respectively. Interestingly, there are two important fatty acids, i.e. linoleic acid (C18:2) and linolenic acid (C18:3), which are generally found in seed oil. Linoleic acid is a PUFA with two double bonds at positions C9 and C12, while linolenic acid has three double bonds at positions C9, C12 and C15. These two fatty acids are also known as essential fatty acids and are needed in the diet of humans since they cannot be synthesized in the body. Therefore, they must be obtained from exogenous sources. Seed oil is very rich in essential fatty acids and may be a valuable source of dietary fat. The essential fatty acids have been implicated in the prevention of various human diseases, including obesity, diabetes, coronary heart disease, the prevalence of strokes, inflammatory and neurologic diseases (Sampath and Ntambi, 2004).

Table 1.3 *Fatty acid composition of different seed oils*

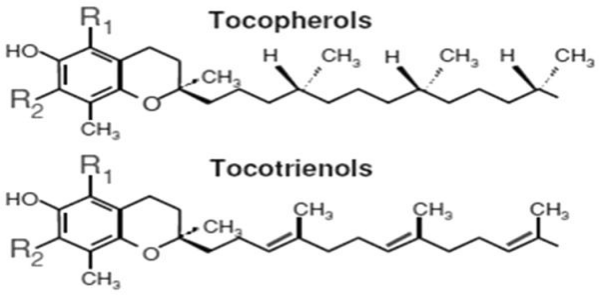
Seed oil	**Fatty acid composition (%)**					**Reference**
	Palmitic acid (C16:0)	**Stearic acid (C18:0)**	**Oleic acid (C18:1)**	**Linoleic acid (C18:2)**	**Linolenic acid (C18:3)**	
Grape	7.4-10.2	3.0-4.7	16.2-21.7	63.3-71.4	0.1-0.4	Baydar *et al.* (2007)
Sesame	9.8	5.4	41.0	42.2	0.3	Lemcke-Norojarvi *et al.* (2001)
Linseed	6.5	4.0	22.7	15.4	51.4	Kelly *et al.* (1998)
Cranberry	7.8	1.9	22.7	44.3	22.3	Parker *et al.* (2003a)
Blackberry	3.7	2.2	14.7	61.2	17.6	Van Hoed *et al.* (2009)
Blueberry	5.7	1.8	21.4	42.5	28.3	
Kiwi	6.0	3.1	14.6	17.6	58.4	
Strawberry	4.3	1.7	14.6	42.2	36.5	
Red raspberry	3.2	1.0	16.9	54.9	24.0	Sucurovic *et al.* (2009)

The fatty acid composition is of great importance for specific end products, e.g. for food, pharmaceutical and cosmetic purposes. Cooking oil is typically liquid at room temperature, generally containing a high proportion of unsaturated fatty acid (UFA), either MUFA or PUFA (Dyer *et al.*, 2008). Some seed oils, for example, berry seed oil (Parker *et al.*, 2003a; Sucurovic *et al.*, 2009; Van Hoed *et al.*, 2009) and grape seed oil (Baydar *et al.*, 2007; Gomez *et al.*, 1996) can be considered as high-value oils with respect to their high amount of essential fatty acids present with the remarkable presence of endogenous antioxidative

components. Such seed oils can be used as a dietary supplement and can be added to cosmetic and nutraceutical products.

1.5.2 Minor component

Minor components in seed oil are micronutrients (i.e. fat-soluble vitamins and minerals) and phytochemical components. Among the micronutrients (e.g. vitamin E, vitamin K and provitamin A or β-carotene), vitamin E plays an important role as an antioxidant in seed oil which is related to the stability of the oil. Vitamin E can be found in most plant-derived foods and exists in eight different forms, four tocopherols and four tocotrienols. They are designated as α-, β-, γ- and δ-forms which differ by the number and position of the methyl groups on the chroman ring (**Figure 1.15**). The α-form consists of three methyl groups at the 5, 7 and 8 positions, whereas the β- and γ-forms have two methyl groups at the 5/8 and 7/8 positions, respectively. The δ-form is methylated at C8 on the chromanol ring. The chemical structure of tocopherols comprises a C16 saturated side chains on a methyl-chroman ring, while tocotrienols have a similar chain, but with three conjugated double bonds at positions C3, C7 and C11 (Brigelius-Flohe and Traber, 1999; Penna and Pogson, 2006).



Tocochromanol type	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Figure 1.15 *Chemical structure of tocopherols and tocotrienols (adapted from Penna and Pogson, 2006)*

In most plant and seed oils, the α- and γ-tocopherols are found to be the principal vitamin E forms. The α-tocopherol is the most abundant tocopherol in grape seed oil (up to 91% of the total tocopherol content) (Baydar *et al.*, 2007). On the other hand, the γ-tocopherol is a predominant tocopherol in some berry seed oils. The γ-tocopherol content varies from a high content, e.g. in red raspberry seed oil (2 g/kg) and cranberry seed oil (1.6 g/kg), to a low content, e.g. in blueberry seed oil (34 mg/kg) and kiwi seed oil (10 mg/kg) (Van Hoed *et al.*, 2009). However, it is suggested that tocopherols are the main antioxidants protecting

the berry seed oil during storage (Van Hoed *et al.*, 2011). The γ-tocopherol is also found as the major tocopherol in sesame oil (amounting up to 570 mg/kg) with a high amount of α-tocopherol (360 mg/kg) and a very small amount of β- and δ-tocopherols (less than 10 mg/kg) (Aued-Pimentel *et al.*, 2006). The variation in tocopherol content may depend mainly on cultivar, different growing, processing and storage conditions.

Tocopherols have been proven to be effective in preventing radical oxidative reactions, i.e. lipid peroxidation. They act as preventative and chain-breaking antioxidants to function against free radicals. Tocopherols primarily function in preventing the initial oxidation and in retarding/stopping the oxidation of UFA by reacting with the lipid peroxyl radicals (Brigelius-Flohe and Traber, 1999). Additionally, the α-tocopherol has been reported to have the highest *in vivo* antioxidative activity, resulting in many health benefits (Krichene *et al.*, 2010; Yoshida *et al.*, 2003). The bioavailability of the γ-tocopherol has also been reported to reduce the risk of human diseases (Jiang *et al.*, 2001). Tocopherols may prevent the occurrence of cancer and tumor growth by functioning as anti-carcinogens, quenching free radicals or reacting with their products (Morrissey *et al.*, 1994). They may also reduce the risk of some chronic diseases, e.g. cardiovascular and atherosclerosis diseases (Brigelius-Flohe and Traber, 1999).

Moreover, some clinically important properties of dietary plant and seed fats may be due to the presence of other minor components such as phytosterols and squalene (a precursor of phytosterols and also an antioxidant), from which the small amounts present in natural foods also appear to be important (Choe and Min, 2006; Gul and Amar, 2006; Ostlund *et al.*, 2002). Phytosterols play a structural role in cellular membranes, participate in the control of membrane-associated metabolic processes and are the precursors of steroid hormones and bile acids in human. They have the potential to reduce the plasma low-density lipoprotein cholesterol level, resulting in a decrease in coronary mortality (Gul and Amar, 2006; Ostlund *et al.*, 2002). Phytosterols can be divided into three groups according to their structural and biosynthetical properties: 4-desmethyl, 4-monomethyl and 4,4-dimethyl phytosterols. The most abundant phytosterols in plant-based foods are 4-desmethyl sterols, i.e. sitosterol, campesterol and stigmasterol (Gul and Amar, 2006). A significant amount of squalene and total desmethylsterol content is noted for kiwi seed oil (826 and 422 mg/100 g, respectively) and cranberry seed oil (672 and 692 mg/100 g, respectively) in which β-sitosterol is the most abundant desmethylsterol (~60% of total desmethylsterols) (Van Hoed *et al.*, 2009). Similar to sesame seed oil, β-sitosterol is also a predominant desmethylsterol (54-64% of total desmethylsterols), followed by campesterol and stigmasterol (Aued-Pimentel *et al.*, 2006).

1.5.3 Oxidative deterioration of oil

The fatty acids in oil are most susceptible to oxidation, resulting in deterioration of the oil during processing and storage. Fatty acid oxidation occurs by three different mechanisms: autoxidation, photosensitized oxidation (or photoxidation) and enzymatic oxidation. Autoxidation occurs spontaneously and involves UFA (at double bonds in fatty acids), atmospheric oxygen and many free radicals. The mechanism of lipid autoxidation is usually described as a three stage process. This process consists of initiation, propagation and termination reactions (Choe and Min, 2006; Sarin *et al.*, 2007).

At the initiation stage of autoxidation, free radicals are formed. The proton (H•) is removed at the double bonds of UFAs resulting in lipid alkyl radicals (R•) (**Eq. 1.1**). This reaction is initiated by temperature, light, metals and other radicals.

$$RH \longrightarrow R\bullet + H\bullet \quad (\mathbf{1.1})$$

Subsequently, the free radical reacts with atmospheric oxygen ($^3O_2$) to form an unstable peroxy free radical (ROO•) (**Eq. 1.2**). The peroxy free radical may in turn remove a proton from another UFA to form lipid hydroperoxide (ROOH) and a new lipid alkyl radical initiating further oxidation and contributing to the chain reaction (**Eq. 1.3**). This free-radical chain reaction is called the propagation stage of autoxidation. The rate of oxidation of fatty acids increases with their degree of unsaturation. Consequently, oils containing high proportions of PUFAs may experience stability problems.

$$R\bullet + {}^3O_2 \longrightarrow ROO\bullet \quad (\mathbf{1.2})$$

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \quad (\mathbf{1.3})$$

In the termination stage of the autoxidation, the free radicals react with themselves and with each other, causing the formation of non-radical components (RR, ROOR) (**Eq. 1.4-1.6**).

$$R\bullet + R\bullet \longrightarrow RR \quad (\mathbf{1.4})$$

$$R\bullet + ROO\bullet \longrightarrow ROOR \quad (\mathbf{1.5})$$

$$ROO\bullet + ROO\bullet \longrightarrow ROOR + O_2 \quad (\mathbf{1.6})$$

Lipid hydroperoxide (ROOH), a well-known primary oxidation product, is formed during lipid oxidation and can be susceptible to further oxidation or decomposition to secondary reaction products, e.g. aldehydes, ketones, carbonic acids and alcohols. In many cases, these components adversely affect flavour, aroma and taste leading to the loss of nutritional value and overall quality (Choe and Min, 2006).

Oxidation also occurs through the photosensitized oxidation mechanism. It is accelerated by light and singlet oxygen ($^1O_2$) in the presence of sensitizers. Chlorophyll is an important sensitizer which is normally present in edible vegetable oil (e.g. olive oil), although, the pigment can be removed during oil processing. The sensitizers absorb energy in the ultraviolet and visible light wavelength regions to go to an excited state. Consequently, they are more efficient in the presence of singlet oxygen which is a source of hydroperoxides (Choe and Min, 2006; Choe and Min, 2009).

Furthermore, oxidation takes place by an enzymatic mechanism. Enzymatic lipid oxidation consists of a non-radical mechanism catalyzed by lipid enzymes. Lipoxygenase is an iron-bound enzyme with Fe in its active centre and oxidizes UFAs containing a 1-*cis*, 4-*cis*-pentadiene system. Oils with a high amount of PUFAs including linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic acid (C20:4) are favoured substrates for this mechanism. It results in deterioration because of off-flavour generation in the oil. The products of this enzymatic reaction are unstable hydroperoxides which are similar to those produced during autoxidation, although the relative proportion of products may vary widely depending on the conditions of the reaction (Choe and Min, 2009; Duque *et al.*, 2011).

Hereto, lipolysis, i.e. the hydrolysis of ester bonds in lipids, by either enzymatic or chemical action (e.g. heating, light and oxygen) may result in the liberation of free fatty acids. The free fatty acids are susceptible to oxidation and may release the short chain fatty acid which is the cause of a rancid flavour (Choe and Min, 2009). The fatty acid oxidation has a significant effect on the oil quality. It has also implicated the pathological processes of some

diseases, e.g. heart disease and ageing (Dhaouadi *et al.*, 2006) and coronary heart disease (Lemcke-Norojarvi *et al.*, 2001). This is due to the occurrence of the oxidized compounds (e.g. free radicals and hydroperoxides) during processing and storage. The reaction may be influenced by several factors such as processing, light, temperature, concentration and type of oxygen, fatty acid composition, free fatty acids, acylglycerol composition, metal agents, peroxide components, thermally oxidized components, pigments, phenolic compounds and antioxidant components (Choe and Min, 2006; Choe and Min, 2009). Amongst all enhanced oxidation parameters, the fatty acid composition in oils is considered to be the most significant factor with regard to oxidation stability of oil. UFA-rich oils, particularly PUFA, are more susceptible to oxidation than less unsaturated oils. As the degree of unsaturation increases, both the rate of formation and the amount of primary oxidation components accumulated at the end of the induction period increase (Choe and Min, 2006). Some authors have found evidence of the impact of fatty acid composition on the rate of oil oxidation. PUFA-rich oils including grape seed and walnut oils are most likely to be susceptible to oxidative deterioration (Kochhar and Henry, 2009). On the other hand, virgin olive oil is considered to be quite resistant to oxidation due to its high content of MUFA together with a remarkable high concentration of naturally occurrence antioxidative components (Krichene *et al.*, 2010).

Oxidation takes place when oxygen is present next to the unsaturated fatty acids and the catalysts. Both type and concentration of the oxygen greatly affect the degree of oxidation. Atmospheric oxygen reacts with lipid radicals and causes autoxidation, whereas singlet oxygen catalyzes the photosensitized oxidation in the presence of light and sensitizers (Choe and Min, 2006; Choe and Min, 2009). Moreover, the oxygen concentration in oil depends on the oxygen partial pressure in the headspace of the oil (Velasco and Dobarganes, 2002). It becomes clear that headspace oxygen is a major factor in controlling the quality of olive oil during storage (Stefanoudaki *et al.*, 2010). The prevention of photoxidation during storage is of great interest to improve the oxidative stability of chlorophyll-rich oils like olive oil. In addition to this, light is much more important than temperature (Choe and Min, 2006). On the other hand, the effect of temperature has significant consequences on the autoxidation and the decomposition of hydroperoxides. The oxidation rate increases with an increasing temperature (Stefanoudaki *et al.*, 2010).

In general, the oxidation rate of fatty acids greatly depends on the number of double bonds and their position. The peroxide value (PV) is the most widely used measure to assess the oxidation. It is a measure for the amount of hydroperoxides, which are the primary oxidation products. The PV value can be measured by a standard method, which is a titration with

sodium thiosulfate or with the use of ferric thiocyanate and ferrous oxidation-xylenol orange (FOX) assays. These assays are associated with lipid oxidation and they are simple and highly reproducible. A ferric thiocyanate assay can be used to assess the level of hydroperoxide using a spectrophotometer at 500 nm (Maqsood and Benjakul, 2010; Moon and Shibamoto, 2009). Oxidants (e.g. hydroperoxide) oxidize Fe^{2+} in ferrous thionate solution to ferric ion, and therefore, the formation of Fe^{3+}-thiocyanate complex (red colour) can be monitored by a spectrophotometer (Moon and Shibamoto, 2009). The principle of FOX assay is illustrated in **Figure 1.16**. Hydroperoxide, which is generated from the lipid oxidation (most commonly from linoleic acid), oxidizes a ferrous ion to a ferric ion which can be monitored as a ferric-xylenol orange complex by a spectrophotometer (Gay *et al.*, 1999; Moon and Shibamoto, 2009). Correct conditions can be easily checked by the colour of the Fe^{3+}-xylenol orange complex which should be orange/brown. A bluish/purple colour indicates the use of insufficient xylenol orange to complex all the Fe^{3+} present. This results in the underestimation of the amount of hydroperoxide in the sample (Gay and Gebicki, 2002).

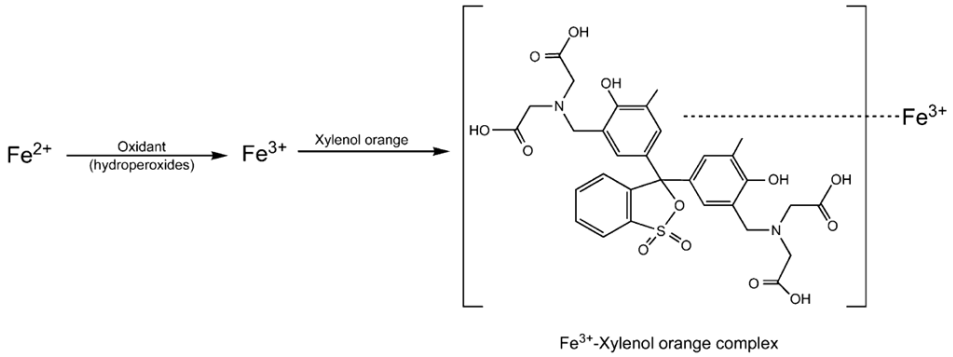


Figure 1.16 *Formation of Fe^{3+}-xylenol orange complex from Fe^{2+} with oxidative component (Moon and Shibamoto, 2009)*

1.5.4 Mechanism of antioxidant action in oil

The naturally occurring antioxidative components in oil, e.g. tocopherol, phytosterol and phenolic compounds, are important for health and nutrition. They react with free radicals (peroxyl radicals: ROO•) counter acting the oxidation process (Choe and Min, 2009; Kamal-Eldin and Appelqvist, 1996; Sies *et al.*, 1992; Wagner and Elmadfa, 2000). Tocopherols can be found at high amounts in various seed oils such as berry seed oil (Van Hoed *et al.*, 2009), grape seed oil (Baydar *et al.*, 2007) and sesame seed oil (Elleuch *et al.*, 2007). They are the

most important antioxidants serving as chain breaking antioxidants and can prevent the propagation of the free radical reaction. Tocopherol (Toc) acts as an antioxidant by donating a proton from the 6-hydroxy group on its chroman ring to the lipid alkyl radical (**Eq. 1.7**) and consequently, scavenges the lipid peroxy radical to produce lipid hydroperoxide and a tocopheroxy radical (Toc•) (**Eq. 1.8**). The lipid peroxy radical can react much faster with tocopherol than with another fatty acid (Choe and Min, 2009; Sarin *et al.*, 2007).

$$Toc + R\bullet \longrightarrow Toc\bullet + RH \quad (\mathbf{1.7})$$

$$Toc + ROO\bullet \longrightarrow Toc\bullet + ROOH \quad (\mathbf{1.8})$$

Subsequently, the tocopheroxy radical is more stable than the lipid peroxy radical and it can interact with other components or with each other depending on the lipid oxidation rate. At a low lipid oxidation rate, the tocopheroxy radicals react with each other and produce tocopheryl quinone and tocopherol (**Eq. 1.9**). On the other hand, at a higher oxidation rate, the tocopheroxy radical may react with the lipid peroxy radical and produce the tocopherol-lipid peroxy complexe (Toc-OOR) which on its turn can be hydrolyzed to tocopheryl quinone and lipid hydroperoxide (**Eq. 1.10**) (Choe and Min, 2009; Liebler *et al.*, 1990; Sarin *et al.*, 2007).

$$Toc\bullet + Toc\bullet \longrightarrow Toc + \text{Tocopheryl quinone} \quad (\mathbf{1.9})$$

$$Toc\bullet + ROO\bullet \longrightarrow \text{Toc-OOR} \longrightarrow \text{Tocopheryl quinone} + ROOH \quad (\mathbf{1.10})$$

The tocopherol-mediated peroxidation can be prevented by vitamin C (ascorbic acid, a water-soluble antioxidant), since ascorbic acid quickly reduces the tocopherol radical to tocopherol and dehydroascorbic acid (**Eq. 1.11**)(Yamamoto, 2001).

$$Toc\bullet + \text{Ascorbic acid} \longrightarrow Toc + \text{Dehydroascorbic acid} \quad (\mathbf{1.11})$$

The relation between the oxidative stability during storage, which is an important indicator of quality and shelf-life, and the natural tocopherol content has been demonstrated amongst others in olive oil (Krichene *et al.*, 2010; Stefanoudaki *et al.*, 2010; Yildirim, 2009) and soybean oil (Yang *et al.*, 2005). Besides tocopherol, there are many other naturally occurring bioactive substances which may influence the lipid oxidation. The antioxidative properties of sesame lignans such as sesamol, sesamin and sesamolin, in sesame seed oil have been reported (Aued-Pimentel *et al.*, 2006; Yoshida and Takagi, 1997) Furthermore, hydroxytyrosol and tyrosol derivatives in olive oil (Krichene *et al.*, 2010) may, next to the tocopherols, limit the progression of oxidation.

1.6 Cell wall polysaccharide

Plant cell walls, consisting of a complex polysaccharide network, characterize the cell's shape and contribute to the structural rigidity of the plant. Plant cell wall polysaccharides, especially pectic substances, are frequently used in a broad range of food products. They may be used as stabilizing agents to modify product's texture and also as dietary fibres adding potential health benefits (Thakur *et al.*, 1997). In spite of pectin availability in a large number of plant species, commercial sources of pectin are very limited. Pectin today is usually extracted from apple, citrus fruits and their co-products (Canteri-Schemin *et al.*, 2005). Besides apple and citrus pectins, alternative pectin sources have been explored. Examples include sugar beet (Mesbahi *et al.*, 2005; Yapo *et al.*, 2007), yellow passion fruit (Kliemann *et al.*, 2009; Yapo and Koffi, 2006; Yapo and Koffi, 2008), murta fruit (*Ugni molinae Turcz*) (Taboada *et al.*, 2010), plum (Renard and Ginies, 2009), acerola (Prato *et al.*, 2005), *Opuntia* spp. (Matsuhiro *et al.*, 2006) and banana peel (Jing-yong, 2006).

1.6.1 Cell wall structure

The overall architecture of monocot and dicot cell walls is similar in that they both consist of a matrix of complex polysaccharides. The matrix comprises cellulosic polysaccharides surrounded by non-cellulosic polysaccharides, e.g. hemicellulosic and pectic substances., The plant cell walls differ considerably in type and relative abundance of non-cellulosic polysaccharides, cross-linking of the polysaccharides, abundance of proteins and phenolic compounds and presence of lignin (Cosgrove, 2005; Harris and Smith, 2006; Vogel, 2008). They are composed of three cell wall layers, i.e. middle lamella, primary cell wall and secondary cell wall. In addition to this, the primary cell wall of flowering plants can be

divided into two broad categories: type I cell walls which are found in dicot plants, including dragon fruit (*Hylocereus* spp.), and type II cell walls which are found only in the commelinoid monocot plants (e.g. grasses, sedges, rushes and gingers). They are composed of cellulose fibres encased in glucuronoarabinoxylans, high levels of hydroxycinnamates, and very low levels of pectin and structural proteins (Carpita, 1996; Vogel, 2008).

During cell division of dicotyledonous plants, the middle lamella is first formed which is mainly composed of pectic components and proteins to bind the cells together. While the cells are enlarging, the primary cell wall is built. In this layer, type I cell walls are formed which are predominantly composed of cellulose (a complex carbohydrate in the form of organized microfibrils) and two groups of polysaccharides, i.e. pectins and cross-linked glycans such as xyloglucan. These polysaccharides are assembled into a network with the cellulose microfibrils (**Figure 1.17**). Lower amounts of phenolic esters, minerals and enzymes are probably present in the primary cell wall (O`Neil and York, 2003). The cross-linked glycans increase the tensile strength, while the coextensive network of pectins provides the ability to resist compression to the cell wall (Buggenhout *et al.*, 2009; Cosgrove, 2005; Harris and Smith, 2006). The apoplast is normally situated between the primary cell wall and the middle lamella (Buggenhout *et al.*, 2009).

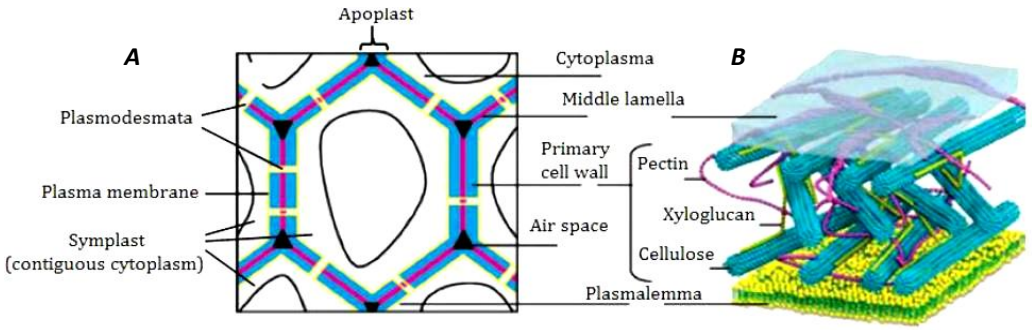


Figure 1.17 *Schematic presentation of (A) parenchyma tissue cell and (B) type I cell walls (adapted from Buggenhout et al., 2009)*

After the completion of cell division and cell enlargement, the secondary cell wall, which basically consists of cellulose, hemicellulose and lignin, is in some plants deposited on the primary cell wall. This process makes the cell wall stronger and usually much thicker compared to the primary cell wall (Cosgrove, 2005; Harris and Smith, 2006). The secondary cell wall can further be grouped into two types: those containing lignin (lignified walls)

which are the more common in plant cell walls and those containing no lignin (non-lignified walls) (Harris and Smith, 2006).

In the edible pulp fruit, the primary cell wall (a relative thin layer) is of central interest, whilst the secondary cell wall is virtually not present in mature fruits (Brummell, 2006). Plant cell walls, which are complex matrices with diverse structures, can be isolated into different polysaccharide fractions. Pectic polysaccharides are solubilized by aqueous buffers, acidic solutions and calcium chelators. They are generally rich in GalA, rhamnose (Rha), arabinose (Ara) and galactose (Gal). Hemicellulosic polysaccharides, on the other hand, require strong alkali for solubilization (after removal the pectin) because hemicelluloses are a group of complex polysaccharides which bind tightly with cellulose to form a hemicelluloses-cellulose network that is strong yet resilient (Brett and Waldron, 1996; Cosgrove, 2005).

1.6.2 Pectin structure

Pectic polysaccharides, which are abundantly found in the plant primary cell wall and middle lamellae, are diverse in their structure and their relative abundance. They mostly consist of acidic hetero-polysaccharides rich in GalA (i.e. galacturonans) structured as a backbone with three pectic domains: homogalacturonan (HG), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (**Figure 1.18**) (Willats *et al.*, 2006). Xylogalacturonan (XG), which is a domain of polyGalA substituted at C3 with residues of xylose (Xyl), also appears to be quite widespread in plant cell walls (Scheller *et al.*, 2007; Willats *et al.*, 2001).

In general, HG and RG-I are the major components, whereas XG and RG-II are the minor components. Many different variations exist, especially on the side chains of RG-I. One of the side chains in RG-II is linked by a borate ester to an apiose residue, thereby forming RG-II dimmers. HG, RG-I and RG-II can be covalently linked to form a pectic network. Furthermore, an alternative macromolecular structure of pectic substances has been reported in which HG and RG-II are considered as the side chains of the RG-I backbone (Sila *et al.*, 2009; Willats *et al.*, 2006). Pectin has considerable potential for modulation of its structure by the action of pectic enzymes (Scheller *et al.*, 2007).

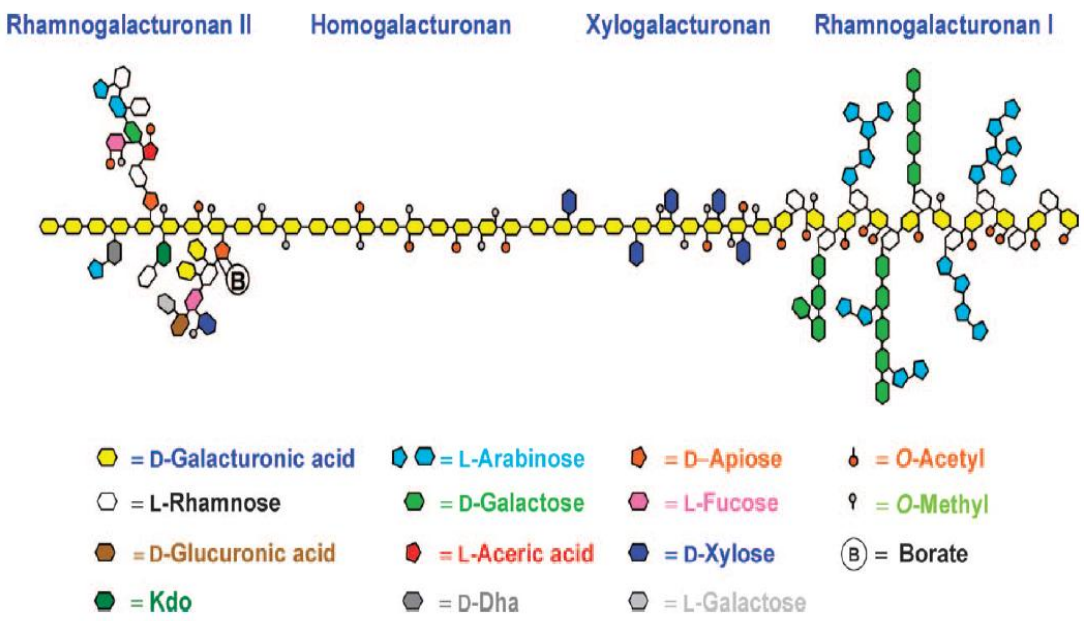


Figure 1.18 *Schematic structure of pectic substances (Scheller et al., 2007)*

Next to the predominant pectic component, i.e. GalA, pectin is also specifically bonded with some specified neutral sugars, e.g. Rha, Ara, Gal and Xyl (Cosgrove, 2005; Harris and Smith, 2006; Willats *et al.*, 2006). Galacturonan in the plant cell walls consists of the unsubstituted HG (smooth region) and the ramified segments (branched RG or hairy regions) which are alternating sequences of 1,2-linked α-L-rhamnosyl and 1,4-linked α-D-galacturonosyl residues with side chains of arabinans, arabinogalactans and galactans (Ovodov, 2009; Scheller *et al.*, 2007). However, the exact structural features of the macromolecular organization of pectin are still under debate.

1.6.2.1 Homogalacturonan

HG is the major component present in pectic substances. **Figure 1.19** illustrates the structure of HG. HG is a linear chain of 1,4-linked α-D-GalA residues in which some carboxylates of GalA residues are esterified with methyl alcohol at C6. Also, acetylation can predominantly occur at C3 of GalA in HG, although C2 substitution may be present in HG (Ridley *et al.*, 2001; Willats *et al.*, 2001). Plants are capable of synthesizing and eventually modifying pectic molecules by themselves due to the action of the related enzymes. High methylated pectin is synthesized in the Golgi apparatus and secreted into the plant cell walls where it is subjected to modifications (Ridley *et al.*, 2001).

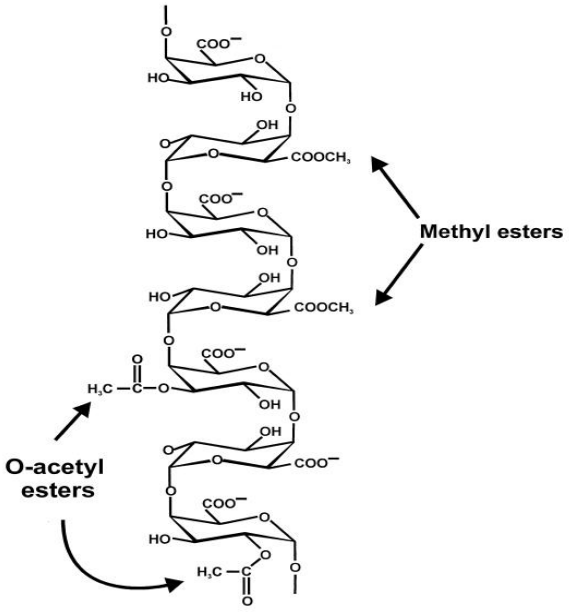


Figure 1.19 *Schematic structure of HG (Ridley et al., 2001)*

The degree and pattern of methyl-esterification depend on plant species, developmental stage, location in the cell wall and pectic enzymes. It largely determines their chemical properties and hence, their suitability for particular industrial applications. In order to achieve the required functionality of pectin, the activity of endogenous PME should be considered. This results in changes of the degree of HG methyl-esterification (Ridley *et al.*, 2001).

1.6.2.2 Rhamnogalacturonan-I

RG-I is an abundant pectic polysaccharide in plant cell walls. The structure of RG-I is shown in **Figure 1.20**. Its acidic pectic domain contains a backbone of the repeating disaccharide 1,4-linked α-D-GalA and 1,2-linked α-L-Rha, and the sugar side chains may be substituted at the Rha residues (Ridley *et al.*, 2001; Willats *et al.*, 2001). About 20-80% of the Rha residues in RG-I are substituted at C4 with side chains in which neutral residues predominate and these can vary in size from a single glycosyl residue to 50 units or more. This results in a large and highly variable family of polysaccharides (Ovodov, 2009; Willats *et al.*, 2001).

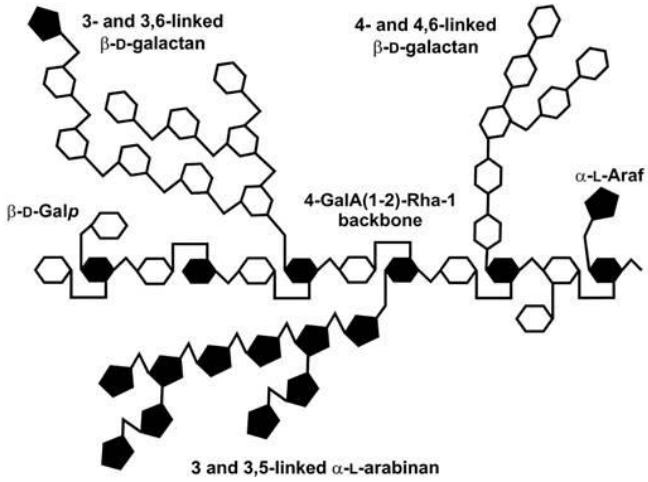


Figure 1.20 *Schematic structure of RG-I (adapted from Ridley et al., 2001)*

A RG-I subunit can have long side chains. The predominant RG-I side chains contain linear and branched α-L-arabinofuranosyl (Araf) and/or β-D-galactopyranosyl (Galp) residues (**Figure 1.20**). These two branched polysaccharide structures can be linked with the C4 position of some Rha residues in RG-I, although their relative proportions and chain lengths may differ depending on the plant source (Ridley *et al.*, 2001; Voragen *et al.*, 2009). They might, however, possess acetyl groups esterified on the position C2 and/or C3 (Willats *et al.*, 2001), and the glycosyl residues α-L-fucosyl, β-D-glucuronosyl and 4-O-methyl β-D-glucuronosyl may also be present (Ridley *et al.*, 2001). The RG-I pectic substances are not depolymerized by the β-elimination, a chemical depolymerization reaction acting on highly methoxylated pectin. Therefore, it is claimed that the GalA residues of RG-I are presumably not methyl-esterified (Voragen *et al.*, 2009).

1.6.2.3 Rhamnogalacturonan-II

RG-II is found as a minor component in primary cell wall and thought not to be present in the middle lamella. It is distinguished by a very complex structure (Vincken *et al.*, 2003). The structure of RG-II differs strongly from that of RG-I. RG-II has a HG-based polymer of approximately nine GalA residues (1,4-linked) as the backbone. The Rha residues are much less abundant in RG-II compared to RG-I and they are present in the side chains of RG-II instead of in the backbone. RG-II is also substituted by four hetero-oligomeric side chains attached to O2 or O3 of the GalA backbone residues. It is generally thought to be heterogeneous glycosidically attached to HG domains, and may carry common sugars and

some rarely peculiar sugar residues, e.g. D-apiose, 3-C-carboxy-5-deoxy-L-Xyl (L-aceric acid), L-Gal, 3-deoxy-D-lyxo-2-heptulosaric acid (Dha) and 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) (Scheller *et al.*, 2007; Vincken *et al.*, 2003; Willats *et al.*, 2001). RG-II is the only polysaccharide known to contain both apiose and aceric acid. Two monomeric units of RG-II cross-link by the apiose residues with borate diester bond (**Figure 1.21**). The borate 1:2 diol ester is formed between OH2 and OH3 of the 30 linked apiosyl residues (Bar-Peled *et al.*, 2012; Ridley *et al.*, 2001). When two apiosyl residues are linked by a borate diester, the boron atom is chiral and two diastereoisomers, i.e. bis[3-*C*-(hydroxymethyl)-β-L-*threo*-furanoside]-(R)-2,3:2',3'borate and bis[3-*C*-(hydroxymethyl)-β-L-*threo*-furanoside]-(S)-2,3:2',3'borate, may form. It is not known if one or both diastereoisomers are formed when RG-II is cross-linked in the cell walls (Bar-Peled *et al.*, 2012).

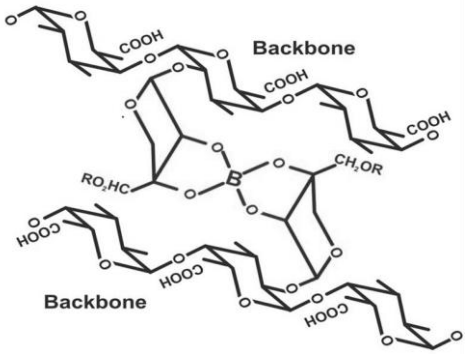


Figure 1.21 *Schematic structure of borate diester bond of two monomeric units of RG-II (adapted from Bar-Peled et al., 2012)*

RG-II is a complex polysaccharide which makes that the relative proportions of the different structural elements of RG-II may vary significantly for different plant tissues. The conservation of the RG-II structure is present in both higher and lower land plants. RG-II must have an important function in the plant cell walls. It is crucial for normal wall formation, control of the wall porosity and wall thickness (Cosgrove, 2005). Therefore, it is not surprising that pectic polysaccharides are considered as one of the most complicated and structurally dynamical classes of biopolymers (Vincken *et al.*, 2003). RG-II appears to be the only major pectic domain that does not have significant structural diversity or modulation of its fine structure, although the distribution of the four side chains in RG-II and the distribution of RG-II in HG still remain to be established (Ovodov, 2009; Vincken *et al.*, 2003; Willats *et al.*, 2001).

1.6.3 Pectin conversion

Despite the possibility of many pectin conversion reactions, only the well-known HG conversion reactions have been widely studied. **Figure 1.22** illustrates how pectin can be subjected to modification and/or degradation by either enzymatic conversions, e.g. PME, PG and pectate lyase (PL), or non-enzymatic reactions, e.g. β-elimination and acid hydrolysis (Buggenhout *et al.*, 2009; Sila *et al.*, 2009).

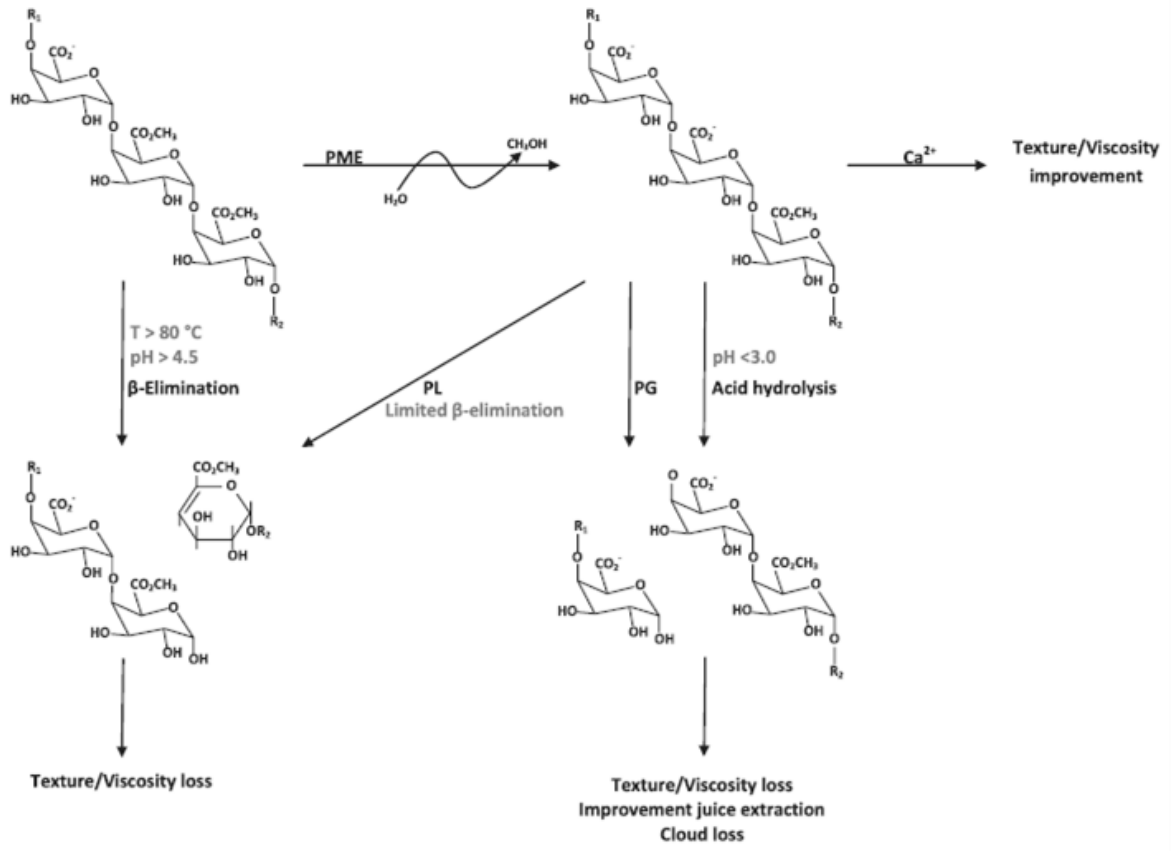


Figure 1.22 *Schematic presentation of HG conversion reaction (Sila et al., 2009)*

Knowledge about pectin conversions can be helpful to elucidate the structural changes in pectin due to these reaction mechanisms, and to figure out the key factors regulating these reactions and the subsequent effects of these changes on pectin functionality. Moreover, it can obtain the characteristics of nature and treated products, and enhance opportunities for pectin application in food and other products (Sila *et al.*, 2009). This knowledge can be used to optimize the reactions of pectin conversion in order to establish desirable pectin functionality in the end products.

1.6.3.1 Non-enzymatic pectin conversion

Pectin can be modified by non-enzymatic pectin conversions either via a depolymerization mechanism (e.g. β-elimination reaction and acid hydrolysis) or a de-methoxylation reaction (e.g. saponification). The β-elimination reaction refers to the eliminative cleavage of the glycosidic linkages in the β-position to the carbonyl function. This results in the formation of a glycosyl anion and an unsaturated bond between the position C5 and C6 of the GalA residues. It is commonly encountered for the pectin with a high degree of methyl-esterification on the GalA residues (Albersheim *et al.*, 1960; Buren, 1979). The β-elimination can occur during heating at temperatures higher than 80 °C, at neutral or alkaline conditions (pH > 4.5) and is possible with the presence of monovalent salts (Buren, 1979). The reaction leads to pectin solubilization, a decrease of cell-cell adhesion and eventually, results in tissue softening. The β-eliminative depolymerization becomes an important issue for modification of pectic substances in plant-based foods during processing (Christiaens *et al.*, 2011b; Sila *et al.*, 2005).

The acid hydrolysis also leads to pectin degradation based on the depolymerization mechanism. The high methyl-esterified pectin is normally susceptible to acid hydrolysis, especially under acidic conditions (at pH below 3). The de-esterified pectin is hydrolyzed at the α-(1,4) glycosidic bonds (Voragen *et al.*, 2009). Since the pH of most plants is between 4 and 6, the acid hydrolysis of pectin is negligible compared to the β-elimination during thermal treatment of plant-based foods. However, the acid hydrolysis reaction is mostly influenced by DM and is less important during regular food processing (Buggenhout *et al.*, 2009).

The saponification of pectic substances is the random de-methoxylation. It hydrolyzes ester bonds at C6 of the GalA residues under alkaline conditions with the formation of alcohols and carboxylic acids (or their salts). The methoxyl groups of pectic substances are rather easily and randomly saponified under mild acid or alkaline conditions (Buren, 1979; Renard and Thibault, 1996). Under alkaline conditions, any increase in temperature leads to an increase in the rate of the β-elimination compared to the saponification, whereas the saponification favourably occurs in a higher rate by increasing the pH (Renard and Thibault, 1996).

1.6.3.2 Enzymatic pectin conversion

The conversion of pectic substances in fruits can be affected by pectic enzymes. This results in either desirable or unfavourable qualities of plant-based foods during plantation, harvesting and processing. Both hairy and smooth regions of pectin are modified and degraded by a number of endogenous and exogenous enzymes (Sila *et al.*, 2009). The pectin side chain in hairy regions is modified by, for example, rhamnogalacturonase and rhamnosidase which are specific for Rha residues of the RG regions (Nakamura *et al.*, 2002). It is also modified by RG α-D-galactopyranosyluronohydrolase or RG-galacturonohydrolase which is able to release the GalA residues from the non-reducing end of RG chains, but not from the HG stretch (Mutter *et al.*, 1998). The enzymatic pectin conversions in smooth regions (HG regions) have been widely studied due to their underlying effect on pectin functional properties (Duvetter *et al.*, 2009). The focus of the following parts is on the pectolytic enzymes, i.e. PME and PG which are, respectively, demethoxylating and depolymerizing enzymes at the HG part of the pectic polymer.

PME catalyzes HG within plant cell walls. It releases methanol and generates the de-methoxylated HG and free acids (**Figure 1.22**). The de-esterified HG regions which refer to the PME catalyzed fragments (particularly free COO^- groups) can further cross-link with calcium ion forming supramolecular assemblies and/or gels that are important for engineering texture/rheological properties (Sila *et al.*, 2009; Willats *et al.*, 2001). Since free acids are generated from PME activity, the pH of the cell walls is locally decreased leading to a control of the activity of other enzymes that are present. The de-methoxylated HG is a susceptible site for depolymerizing enzymes, including lyases (i.e. PL) and hydrolases (i.e. PG), associated with texture/viscosity loss. However, it is useful in the improvement of juice extraction yields and in controlling cloud stability in juice products (Ribeiro *et al.*, 2010; Sila *et al.*, 2009). The lyases can form a double bond between C4 and C5 at the newly formed non-reducing end via the β-elimination reaction, whereas the hydrolases cleave glycosidic bonds via acid/base-assisted catalysis (Sila *et al.*, 2009).

PMEs of different origins can remove the methoxyl groups from HG to obtain different typical patterns of the de-methoxylate pectin, i.e. blockwise, non-blockwise and random patterns (**Figure 1.23**). Most plant PMEs have a blockwise action. This action is displayed when the enzyme removes methyl groups from contiguous GalA residues. This results in relatively long stretches or blocks of de-esterified GalA residues, while PMEs from microbial (fungal) origin usually have non-blockwise action. Fungal PMEs produce HG with a dispersed arrangement of methyl-esters along the HG chain (Fraeye *et al.*, 2010; Willats *et al.*, 2006). The random chemical de-methoxylation (i.e. from saponification) of pectin is also

comparable with the PME de-methoxylation mechanisms. The mode of PME action on HG remains controversial and seems influenced by a wide range of factors, e.g. pH of plant cell walls, DM and the pattern of methyl-esterification (Willats *et al.*, 2001; Wolf *et al.*, 2009).

Due to the sensitivity of PME to their ionic environment, their activity can be manipulated by altering the pH. The optimum pH for PME activity in most plants is normally between 6 and 8 (Duvetter *et al.*, 2009). A thermal treatment also greatly affects PME activity. Plant PMEs are rather heat labile enzymes at atmospheric pressure, for example, tomato PME can be inactivated at 70 ˚C for 5 min (Plaza *et al.*, 2007), while heating at 80 ˚C for 5 min allows complete inactivation of banana PME (Ly-Nguyen *et al.*, 2002a). The residual PME activity of acerola PME at 98 °C for 60 min is greater than 66% compared to the initial PME activity (Assis *et al.*, 2007), whereas the activity of grapefruit PME retains 87% after heating at 80 °C for 10 min (Guiavarc'h *et al.*, 2005).

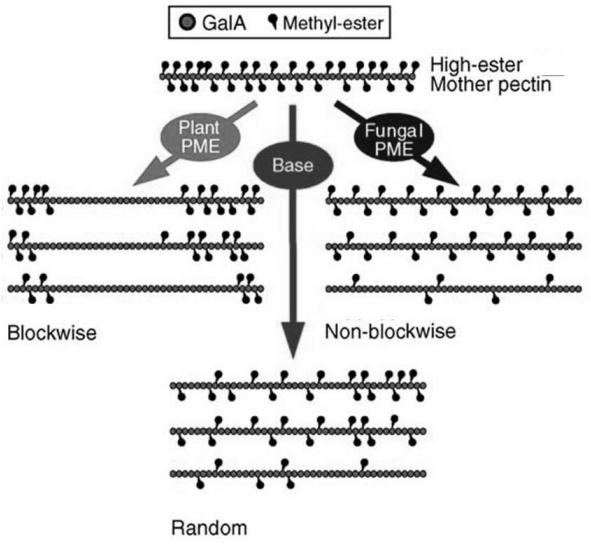


Figure 1.23 *Pattern of de-methoxylate pectins as affected by different PME sources (adapted from Willats et al., 2006)*

PG is one of the important HG-related enzymes. It is a depolymerizing enzyme that catalyzes the hydrolytic cleavage of 1,4-linked α-D-galacturonan linkages in the de-esterified HG backbone (**Figure 1.22**). PME activity triggers PG activity. In higher plants, most PGs are generally present in more than one isoform and are electrostatically bound to the cell wall (Duvetter *et al.*, 2009). PG causes a rapid decrease in the viscosity of pectin solutions without a marked increase in reducing groups. Two free carboxyl groups of the polyGalA

chain should be positioned at a certain distance to form binding sites for the enzyme molecule. Because of the action of PG, it is considered to participate in the softening of fruit tissue during ripening (Labib and El-Asbwab, 1995). The optimum pH for PG activity depends on the source and the PG isoforms within the source. It is generally between 4 and 6, and enzyme activity is almost inhibited below a pH of 3.5 (Duvetter *et al.*, 2009).

In the fruit juice industry, thermal treatments may affect the activity of desired and undesired enzymes. Up to the optimum temperature for enzyme activity, the catalytic activity of enzymes is enhanced by thermal processing due to the increasing kinetic energy of the molecules. However, intra- and intermolecular bonds in the protein structure of enzymes are probably broken down at high temperature regions and the rate of enzyme inactivation dominates the catalytic activity (Duvetter *et al.*, 2009). Cloud stability is a major quality attribute of fruit juice and can either be a favourable attribute (e.g. cloudy juice) or be an undesirable quality (e.g. clarified juice). Thus, pectic enzymes are used as a processing aid in juice extraction to facilitate juice recovery by removing the suspended matters in fruit pulp. Prior to juice processing, PME activity can be used, followed by PG activity. However, a complete breakdown of pectin requires a correct proportion of different enzymes. Therefore, PME and PG activities have to be stimulated during the production of clarified juices, but they should be prevented in the production of cloudy juices. These enzymatic reactions can result in cloud loss, negatively affecting product acceptability (Sila *et al.*, 2009).

1.6.4 Pectin functionality

Pectin is used in a number of foods as, for example, gelling agent, stabilizer, texturizer, thickening agent, adhesive agent and emulsifier. Its multifunctionality results from the different structures of a pectic molecule (Ovodov, 2009). The GalA residues in pectin are commonly partly esterified with a methyl group. Pectin is, therefore, mainly classified as a function of DM, the main parameter influencing the physical properties of pectin. Pectin gelation is an important property in the food industry. Two main gelation mechanisms are distinguished depending mostly on the DM of pectin, i.e. high methyl-esterified pectin (HME) and low methyl-esterified pectin (LME) (Fraeye *et al.*, 2010). Pectins containing 50% or above of their DM are classified as HME (Thakur *et al.*, 1997). The gel formation of HME is based upon the presence of high amount of soluble solids (normally sucrose in food applications) at a low pH value. The gel resulting from the HME gelation is also known as a sugar-acid pectin gel which can stabilize the structure of junction zones of gel formation by

hydrophobic interactions between methyl esters (Ovodov, 2009; Sharma *et al.*, 2006). On the other hand, LME has less than 50% of DM. It contains sufficient acid groups to form a gel in the presence of divalent cations (particularly calcium ion) at low sugar contents (or even no sugar) and a wide range of pH values (Ovodov, 2009; Thakur *et al.*, 1997). The gel forming ability of LME decreases with higher levels of DM. Some blockwise distributions of carboxyl groups in HG are very sensitive to calcium levels (Sharma *et al.*, 2006). The gelling interactions between calcium ions and carboxyl groups of the pectin are described by the egg-box model (Fraeye *et al.*, 2010; Thakur *et al.*, 1997). The pectin gelation resulting from ionic linkages via calcium bridges between two carboxyl groups belonging to two different chains in close contact is illustrated in **Figure 1.24**. It is suggested that two-fold symmetrical galacturonans are cooperatively bound by calcium ions that are packed in the interstices of the twisted chains, in analogy with a corrugated egg-box (Fraeye *et al.*, 2010). The adjacent chains are linked intermolecularly through electrostatic and ionic bonding of the carboxyl groups (Sharma *et al.*, 2006).

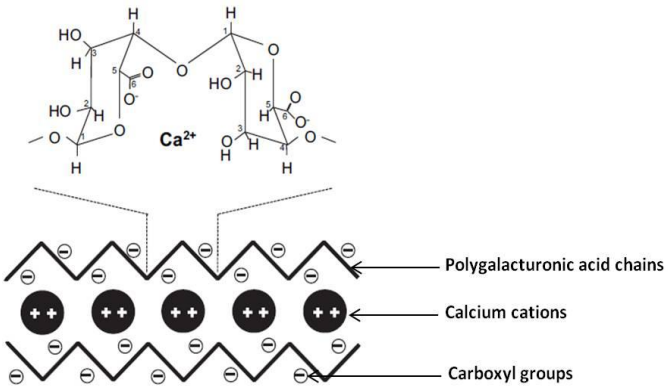


Figure 1.24 *Schematic representation of egg-box model in low methyl-esterified pectin (adapted from Fraeye et al., 2010)*

Commercial apple and citrus pectins are the most important sources for producing HME (Canteri-Schemin *et al.*, 2005). They may be further de-esterified under particular controlled conditions (i.e. mildly acidic conditions) to obtain LME. Both HME and LME are widely used in various commercial foods, e.g. jam, jelly, confectionary, beverage and pharmaceutical products. LME is of great interest for applications in low-sugar or low-calorie foods. This is due to the limited gel formation of HME in the presence of high amounts of sugar. However, gums (e.g. carrageenan and guar gum) can be used in such products, but the advantage of LME over these gums is to form pectin-calcium gels with greater gel stability under acid

conditions (Thakur *et al.*, 1997). Besides DM, various parameters also have a significant effect on the pectin gel network such as internal factors (e.g. pattern of methyl-esterification, neutral sugar composition and molecular mass of pectins) and external factors (e.g. temperature, pH, sugar, calcium and other solutes) (Fraeye *et al.*, 2010; Thakur *et al.*, 1997; Willats *et al.*, 2006).

Moreover, pectic substances can reduce the interfacial tension between oil and water phases and therefore, they can be used as an emulsifier in many food products. Sugar beet pectin has the ability to act as a surface-active agent and is very effective in oil/water emulsions. The surface activity of pectin is affected by many factors such as its molar mass, the presence of acetyl groups and/or protein residues and the concentration of pectin (Yapo *et al.*, 2007). Pectic substances also have an interesting function as food fibre components. They contribute to the nutrition of humans (Voragen *et al.*, 2009; Willats *et al.*, 2006). Since pectin is a soluble dietary fibre, its solubility property needs to be understood. Water solubility relates to DM, the methyl-esterification pattern and the degree of polymerization. The solubility increases with increasing number of esterified carboxyl groups and decreasing molecular weight, although external factors (e.g. conditions of solution used and pectin concentration) have a significant effect on pectin solubility (Thakur *et al.*, 1997).

1.6.5 Hemicellulose structure

Hemicellulosic polysaccharides are defined as non-cellulosic cell wall polysaccharides. They have a strong hydrogen bond between hemicellulose and cellulose microfibrils. Their composition can differ greatly in different cell types and plant species (Brett and Waldron, 1996; Scheller *et al.*, 2007). Hemicellulose can be present in both the primary and secondary cell walls (Brett and Waldron, 1996; Caffall and Mohnen, 2009). Hemicellulose is an indigestible complex fibre comprising several heteropolymers together with cellulose microfibrils in most plant cell walls. It forms a cellulose-hemicellulose network by hydrogen bonding that provides the main structural strength in growing cell walls (Brett and Waldron, 1996; Cosgrove, 2005). Hemicellulose can be classified according to the main sugar present in the backbone of the polymers such as xylan (1,4-linked β-D-Xyl), mannan (1,4-linked β-D-Man) and xyloglucan (1,4-linked β-D-Glc), as shown in **Figure 1.25**.

The backbone of hemicellulose may have many branches composed of monomers, e.g. D-Gal, D-Xyl, L-Ara and D-GalA (Scheller *et al.*, 2007). Amongst the diverse structures of hemicellulose, xyloglucan is the most abundant hemicellulose present in hemicellulosic polysaccharides (Cosgrove, 2005). Xyloglucan has the structure of an unbranched 1,4-linked

β-D-glucan similar to cellulose, but it is extensively substituted at the C6 position of Glc with 1,6-linked α-D-Xyl. The Xyl residues can be further serially appended with Gal and fucose (Fuc) residues (Brett and Waldron, 1996; Cosgrove, 2005). The most important feature of xyloglucan is its characteristic capacity to form strong and non-covalent associations through hydrogen bonds with cellulose (O`Neil and York, 2003). Besides xyloglucan, arabinoxylan can also be found in hemicellulosic substances that consist of a 1,4-linked β-D-xylan backbone decorated with Ara branches. Arabinoxylan might bind cellulose and be cross-linked by ferulic acid esters (Cosgrove, 2005).

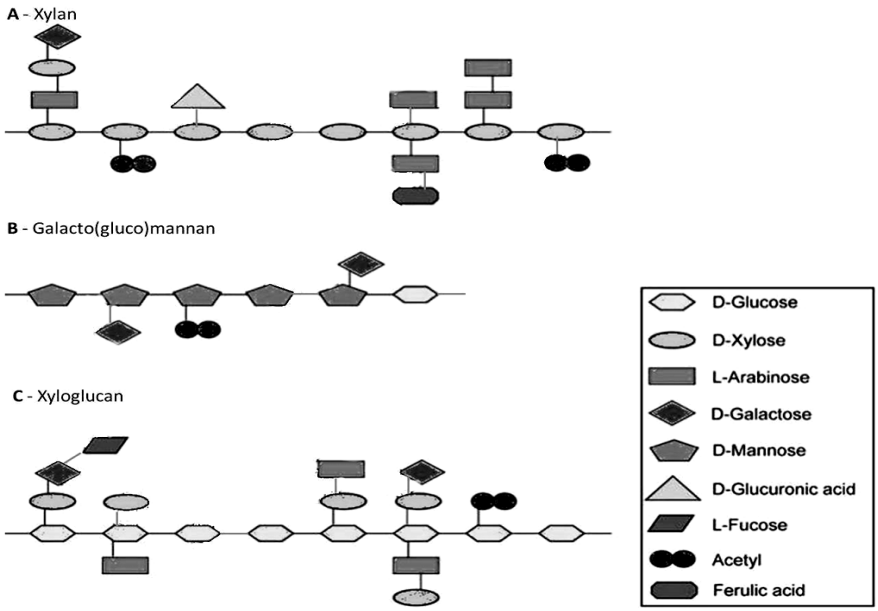


Figure 1.25 *Schematic structures of hemicelluloses: (A) xylan, (B) galacto (gluco) mannan and (C) xyloglucan (adapted from Brink and Vries, 2011)*

Besides xyloglucan and xylan, other hemicelluloses are present in cell walls. Mannan is also found in the primary cell wall and probably function in the same way as xyloglucan and arabinoxylan (Cosgrove, 2005). Galactoglucomannan may be abundant in the primary cell wall. It is a mannan-type hemicellulose characterized by a backbone of alternating 1,4-linked β-D-Glc and 1,4-linked β-D-Man residues. The Man residues can be substituted at O6 with α-D-Gal or β-D-Gal-(1,2-linked)-α-D-Gal side chains (O`Neil and York, 2003).

1.6.6 Isolation of cell wall polysaccharide

Plant cell wall materials can be isolated by the organic solvents such as ethanol and acetone. These organic solvents used are employed to dehydrate the cell wall components and may modify hydrophobic interactions and hydrogen bonds as well as denature proteins. This results in a decreased accessibility to cell wall-related enzymes (Koch and Nevins, 1989). The isolated cell wall is referred to as the alcohol-insoluble residue (AIR). The AIR can further be sequentially extracted using various solvents to obtain different fractions of the cell wall polysaccharides. The pectic substances in the water-soluble fraction (WSF) are slightly bound (i.e. van der Waals forces, intensive hydrogen bonds) in the primary cell wall and middle lamella of plant cell walls (Renard and Ginies, 2009; Sila *et al.*, 2009). They normally contain highly-methoxylated pectin and are rich in HG regions (Thimm *et al.*, 2009). The pectic substances in the chelator-soluble fraction (CSF) mostly originate from the middle lamella, encompasses less-methoxylated pectins held in the cell wall network by calcium bridges and have a lower neutral sugar content compared to the WSF pectin (Thimm *et al.*, 2009). On the other hand, the sodium carbonate-soluble fraction (NSF) is composed of pectic substances originating from the primary cell wall, has long side chains and is bound to other cell wall polymers by ester-bonds. The hemicellulosic fraction (HF) is composed of hemicellulosic polysaccharides and possibly some branched pectins which are strongly bound to non-pectic substances, e.g. celluloses or hemicelluloses (Renard and Ginies, 2009; Thimm *et al.*, 2009). The final residue which could not be solubilized by the procedure used is thought to consist mainly of celluloses and remaining hemicelluloses. This fraction probably contains complex pectins which are strongly attached to celluloses (Thimm *et al.*, 2009).

1.6.7 Structural elucidation of cell wall polysaccharide

The knowledge to elucidate the chemical structure of cell wall polysaccharides is necessary to better understand their properties towards their possible applications and their roles in plant growth during fruit ripening as well as their modification during plantation and processing. The cell wall polysaccharide composition can vary with the source, extraction conditions, location and other environmental factors. In order to elucidate the structure of polysaccharides, a combination of different analytical methods should be considered.

1.6.7.1 Assessment of monosaccharide composition

High-performance anion-exchange chromatography (HPAEC) equipped with a pulsed amperometric detection (PAD) is an accurate technique to separate all classes of carbohydrate polymers. After acid hydrolysis, the composition of polysaccharides is determined according to structural features, e.g. size, composition, anomericity and linkage isomerism (Arnous and Meyer, 2008; Corradini *et al.*, 2012; Daas *et al.*, 1998). Polysaccharides are weak acids and consequently, their hydroxyl groups are partially or totally transformed into oxyanions under high alkaline conditions. Carbohydrate compounds can be selectively eluted by using HPAEC (Corradini *et al.*, 2012). HPAEC is often equipped with a CarboPac PA20 column for high selectivity resolution of monosaccharides, enabling the accurate determination of the common sugars such as Gal, Glc and Man (Weitzhandler *et al.*, 2004). The separation of closely related carbohydrates (i.e. Glc and Man which differ only in the axialequatorial configuration of their hydroxyl groups) can be enhanced significantly by lowering the pH of the alkaline eluent to a value that is comparable to the pK_a of the sugar molecules (pH 12) with the gold working electrode of the detector (Corradini *et al.*, 2012). The approach of PAD is based on the oxidation of sugars in alkaline media on the surface of a gold electrode by application of a positive potential. The generated current is proportional to the monosaccharide concentration, making the detection and quantitation of carbohydrates possible. If only the positive potential would be applied for detection of compounds, oxidation products would gradually poison the electrode surface causing a loss of signal. PAD also employs a series of potential waveforms applied at defined time periods (Arnous and Meyer, 2008; Corradini *et al.*, 2012).

Due to an improvement of PAD reproducibility, a quadruple potential waveform has been applied together with the working gold electrode in the flow-through detector cell. A positive detection potential (E_1) is applied for the time period corresponding to both the delay and the detection periods. The delay period is the time required for the charging current to decay, so that only the current originating from the oxidation of the analytical components is measured during the detection period. After detection, a negative potential (E_2) is applied to clean the electrode surface. Subsequently, a small amount of gold oxide, necessary to maintain an active working electrode surface, is generated by applying a positive potential (E_3). The final potential of this waveform (E_4) is applied to reduce the small amount of gold oxide formed by E_3 back to gold, permitting detection during the next cycle. The potential signal as a function of time is reported in the chromatogram and then, the monosaccharide composition can be qualified (Arnous and Meyer, 2008).

1.6.7.2 Assessment of molar mass distribution

The molar mass (MM) distribution is a common parameter for size determination of carbohydrate polymers (Christiaens *et al.*, 2011b; Yapo *et al.*, 2007). High-performance size-exclusion chromatography (HPSEC) is a standard technique for determination of the MM distribution of the polymers towards an average-molecular weight. This separation method, however, depends on the shape of the polymers (i.e. hydrodynamic volume) rather than the molecular weight. The gel column of HPSEC is used as a molecular probe filled with polymer beads with various particle sizes, enabling the perfect separation of the polymers (Kravtchenko *et al.*, 1992; Trathnigg, 2000). **Figure 1.26** shows the HPSEC column for determination of the MM distribution. Small particles can penetrate into the pores, but they can be verified as their elution velocity is similar to that of the elution buffer. This is an indication that there is no retention of these molecules by the gel matrix. However, large particles elute much faster than the elution buffer because they can only pass through the spaces between the beads. The larger particles are normally eluted quicker than the smaller particles (Trathnigg, 2000).

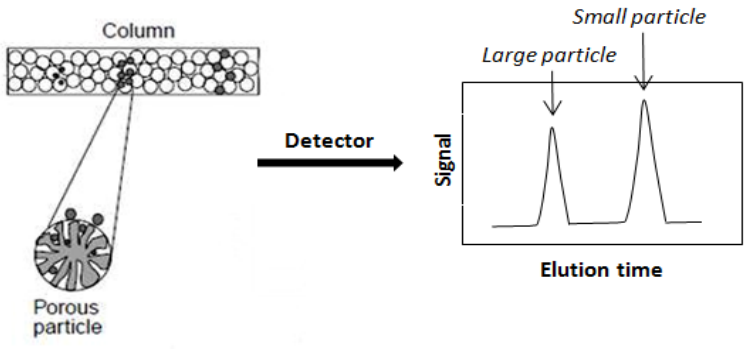


Figure 1.26 *Schematic representation of HPSEC column providing the size-exclusion chromatogram (adapted from Trathnigg, 2000)*

For better interpretation of the chemical structure of plant cell wall polysaccharides, the combination between the MM distribution results and the monosaccharide composition data should be considered (Houben *et al.*, 2011). The MM distribution may be used to investigate the pectin modification as affected by thermal processing, for example, in broccoli (Christiaens *et al.*, 2011b).

1.6.7.3 Probing of anti-pectin antibodies

Monoclonal antibodies are one of the possible state-of-the-art techniques to elucidate the structure of pectin (Willats *et al.*, 2006). They are antibodies produced by only one clone and therefore, they have only one type of antigen-binding site, resulting in their specificitivity for the type of antigen they recognize (VandenBosch *et al.*, 1989). The number of anti-pectin antibodies has recently increased due to a diversity of pectin structures. Monoclonal antibodies with specificities for numerous side chains and backbone HG domains can recognize pectic molecules with different DM. They can be used to analyze HG structure in context of cell wall architecture and plant cell development (Willats *et al.*, 2006; Willats *et al.*, 2001). **Figure 1.27** shows pectic polysaccharides recognized by some anti-pectin antibodies, i.e. LM7, JIM5, JIM7, 2F4 and PAM1. The epitopes of antibodies LM7, JIM5 and JIM7 bind to partially methyl-esterified HGs, but have different specificities with respect to DM and pattern of methyl-esterification. JIM5 perfectly binds with the pectin in a medium range of DM (DM greater than 40%). The binding strength seems to increase with decreasing DM and it binds weakly to a de-esterified pectin (DM $\leq$ 20%) (Willats *et al.*, 2000). Hence, the JIM5 epitope probably contains both methyl-esterified and non-methyl-esterified GalA residues (Christiaens *et al.*, 2011c). In contrast, JIM7 can be used as a general anti-HG probe due to its binding with a broad DM range of pectins. Moreover, no remarkable differences of the JIM7 binding strength with pectin containing a wide range of DM values (15-80% DM) are found (Christiaens *et al.*, 2011a; Willats *et al.*, 2000).

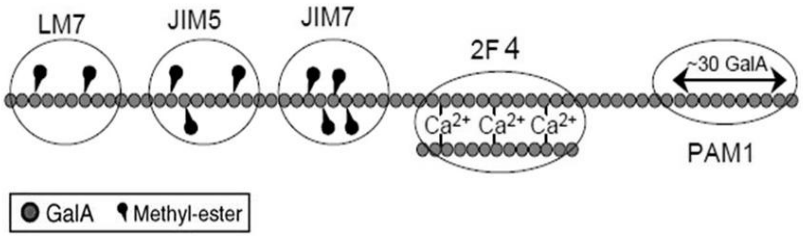


Figure 1.27 *Recognition of some anti-pectin antibodies against specific HG structure (adapted from Willats et al., 2006)*

2F4 binds specifically to HG that is crossed linked via calcium ions (Willats *et al.*, 2006), whilst the PAM1 epitope recognizes the long stretches of unesterified HG resulting from the blockwise action of plant PMEs (Willats and Knox, 1999). PAM1 is the most specific anti-HG antibody because it binds to long blocks of approximately 30 non-esterified GalA residues.

The binding capacity of PAM1, however, correlates to the DM, the pattern of methyl-esterification and the degree of blockiness of the pectin. PAM1 can be very useful to investigate methyl-esterification of pectin as affected by processing (Willats *et al.*, 1999).

To fully explore the HG structures, other monoclonal antibobies such as LM18, LM19 and LM20 which recognize the specific type of HG domain structure, have been prospected. LM20 binds to an epitope similar to JIM7, while LM18 and LM19 bind preferentially to de-esterified HG, similar to JIM5 (Verhertbruggen *et al.*, 2009). Some blocks of non-methyl-esterified GalA residues are necessary for LM18 recognition (Christiaens *et al.*, 2011c). So far, immunoprofiling from anti-HG antibodies has a considerable potential in applications of pectic polysaccharides in industrial and commercial uses (Willats and Knox, 1999).

CHAPTER 2

Characterization of Dragon Fruit

This chapter is based on:

Liaotrakoon, W., De Clercq, N., Lewille, B. and Dewettinck, K. (2012) Physicochemical properties, glass transition state diagram and colour stability of pulp and peel of two dragon fruit varieties (Hylocereus spp.) as affected by freeze-drying. ***International Food Research Journal****, 19(2): 743-750.*

Liaotrakoon, W., De Clercq, N., Van Hoed, V., Van de Walle, D., Lewille, B. and Dewettinck, K. (2013) Impact of thermal treatment on physicochemical, antioxidative and rheological properties of white-flesh and red-flesh dragon fruit (Hylocereus spp.) purees. ***Food and Bioprocess Technology****, 6(2): 416-430.*

2. Characterization of dragon fruit

2.1 Introduction

Dragon fruit (*Hylocereus* spp.) is a rich source of vitamins and minerals (To *et al.*, 1999) as well as an excellent source of antioxidative components (Jaafar *et al.*, 2009; Lim *et al.*, 2007; Mahattanatawee *et al.*, 2006; Wu *et al.*, 2006). Dragon fruit with red flesh (*H. polyrhizus*) contains betacyanin which is known as a powerful antioxidative pigment (Phebe *et al.*, 2009; Stintzing *et al.*, 2002; Wybraniec *et al.*, 2001). These components are present in the fruit and are related to positive health effects. Dragon fruit is usually consumed fresh and can be processed in fruit juice and puree. Processed red-flesh dragon fruit is the most popular processed dragon fruit product because of its attractive colour (red/purple). Additionally, dragon fruit puree, which leaves the seeds intact, also gives the product a unique appearance with small black seeds embedded in the flesh. Dragon fruit products may be applied in various foods, e.g. dairy, beverage, ice cream, jam and jelly industries (Barbeau, 1993; Gibson and Nobel, 1986; Tepora, 2009). The unique colour and appearance of dragon fruit are believed to have a great effect on consumer preferences (Sabbe *et al.*, 2009). As it is, the fruit also gains popularity in the overall global market.

Knowledge regarding the quality of dragon fruit is essential for the efficient handling of fruit production for equipment design, processing conditions and quality control. Currently, literature on the characterization of dragon fruit is only partially available. Studies on the possible value-added components of the fruit and its applications are also lacking. In this chapter, the experimental work provides a comprehensive insight into the characteristics (e.g. physicochemical, antioxidative, microbiological and rheological properties) of the whole fruit, peel, pulp and puree of white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). The obtained results might be correlated with the results from subsequent experiments.

2.2 Research strategy

A schematic layout of the experimental set-up of this chapter is presented in **Figure 2.1**. The experimental data for the properties of the whole fruit, peel, pulp (flesh without seeds) and puree (flesh containing seeds) of the two species of dragon fruit are discussed separately. The dimension and weight of the whole dragon fruit were measured. The weight proportion

of peel, pulp and seed was also estimated based on the whole fruit. The physicochemical properties such as dry matter, pH, vitamin C content, total betacyanin content and colour parameters of dragon fruit peel were determined. Besides these attributes, a whole set of nutritional properties (e.g. protein, fat, ash, carbohydrates and total dietary fibre contents), total plate count, antioxidative properties (i.e. total phenolic content, FRAP index and DPPH value) and rheological parameters of dragon fruit pulp and puree were investigated to gain a better understanding of the characteristics of the fruit.

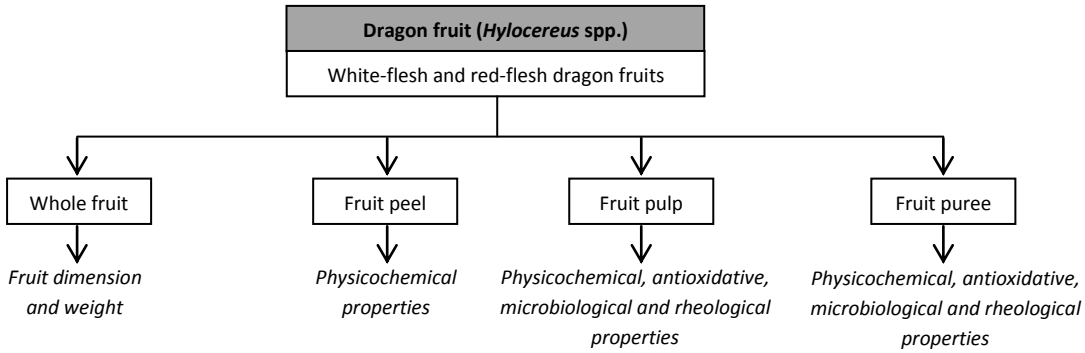


Figure 2.1 *Schematic overview of the experimental set-up to characterize the dragon fruit*

The outcome of this research may provide a clearer understanding of the characteristics of dragon fruit which could possibly have benefits for farmers, manufacturers and food scientists. Moreover, the data obtained from the current research is also needed for following experiments. The informative results of dragon fruit peel and pulp are further discussed and compared with freeze-dried dragon fruit. The experiment is mainly focusing on their utilization as a food colouring (see **Chapter 3**). The results of the dragon fruit puree are necessary as a reference (unheated) sample for a study of thermal processing of dragon fruit puree (see **Chapter 4**). Additionally, the obtained data may also be used as a base to understand the results of the further study on the possible value-added fragments from the dragon fruit such as dragon fruit seed oil and extracted cell wall polysaccharides which are discussed extensively in **Chapter 5** and **Chapter 7**, respectively.

2.3 Materials and methods

2.3.1 Preparation of dragon fruit

Two species of dragon fruit, i.e. white-flesh with red-peel (*H. undatus*) and red-flesh with red-peel (*H. polyrhizus*) originating from Thailand, were obtained from a Thai shop in Ghent, Belgium. Fruit was washed under running tap water and hand-peeled. To obtain the homogeneous dragon fruit pulp (without seeds), the dragon fruit flesh was cut into pieces and extracted by using a juice extractor (JF 2750 Fritel, Belgium). In contrast, small pieces of the dragon fruit flesh were homogenized in a food blender (BL 9170 Fritel, Belgium) for 30 sec, and then passed through a 38-mesh sieve and a 32-mesh sieve, respectively, to obtain the consistency of dragon fruit puree (with seeds). The dragon fruit samples were then stored at -18 °C until further characterization.

2.3.2 Determination of physicochemical properties

The chemical properties of the dragon fruit samples were determined according to standard methods, as described by AOAC (1995). Dry matter, ash, total dietary fibre, crude fat, protein and vitamin C contents of the samples were determined by oven drying, furnace drying, an enzymatic-gravimetric method, soxhlet extraction, kjeldahl and 2,6-dichloroindophenol titrimetric methods, respectively. In addition, carbohydrates of the dragon fruit samples were estimated by difference from the proximate composition.

The density of dragon fruit pulp and puree was measured by using a pycnometer at 20 °C after resting for 30 min with water as a reference sample (Bradley, 2010; Ramos and Ibarz, 1998). The determination of the Hunter colour parameters (L*, a* and b* values) of the dragon fruit was also carried out by a spectrophotometer colorimeter (CM-2500D, Minolta) where the L* value corresponds to lightness and varies from 0 for black to 100 for perfect white, the a* value measures redness when positive, gray when zero, and greenness when negative, and the b* value means yellowness when positive, gray when zero, and blueness when negative.

The fruit puree consists of three phases: flesh, juice and seed. The yield of each phase of the fruit puree can be determined by the modified method of Ariffin *et al.* (2009). The puree sample was centrifuged at 12000 g for 20 min. Subsequently, the weight of the supernatant phase can be used to calculate the yield of juice, as shown in **Eq. 2.1**.

$$\text{Yield of juice (\%)} = \frac{\text{supernatant weight (g) x 100}}{\text{original sample weight (g)}} \quad (\mathbf{2.1})$$

After centrifugation, the pellet was then placed into a beaker. A large amount of water was added, vigorously agitated and then sieved to separate the seeds. The recovered seeds were dried overnight at 60 °C in a hot air oven. The yield of seed can be estimated by a weight percentage of dried seed to the original sample (**Eq. 2.2**). Eventually, the yield of flesh can be simply computed by difference from the yield of juice and seed.

$$\text{Yield of seed (\%)} = \frac{\text{dried seed weight (g) x 100}}{\text{original sample weight (g)}} \quad (\mathbf{2.2})$$

All quantitative analyses of the physicochemical properties were performed with three replicates for each species of dragon fruit.

2.3.3 Determination of total betacyanin content

The total betacyanin content of the dragon fruit sample was determined in triplicate by a colorimetric method (Rebecca *et al.*, 2008; Stintzing *et al.*, 2003). The dragon fruit sample was weighted and diluted in water. The mixture was filtrated using a Whatman No.1 filter paper. The absorption values of the filtrate were measured using a spectrophotometer at a wavelength of 538 nm. Afterwards, the total betacyanin content can be estimated and expressed as mg of the betanin equivalents per 100 g of sample (fresh weight), as shown in **Eq. 2.3**. This is basically due to the fact that betanin is considered as the representative component of betacyanin pigments.

$$\text{Total betacyanin content (mg/100 g fresh weight)} = \frac{\text{Abs x DF x MW x 100}}{\varepsilon \text{ x LC}} \quad (\mathbf{2.3})$$

In **Eq. 2.3**, Abs is the absorbance value at a wavelength of 538 nm, DF is the dilution factor, MW is molecular weight of betanin (550 g/mol), ε is the molar extinction coefficient of betanin in water (60,000 l/mol.cm) and LC is the path length of the cuvette (1 cm).

2.3.4 Determination of total plate count

The total plate count was obtained using the pour plate technique on plate count agar, which is a standard method. The dragon fruit samples were taken immediately after sample preparation (**Section 2.3.1**), weighted in a sterilized plastic bag, vacuum sealed and stored at -18 °C for microbiological testing. Each sample was diluted in a peptone solution and then, one millilitre of each dilution was used for microbial count determination. The plates were inverted and incubated at 35 $\pm$ 1 °C for 48 h. Duplicates were done for each dilution. The number of micro-organisms was expressed in colony forming units per 1 g of fruit sample (CFU/g).

2.3.5 Determination of antioxidative properties

2.3.5.1 Extraction of polar compounds

The polar compounds from the dragon fruit sample were extracted using the modified method of Wu *et al.* (2006). Twenty gram of sample was extracted with 80 ml of chilled 80% acetone solution, at 4 °C, in a blender for 10 min. The slurry was vacuum filtered over a Whatman No. 1 filter paper and then, the extract (supernatant phase) was stored at -18 °C for the determination of antioxidative properties. All assays for antioxidative properties were carried out in triplicate.

2.3.5.2 Total phenolic content

Folin-Ciocalteu's reagent was used for total phenolic content determination (Lim *et al.*, 2007). An aliquot of 0.3 ml of the polar extract was added to 1.5 ml of Folin-Ciocalteu's reagent, which was diluted 10 times in distilled water, and 1.2 ml of 7.5% (w/v) sodium carbonate. The mixture solution was vortexed, covered with parafilm and left for 30 min. The total phenolic content was determined by using a spectrophotometer at a wavelength of 765 nm. The standard curve was prepared using solutions of GA. The total phenolic content was expressed in GA equivalents (mg GA/100 g fresh weight).

2.3.5.3 Ferric reducing antioxidant power

The potassium ferricyanide-ferric chloride method was used for the FRAP determination of the polar extract (Lim *et al.*, 2007). One millilitre of the polar extract was mixed with 2.5 ml

0.2 M phosphate buffer at pH 6.6 and 2.5 ml 1% potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min in a water bath, and then 2.5 ml 10% trichloroacetic acid was added. 2.5 ml of this mixture was taken and 2.5 ml water and 0.5 ml 1% $FeCl_3$ were added. The absorbance at 700 nm measured after standing for 30 min at room temperature, is the FRAP index. Phosphate buffer served as a blank solution. An increasing FRAP index value indicates an increase in the reducing power.

2.3.5.4 DPPH radical scavenging activity

The DPPH radical scavenging activity of the polar extract was spectrophotometrically quantified following the modified method of Wu *et al.* (2006) that can be used to determine the radical scavenging capacity of the sample. One millilitre of the polar extract was mixed with 4 ml of 80% ethanolic 0.6 mM of 2,2-diphenyl-1-picrylhydrazyl solution and vortexed for 15 sec. The absorbance was measured at 515 nm after standing for 3 h at room temperature. An 80% ethanol served as a blank solution. For this assay, GA solutions were prepared for the standard curve and the DPPH value was expressed in µg of GA equivalents per 1 g of fruit sample (µg GA/g fresh weight).

2.3.6 Determination of rheological parameters

Dragon fruit pulp and puree were submitted to a rheological determination. Due to the seed particles in the puree, the puree was centrifuged at 12000 g for 20 min, and then, the supernatant phase was collected and used for the rheological measurement to avoid interferences of the seeds. After that, the apparent viscosity of the (origin) dragon fruit puree can be estimated by recalculating from the apparent viscosity of the supernatant phase, as described by Einstein viscosity equation (**Eq. 2.4**). This equation gives the predicted viscosity of concentrated solutions or suspensions (Toda and Furuse, 2006).

$$\eta_p = \eta_s (1 + 2.5\varphi) \quad \textbf{(2.4)}$$

Where η_p and η_s are the apparent viscosity (mPa.s) of dragon fruit puree and supernatant phase (pulp and juice fractions), respectively, and φ is the volume fraction estimated by the ratio of the volume of the supernatant over the volume of the supernatant plus the volume

of pellet (seed fraction) in which the volume is computed by taking the ratio of weight (g) over density (g/cm^3).

Prior to rheological measurement, the dragon fruit sample was kept in a water bath at 25 °C to ensure a uniform temperature in the sample. The measurement was carried out using an AR2000 Rheometer equipped with a 28-mm conical concentric cylinder. Stepped flow curves were recorded at 25 °C within a shear rate range from 0.06 to 500 s^{-1} (Vasquez-Caicedo *et al.*, 2007). The measurement was replicated three times. The data of the rheological measurements and the suitability of the fitted model were analyzed with the supporting rheometer software (TA Rheology Advantage Data analysis software).

2.3.7 Statistical analysis

In this study, a two-sample *t*-test analysis was conducted to differentiate between the means of the two different species of dragon fruit. The reported results are expressed as mean $\pm$ standard deviation (SD) with the number of measurements (N). The significance value for all of the analyses was defined at $p < 0.05$. The statistical analyses were carried out using S-PLUS 8.0 software.

2.4 Results and discussion

2.4.1 Characteristics of whole dragon fruit

The physical properties of two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), were determined. Thirty fruits of each species of dragon fruit were selected to investigate the appearance and to determine the average dimension, weight and proportion of the fruit. The appearance of the whole and half-cut of the studied white-flesh and red-flesh dragon fruits is shown in **Figure 2.2**. The appearance of the whole white-flesh dragon fruit was similar to the red-flesh dragon fruit. Both white-flesh and red-flesh species are ovary fruit with brilliantly red peel. They have reddish scales (special leaves) with greenish at the end of the scales, whereas the scales of red-flesh dragon fruit were obviously shorter than the white-flesh dragon fruit. This unique appearance gives the fruits an interestingly exotic and oriental looks. The numerous edible small black seeds contribute with white-flesh for white-flesh dragon fruit (**Figure 2.2A**) and with red/purple-flesh for red-flesh dragon fruit (**Figure 2.2B**) were clearly observed.

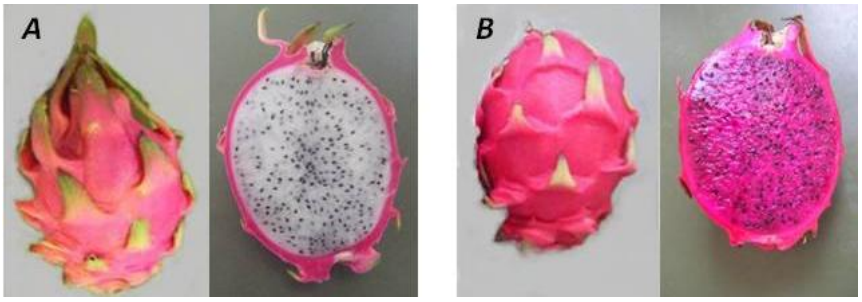


Figure 2.2 *The whole and half-cut of (A) the white-flesh dragon fruit (H. undatus) and (B) the red-flesh dragon fruit (H. polyrhizus)*

The physical properties in terms of dimension, weight and proportion of both white-flesh and red-flesh species are given in **Table 2.1**. The average length, diameter and weight of the white-flesh dragon fruit were significantly higher than that of the red-flesh dragon fruit (14 cm compared to 13 cm, 11 cm compared to 7 cm, and 445 g compared to 358 g, respectively). The results are comparable to the values found by Wichienchot *et al.* (2010).

Table 2.1 *Physical properties of the dragon fruit*

Characteristic	White-flesh dragon fruit	Red-flesh dragon fruit
Fruit dimension (cm)		
Length	14.0 ± 1.2^{a}	12.8 ± 0.9^{b}
Diameter	10.9 ± 1.7^{a}	7.1 ± 1.6^{b}
Fruit weight (g/fruit)		
Peel	108.9 ± 3.2^{a}	90.2 ± 1.0^{b}
Pulp	300.1 ± 8.8^{a}	237.1 ± 2.7^{b}
Seed	35.2 ± 1.0^{a}	30.7 ± 0.4^{b}
Whole fruit	445.1 ± 13.0^{a}	358.2 ± 4.1^{b}
Fruit proportion (%, w/w)		
Peel	24.5 ± 0.7^{a}	25.2 ± 0.9^{a}
Pulp	67.4 ± 0.7^{a}	66.2 ± 0.8^{a}
Seed	7.9 ± 0.8^{a}	8.6 ± 0.9^{a}

Values are given as mean ± SD (N=30)

and the superscripts within each row indicate significant differences ($p < 0.05$)

Additionally, the weight proportion of the peel, pulp and seed of dragon fruit was also estimated based on the weight of the whole fruit and expressed as percentage (w/w). Despite the difference in dragon fruit size, no significant differences in the ratio of the peel, pulp and seed between the two species of dragon fruit were observed. Both white-flesh and

red-flesh species contained about two-thirds of pulp (~67%) and around 8% of seeds. There was also a sticky mucilage layer between the flesh and seed of dragon fruit. The dragon fruit peel accounted for approximately 25% of the whole fruit weight. It is normally discarded during dragon fruit processing. The results are slightly different from the data found by Lim *et al.* (2010a) where dragon fruit pulp is about half and dragon fruit peel is closely to one-third of the whole fruit. This is probably due to the differences in the origin, plantation and harvesting conditions.

2.4.2 Characteristics of dragon fruit peel

Dragon fruit peel is the major by-product left over from either fresh consumption or fruit processing. It is accounted for about one-fourth of the whole fruit (**Table 2.1**). The dragon fruit peel may contain some valuable components, thus, its properties are needed to be exploited and understood. The physicochemical properties of dragon fruit peel are displayed in **Table 2.2**. The pH value (~4.7) and dry matter (7-9 g/100 g fresh weight) of both dragon fruit peels were comparable values. Vitamin C content of the red-flesh dragon fruit peel was about two and half times compared to the white-flesh dragon fruit (13 mg/100 g compared to 5 mg/100 g). No significant differences between L* (lightness) and a* (redness) values of both dragon fruit peels were observed, whereas b* value (yellowness) of the red-flesh dragon fruit peel was higher than that of the white-flesh dragon fruit.

Table 2.2 *Physicochemical properties of the dragon fruit peel*

Characteristic	White-flesh dragon fruit	Red-flesh dragon fruit
pH	4.68 ± 0.005^{a}	4.65 ± 0.004^{a}
Dry matter (g/100 g)	9.19 ± 0.24^{a}	6.55 ± 0.28^{b}
Vitamin C (mg/100 g)	5.06 ± 0.17^{a}	12.95 ± 0.09^{b}
Betacyanin (mg/100 g)	10.19 ± 0.24^{a}	19.98 ± 0.32^{b}
Colour parameter		
L* (lightness)	31.05 ± 0.28^{a}	30.48 ± 0.23^{a}
a* (redness)	17.98 ± 0.54^{a}	17.64 ± 0.72^{a}
b* (yellowness)	-2.41 ± 0.12^{a}	-0.99 ± 0.16^{b}

Values are given as mean ± SD (N=3) and the contents are based on fresh weight basis.

The superscripts within each row indicate significant differences ($p < 0.05$)

In the work, both dragon fruit peels also contained a significant amount of betacyanin (red/purple pigment). According to **Table 2.2**, total betacyanin content in the red-flesh

dragon fruit peel was, however, double the amount of the white-flesh peel (20 mg/100 g compared to 10 mg/100 g). It is suggested that dragon fruit peel of two species is a possible natural source of betacyanin. The betacyanin pigment in dragon fruit has also been identified to consist mainly of betanin, phyllocactin, hylocerenin and their derivatives (Kim *et al.*, 2011). They may be applied in food industry as a promising food colouring. To emphasize this hypothesis, both dragon fruit peels were further studied by freeze-drying and the characteristics of freeze-dried dragon fruit were examined along with the study of colour shift of the freeze-dried product. The results are discussed extensively in **Chapter 3**. Moreover, betacyanin present in dragon fruit can be simply extracted by heating at 100 °C for 5 min at pH 5 to obtain the high yield of the pigment (Harivaindaran *et al.*, 2008). However, the degradation of betacyanin through thermal treatment can naturally occur. It is possible that the existing compounds after heating are isobetanin and its derivatives. These compounds are reported to give the same wavelength colour which can be detected at 538 nm using a spectrophotometric method (the common procedure for measuring betacyanin content) (Harivaindaran *et al.*, 2008; Herbach *et al.*, 2006a; Herbach *et al.*, 2006b).

2.4.3 Characteristics of dragon fruit pulp

In this part of the current work, the characterization of dragon fruit pulp (flesh without seeds) was studied to gain a better insight into its quality. The physicochemical, microbiological and antioxidative properties of dragon fruit pulp were determined and the results are given in **Table 2.3**. The dry matter content of both dragon fruit pulps were relatively low (about 10 g/100 g), indicative of the juicy attribute of the fruit. The pH values of both white-flesh and red-flesh pulps were similar (4.4-4.6). However, they were slightly lower than that of their peel. No significant differences between density and fat content of both dragon fruit pulps were found. On the other hand, the content of protein, ash and total dietary fibre of the red-flesh dragon fruit pulp were significantly higher than that of the white-flesh pulp. The vitamin C content of the red-flesh dragon fruit pulp was also twice the amount of the white-flesh pulp (9.85 mg/100 g compared to 4.90 mg/100 g). On the contary, carbohydrates of the white-flesh dragon fruit pulp were higher than that of the red-flesh dragon fruit pulp (9.34 g/100 g compared to 8.15 g/100 g).

Obviously, the pulp of white-flesh dragon fruit showed white colour, while of red-flesh dragon fruit represented red/purple colour due to the presence of betacyanin pigment. It resulted in a marked high value of a* value (redness) in the red-flesh dragon fruit sample. According to Stintzing *et al.* (2002), betacyanin content of white-flesh dragon fruit pulp

could not be determined by a spectrophotometer due to its very low amount. Therefore, the direct comparison of colour parameters is a reasonable method for colour measurement of white-flesh pulp (Stintzing *et al.*, 2003). **Table 2.3**, total betacyanin content of the red-flesh pulp was relatively high (16.5 mg/100 g). However, it was slightly lower than that of its peel (20.0 mg/100 g). It is indicated that it probably can be applied as natural betacyanin source of food additive. Betacyanin in the studied dragon fruit is lower than that in an extracted acidic red beetroot (29 mg/100 g) (Azeredo *et al.*, 2007), known as a commercially natural source of betacyanin. In order to improve the concentration of betacyanin in dragon fruit, it is strongly recommended that the pigment could be concentrated or extracted. Moreover, the utilization of the red-flesh dragon fruit pulp as food colouring was investigated and the results are discussed in **Chapter 3**.

Table 2.3 *Characteristics of the dragon fruit pulp*

Characteristic	White-flesh dragon fruit	Red-flesh dragon fruit
pH	4.57 ± 0.006^{a}	4.40 ± 0.003^{b}
Density (g/cm^3)	1.00 ± 0.02^{a}	0.97 ± 0.02^{a}
Dry matter (g/100 g)	10.93 ± 0.24^{a}	10.01 ± 0.17^{a}
Protein (g/100 g)	0.18 ± 0.02^{a}	0.26 ± 0.004^{b}
Ash (g/100 g)	0.96 ± 0.01^{a}	1.18 ± 0.01^{b}
Crude fat (g/100 g)	0.45 ± 0.02^{a}	0.42 ± 0.02^{a}
Carbohydrates (g/100 g)	9.34 ± 0.07^{a}	8.15 ± 0.07^{b}
Total dietary fibre (g/100 g)	0.45 ± 0.03^{a}	0.87 ± 0.06^{b}
Vitamin C (mg/100 g)	4.90 ± 0.32^{a}	9.85 ± 0.53^{b}
Betacyanin (mg/100 g)	Not detected	16.53 ± 0.17
Colour parameter		
L* (lightness)	39.09 ± 0.77^{a}	26.25 ± 0.65^{b}
a* (redness)	-0.34 ± 0.06^{a}	9.15 ± 0.68^{b}
b* (yellowness)	-3.55 ± 0.13^{a}	-1.93 ± 0.42^{b}
Total plate count (10^4 CFU/g)	2.89 ± 0.15^{a}	3.51 ± 0.18^{b}
Total phenolic (mg GA/100 g)	10.21 ± 0.13^{a}	16.33 ± 0.49^{b}
FRAP index	0.3467 ± 0.0058^{a}	0.5533 ± 0.0057^{b}
DPPH (μg GA/g)	17.56 ± 0.56^{a}	22.65 ± 0.67^{b}

Values are given as mean ± SD (N=3) and the contents are based on fresh weight basis.

The superscripts within each row indicate significant differences ($p < 0.05$)

The antioxidative properties (i.e. total phenolic content, FRAP index and DPPH value) of the red-flesh dragon fruit pulp were significantly higher than that of the white-flesh pulp ($p < 0.05$). They were approximately 2 times higher than that of the white-flesh dragon fruit (**Table 2.3**). It is suggested that the antioxidative activity in the red-flesh dragon fruit is

probably related to the presence of betacyanin pigment. Betacyanin contributes to the total phenolic content due to a phenol structure in the pigment molecule (Zainoldin and Baba, 2009). Thus, the pigment can also act as an antioxidative component. The results are useful in promoting dragon fruit as a healthy fruit due to the remarkable amounts of nutritional and functional components it contains.

The microbial load of both dragon fruit pulps was comparable (~$3x10^4$ CFU/g). It is lower compared to some fresh tropical fruits, for example, mango, guava and pineapple (total plate counts $\geq 10^5$ CFU/ml) (Al-Jedah and Robinson, 2002). From a food safety point of view, the observed microbial load of fresh dragon fruit and other fresh tropical fruits may greatly depend on the fruit's own inherent quality as well as the process hygiene. Since fruits are frequently consumed fresh without having been exposed to a process that can reliably eliminate pathogens. The plantation, harvesting, pre-processing (e.g. cleaning, sorting, packing and initial storage), logistic systems and processing (e.g. peeling, cutting, juicing/pulping and filling) of the fresh fruits may affect the initial micro-organisms. Fruits from tropical areas are more susceptible to microbial contamination than those grown in subtropical or temperate climates during plantation and harvesting. Operations such as peeling remove important natural barriers to contamination. Likewise, the cutting of the fruits exposes new surface to microbial contamination and generally releases nutrients that enhance the growth of micro-organisms. Therefore, good sanitation of equipments and processing environments might be applied. Furthermore, heat process is used for nearly all thermally fruit products. The process (e.g. pasteurization and sterilization) aims to reduce the microbial load of the products and results in shelf-life expansion.

For the rheological data of the dragon fruit pulp, the plots between shear rate and shear stress as well as shear rate and viscosity of two species of dragon fruit are represented in **Figure 2.3**. The rheological profile showed that shear stress increased with increasing shear rate for both dragon fruit pulps (**Figure 2.3A**). The apparent viscosity of the pulps also decreased with increasing shear rate, particularly at the low shear rate region (**Figure 2.3B**). It showed shear-thinning behaviour like found in mango pulp (Vidal *et al.*, 2006). At high shear rate region, the white-flesh dragon fruit pulp was, on the other hand, likely to be a Newtonian behaviour fluid. The similar behaviour is also found in pineapple juice (Shamsudin *et al.*, 2009).

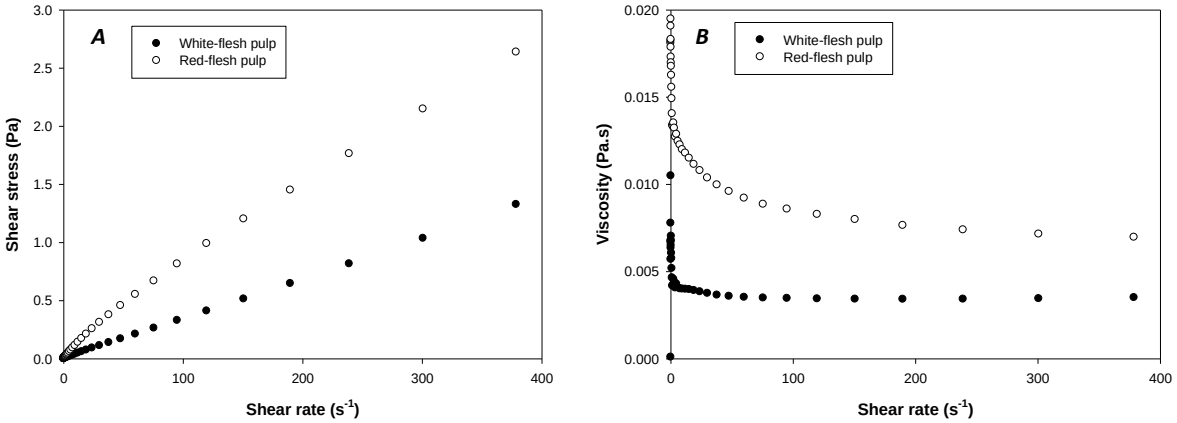


Figure 2.3 *Rheological flow curves: (A) shear rate vs. shear stress and (B) shear rate vs. viscosity of the dragon fruit pulp*

The apparent viscosity of the red-flesh dragon fruit pulp was about five times higher than that of the white-flesh pulp (17.1 mPa.s compared to 3.5 mPa.s). It is because the red-flesh pulp contains higher mucilage materials (viscous substances) compared to the white-flesh pulp, e.g. dietary fibre (**Table 2.3**), oligosaccharides (Wichienchot *et al.*, 2010) and pectin (Mahattanatawee *et al.*, 2006). Moreover, the viscosity of water, an ideal sample of Newtonian behaviour fluid, was also determined (1.0 mPa.s) and found that the viscosity of water was lower than that of both dragon fruit pulps.

Moreover, the rheological data of both dragon fruit pulps fitted very well with the power-law model, as shown in **Eq. 2.5** (Ditchfield *et al.*, 2004; Shamsudin *et al.*, 2009). The model also showed a perfect R^2 value (0.9997-0.9999) for all dragon fruit samples.

$$\tau = K\gamma^{n} \qquad (\mathbf{2.5})$$

In the power-law model (**Eq. 2.5**), τ is the shear stress (mPa) and γ is the shear rate (s^{-1}). K and n are required to characterize the flow behaviour. K is the consistency coefficient ($mPa.s^n$) that is equivalent to apparent viscosity, whereas n is the flow behaviour index (dimensionless). The 2 parameters (K and n values) from the power-law model can also be used for flow behaviour prediction. In theory, non-Newtonian foods are normally pseudoplastic-behaviour materials or shear-thinning fluids with $n < 1$, while very few foods are dilatant-behaviour materials or shear-thickening fluids with $n > 1$. The apparent viscosity

of Newtonian foods does not change when shear rate is increased thus n value equals 1. Krokida *et al.* (2001b) reported that the n value is close to 0.5 for pulpy products and near 1.0 for clear juices.

In the current study, the K values of the white-flesh and red-flesh dragon fruit pulp were 3.5 and 17.1 mPa.s^n, whilst n values showed 0.9996 and 0.8479, respectively. Therefore, it can be noted that the white-flesh dragon fruit pulp seemed to behave like a Newtonian behaviour fluid (due to the n value being close to 1). On the other hand, the red-flesh dragon fruit pulp showed shear-thinning behaviour due to a high K value (and also further refers to a high apparent viscosity) and low n value. This is possible for red-flesh dragon fruit due to their containing large amounts of mucilaginous materials.

2.4.4 Characteristics of dragon fruit puree

The characteristics of the puree (flesh containing seeds) of the two species of dragon fruit were determined in terms of physicochemical, microbiological, antioxidative and rheological properties. An overview of the results of the different physicochemical, microbial and antioxidative analyses is given in **Table 2.4**. Most of the characteristic attributes such as the yield of flesh, total dietary fibre, vitamin C content, total phenolic content and antioxidative activities, of the white-flesh and red-flesh dragon fruit purees were significantly different ($p < 0.05$). For all parameters, the values of the red-flesh puree were about two times higher compared to the white-flesh puree. On the other hand, no significant differences in seed yield, density, dry matter and crude fat content of different species of dragon fruit were found ($p > 0.05$). Hence, the yield of juice of the white-flesh puree was 17% higher than that of the red-flesh puree, suggesting the more viscous puree in the red-flesh puree. The pH value of the white-flesh puree and the red-flesh puree was similar (4.5-4.6, **Table 2.4**) and comparable to the pH value of the dragon fruit pulp (4.4-4.6, **Table 2.3**). The dragon fruit puree and pulp are considered as an acidic food, pasteurization can be further applied. Additionally, the initial microbial load of both fresh dragon fruit purees was comparable to their pulp (~2-3x10^4 CFU/g).

***Table 2.4** Characteristics of the dragon fruit puree*

Characteristic	White-flesh dragon fruit	Red-flesh dragon fruit
Yield of flesh (%)	19.86 ± 0.66[a]	37.02 ± 0.74[b]
Yield of juice (%)	79.10 ± 0.64[a]	62.07 ± 0.71[b]
Yield of seed (%)	1.03 ± 0.01[a]	0.91 ± 0.03[a]
pH	4.50 ± 0.001[a]	4.64 ± 0.001[b]
Density (g/cm^3)	1.02 ± 0.05[a]	0.98 ± 0.06[a]
Dry matter (g/100 g)	14.56 ± 0.09[a]	16.06 ± 0.13[a]
Protein (g/100 g)	0.63 ± 0.04[a]	0.83 ± 0.05[b]
Ash (g/100 g)	1.25 ± 0.001[a]	1.55 ± 0.001[b]
Crude fat (g/100 g)	0.62 ± 0.03[a]	0.57 ± 0.03[a]
Carbohydrates (g/100 g)	12.06 ± 0.04[a]	13.11 ± 0.05[b]
Total dietary fibre (g/100 g)	0.95 ± 0.01[a]	2.06 ± 0.09[b]
Vitamin C (mg/100 g)	7.32 ± 0.21[a]	15.00 ± 0.56[b]
Betacyanin (mg/100 g)	Not detected	14.82 ± 0.19
Colour parameter		
L* (lightness)	39.48 ± 0.67[a]	26.15 ± 0.29[b]
a* (redness)	0.38 ± 0.08[a]	9.13 ± 0.71[b]
b* (yellowness)	0.14 ± 0.11[a]	-2.45 ± 0.10[b]
Total plate count (10^4 CFU/g)	2.11 ± 0.12[a]	3.70 ± 0.27[b]
Total phenolic (mg GA/100 g)	18.75 ± 0.31[a]	35.82 ± 0.67[b]
FRAP index	0.3900 ± 0.0079[a]	0.5827 ± 0.0063[b]
DPPH (µg GA/g)	30.43 ± 0.07[a]	63.53 ± 0.89[b]

Values are given as mean ± SD (N=3) and the contents are based on fresh weight basis.

The superscripts within each row indicate significant differences ($p < 0.05$)

The contents of protein, fat and vitamin C in the red-flesh puree are in correspondence with the values reported by Jaafar *et al.* (2009). Other researchers found that the vitamin C content of *Hylocereus* spp. varies from 3 to 170 mg/100 g fresh weight (Lim *et al.*, 2007; Mahattanatawee *et al.*, 2006; To *et al.*, 1999; Vaillant *et al.*, 2005; Wu *et al.*, 2006) depending on the fruit species, regional and seasonal differences, harvesting period and storage time. It is also interesting to see that the crude fat content of the dragon fruit puree (with seeds) was slightly higher compared to the pulp (0.6 g/100 g vs. 0.4 g/100 g). The dragon fruit seeds are indeed suggested to contain significant amount of oil like found in berry and grape seed oils. The ash and protein contents of the studied dragon fruit puree are higher than reported for ripe mango puree (Kansci *et al.*, 2008), but the pH value of the mango (Ahmed *et al.*, 2002) is similar to the values observed for the dragon fruit puree.

The colour of the white-flesh and red-flesh dragon fruit purees was completely different, showing respectively gray and deep red/purple with a large amount of black spot coming from the seeds. As can be seen in **Table 2.4**, the L* (lightness) and b* (yellowness) colour

parameters of the white-flesh puree were significantly higher than that of the red-flesh puree, although the a* (redness) value of the red-flesh puree was significantly higher compared to the white-flesh puree due to its red pigmentation.

Betacyanin is the dominant pigment present in red and purple fruits, including red-flesh dragon fruit. In the work, the total betacyanin content of the red-flesh puree was also high (15 mg/100 g fresh weight, **Table 2.4**). However, it is lower than that of an extracted acidic red beetroot (Azeredo *et al.*, 2007). The total betacyanin content in the red-flesh dragon fruit varies from a very small amount to up to 80 mg/100 g based on wet basis. This is due to the differences of fruit species, level of fruit maturity, extraction procedures and analysis methods (Esquivel *et al.*, 2007; Harivaindaran *et al.*, 2008; Phebe *et al.*, 2009; Rebecca *et al.*, 2008). In addition, betacyanin can be easily degraded when it is exposed to oxygen, light, high temperature, and excessive acidity and alkalinity solutions (Herbach *et al.*, 2006b; Herbach *et al.*, 2007).

In the case of the antioxidative properties (**Table 2.4**), the total phenolic content, FRAP index and DPPH value of the red-flesh puree were significantly different compared to the white-flesh puree ($p < 0.05$). They were up to 2 times higher than that of the white-flesh puree. The results are similar to what Mahattanatawee *et al.* (2006) reported. The antioxidative activities of the red-flesh puree are higher compared to, for example, lychee, mango and longan, while the antioxidative activities of the white-flesh puree are lower than that of such as guava, mangosteen, papaya, starfruit and water apple (Lim *et al.*, 2007). However, the total phenolic content of strawberries (Aaby *et al.*, 2005) is eight-fold that of the studied red-flesh puree. Based on the results, the dragon fruit purees can be considered as a healthy fruit puree, particularly the red-flesh puree. This is due to a relative high nutritional values and antioxidative activity. However, it can be stated that the total phenolic content and antioxidative activities can vary depending on the fruit source.

The determination of the rheological properties of both white-flesh and red-flesh dragon fruit purees were investigated at 25 °C within a considered shear rate range and recorded as the stepped flow curves. In this case, the supernatant phase obtained from the centrifuged puree was used as the samples (instead of the origin purees) to prevent the interruption from the seed particles during measurement. The rheological behaviour of the supernatant samples showed the same behaviour of the dragon fruit pulp and the data also fitted very well with the power-law model with R^2 values greater than 0.9997 for all dragon fruit samples. Both dragon fruit purees showed shear-thinning behaviour, particularly at the low shear rate region. The viscosity of the dragon fruit puree was calculated using **Eq. 2.4**. In the equation, the volume fractions of the white-flesh and red-flesh dragon fruit purees were

determined and found to be 0.83 and 0.59, respectively. Consequently, the estimated values of apparent viscosity of these two dragon fruit purees were 10.7 and 42.3 mPa.s, respectively. The viscosity of the white-flesh puree is similar to, for example, apple, raspberry and orange (Krokida *et al.*, 2001b), whereas the red-flesh puree is similar to, for example, pineapple, guava and apricot (Krokida *et al.*, 2001b).

Since many fruits (and co-products) are considered for applications in dairy products, the activity of proteolytic enzymes either from lactic acid bacteria or from fruit is of great interest. This is due to their consequence for processing and storage. The proteolysis which is a hydrolysis reaction of proteins (caseins) into peptides and amino acids has been perfectly linked to dairy product's attributes, e.g. texture and sensory characteristics. The texture of dairy products may change due to breakdown of the protein network, whereas peptides and amino acids can taste bitter (Hassan and Amjad, 2010; Zainoldin and Baba, 2009). Some tropical fruits contain high amounts of proteolytic enzymes such as papain in papaya and bromelain in pineapple (Chye *et al.*, 2012; Ketnawa *et al.*, 2009). They probably have a significant effect on texture of the products, thus, an appropriate heating process is applied during sample preparation to avoid the hydrolytic digestion of milk protein in the products (Chye *et al.*, 2012). On the contrary, the addition of white-flesh and red-flesh dragon fruits (up to 30%) has no significant effect on the proteolysis during yogurt fermentation (Zainoldin and Baba, 2009).

Hereto, the results of the characterization for dragon fruit puree in this chapter give an idea of how the puree behaves. Accordingly, the results are very important for the study of the quality changes of dragon fruit puree during thermal processing. The obtained data is applied for the reference (unheated) sample compared with the thermally dragon fruit puree (see **Chapter 4**).

2.5 Conclusion

The work provides further insight into the important characteristics of the different fractions (the whole, peel, pulp and puree) of the two species of dragon fruit: white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). About two-thirds of the whole dragon fruit was found to be the dragon fruit pulp. The dragon fruit peel and seed (~33% of the whole fruit) are considered as by-products left over from juice processing. They are of great interest because they may contain some beneficial components. Betacyanin is the red/purple pigment present in both dragon fruit peels, particularly the red-flesh peel (10-20 mg/100 g) as well as in the pulp and puree of the red-flesh dragon fruit

(15-17 mg/100 g). The dragon fruit could possibly be applied as a promising source of natural food colouring. Its potential to be used as colouring is discussed deeply in **Chapter 3**. Significant amounts of antioxidative properties of the dragon fruit pulp and puree were observed. The antioxidative properties of the red-flesh dragon fruit were around twice the amount compared to the white-flesh dragon fruit, suggesting due to betacyanin pigment. On the other hand, the antioxidative properties of the dragon fruit puree were considerably higher compared to that of the dragon fruit pulp, especially total phenolic content and DPPH value. It is probably due to a potential antioxidative activity in the dragon fruit seeds. Thus, oil from dragon fruit seeds was extract and its characteristics is extensively discussed further in **Chapter 5**. Moreover, the dragon fruit pulp and puree showed shear-thinning behaviour, although the white-flesh dragon fruit was likely to be a Newtonian fluid at high shear rate range. The viscosity of the dragon fruit puree was higher than that of the dragon fruit pulp, particularly the red-flesh dragon fruit. This is due to the presence of mucilage materials (viscous substances) in the fruit. Therefore, the characterization of cell wall polysaccharides (pectic and hemicellulosic substances) of dragon fruit was investigated to gain better insight into a structure of dragon fruit polysaccharides. The results are addressed and discussed in **Chapter 7**.

CHAPTER 3

Characterization of Freeze-Dried Dragon Fruit

This chapter is redrafted after:

Liaotrakoon, W., De Clercq, N., Lewille, B. and Dewettinck, K. (2012) Physicochemical properties, glass transition state diagram and colour stability of pulp and peel of two dragon fruit varieties (Hylocereus spp.) as affected by freeze-drying. ***International Food Research Journal****, 19(2): 743-750.*

3. Characterization of freeze-dried dragon fruit

3.1 Introduction

Many factors (e.g. food composition of raw material, reaction of food mixture, processing conditions and the addition of food colouring) may contribute to colour, which has great effects on the sensorial characteristic and consumer preferences, of the product. In the case of food colouring, it can be either produced naturally or derived synthetically. Besides being colour function of natural pigments from plant-based foods, they also have positive health effects. It leads to an increase in consumer demand (Stintzing and Carle, 2004). In general, the natural pigments are easily degraded during processing and storage depending on several factors such as structure and concentration of the pigment, pH, temperature, light intensity, presence of metal ion, enzyme, oxygen, ascorbic acid, sugar and their degradation products (Herbach *et al.*, 2006b; Moreno *et al.*, 2008).

Despite a large number of natural pigments in various plants, dragon fruit (*Hylocereus* spp.) can be considered as a promising source of betacyanin (Phebe *et al.*, 2009; Stintzing *et al.*, 2002; Wybraniec *et al.*, 2001). Betacyanin is a group of water-soluble reddish to violet betalain pigment which has been approved to be used as an additive for food industry (Moreno *et al.*, 2008). The pigment can be found in many fruits and vegetables, for example, amaranth, beetroot, Swiss chard and cactus fruit. Currently, only red beetroot is the commercial source of betacyanin and is usually used in food industry (Delgado-Vargas *et al.*, 2000; Stintzing and Carle, 2007). Therefore, betacyanin in dragon fruit is expected to be used as an alternative food colouring with the presence of multinutritional values of the fruit (Jaafar *et al.*, 2009; Lim *et al.*, 2007; Mahattanatawee *et al.*, 2006; Wu *et al.*, 2006).

Freeze-drying is a drying method to produce a highly dried product with superior quality. Freeze-dried products normally retain high amount of nutrients and provide fresh-like properties, especially colour. This process aims to dehydrate the frozen material, which normally refers to water in food matrix, at very low temperature under vacuum condition which sublimed directly from a solid phase into a vapour phase (Khalloufi and Ratti, 2003; Marques *et al.*, 2007). Freeze-dried products are generally very sensitive to change with temperature and moisture content, thus, the thermal properties of the products should be considered. This is strongly relevant to chemical and physical changes during food processing and storage. The thermal properties are unique for each material and can be determined by using a DSC. DSC monitors heat effects associated with phase transitions and

chemical reactions as a function of temperature. The measurement provides DSC thermogram which is useful for determining T_g value. Glass transition state takes place when a glassy state changes to a rubbery state which probably is used to indicate quality and stability of food product (Marques *et al.*, 2007).

Currently, there are only few studies on the quality characteristics of tropical fruits as affected by freeze-drying. As a result, research regarding the qualities and utilization of freeze-dried dragon fruit is missing from the literature. Therefore, the aim of this research was to experimentally determine the characteristics of peel (by-products) and pulp of two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), after freeze-drying and rehydration. Moreover, the experiment is pointed on utilization of freeze-dried dragon fruit as food colouring (and possible related products). Due to pH-sensitivity of betacyanin pigments in dragon fruit, the influence of pH on colour changes of freeze-dried dragon fruit was also studied.

3.2 Research strategy

Dragon fruit has generated a lot of interest as a new source of natural red colouring. In **Chapter 2**, the results showed that dragon fruit contains a high content of betacyanin. As expected, freeze-drying is supposed to be a suitable drying method to preserve fruit's colour as well as other components of the fruit. In the current chapter, a schematic overview of the experimental set-up is presented in **Figure 3.1**. The pulp and peel of the two dragon fruit species (white-flesh and red-flesh dragon fruits) were subjected to freeze-drying. The characteristics of the freeze-dried dragon fruit samples were also determined.

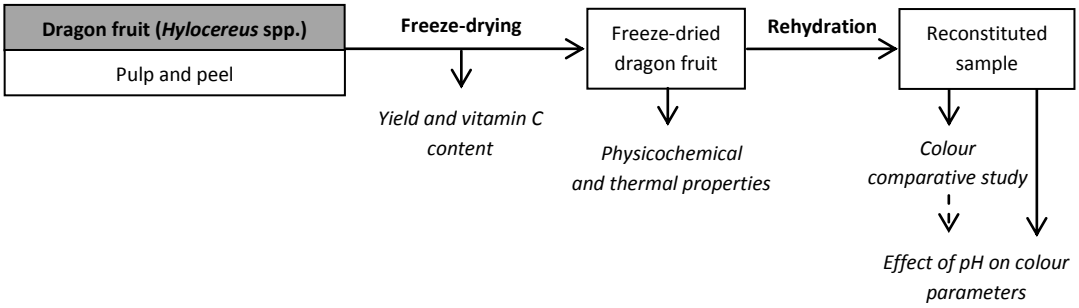


Figure 3.1 *Schematic overview of the experimental set-up to characterize the freeze-dried dragon fruit*

The yield and vitamin C content of the pulp and peel of the two dragon fruit species were monitored upon freeze-drying. After freeze-drying, the physicochemical properties (e.g. dry matter, pH, water solubility, water absorption, bulk density, vitamin C, total betacyanin and colour parameters) and thermal properties (i.e. T_g by using DSC) of freeze-dried dragon fruit samples were investigated. The results can be used to evaluate their potential for recovery of any value-added materials. To gain insight into utilization of dragon fruit as food colouring, the colour of the reconstituted dragon fruit was taken into account. The colour parameters (L*, a* and b* values) of the reconstituted sample were compared with the natural fruit. In addition, the colour shift of the reconstituted dragon fruit as affected by pH values was also investigated. It is because betacyanin is very sensitive to pH and it greatly results in colour changes.

3.3 Materials and methods

3.3.1 Preparation of dragon fruit

White-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*) originating from Thailand were used in this study. The fruit was gently washed under running tap water and the peel was removed. The homogenized pulp (without seeds) samples were obtained using a juice extractor (JF 2750 Fritel, Belgium) in which some fibre fractions may loss during this step. The dragon fruit peel was cut into small pieces and homogenized in a food blender (BL 9170 Fritel, Belgium). Prior to freeze-drying, the dragon fruit pulp and peel samples were poured into a round bottom laboratory flask (about one-third of the volume of the flask) and frozen at -40 °C for at least 48 h.

3.3.2 Freeze-drying process

Dragon fruit sample was freeze-dried by using a laboratory freeze-dryer (Heto Powerdry PL 3000 Thermo, Denmark). During processing, the total pressure and temperature inside the vacuum chamber were about 4.6 Pa and -54 °C, respectively. Average freeze-drying time was closely to 72 h for all dragon fruit samples. After stage of drying, the final product reached a final temperature of about 25 °C. Eventually, the freeze-dried product was ground by a coffee grinder, sieved through a 60-mesh sieve to obtain the uniform freeze-dried powder and stored at -40 °C in the dark until further analysis.

3.3.3 Determination of physicochemical properties

The determinations of dry matter and vitamin C contents of the sample were conducted by oven drying and 2,6-dichloroindophenol titrimetric methods, respectively, as described by the standard AOAC procedure (1995). To measure the bulk density, around 2 g of the freeze-dried sample was taken into a graduated cylinder and the bulk density was calculated by mass to volume of the sample (Goula and Adamopoulos, 2005). The water solubility and water absorption of the freeze-dried dragon fruit were also determined according to the method of Que *et al.* (2008) as follows: one gram of the sample was put in a centrifuge tube, 10 ml of distilled water was added and homogeneously mixed. Afterwards, the mixture solution was incubated in a water bath at 37 °C for 30 min and centrifuged at 3000 g for 10 min. The water absorption can be computed by the weight of pellet to original sample, as shown in **Eq. 3.1**.

$$\text{Water absorption (g gel/g)} = \frac{\text{pellet weight (g)}}{\text{original sample weight (g)}} \quad (\mathbf{3.1})$$

On the other hand, the supernatant phase was dried at 105 °C for 3 h to obtain the residue fraction and the water solubility was estimated by the weight of the residue to the original sample, as illustrated in **Eq. 3.2**.

$$\text{Water solubility (g/100 g)} = \frac{\text{residue weight (g) x 100}}{\text{original sample weight (g)}} \quad (\mathbf{3.2})$$

Moreover, total betacyanin content of the dragon fruit sample was measured using a spectrophotometer at a wavelength of 538 nm (Rebecca *et al.*, 2008; Stintzing *et al.*, 2003). It can be computed by using **Eq. 2.3** and expressed in mg per 100 g of freeze-dried sample (fresh weight). All of these physicochemical properties were performed in triplicate for each sample.

3.3.4 Determination of colour parameters and total colour change

The Hunter colour parameters (L*, a* and b* values) of the dragon fruit sample were measured in triplicate by a spectrophotometer colorimeter (CM-2500D, Minolta). Furthermore, TCC was calculated using **Eq. 3.3**, a CIE2000 colour difference formula. The equation is a correct approach to characterize the variation of colour. It appropriately investigates small to medium colour difference and fits for human observer responses (Luo *et al.*, 2001).

$$TCC = \sqrt{\left(\frac{\Delta L^*}{K_L S_L}\right)^2 + \left(\frac{\Delta C^*}{K_C S_C}\right)^2 + \left(\frac{\Delta H^*}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C^*}{K_C S_C}\right)\left(\frac{\Delta H^*}{K_H S_H}\right)} \quad (\mathbf{3.3})$$

In **Eq. 3.3**, K_L, K_C and K_H values are the parametric factors to be adjusted according to different viewing parameters of the lightness, chroma and hue components, respectively (the default values are normally set as 1.0, however, it depends on the application), whereas S_L, S_C and S_H are the weighting functions of the lightness, chroma, and hue components, respectively. R_T is an interactive term between chroma and hue differences. To compare the colour differences between natural and reconstituted samples, ΔL^*, ΔC^* and ΔH^* are the differences of L*, a* and b* colour parameters between the natural dragon fruit (reference sample) and the freeze-dried dragon fruit after rehydration (reconstituted sample). It is necessary to note here that the reconstituted sample was obtained by rehydrating the freeze-dried powder with water until it reached the same moisture content as its fresh fruit.

3.3.5 Determination of glass transition state

Glass transition state of the freeze-dried dragon fruit was monitored by using a DSC (Q1000 V9.8 Build 296, USA) with three replicates. An average of 4 mg of the freeze-dried dragon fruit pulp and 2 mg of the freeze-dried dragon fruit peel was put on an aluminium pan and perfectly sealed. The heat profile of the dragon fruit sample was programmed as follows: equilibrium to -60 °C, heated up to 100 °C, cooled down to -60 °C and reheated up to 100 °C. The constant heating and cooling rates at 20 °C/min were applied. Hereto, DSC produces heat flow (watt/g) versus temperature thermogram. Glass transition state of the freeze-dried sample was analyzed by DSC thermogram using a software universal analysis V4.5A

(TA Instruments, USA). The T_g values can be considered to consist of three T_g values: the onset (T_{go}), midpoint (T_{gm}) and endpoint (T_{ge}) values. These values as well as specific heat changes or enthalpy (ΔH) are identified as a vertical shift in the heat flow curve of the DSC thermogram.

3.3.6 Determination of colour changes at varying pH

The reconstituted sample of the freeze-dried dragon fruit was obtained after rehydration. It was placed on a pH meter with stirrer and adjusted to the pH value at 1, 3, 5, 7, 9 and 11 ($\pm$ 0.005) by titrating with a few drops of either concentrated HCl or 5 M NaOH. The colour parameters (L*, a* and b* values) of the reconstituted sample at different pH values were measured three times by a spectrophotometer colorimeter (CM-2500D, Minolta). In the meantime, the TCC value of the sample was also evaluated by using **Eq. 3.3** where ΔL^*, ΔC^* and ΔH^* are referred to the differences of L*, a* and b* colour parameters of the reconstituted sample at initial pH of the fruit compared to the sample at varying pH values.

3.3.7 Statistical analysis

The data are shown as mean $\pm$ SD. The significance differences among means of each property of four dragon fruit samples (i.e. white-flesh peel, white-flesh pulp, red-flesh peel and red-flesh pulp) were determined by analysis of variance (ANOVA). On the other hand, a paired-sample *t*-test was carried out to differentiate between the colour of dragon fruit before (natural fruit) and after processing (reconstituted sample). The significance value for all of the analyses was defined at $p < 0.05$ by using S-PLUS 8.0 software.

3.4 Results and discussion

3.4.1 Yield and vitamin C degradation during freeze-drying

Yield, an important factor in production, of the dragon fruit samples was investigated upon freeze-drying. The yield of the red-flesh dragon fruit after fruit preparation was lower than that of the white-flesh dragon fruit. On the other hand, the yield of the dragon fruit pulp of both white-flesh and red-flesh species was higher than that of the peel sample (67.7-71.5% compared to 20.3-26.7%) based on the initial weight of the fruit. It is because a large amount of peel substance was discarded during extraction to obtain peel slurry. This may

improve water solubility of the peel sample. After freeze-drying, the yield of the dragon fruit pulp was also higher than that of the peel sample (7.8-8.8% compared to ~3.5%) based on the initial weight of the fruit. It is supposed to depend on the preparation procedure. The weight loss from frozen state to freeze-dried state of the dragon fruit sample accounted up to 90%. It is suggested that almost all water present in the fruit were perfectly removed by the process.

In this work, vitamin C, a nutrition loss index, of the pulp and peel of the two species of dragon fruit was monitored during freeze-drying. Therefore, the vitamin C contents of untreated, homogenized, frozen and freeze-dried dragon fruits were determined and the results are illustrated in **Figure 3.2**. The vitamin C content of the pulp and peel of dragon fruit was on a decrease from the untreated dragon fruit to the finished product upon processing. The vitamin C retention of the homogenized, frozen and freeze-dried dragon fruits was found up to 78.0, 53.9 and 37.7%, respectively, based on their untreated dragon fruits as 100% vitamin C retention. The loss of vitamin C is possibly due to the instability of vitamin C to processing, moisture content, light and oxygen.

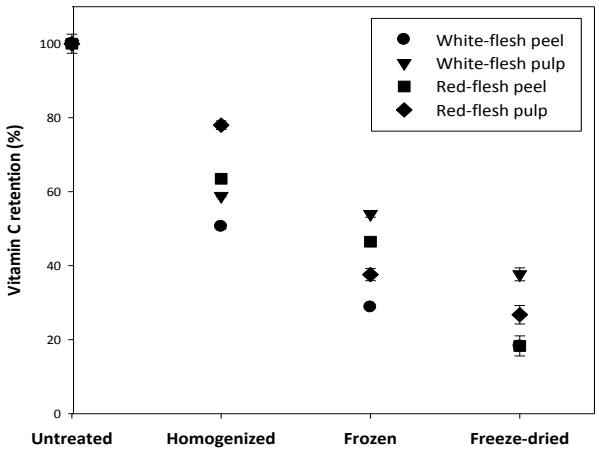


Figure 3.2 *Vitamin C retention of the dragon fruit upon freeze-drying*

However, the vitamin C content of the freeze-dried samples showed only a small decrease (counting up to 71.1% of vitamin C retention) compared to the frozen fruit before freeze-drying. It is due to low temperature and the use of vacuum in the process. The results are comparable values to previous studies, for example, freeze-dried papaya and guava (Hawlader *et al.*, 2006), freeze-dried acerola (Marques *et al.*, 2007) and freeze-dried carrot (Lin *et al.*, 1998).

3.4.2 Characteristics of freeze-dried dragon fruit

3.4.2.1 Physicochemical properties

In this work, the pulp and peel of the white-flesh and red-flesh dragon fruits were subjected to freeze-drying and their properties were determined. **Figure 3.3** shows the freeze-dried pulp and peel of the two species of dragon fruit. Obviously, both freeze-dried dragon fruit peels showed red/purple colour as well as the red-flesh pulp with brilliant red/purple colour. On the other hand, the freeze-dried white-flesh pulp showed white colour. They are greatly depending on the natural colour of the fruit.

Figure 3.3 *The freeze-dried dragon fruit: (A) the white-flesh peel, (B) the white-flesh pulp, (C) the red-flesh peel and (D) the red-flesh pulp*

The physicochemical properties of the freeze-dried dragon fruit (i.e. white-flesh pulp and peel as well as red-flesh pulp and peel) were determined and the experimental data are given in **Table 3.1**. The dry matter of all freeze-dried dragon fruit samples increased from about 10 g/100 g of fresh fruit up to 95 g/100 g of freeze-dried fruit (wet basis). It is clearly indicated that freeze-drying is an efficient drying process to eliminate water (frozen material) from fresh dragon fruit by sublimation at low temperature and pressure. However, the process had no effect to pH value of the product. The pH value of the freeze-dried samples (**Table 3.1**) was comparable with their natural fruits (**Table 2.2-2.3**). The pH values of these samples varied between 4.4 and 4.7. The vitamin C content of the red-flesh dragon fruit was considerably higher than that of the white-flesh dragon fruit (24.6-34.5 mg/100 g compared to 9.5-15.7 mg/100 g, **Table 3.1**). The higher vitamin C content was also found in the dragon fruit peel compared to the pulp sample. The results are in good agreement with the initial vitamin C content (**Table 2.2-2.3**).

Table 3.1 *Characteristics of the freeze-dried dragon fruit*

Characteristic	White-flesh pulp	White-flesh peel	Red-flesh pulp	Red-flesh peel
pH	4.51 ± 0.002^{c}	4.67 ± 0.002^{b}	4.39 ± 0.001^{d}	4.69 ± 0.006^{a}
Dry matter (g/100 g)	93.15 ± 0.64^{b}	94.63 ± 0.18^{a}	93.41 ± 0.10^{b}	95.37 ± 0.18^{a}
Vitamin C (mg/100 g)	9.52 ± 0.27^{d}	15.68 ± 0.74^{c}	24.57 ± 0.50^{b}	34.50 ± 0.72^{a}
Water solubility (g/100 g)	81.29 ± 0.66^{a}	15.15 ± 0.56^{c}	75.00 ± 0.68^{b}	10.19 ± 0.77^{d}
Water absorption (g gel/g)	0.91 ± 0.13^{c}	8.92 ± 0.32^{b}	1.39 ± 0.04^{c}	10.51 ± 0.70^{a}
Bulk density (g/cm^{3})	0.51 ± 0.01^{a}	0.12 ± 0.01^{c}	0.45 ± 0.01^{b}	0.10 ± 0.01^{d}
Betacyanin (mg/100 g)	Not detected	185.52 ± 0.15^{c}	224.98 ± 0.07^{b}	360.14 ± 0.54^{a}
Colour parameter				
L* (lightness)	84.65 ± 0.14^{a}	28.69 ± 0.50^{c}	40.14 ± 0.37^{b}	21.65 ± 0.42^{d}
a* (redness)	1.41 ± 0.06^{c}	35.88 ± 0.72^{a}	19.46 ± 0.43^{b}	34.64 ± 0.43^{a}
b* (yellowness)	10.83 ± 0.80^{a}	-5.24 ± 0.37^{b}	-8.23 ± 0.21^{c}	-8.52 ± 0.36^{c}

Values are given as mean ± SD (N=3) and the contents are based on fresh weight basis.

The superscripts with the different letters in a same row are significantly different at a probability ($p < 0.05$)

The water absorption of the freeze-dried dragon fruit peel was considerably higher compared to the pulp sample (8.9-10.5 g gel/g compared to 0.9-1.4 g gel/g), whereas the water solubility of the freeze-dried dragon fruit peel was, on the other hand, significantly lower than that of the pulp sample (10.2-15.2 g/100 g compared to 75.0-81.3 g/100 g). This suggests that the water insoluble components are present mostly in the dragon fruit peel, while the dragon fruit pulp consists mainly of water-soluble substances. The water solubility of the red-flesh dragon fruit was also slightly lower than that of the white-flesh dragon fruit. It may be suggested that the red-flesh dragon fruit contains a high amount of mucilage material which may difficult to be dissolved in water. The term water solubility can be used to indicate quality of an easy-to-use product. The bulk density of the freeze-dried dragon fruit pulp was noticeably higher than that of the peel sample (0.5 g/cm^{3} compared to 0.1 g/cm^{3}). The results are in between the bulk density of freeze-dried pumpkin (0.3 g/cm^{3}) (Que *et al.*, 2008).

Total betacyanin content of the freeze-dried red-flesh peel was the highest (360 mg/100 g), followed by the red-flesh pulp and white-flesh peel (225 and 186 mg/100 g, respectively, **Table 3.1**). It is in accordance with their initial betacyanin content (**Table 2.2-2.3**). The white-flesh pulp assumes not to contain betacyanin because it could not be detected by the procedure used. The betacyanin content of the freeze-dried red-flesh peel was much higher compared to the results found by Jamilah *et al.* (2011) (150 mg/100 g of dried powder). However, the pigment concentration in dragon fruit pulp and peel is lower compared to dried red beetroot (860 mg/100 g of dried tissue sample) (Castellanos-Santiago and Yahia,

2008). It is recommended that the concentration of betacyanin in dragon fruit may enhance by extracting and concentrating (Harivaindaran *et al.*, 2008) prior to freeze-drying.

The colour parameters (L*, a* and b* values) of both freeze-dried peel samples gave similar values (**Table 3.1**). The high a* values were apparently shown in both peel samples due to the presence of betacyanin in the fruit. On the other hand, remarkable differences between the colours of both freeze-dried dragon fruit pulps were clearly observed. The high L* and b* values of the white-flesh pulp were found, whereas the high a* value was observed in the red-flesh pulp. Additionally, the L* value of both dragon fruit peels slightly decreased from ~31 for the natural samples to 22-29 for the freeze-dried samples, while the L* value of both dragon fruit pulps, on the other hand, increased from 26-39 to 40-85. An increase of L* value was more pronounced in the white-flesh pulp. The a* value of all dragon fruit samples generally increased after freeze-drying from 18 to 35 for both dragon fruit peels, from 9 to 19 for the red-flesh pulp and from -0.3 to 1.4 for the white-flesh pulp. On the contrary, the b* value of the red-flesh pulp and both dragon fruit peel samples showed only a small decrease, while the b* value of the white-flesh pulp increased from -4 up to 11 upon freeze-drying.

Overview, the freeze-dried dragon fruit had good qualities with high vitamin C content and well-preserved colour. The results are also consistent with the results found in other freeze-dried tropical fruits, e.g. pineapple, guava, papaya and mango (Marques *et al.*, 2006). From an application point of view, the freeze-dried dragon fruit pulp can be claimed as an easy-to-use product which is possibly served as both a juice instant powder and a semi-processed fruit product. Moreover, the freeze-dried red-flesh pulp has the potential to be used in food colouring because it rich in betacyanin and easily dissolved in water. The freeze-dried dragon fruit peels also contained relatively high betacyanin content, but they were very difficult to dissolve in water. Obón *et al.* (2009) found that betacyanin in fruit of *Opuntia stricta* is successfully spray dried and the spray dried pigment can profitably be applied in food products such as yogurt and soft drink.

3.4.2.2 Glass transition state

Glass transition state of the freeze-dried dragon fruit was analyzed by DSC. The thermogram obtained from DSC measurement was drawn according to heat flow as a function of temperature. The T_{go}, T_{gm}, T_{ge} and ΔH occur during glass transition state from a glassy state to a rubbery state. These parameters of the freeze-dried dragon fruit samples can be estimated from their DSC thermogram, as shown in **Figure 3.4**. They are specific for each

material and could be used to indicate the quality of product. The obtained DSC thermogram may be helpful in developing better product made of the freeze-dried dragon fruit.

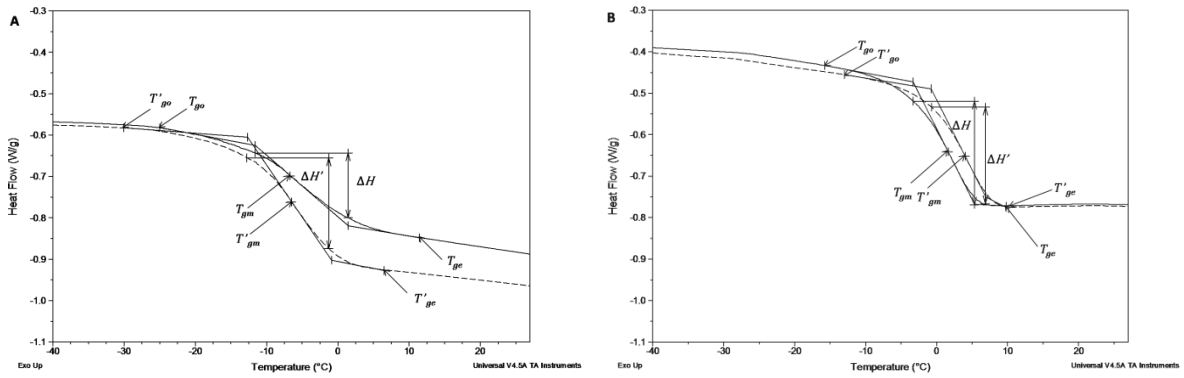


Figure 3.4 *DSC thermogram of (A) the freeze-dried dragon fruit peel and (B) the freeze-dried dragon fruit pulp. The dashed line corresponds to the white-flesh species, whereas the smoothed line represents the red-flesh species*

The average value of T_{go}, T_{gm}, T_{ge} and ΔH of triplicate analysis for the freeze-dried dragon fruit is given in **Table 3.2** where a reported value of T_g is often taken from the midpoint temperature of the glass transition. All freeze-dried dragon fruit samples were considered as a low T_g material (T_g values varied between -6.7 °C and 4.8 °C). This is because dragon fruit is rich in sugars and acids. The fruit consists mainly of malic acid (Nomura *et al.*, 2005), fructose and Glc (To *et al.*, 1999; Wichienchot *et al.*, 2010). The T_g values of these components are relatively low (-21, 5 and 31 °C, respectively) (Bhandari and Howes, 1999; Bhandari *et al.*, 1993). Furthermore, no significant differences of ΔH values in all dragon fruit samples were found in this study.

Table 3.2 *Heat flow properties at glass transition state of the freeze-dried dragon fruit*

Heat flow properties	White-flesh pulp	White-flesh peel	Red-flesh pulp	Red-flesh peel
T_{go} (°C)	-0.15 ± 1.92^{a}	-9.96 ± 3.81^{b}	-2.82 ± 1.10^{a}	-12.94 ± 1.20^{b}
T_{gm} (°C)	4.83 ± 2.11^{a}	-2.41 ± 5.79^{bc}	2.60 ± 1.69^{ab}	-6.70 ± 0.22^{c}
T_{ge} (°C)	8.15 ± 1.94^{a}	1.39 ± 3.22^{b}	5.91 ± 1.23^{a}	0.70 ± 0.93^{b}
ΔH (x 10^{-2} watt/g)	22.09 ± 0.85^{a}	22.14 ± 0.35^{a}	24.20 ± 0.97^{a}	23.39 ± 0.52^{a}

Values are given as mean ± SD (N=3) and the superscripts with the different letters in a same row are significantly different at a probability ($p < 0.05$)

Since moisture content of the sample may affect the T_g value, the moisture content of the freeze-dried dragon fruit was also determined. The moisture content of all dragon fruit samples was relatively low. The moisture content of the pulp of white-flesh and red-flesh dragon fruits was slightly higher compared to their peel samples (6.6-6.8 g/100 g compared to 4.6-5.4 g/100 g), whilst T_g value of the pulp samples was significantly higher than that of the peel samples (2.6-4.8 °C compared to -2.4 to -6.7 °C). Thus, the moisture content of the dragon fruit may not be directly related to the low and differing T_g values of the sample. The T_g value of the dragon fruit is higher compared to, for example, freeze-dried acerola (-32 °C) (Marques *et al.*, 2007), Chinese gooseberry (-57 °C) (Wang *et al.*, 2008), persimmon (-57 °C) (Sobral *et al.*, 2001) and guava (-18 °C) (Marques *et al.*, 2009). However, it is lower than mango (46 °C), papaya (37 °C) and pineapple (20 °C) (Marques *et al.*, 2009). This clearly shows that T_g value can vary from fruit to fruit depending on original source, cultivar, fruit composition and processing conditions.

The low T_g value of dried products leads to stickiness and agglomeration problems (Bhandari and Howes, 1999). Stickiness is a characteristic that probably causes the over-heating of thermal-sensitive substances. It results in product degradation during processing and storage towards undesirable sensorial characteristics of the product (Bhandari *et al.*, 1997). Both an addition of a high molecular drying aid (e.g. maltodextrin) and a reduction of moisture could be applied to enhance the T_g value of the product (Khalloufi *et al.*, 2000; Silva *et al.*, 2006; Sobral *et al.*, 2001; 2009). On the other hand, lower molecular weight solutes (e.g. sugars and acids) result in lower T_g value (Silva *et al.*, 2006).

3.4.3 Characteristics of freeze-dried dragon fruit after rehydration

3.4.3.1 Colour attribute

Betacyanin is present in both dragon fruit peels and in red-flesh dragon fruit pulp, as discussed in the previous chapter. Thus, the a* value (redness) of these dragon fruit samples is of great interest. In this work, the colour parameters (L*, a* and b* values) of the reconstituted sample were compared with the natural (untreated) dragon fruit and the results are shown in **Figure 3.5**.

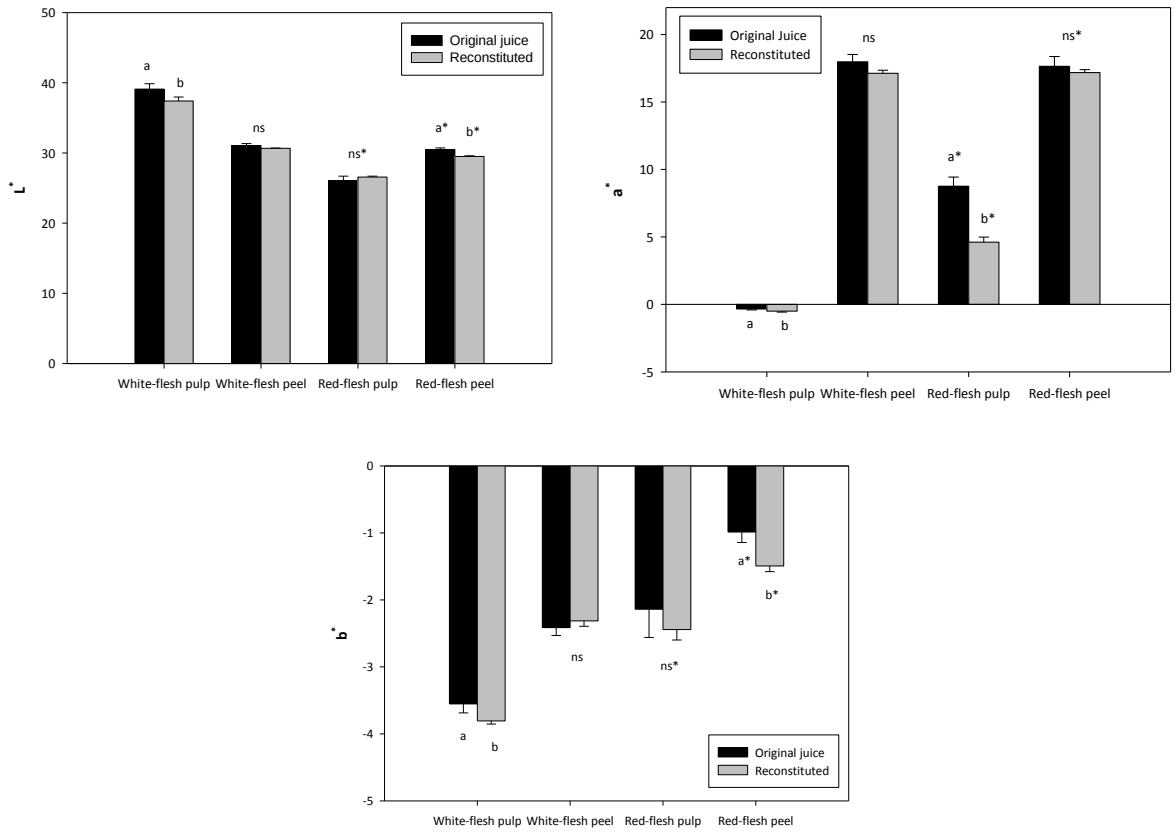


Figure 3.5 *Comparison of colour parameters (L*, a* and b* values) between the natural and the reconstituted of dragon fruit. Bar groups with difference letters indicate a significant difference ($p < 0.05$), while bars marked with ^{ns} indicate no significant differences*

No significant differences of the a* values between the natural and reconstituted samples of both dragon fruit peels were observed ($p > 0.05$), whereas the a* value of the red-flesh pulp showed a marked decrease from 8.8 for the natural samples to 4.6 for the reconstituted sample. On the contrary, there were no remarkable differences of all colour parameters of the white-flesh peel, either the natural or reconstituted samples. A small decrease of L* and b* values of the red-flesh peel between the natural and reconstituted samples was observed (30.5 compared to 29.5, and -1.0 compared to -1.5, respectively). The similar results were also found in the white-flesh pulp. All colour parameters (L*, a* and b* values) of the white-flesh pulp were on a limited decrease after rehydration (39.1 compared to 37.4, -0.3 compared to -0.5, and -3.6 compared to -3.8, respectively).

In addition, TCC values of the reconstituted sample were estimated based on the colour of natural dragon fruit. The TCC values of both dragon fruit peels were comparable and gave only a small value (0.64-0.89), whereas the TCC value of the red-flesh pulp was higher

compared to the white-flesh pulp (4.32 compared to 1.48). Obviously, the reconstituted white-flesh pulp became slightly brighter, while the other reconstituted samples became slightly darker and redder than the natural dragon fruit. However, the reconstituted freeze-dried dragon fruit showed only a small value of TCC compared to the fresh fruit sample. According to Krokida *et al.* (2001), freeze-drying is the best method to improve colour compared to convectional, vacuum, microwave and osmotic drying methods.

3.4.3.2 Influence of pH on colour shift

Due to pH-sensitivity of betacyanin, it may degrade undergo extreme pH conditions and basically results in colour changes of the pigment. In the current work, the colour changes of three reconstituted dragon fruit samples (the white-flesh peel, the red-flesh peel and the red-flesh pulp) were investigated at varying pH values ranging from 1 to 11 (interval 2). These three samples of dragon fruit are reported to contain a significant amount of betacyanin (**Table 3.1**), whereas the white-flesh pulp is excluded in this experimental work because of an absence of the pigment in the sample. The colour parameters (L*, a* and b* values) of the reconstituted dragon fruits at desired pH range are shown in **Figure 3.6**. The L* and b* values of all samples gave a small change, whereas the a* values (redness) showed a remarkable change over a whole pH range (pH 1-11). A high a* value was clearly observed and it was found to be stable over pH between 3 and 7, while a marked decrease of the a* value was visible at out of this pH range, especially at high pH values (pH 9 and 11).

The deformation and/or degradation of betacyanin may occur as affected by pH values. It can be explained by the fact that the electrophilic ammonium conjugates (a zone of weakness of betacyanin molecule) within betalamic acid and *cyclo*-Dopa-5-*O*-β-glucoside would be destroyed. It is probably that the aldimine bond of betanin and isobetanin leads to creation of betalamic acid and isobetalamic acid (bright yellow compounds), and *cyclo*-Dopa-5-*O*-β-glucoside (colourless compound) at excessive either acidity or alkalinity conditions (Herbach *et al.*, 2004b). Also, 14,15-dehydrobetanin or neobetanin (yellow pigment) possible generates from betanin and isobetanin (Herbach *et al.*, 2006a). The mechanism is underlying the red/purple to yellow transitions, particularly upon the excessive addition of alkali to a betacyanin solution (Delgado-Vargas *et al.*, 2000).

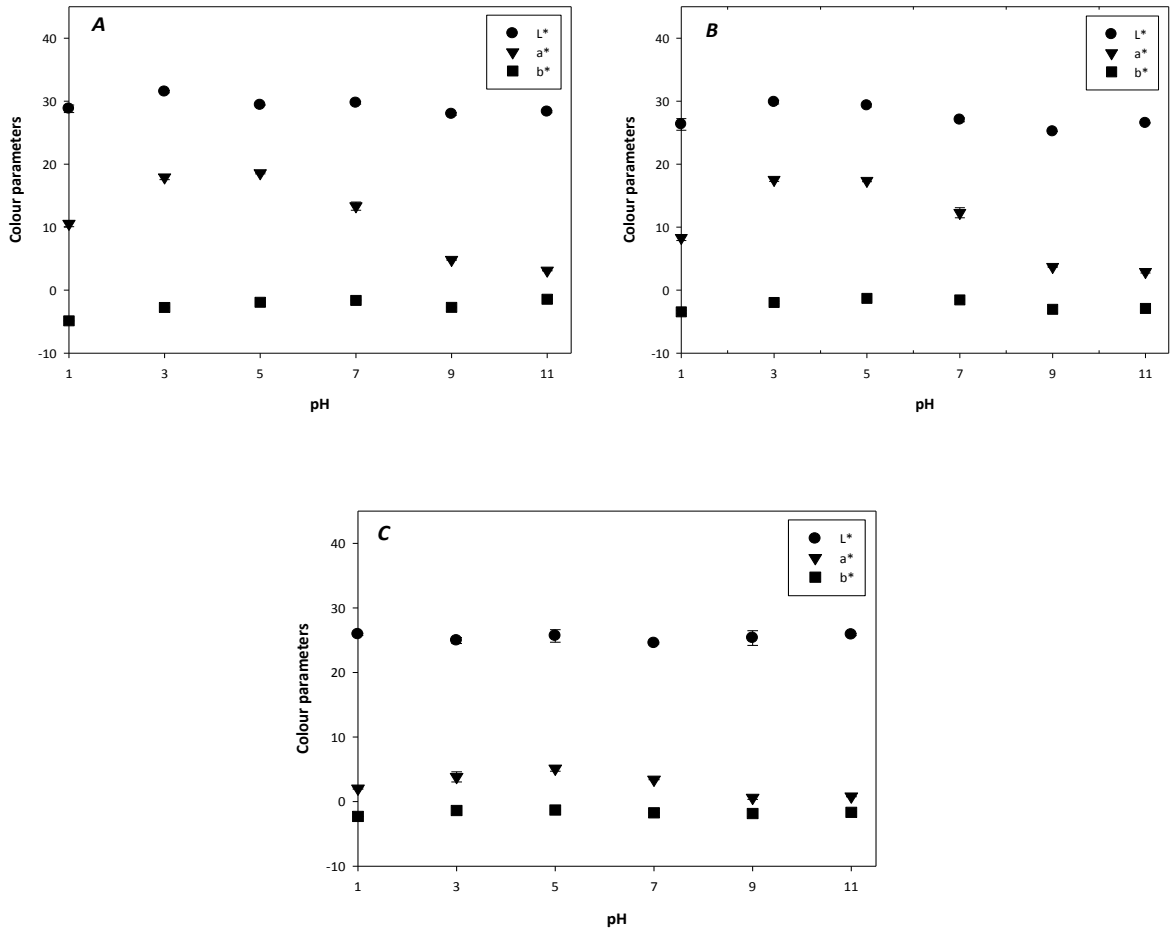


Figure 3.6 *Colour parameters at varying pH values of (A) the white-flesh peel, (B) the red-flesh peel and (C) the red-flesh pulp*

The TCC value, an index of colour stability, of the dragon fruit sample at varying pH values is illustrated in **Figure 3.7**. A small TCC value of all reconstituted dragon fruits was observed at pH between 3 and 7 (0.2-4.0), whereas the TCC value dramatically increased at extreme acid condition (pH 1) up to 7.7. It was more pronounced at high basis condition (pH 9 and 11) that the TCC value reached up to 13.0. No remarkable differences between TCC of both peel dragon fruits were observed over the studied pH range, while the colour changes of the red-flesh pulp showed the most colour stability over a whole pH range (TCC values $\leq$ 5.2) compared to both dragon fruit peel samples. It is suggested that mucilage material in the pulp may prevent the degradation of betacyanin upon aggressive pH conditions.

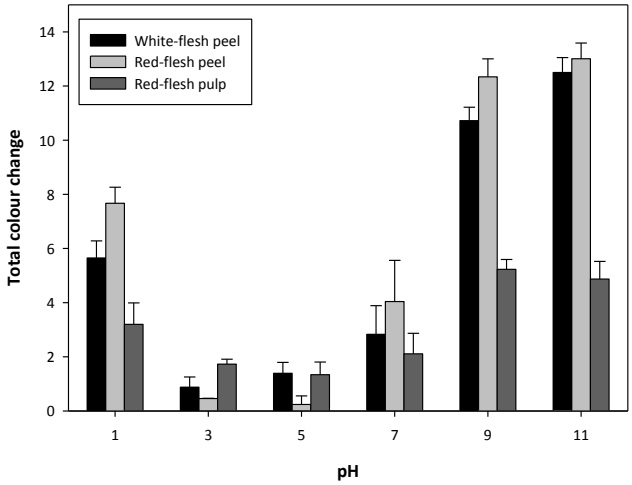


Figure 3.7 *Total colour change of the reconstituted dragon fruit as affected by pH values*

In this current work, these three reconstituted dragon fruits may show the highest red/purple shame at their natural pH values (pH ~5) and show the most colour stability at pH range between 3 and 7. Thus, they are probably suitable for application in low-acid and neutral foods which are mostly seen in food products. However, they became colourless or pink colour at strong acid condition (pH 1), whereas they became and yellow/brown at strong basis condition (pH 9 and 11). It is also noted that a reversible reaction of colour change of the samples could be observed in the experiment. In addition, the results of the TCC (**Figure 3.7**) are in accordance with their colour parameters (**Figure 3.6**) and also in good agreement with the result found by Herbach *et al.* (2006b).

3.5 Conclusion

The pulp and peel of two dragon fruit species, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), were successfully freeze-dried. The process produced the freeze-dried dragon fruits with fresh-like quality, e.g. high nutritional values and colour appearance. The freeze-dried dragon fruit pulps are an excellent source of vitamin C, well-dissolved in water and retain natural colour. On the other hand, the freeze-dried dragon fruit peels are a potential source of betacyanin, but the major disadvantage is the difficulty to dissolve in water. Due to fruit composition, all freeze-dried dragon fruits had low T_g values (below 5 °C). Additionally, no remarkable differences were found between the colours of all reconstituted samples and their natural fruits. The perfect colour stability of

the dragon fruit sample, i.e. the white-flesh peel, the red-flesh peel and the red-flesh pulp, was found to be optimum between pH 3 and pH 7, while the colour of the sample drastically changed when out of this pH range, excluding the red-flesh pulp. The colour change of the dragon fruit is due to the pH-sensitivity nature of betacyanin (red/purple pigment) which is present in the fruit.

Hereto, the freeze-dried dragon fruit may be recommended to explore its potential uses for food applications. The freeze-dried dragon fruit pulp possible to serve as an instant juice powder and a semi-processed fruit product in food industry. The freeze-dried red-flesh pulp can probably be used as a natural colouring. It is due to high betacyanin level and being water-soluble powder as well as providing colour stability over a wide pH range. However, the freeze-dried dragon fruit peel may not suitable to be applied as food colouring because it was not an easy-to-use product, even though it contained a remarkable high content of betacyanin.

CHAPTER 4

Characterization of Thermally Processed Dragon Fruit Puree

This chapter is redrafted after:

Liaotrakoon, W., De Clercq, N., Van Hoed, V., Van de Walle, D., Lewille, B. and Dewettinck, K. (2013) Impact of thermal treatment on physicochemical, antioxidative and rheological properties of white-flesh and red-flesh dragon fruit (Hylocereus spp.) purees. ***Food and Bioprocess Technology****, 6(2): 416-430.*

4. Characterization of thermally processed dragon fruit puree

4.1 Introduction

Thermal processing is an important method for fruit preservation and shelf-life prolongation due to inactivation of micro-organisms and enzymes. However, this process leads to the loss of nutrients, degradation of colour and formation of browning pigment of food items (Awuah *et al.*, 2007). Betacyanin pigments are present in dragon fruit. They are degraded by temperature, oxygen, pH and different co-factors during processing or storage (Herbach *et al.*, 2006a). Obviously, degradation results in colour changes of the processed products. Thus, the pigment compounds can probably be used for the authenticity and quality control of the processed fruit products (Fugel *et al.*, 2005). On the other hand, the heat treatment may enhance the bioavailability of antioxidative components (e.g. phenolic compounds) in some fruits and vegetables (Choi *et al.*, 2006; Kusznierewicz *et al.*, 2008; Nicoli *et al.*, 1997a; Nicoli *et al.*, 1997b; Patras *et al.*, 2009; Rickman *et al.*, 2007b; Yang and Gadi, 2008). Insights in the impact thermal processing are necessary to design new food processes for the development of safe end products with superior quality, e.g. maximum retention of nutrients and fresh-like appearance.

Dragon fruit (*Hylocereus* spp.) puree, especially red-flesh dragon fruit (*H. polyrhizus*), has been thermally processed and widely used as a semi-processed product in concentrated juice, beverage, ice cream, jam and jelly processing (Barbeau, 1993; Gibson and Nobel, 1986; Tepora, 2009). Nowadays, tropical fruits including dragon fruit are used as ingredients for innovative food products that respond to the consumers' interest (Le Bellec *et al.*, 2006; Sabbe *et al.*, 2009). So, the chemical properties and rheological parameters of fruit purees are important factors in the design of equipments for such operations.

However, no studies are available on the quality changes of dragon fruit puree during thermal processing. Therefore, the purees of two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), were submitted to a heat treatment of maximum 60 min. The temperatures varied between 50 °C and 90 °C, in steps of 10 °C. The aim of this research was to study the influence of the heat treatment on a wide variety of quality parameters. More particularly, the physicochemical, microbiological, antioxidative and rheological properties of dragon fruit purees were investigated upon thermal treatments.

4.2 Research strategy

A schematic overview of the experimental set-up of this chapter is presented in **Figure 4.1**. Fruit purees (with seeds) of white-flesh and red-flesh dragon fruits were studied in the current work. Dragon fruit purees were subjected to a thermal treatment at specific temperatures, ranging from 50 to 90 °C, for 0 to 60 min. Subsequently, the physicochemical properties (e.g. vitamin C content, betacyanin concentration, colour parameters and TCC), microbial count, antioxidative properties (i.e. total phenolic content, FRAP index, DPPH value and browning formation pigment value) and rheological parameters of the processed purees were determined as a function of thermal treatment. The properties of the unheated puree (see **Section 2.4.4**) were determined to have reference data.

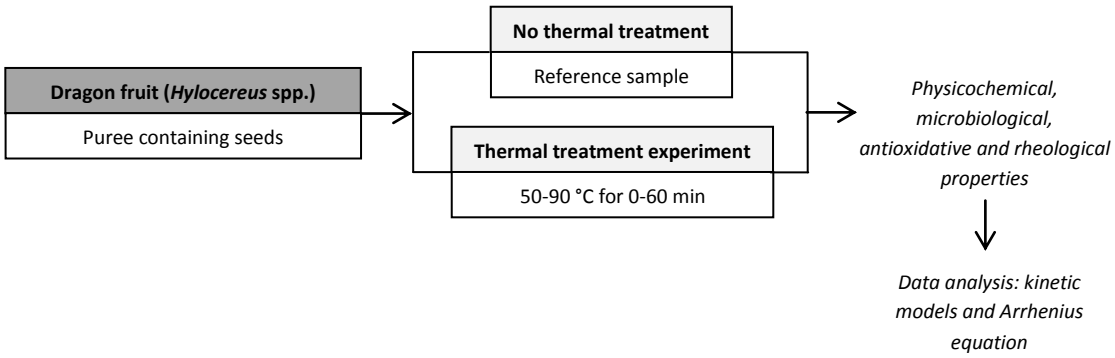


Figure 4.1 *Schematic overview of the experimental set-up to characterize the thermally processed dragon fruit puree*

Moreover, various kinetic models (i.e. the zero-, first- and second-order models) were used to analyze changes of dragon fruit properties (i.e. degradation of colour and betacyanin as well as the changes of antioxidative properties) during thermal processing. In addition, the Arrhenius equation allowed computing the E_a value. The outcome of this research gives an overview of the behaviours of dragon fruit puree during thermal processing. So, the results probably can be further applied in dragon fruit processing to obtain high quality products.

4.3 Materials and methods

4.3.1 Preparation of dragon fruit puree

Two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*) originating from Thailand, were investigated separately. After cleaning and peeling, the flesh of dragon fruit was cut into small pieces and homogenized in a food blender (BL 9170 Fritel, Belgium) for 30 sec. The homogenized sample was then passed through a 38-mesh sieve and a 32-mesh sieve, respectively, to obtain a puree product of around 1.25-mm size of uniform consistency. The dragon fruit puree, containing the seeds, was then immediately submitted to a thermal treatment.

4.3.2 Thermal treatment

The studied white-flesh and red-flesh dragon fruit purees were submitted to the thermal treatment at selected temperatures (50 °C, 60 °C, 70 °C, 80 °C and 90 °C) for 0-60 min, in steps of 10 min. About 150 g of the dragon fruit puree was poured into a 250-ml sterile glass container and covered with a sterile plastic screw cap. For each temperature, eight bottles with puree were prepared. Seven bottles were used as the sample for different heating times (at 0, 10, 20, 30, 40, 50 and 60 min) and a fixed thermometer in another one bottle was used to monitor temperature thought the experiment. The containers were then placed in a thermostatic water bath (MP-Basis, Julabo, Germany) at desired temperatures and agitated by magnetic stirrer to ensure a uniform temperature profile during thermal treatment. The puree was heated for 0, 10, 20, 30, 40, 50 and 60 min, respectively, after reaching the desired temperature. A heating time of 0 min corresponds with a puree that was heated from room temperature to the preset temperature. When the desired temperature was reached, the bottle was instantly taken out and immediately cooled in an ice-water bath to stop the reactions. All samples were stored at -18 °C prior to further study, while the samples for phenolic and antioxidative properties assays were directly extracted before storing them at -18 °C.

4.3.3 Determination of chemical properties

The chemical properties of the dragon fruit puree were determined according to standard AOAC methods (1995). Dry matter of the sample was determined by using oven drying at 105 °C for 3 h and expressed in g/100 g fresh weight. Total dietary fibre of the dragon fruit

sample was performed by enzymatic-gravimetric assay using heat stable α-amylase, protease and amyloglucosidase, respectively. The content of ash, protein and vitamin C of the puree sample was also analyzed by furnace, kjeldahl and 2,6-dichloroindophenol titrimetric (using a standard ascorbic acid solution) methods, respectively. Carbohydrate content of the sample was calculated by difference from the proximate composition. In addition, total betacyanin content of the puree sample was determined by using a spectrophotometer at a wavelength of 538 nm (Rebecca *et al.*, 2008; Stintzing *et al.*, 2003). The total betacyanin content was expressed in mg/100 g fresh weight and further calculated as percentage of retention. All analyses of chemical properties were performed in triplicate.

4.3.4 Determination of colour parameters and total colour change

The L*, a* and b* colour parameters of the heated purees were measured five times by using a spectrophotometer colorimeter (CM-2500D, Minolta). In this chapter, TCC was also calculated using **Eq. 3.3** (Luo *et al.*, 2001) where ΔL^*, ΔC^* and ΔH^* are the differences of L*, a* and b* colour parameters between the unheated dragon fruit (reference sample) and the thermally processed dragon fruit sample at different heating temperatures.

4.3.5 Determination of microbiological property

The total plate count was performed using the regular pour plate technique on plate count agar. The puree sample was diluted to 10^{-1}, 10^{-2} and 10^{-3} in peptone solution. One millilitre of each dilution was pipette and poured with agar for microbial count measurement. The inverted plates were incubated at 35 $\pm$ 1 °C for 48 h. Duplicates were done for each dilution and the total plate count was expressed in CFU/g.

4.3.6 Determination of antioxidative properties

The polar compounds of the dragon fruit puree was extracted with the chilled acetone using the modified method of Wu *et al.* (2006). After that, the acetone extract was then subjected to determination of antioxidative properties as follows: total phenolic content was conducted by the Folin-Ciocalteu's method and expressed in GA equivalents (mg GA/100 g fresh weight) (Lim *et al.*, 2007), FRAP index was determined according to the potassium ferricyanide-ferric chloride method (Lim *et al.*, 2007), and the DPPH radical scavenging activity of the samples was also spectrophotometrically quantified by using the modified

method of Wu *et al.* (2006) and expressed in GA equivalents (µg GA/g fresh weight). The details of these analyses are explained in **Section 2.3.5**. Moreover, browning formation pigment index was also carried out. The acetone extract was diluted in 80% acetone to obtain the absorption value between 0.1 and 0.8 by using a spectrophotometer at a wavelength of 420 nm (modified from Kusznierewicz *et al.*, 2008).

4.3.7 Determination of rheological properties

In the work, it was impossible to measure the apparent viscosity of the dragon fruit puree directly because the puree contains a lot of seed particles that might interrupt the viscosity measurement. Prior to the rheological determination, the heated puree sample was centrifuged at 12000 g for 20 min. The supernatant phase was then collected to perform the rheological measurement. Consequently, the viscosity of the dragon fruit puree can be calculated based on the properties of the supernatant phase of the heated puree by using **Eq. 2.4**. Before measuring, the supernatants were kept at 25 °C to ensure a uniform temperature. The rheological measurements were done using an AR2000 Rheometer equipped with a 28-mm conical concentric cylinder. Stepped flow curves were recorded at 25 °C within a shear rate range from 0.06 to 500 s^{-1} (Vasquez-Caicedo *et al.*, 2007). The measurements were done in triplicate. The data of the rheological measurements and the suitability of the fitted model, the power-law model (**Eq. 2.5**), were analyzed with the supporting rheometer software (TA Rheology Advantage Data analysis software).

4.3.8 Data analysis

The kinetics of the colour changes, degradation of betacyanin and changes of antioxidative properties of the heated dragon fruit puree during thermal treatments were analyzed. The zero-order kinetic model (**Eq. 4.1**) (Kaymak-Ertekin and Gedik, 2005), the first-order kinetic model (**Eq. 4.2**) (Ahmed *et al.*, 2002) and the second-order kinetic model (**Eq. 4.3**) (Kaymak-Ertekin and Gedik, 2005) were employed to find the best fit kinetic model through a series of the experimental data. In these equations, A is measured value of the heated dragon fruit puree, A_0 is measured value of the unheated dragon fruit sample, k is the rate constant (min^{-1}) and t is heating time (min).

The zero-order kinetic: $$A = A_0 - kt \quad (\mathbf{4.1})$$

The first-order kinetic: $$\ln \frac{A}{A_0} = -kt \quad (\mathbf{4.2})$$

The second-order kinetic: $$\frac{1}{A} = \frac{1}{A_0} + kt \quad (\mathbf{4.3})$$

Hence, the temperature dependence of all reaction rate constants was determined using the Arrhenius equation (**Eq. 4.4**) (Ahmed *et al.*, 2002). Where k_0 is frequency or pre-exponential factor (min^{-1}), E_a is activation energy (kJ/mol), R is universal gas constant (8.314 J/mol.K) and T is absolute temperature (K).

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \quad (\mathbf{4.4})$$

In theory, an accurate determination of E_a can be calculated from the Arrhenius equation. It requires at least 3 runs completed at different reaction temperatures and the temperature intervals should be at least 5 °C. In this current study, it contains 5 different reaction temperatures and temperature interval is 10 °C, then the data is ensure to susceptible to the accurate procedure.

4.3.9 Statistical analysis

The results are reported as mean ± SD. The significance of differences among treatment means was determined by ANOVA with 5% level of significant ($p < 0.05$) using S-PLUS 8.0 software. Hence, linear correlations from regression analysis between the parameters were also analyzed with the same software.

4.4 Results and discussion

4.4.1 Influence of thermal treatment on chemical properties

In the work, the puree was warmed up from room temperature (~20 °C, the unheated sample) to the desired temperature (the heated sample at 0 min), it was pre-heating process. After that, it was heated at the set temperature for different heating times at maximum 60 min. The time necessary to warm up the puree varied depending on the heating temperature, however, it was between 8 and 15 min to reach the set temperatures at 50-90 °C. The properties of the unheated puree, the heated puree at time 0 min and the heated puree at time 10-60 min (interval 10 min) are clearly shown in order to investigate the effect of the pre-heating and heating processes on properties of the puree.

The chemical properties of the heated puree were evaluated as function of time and temperature. The dry matter and pH of all dragon fruit purees were not observably changed during heating due to the close system experiment. The dry matter of the heated samples was about 14-16 g/100 g through the heating treatments. The pH values of the heated white-flesh and red-flesh dragon fruits were also comparable values (4.5-4.8). Due to being an acid food, the dragon fruit puree can be pasteurized for product safety.

Vitamin C is normally used as a nutrient index for thermal processing. The vitamin C content was determined and the percentage of vitamin C retention was calculated by considering the unheated dragon fruit puree as 100% vitamin C retention. The percentage of the vitamin C retention of the white-flesh and red-flesh purees are represented in **Figure 4.2** and the value at time 0 min corresponds with time that the dragon fruit puree reached the preset temperature. The depletion of vitamin C of the puree occurred during pre-heating from temperature of the unheated sample to the preset temperature. It was more pronounced at the high temperature region.

During thermal treatment, the vitamin C retention of the white-flesh puree remained stable at a level of about 20% after 20 min of heating at different temperatures, whereas vitamin C of the heated red-flesh puree decreased continuously during heating. An increase temperature and time corresponds with decreasing of the vitamin C content due to heat sensitivity of vitamin C. After 60 min heating at 50 °C, 33% of the vitamin C of the red-flesh puree was retained, but at higher temperatures, the vitamin C retention was less than 11%. Comparing the vitamin C retention of the two species of dragon fruit after 90 °C for 60 min, it was about two times higher in the white-flesh puree than that of the red-flesh puree. These observations are in accordance with the vitamin C degradation reported by other authors who found that the thermal processing of various food products is causing a

decrease of heat-sensitivity nutrients like vitamin C as illustrated for papaya puree (Parker *et al.*, 2010), apple puree (Picouet *et al.*, 2009) and tomato puree (Rickman *et al.*, 2007b; Zanoni *et al.*, 2003).

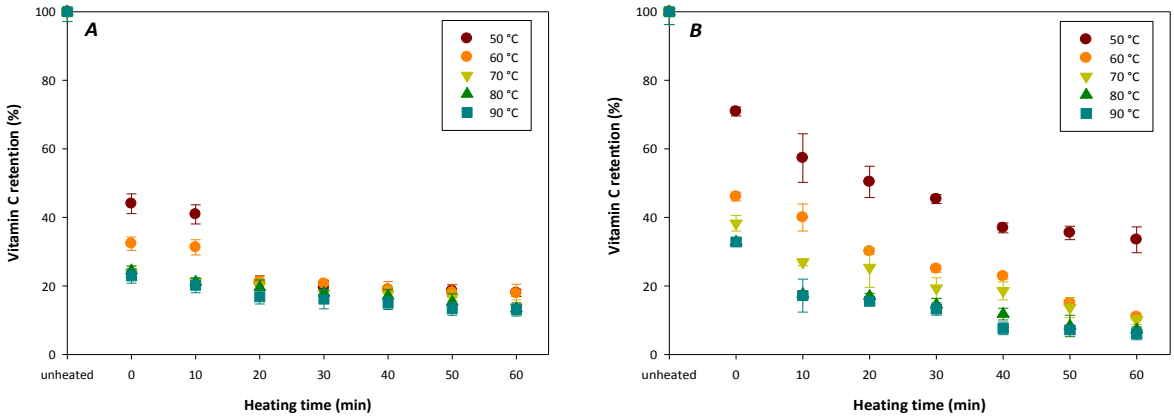


Figure 4.2 *Vitamin C retention vs. heating time at different heating temperatures: (A) the white-flesh dragon fruit puree and (B) the red-flesh dragon fruit puree*

The influence of heat treatment on total dietary fibre, an important component in plant-based food, at different temperatures after 60 min in the white-flesh and the red-flesh purees was also determined. **Table 4.1** shows the results of total dietary fibre in the unheated and heated dragon fruit purees.

Table 4.1 *Influence of heating temperatures on total dietary fibre of the dragon fruit puree*

Thermal treatment	White-flesh puree	Red-flesh puree
Unheated	0.95 ± 0.01^{a}	$2.06 \pm 0.09^{a*}$
50 °C	1.19 ± 0.20^{a}	$2.09 \pm 0.05^{a*}$
60 °C	1.13 ± 0.03^{a}	$2.04 \pm 0.18^{a*}$
70 °C	1.20 ± 0.04^{a}	$2.33 \pm 0.01^{a*}$
80 °C	1.84 ± 0.10^{b}	$2.10 \pm 0.10^{a*}$
90 °C	1.98 ± 0.09^{b}	$3.00 \pm 0.03^{b*}$

*Given values are total dietary fibre content (g/100 g fresh weight) as mean ± SD (N=3), the superscripts within each column are significantly different ($p < 0.05$) and * indicates the significance among treatments of the red-flesh dragon fruit puree*

According to **Table 4.1**, the amount of total dietary fibre of the white-flesh puree at 80 °C and 90 °C increased significantly ($p < 0.05$) compared to the other heated white-flesh purees, while the total dietary fibre content of the red-flesh puree after heat treatment at 90 °C increased significantly ($p < 0.05$) among all heated red-flesh purees. After 90 °C, the total dietary fibre content of the white-flesh and red-flesh purees remarkably increased from 0.95% to 1.98% and from 2.06% to 3.00%, respectively. Additionally, the term dietary fibre can be defined as the edible parts of plant-based foods that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Total dietary fibre contains both soluble and insoluble fibres in which it is suggested to include polysaccharides, oligosaccharides, lignin and associated plants substances. Analytically, the dietary fibre in plant sample is digested by α-amylase, protease and amyloglucosidase, respectively. This enzymatic procedure simulating human digestive systems is the basis for determination of total dietary fibre by the enzymatic-gravimetric method (Mehta, 2009).

An increase in dietary fibre in thermally product is related to changes in cell walls during heating. Cell wall materials are added to food product to increase their dietary fibre content, but they can be degraded by thermal processing. The changes occurring during the processing are determined by mechanical and thermal stresses and by its composition (Kunzek *et al.*, 1999). An increase in dietary fibre content during processing is also previously observed for wheat, rice, mung bean and soy bean (Azizah and Zainon, 1997), mashed potato and french fries (Thed and Phillips, 1995) and carrot (Rickman *et al.*, 2007a). So, it may be concluded that a high temperature and long time thermal treatment might probably enhance the accessibility of the total dietary fibre. In contrast, Azizah and Zainon (1997), however, reported that the total dietary fibre of groundnut decreases after heat treatment.

4.4.2 Influence of thermal treatment on colour and total betacyanin content

As the colour of the processed foods is an important factor for consumer acceptability, colour degradation during heat treatment has been widely studied. Several authors (Ahmed *et al.*, 2002; Ahmed *et al.*, 2004; Chutintrasri and Noomhorm, 2007) have studied the colour parameters (L*, a* and b* values) while working on thermal processing of pureed foods. They reasoned that all the three colour parameters can be used to describe the colour degradation of pureed foods during heating. In the work, three colour parameters (L*, a* and b* values) and TCC value of the dragon fruit puree were evaluated. The heating time and temperature affected the colour of the purees. During heating, both dragon fruit purees

became browner and more pronounced when they were heated for a longer time at higher temperatures. All colour parameters (L*, a* and b* values) of the puree samples were showing an upward trend during heating, excluding a* value of the white-flesh puree that it was on the decrease. **Figure 4.3** represents TCC as a function of processing time for the two dragon fruit purees. The TCC values of all puree samples considerably increased during pre-heating from the unheated samples to the set temperature (the heated puree at 0 min), particularly at high temperature region. The total betacyanin content of the red-flesh puree was determined and its retention at different heating treatments is shown in **Figure 4.4**.

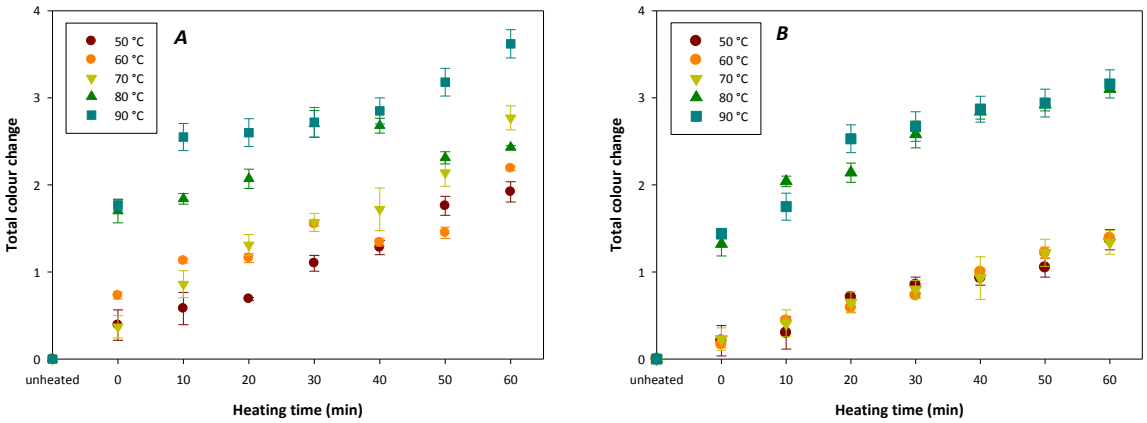


Figure 4.3 *Total colour change vs. heating time at different heating temperatures: (A) the white-flesh dragon fruit puree and (B) the red-flesh dragon fruit puree*

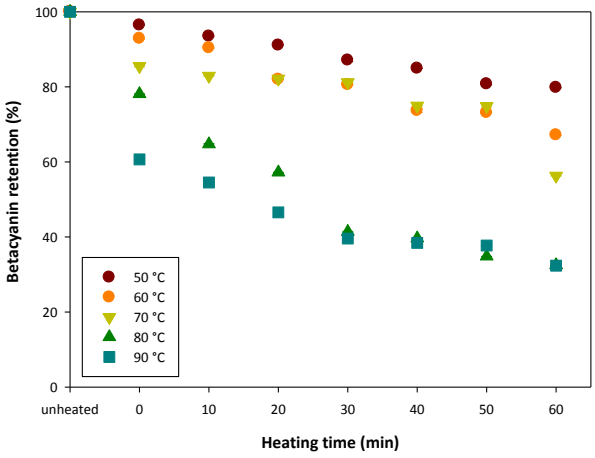


Figure 4.4 *Retention of betacyanin content of the red-flesh dragon fruit puree vs. heating time at different heating temperatures*

As shown in **Figure 4.4**, the retention of total betacyanin content of the red-flesh puree was calculated by using the unheated red-flesh puree as 100% betacyanin retention, whereas the amount of betacyanin of the white-flesh puree was too small to be used as a parameter during the thermal treatment. Betacyanin content of all heated red-flesh purees decreased with heating time and temperature. The retention of total betacyanin content after heating at 50 °C to 90 °C for 60 min varied from 80% to 32%, respectively, so the higher temperature and time resulted in more betacyanin degradation. The heating at temperatures higher than 80 °C resulted in more drastic colour changes and betacyanin content compared to the lower temperature regions. The TCC of both purees and betacyanin content of the red-flesh puree during thermal treatment could be clearly divided into 2 groups, at low temperature (50 °C to 70 °C) with gradual change and at high temperature (80 °C and 90 °C) with substantial increase. It was more pronounced in the case of the red-flesh puree due to the sensitivity of red/purple pigment (betacyanin) in the fruit. The betacyanin content was strongly influenced at temperatures higher than 80 °C. Up to 70 °C, 56% of the betacyanin were retained. At temperatures higher than 80 °C, a more drastic influence was observed as 32% of the original amount of betacyanin was left. It can be explained by the fact that betacyanin is heat-sensitivity compounds due to the instability of the pigment structure. Also, it is possible that the mucilage components in red-flesh dragon fruit puree may contribute to minimize betacyanin degradation upon a heat treatment. However, some mucilage materials in the puree may be destroyed at high temperature and it probably results in low betacyanin content (Herbach *et al.*, 2006b; Herbach *et al.*, 2007).

Moreover, the correlation between colour parameters, TCC and betacyanin of the heated puree was estimated and it is given in **Table 4.2**. The L* and b* values of both purees strongly influenced the TCC during heating that can be described by a correlation equation with a relative high coefficient (R^2 values were between 0.7733 and 0.8680), while the a* value was only slightly changed with no relation to the TCC. Therefore, the L* and b* values may be used to indicate the colour degradation for the heated dragon fruit puree. It has been previously reported in other tropical fruit purees, for example, the L* parameter may be used for quality control of pineapple puree (Chutintrasri and Noomhorm, 2007), while the b* value was used for mango puree (Ahmed *et al.*, 2002). This greatly depends on pigments in fruit purees.

Table 4.2 *Regression equation and correlation coefficient (R^2) of colour parameters (L*, a* and b* values), TCC and betacyanin of the dragon fruit puree*

White-flesh dragon fruit puree		**Red-flesh dragon fruit puree**	
Correlation equation	***R^2***	***Correlation equation***	***R^2***
TCC= 1.2431L* - 48.8343	0.7733	TCC= 1.0843L* - 28.1452	0.8680
TCC= -1.8079a* + 1.0875	0.3867	TCC= 0.6780a* - 5.6910	0.3028
TCC= 1.1328b* + 0.1134	0.8445	TCC= 0.8515b* + 2.6701	0.8122
		TCC= -3.1524betacyanin + 14.8147	0.9442

In the case of the red-flesh puree, it was also observed that the TCC increased through thermal treatment, while betacyanin content decreased. Dependence of the TCC on the betacyanin content was described using the linear relationship. The degradation of betacyanin showed high and negative correlation with TCC (R^2 = 0.9442, **Table 4.2**). Betacyanin pigments, being most heat-sensitivity, may preferably be used as an index of food product quality during thermal processing. The excellent linear correlation between the TCC and betacyanin content of the red-flesh puree indicates that the TCC can probably be used to evaluate the betacyanin content of the puree during heating process. The results are in agreement with the results found by Ahmed *et al.* (2004) that anthocyanin pigment and TCC may preferably be used as an index of product quality for plum puree.

The kinetic of colour changes and betacyanin degradation of the dragon fruit puree was also analyzed. It was found that the second-order kinetic model (**Eq. 4.3**) was the best fitted for TCC and degradation of betacyanin of both dragon fruit purees during thermal treatment. The E_a was also estimated by plotting the natural logarithm of the rate constant against the reciprocal of the respective temperature (the Arrhenius equation: **Eq. 4.4**). The E_a is a measure for the temperature dependence of the rate constants. A high absolute value of E_a signifies the rate constants depend strongly on temperature. **Table 4.3** shows the kinetic parameters (i.e. rate constant, activation energy, pre-exponential factor of the Arrhenius equation and coefficient of correlation) corresponding to the five temperatures. The magnitudes of the E_a value give an indication of the heat sensitivity of the TCC and betacyanin content of the dragon fruit puree during thermal processing. The TCC and betacyanin were accelerated by temperature, particularly at high temperatures. The computed values of E_a for the TCC of the white-flesh and red-flesh purees were 44.8 and 83.2 kJ/mol, respectively. This is indicated that the TCC of the red-flesh puree has more sensitive to the change of heating temperatures between 50 °C and 90 °C than that of the white-flesh puree. This could be explained by the fact that the red-flesh puree contains more heat-sensitivity compounds (e.g. betacyanin) compared to the white-flesh puree. The

results are higher compared to the result found for TCC of mango puree (36.8 kJ/mol) during heating from 50 °C to 90 °C (Ahmed *et al.*, 2002).

Table 4.3 *Second-order kinetic parameters for total colour change and betacyanin content of the dragon fruit puree during heat treatment at five temperatures*

Parameter	Temperature (°C)	k (min^{-1}) x 10^{-2}	R^2	E_a (kJ/mol)	k_0 (min^{-1})	R^2
White-flesh puree						
TCC	50	1.59	0.8340	44.77	9.35 x 10^8	0.7036
	60	1.00	0.7705			
	70	1.47	0.8352			
	80	0.26	0.6544			
	90	0.31	0.9219			
Red-flesh puree						
TCC	50	2.11	0.9360	83.17	5.88 x 10^{14}	0.7321
	60	3.65	0.9627			
	70	2.16	0.8357			
	80	0.17	0.7579			
	90	0.13	0.8087			
Betacyanin	50	0.25	0.9917	38.50	4.17 x 10^3	0.7595
	60	0.47	0.8704			
	70	0.54	0.7458			
	80	0.49	0.6964			
	90	1.79	0.9439			

Moreover, the computed value of E_a for betacyanin degradation of the red-flesh puree was estimated (38.5 kJ/mol). The results are comparable with the result found for degradation of anthocyanin pigment present in plum puree (37.5 kJ/mol) during thermal processing from 50 °C to 90 °C (Ahmed *et al.*, 2004).

4.4.3 Influence of thermal treatment on microbiological property

The number of micro-organisms is an important factor in thermal processing as it can be used to evaluate the product safety. The total plate counts of the heated purees at the different temperatures during the heat treatment were determined. **Figure 4.5** shows the changes of microbial count of both dragon fruit purees at different heating temperatures (50-90 °C) for maximum 60 min. As expected, an increase of temperature and time resulted in a decrease of the microbial count of both dragon fruit purees. The microbial load of all heated puree samples decreased during pre-heating, especially at high temperature regions

(80-90 °C). After 60 min of heating, all the dragon fruit purees contained less than 7 x 10^2 CFU/g compared to the unheated purees (~3 x 10^4 CFU/g). The results were more pronounced at the high temperature and prolonged time that at temperature greater than 80 °C for more than 50 min, the number of microbial count was less than 80 CFU/g. The microbial count, however, depleted down to 15 CFU/g after 90 °C for 60 min.

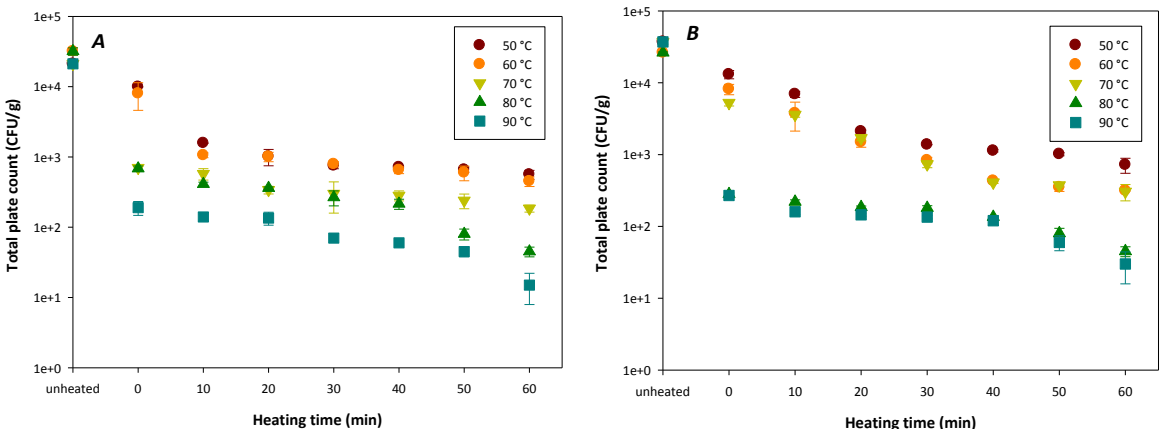


Figure 4.5 *Changes of microbial count vs. heating time at different heating temperatures: (A) the white-flesh dragon fruit puree and (B) the red-flesh dragon fruit puree*

Additionally, the dragon fruit purees are ordinarily used as a semi-processed fruit product for a wide variety of products such as beverage and confectionary producing, so usually an extra heating step is applied during the processing of such these products. It has been shown that the microbial count in banana puree after pasteurization is sufficiently reduced (Ditchfield *et al.*, 2006). Some non-pathogenic micro-organisms in papaya puree are also eliminated by heating (Parker *et al.*, 2010).

4.4.4 Influence of thermal treatment on antioxidative properties

As antioxidative compounds are heat-sensitivity, it was expected that thermal processing would influence the antioxidative properties of the fruit puree. Several approaches have been used to measure antioxidative activity such as total phenolic content, FRAP (Lim *et al.*, 2007), trolox equivalent antioxidant capacity (Wu *et al.*, 2006), DPPH and oxygen radical absorbance capacity (Mahattanatawee *et al.*, 2006). In the research, total phenolic content, FRAP index and DPPH value were used to evaluate the antioxidative activity of the dragon

fruit puree during heating because these methods are technically simple, rapid, accuracy, stable and reproducible. Furthermore, the Maillard reaction products, represented by the browning formation pigment value, are related to the antioxidative activity, thus, the value was also investigated (Kusznierewicz *et al.*, 2008).

The changes in antioxidative properties of the unheated puree (at room temperature), the heated puree at time 0 min (pre-heating from room temperature to the set temperature) and the heated puree at time 10-60 min (interval 10 min) are shown in **Figure 4.6** in order to investigate the effect of the pre-heating and heating processes on antioxidative properties of the puree. Surprisingly, the thermal treatment resulted in an increase of all the antioxidative properties of both dragon fruit puree species. After heating at 90 °C for 60 min, the total phenolic content and FRAP index of the red-flesh puree were about 2 times higher compared to the white-flesh puree. This can be explained by the fact that the initial value of the unheated dragon fruit red-flesh puree was higher than that of the white-flesh puree (**Table 2.4**) and also suggested that the betacyanin in the red-flesh puree could play as an antioxidative component (Moreno *et al.*, 2008). The changes of total phenolic content and FRAP index of the heated red-flesh puree were also bigger than that of the heated white-flesh puree as a function of heating time and temperature.

In contrast, the DPPH changes of both dragon fruit purees increased up to 34 µg GA/g after heating at 90 °C for 60 min. It increased from 30 to 62 µg GA/g for the white-flesh puree and from 64 to 98 µg GA/g for the red-flesh dragon fruit puree compared to the unheated puree. The antioxidative properties of the heated white-flesh puree at each temperature showed the same trend: a steady increase as a function of temperature and time. For the red-flesh puree, on the other hand, resulted in the higher temperature region (80-90 °C) differed from the lower temperature region. It is suggested that the increase in antioxidative properties of the heated purees exposed to thermal treatment can be explained by the inevitable presence of the seeds in the dragon fruit puree. Therefore, an additional experiment was performed to verify the influence of the seeds in the dragon fruit puree (see **Section 4.4.4.1**).

In general, the antioxidative activities of plant-based foods decrease with thermal processing as Murcia *et al.* (2009) reported that the antioxidative properties for pea, garlic and beetroot lost around 50% of their unprocessed counterpart after thermal processing. However, it has also been reported that thermal processing can lead to higher antioxidative activities of plant-based foods due to the changes of the compounds during heat treatment (Choi *et al.*, 2006; Kusznierewicz *et al.*, 2008; Nicoli *et al.*, 1997a; Nicoli *et al.*, 1997b; Patras *et al.*, 2009; Rickman *et al.*, 2007b; Yang and Gadi, 2008).

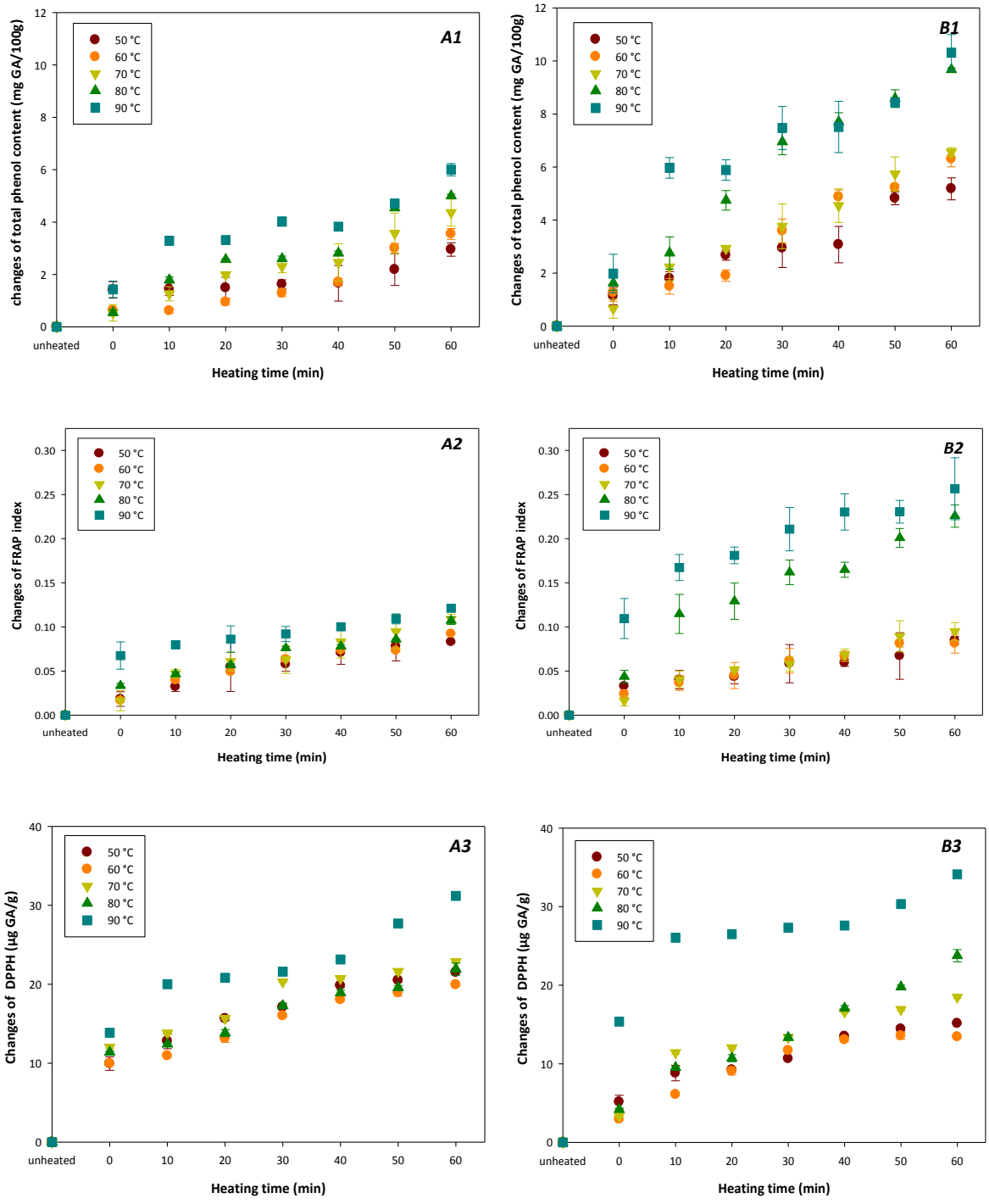


Figure 4.6 *Changes of antioxidative properties vs. heating time at different heating temperatures: (A) the white-flesh dragon fruit puree and (B) the red-flesh dragon fruit puree*

In the current work, the browning formation pigment values in the heated purees during heating were also measured. Similar to the antioxidative properties, the browning formation pigment values also increased with thermal treatment for all cases, as shown in **Figure 4.7**.

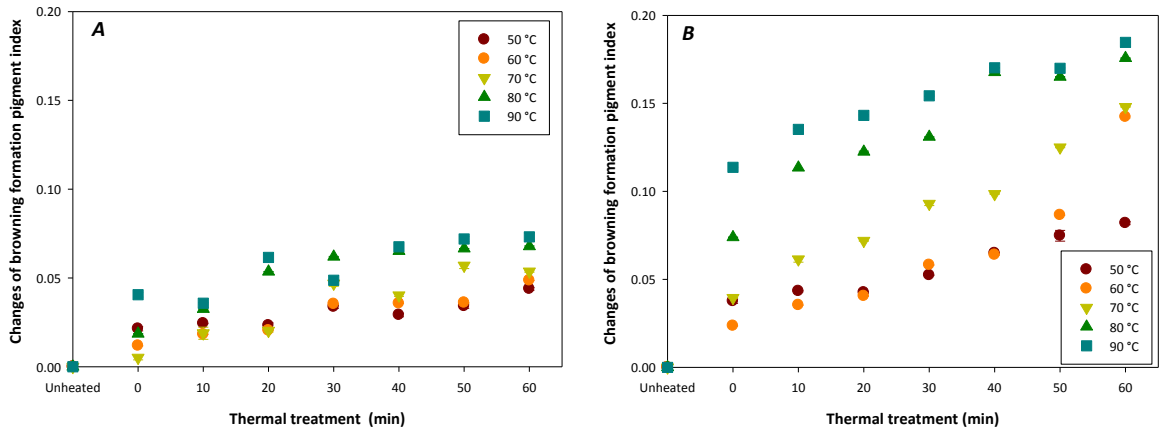


Figure 4.7 *Changes of browning formation pigment index vs. heating time at different heating temperatures: (A) the white-flesh dragon fruit puree and (B) the red-flesh dragon fruit puree*

When the dragon fruit purees were heated, they became browner due to the occurrence of some browning compounds which can be referred to as Maillard reaction products. These products lead to an increasing of browning formation pigment values, particularly in the red-flesh puree. It is suggested to occur accompanied by the degradation of betacyanin. The development of the Maillard reaction products as a consequence of heating caused the formation of some antioxidative components (Nicoli *et al.*, 1997b). A similar result is also found in sauerkraut. The increase of antioxidative properties during heat treatment are probably due to the formation of novel compounds having antioxidative activity (Kusznierewicz *et al.*, 2008).

Furthermore, an excellent positive relationship batween total phenolic content, FRAP index and DPPH value was noticed with R^2 values $\geq$ 0.8315 (**Table 4.4**). It is suggested that phenolic compounds present in the dragon fruit probably are major contributors to antioxidative properties. A strong positive linear correlation between browning formation pigment index and antioxidative properties of the thermally processed dragon fruit purees was also observed with high R^2 values (0.7585-0.9369). It may be suggested that the browning formation pigment index can probably be used to evaluate the antioxidative

properties due to a perfect correlation. Time can be saved as the browning formation pigment analysis that is much more simple and rapid than the methods to assess the antioxidative properties.

Table 4.4 *Correlation coefficient (R^2) for relationships among antioxidative properties of the dragon fruit puree undergoes thermal treatments*

Parameter	White-flesh dragon fruit puree				Red-flesh dragon fruit puree			
	Total phenolic	**FRAP index**	**DPPH**	**Browning pigment**	**Total phenolic**	**FRAP index**	**DPPH**	**Browning pigment**
Total phenolic	1.0000	-	-	-	1.0000	-	-	-
FRAP index	0.8706	1.0000	-	-	0.8315	1.0000	-	-
DPPH	0.8487	0.8799	1.0000	-	0.9141	0.9304	1.0000	-
Browning pigment	0.9068	0.8792	0.7961	1.0000	0.9369	0.8765	0.7585	1.0000

The kinetic parameters of the changes of antioxidative properties (total phenolic content, FRAP index and DPPH value) was analyzed. The increase of antioxidative properties with thermal treatments was the best fitted to the zero-order kinetic (**Eq. 4.1**), excluding DPPH value of the white-flesh puree which was better fitted with the first-order kinetic model (**Eq. 4.2**). The temperature dependence of the rate constant was expressed by the Arrhenius equation (**Eq. 4.4**). The kinetic parameters such as rate constant, activation energy, pre-exponential factor of the Arrhenius equation and coefficient of correlation, for antioxidative properties of the white-flesh and red-flesh dragon fruits are given in **Table 4.5**.

The computed values of E_a of total phenolic content for both purees were comparable values (17-21 kJ/mol), whereas a significant difference between the E_a of FRAP index and DPPH value for the white-flesh and red-flesh dragon fruit purees was observed. The E_a values of FRAP index and DPPH value for the red-flesh were significantly higher than that of the white-flesh puree (31.5 kJ/mol compared to 9.2 kJ/mol and 16.9 kJ/mol compared to 6.2 kJ/mol, respectively). It can be deduced that the enhancing of the antioxidative properties of the red-flesh puree was more susceptible to a heating than that of the white-flesh puree. Also, the coefficient of correlation (R^2) of FRAP index and DPPH value of the red-flesh puree was higher compared to the white-flesh dragon fruit. It is probably indicated that the antioxidative properties of the red-flesh puree are more dependent on the rate of heating compared to the white-flesh dragon fruit.

Table 4.5 *Kinetic parameters for antioxidative properties of the dragon fruit puree during heat treatment at five temperatures*

Parameter	Temperature (°C)	k (min^{-1}) x 10^{-2}	R^2	E_a (kJ/mol)	k_0 (min^{-1})	R^2
White-flesh dragon fruit puree						
Total phenolic	50	30.19	0.7955	20.85	8.05 x 10^2	0.9035
	60	47.96	0.9102			
	70	59.07	0.9762			
	80	69.17	0.9516			
	90	72.29	0.8980			
FRAP index	50	0.83	0.9498	9.18	2.78 x 10^{-1}	0.6128
	60	1.08	0.9521			
	70	1.14	0.9619			
	80	1.41	0.9718			
	90	1.15	0.9883			
DPPH value	50	6.41	0.7951	6.21	6.17 x 10^{-1}	0.6643
	60	6.51	0.8030			
	70	6.68	0.7550			
	80	6.83	0.7748			
	90	8.66	0.8003			
Red-flesh dragon fruit puree						
Total phenolic	50	70.02	0.9614	16.85	3.79 x 10^2	0.8794
	60	90.18	0.9718			
	70	94.19	0.9934			
	80	142.28	0.9806			
	90	131.74	0.8987			
FRAP index	50	1.00	0.9133	31.50	1.08 x 10^3	0.8706
	60	1.15	0.9580			
	70	1.33	0.9747			
	80	3.03	0.9432			
	90	3.12	0.8377			
DPPH value	50	199.82	0.9164	16.89	9.92 x 10^2	0.9433
	60	203.12	0.9136			
	70	254.95	0.8899			
	80	320.87	0.9864			
	90	379.87	0.7350			

4.4.4.1 Contribution of dragon fruit seeds to antioxidative properties

The experiment aimed to explore the influence of the presence of seeds in the dragon fruit puree on the antioxidative properties during heating. In this set-up, the red-flesh dragon fruit puree with and without seeds were used due to a higher level of antioxidative activities compared to the white-flesh puree. Therefore, the property changes using the red-flesh dragon fruit puree should be more pronounced. To obtain the dragon fruit puree without seeds, the red-flesh dragon fruit was gently mashed using a potato masher and centrifuged

at 12000 g for 20 min. It is noted that there is mucilage material between the seeds and flesh of dragon fruit, resulting in difficulties to separate the seeds. Nevertheless, the physicochemical and antioxidative properties of the unheated dragon fruit puree without seeds were determined. The average value and SD from triplicate analyses of these properties of the puree without seeds are given in **Table 4.6** in comparison with the puree with seeds. The pH values of the puree with and without seeds were comparable, while dry matter, fat, carbohydrates, total dietary fibre, protein, ash and vitamin C contents of the puree with seeds were remarkably higher than that of the sample without seeds. It is suggested that these higher values are due to the contribution from the seeds. In contrast, the betacyanin content of the puree with seeds was lower than that of the puree without seeds. This is due to the fact that the dragon fruit seeds do not contain betacyanin, unlike the flesh. Additionally, the antioxidative properties (particularly total phenolic content and DPPH value) of the unheated dragon fruit puree with seeds were remarkably higher than that of the untreated sample without seeds. It is claimed that the dragon fruit seeds are a potential source of these antioxidative activities.

Table 4.6 *Characteristics of the red-flesh dragon fruit puree with and without seeds*

Characteristic	Puree without seeds	Puree with seeds
pH	4.59 ± 0.001	4.64 ± 0.001
Dry matter (g/100 g)	10.40 ± 0.11	16.06 ± 0.13
Protein (g/100 g)	0.26 ± 0.01	0.83 ± 0.05
Ash (g/100 g)	1.18 ± 0.001	1.55 ± 0.001
Crude fat (g/100 g)	0.39 ± 0.04	0.57 ± 0.03
Carbohydrates (g/100 g)	8.57 ± 0.02	13.11 ± 0.05
Total dietary fibre (g/100 g)	0.87 ± 0.04	2.06 ± 0.09
Vitamin C (mg/100 g)	7.40 ± 0.58	15.00 ± 0.56
Betacyanin (mg/100 g)	26.18 ± 0.10	14.82 ± 0.19
Colour parameter		
L* (lightness)	26.46 ± 0.14	26.15 ± 0.29
a* (redness)	2.67 ± 0.10	9.13 ± 0.71
b* (yellowness)	-1.02 ± 0.11	-2.45 ± 0.10
Total phenolic (mg GA/100 g)	16.13 ± 0.43	35.82 ± 0.67
FRAP index	0.5502 ± 0.0064	0.5827 ± 0.0063
DPPH (μg GA/g)	22.29 ± 0.83	63.53 ± 0.89

Values are given as mean ± SD (N=3) and the contents are based on fresh weight basis

The antioxidative properties of the heated puree at 70 °C for maximum 60 min were determined. **Figure 4.8** shows their changes in the dragon fruit purees during the thermal treatment. Heating puree without seeds, in contrast to the puree with seeds, results in a decrease of the antioxidative properties as a function of processing time.

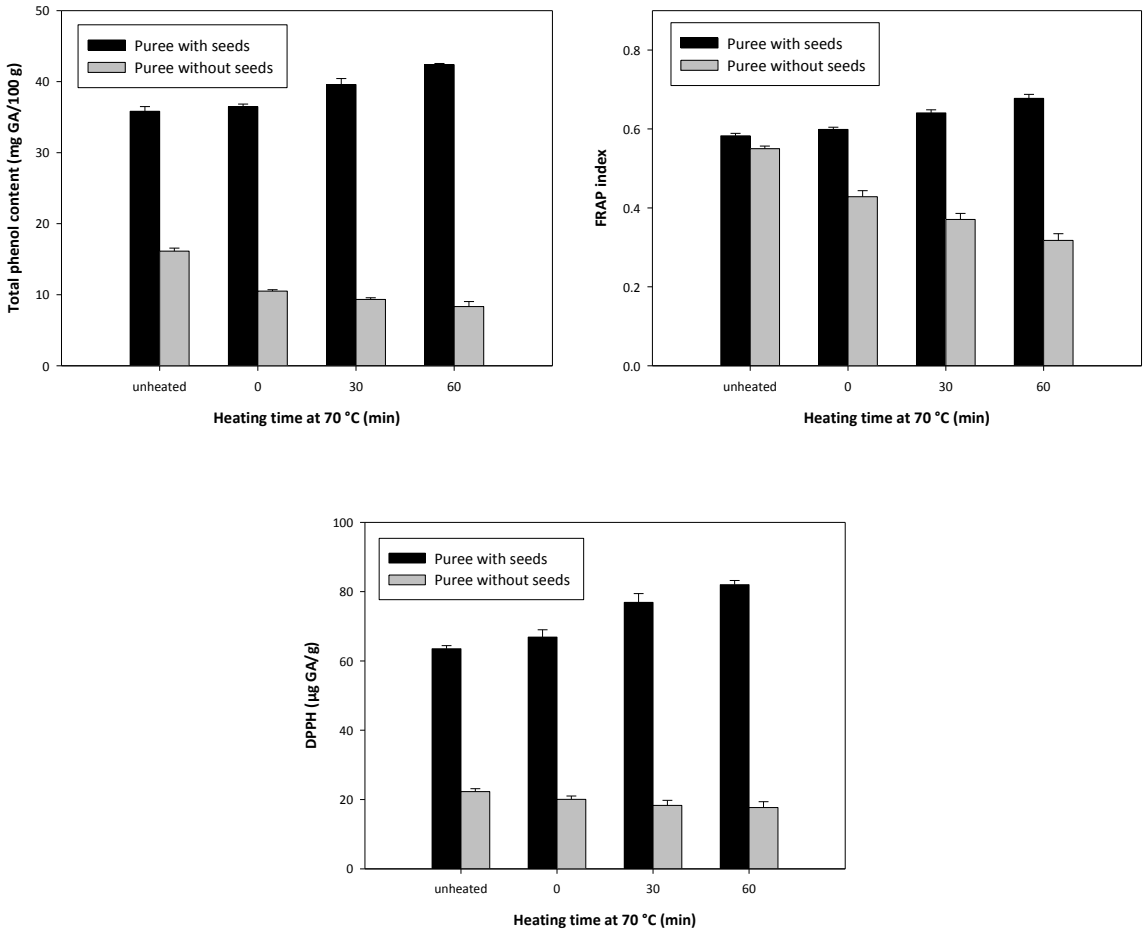


Figure 4.8 *Changes of antioxidative properties of the red-flesh dragon fruit puree with and without seeds at 70 °C as a function of heating time*

These results confirmed that the seeds in dragon fruit puree consist of some antioxidative components which can be released during thermal treatment. It is suggested that the heat treatment likely breaks the cell walsl and then release the antioxidants. The antioxidative components in dragon fruit seeds supposes to be mainly tocopherols with a remarkable amount of phytosterols and phenolic compounds (Lim *et al.*, 2010b). To further elucidate the antioxidative potential of the dragon fruit seeds, their oil was extracted and further characterized. The results are discussed extensively in **Chapter 5**.

Next, the browning formation pigment index of puree without seeds was determined. This parameter increased from 0.3122 (unheated) to 0.5627 (after heating at 70 °C for 60 min) due to the Maillard reaction and colour degradation. In contrast to the puree with seeds, a strong negative linear correlation between the browning formation pigment index and antioxidative properties was found ($R^2 \geq 0.8133$) in the puree without seeds.

4.4.5 Influence of thermal treatment on rheological properties

The influence of heating time at a constant temperature on rheological properties of the dragon fruit purees was investigated. It is noted that the supernatant of the puree was used for rheological determination to prevent the interruption from the seed particles of the puree during measurement. **Figure 4.9** shows the plot between viscosity and shear rate as a function of heating time for both dragon fruit purees at 70 °C. During heating, the apparent viscosity of the heated purees increased with increasing heating time. This may suggest that a dragon fruit has slime like oligosaccharides which probably form a weak gel with pseudonetwork-like behaviour when heating up. There is an implication that a structural change happens while the thermal processing is taking place.

Similar results are seen for the effect of heating temperatures on the rheological properties (**Figure 4.10**). It was found that the apparent viscosity of both purees increased with increasing temperature, particularly for the red-flesh puree. These results are in agreement with the results found by Ditchfield *et al.* (2004) that the viscosity of banana puree clearly increases during heating from 50 °C to 60 °C probably due to starch gelation of the puree. However, the apparent viscosity can also decrease with increasing heating temperature and time as found for raspberry, strawberry, peach and prune purees (Maceiras *et al.*, 2007) as well as for Thai seedless guava juice (Shamsudin *et al.*, 2005).

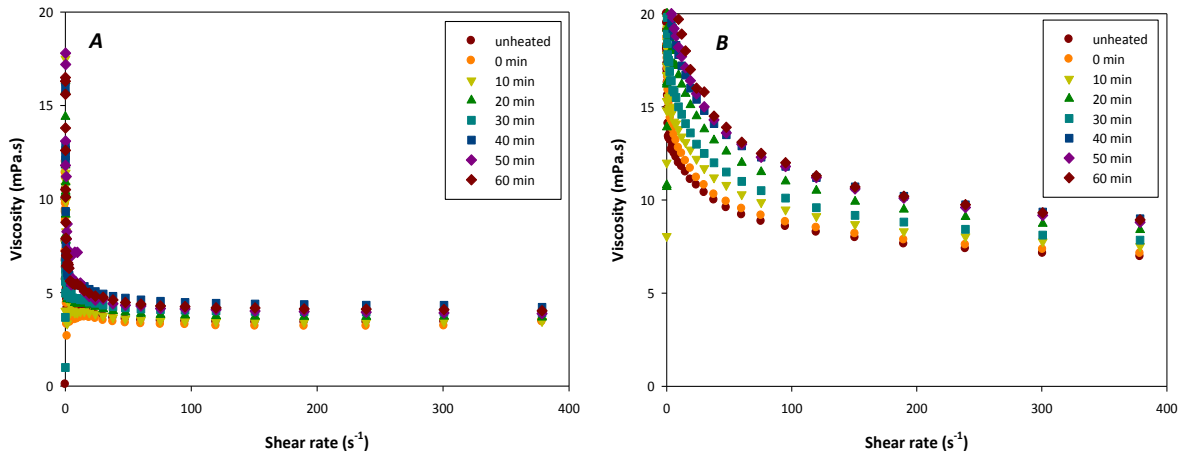


Figure 4.9 *Viscosity vs. shear rate of (A) the white-flesh and (B) the red-flesh dragon fruits at 70 °C for different heating times*

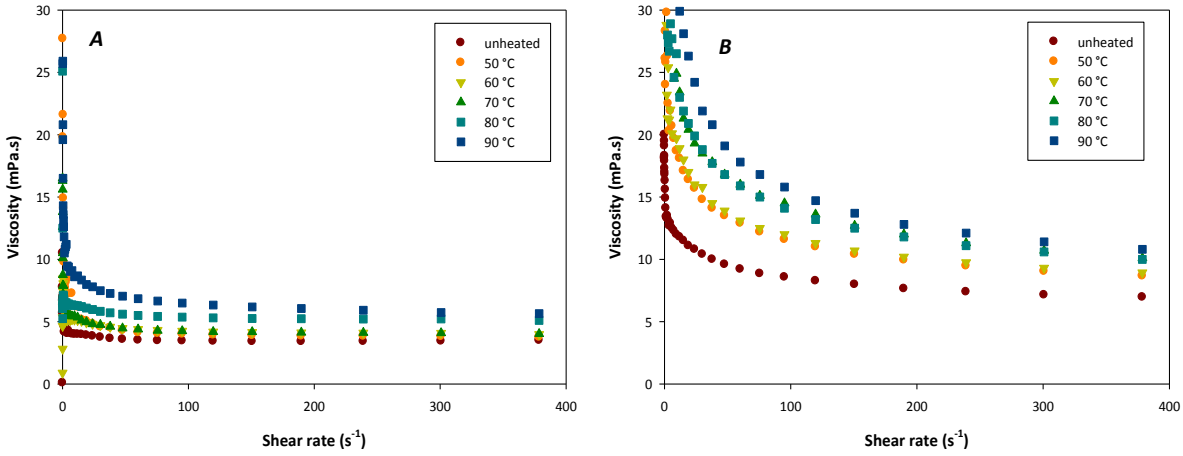


Figure 4.10 *Viscosity vs. shear rate of (A) the white-flesh and (B) the red-flesh dragon fruits after 60 min at different heating temperatures*

The influence of thermal treatment was investigated for the two parameters from the power-law model (**Eq. 2.5**): consistency coefficient (K, mPa.s^n) and flow behaviour index (n, dimensionless). The rheological data of the dragon fruit puree fitted very well with the power-law model ($R^2 \geq 0.9988$). The K and n values of two dragon fruit purees (the white-flesh and the red-flesh dragon fruits) are shown in **Figure 4.11**. For all dragon fruit purees, the K values increased, while the n values decreased as a function of heating time and temperature. The K and n values of the white-flesh puree were slightly changed, whereas

the red-flesh puree showed a big change when they were heated up. In the current study, the n values of the heated purees varied between 0.89 and 0.99 for the white-flesh puree and between 0.69 and 0.85 for the red-flesh puree during thermal treatment. Thus, it can be confirmed that the dragon fruit purees showed shear-thinning behaviour, whilst the red-flesh puree seemed to be more viscous compared to the white-flesh puree. The current results are in agreement with other studies for blueberry puree (Nindo *et al.*, 2007) and pumpkin puree (Dutta *et al.*, 2006).

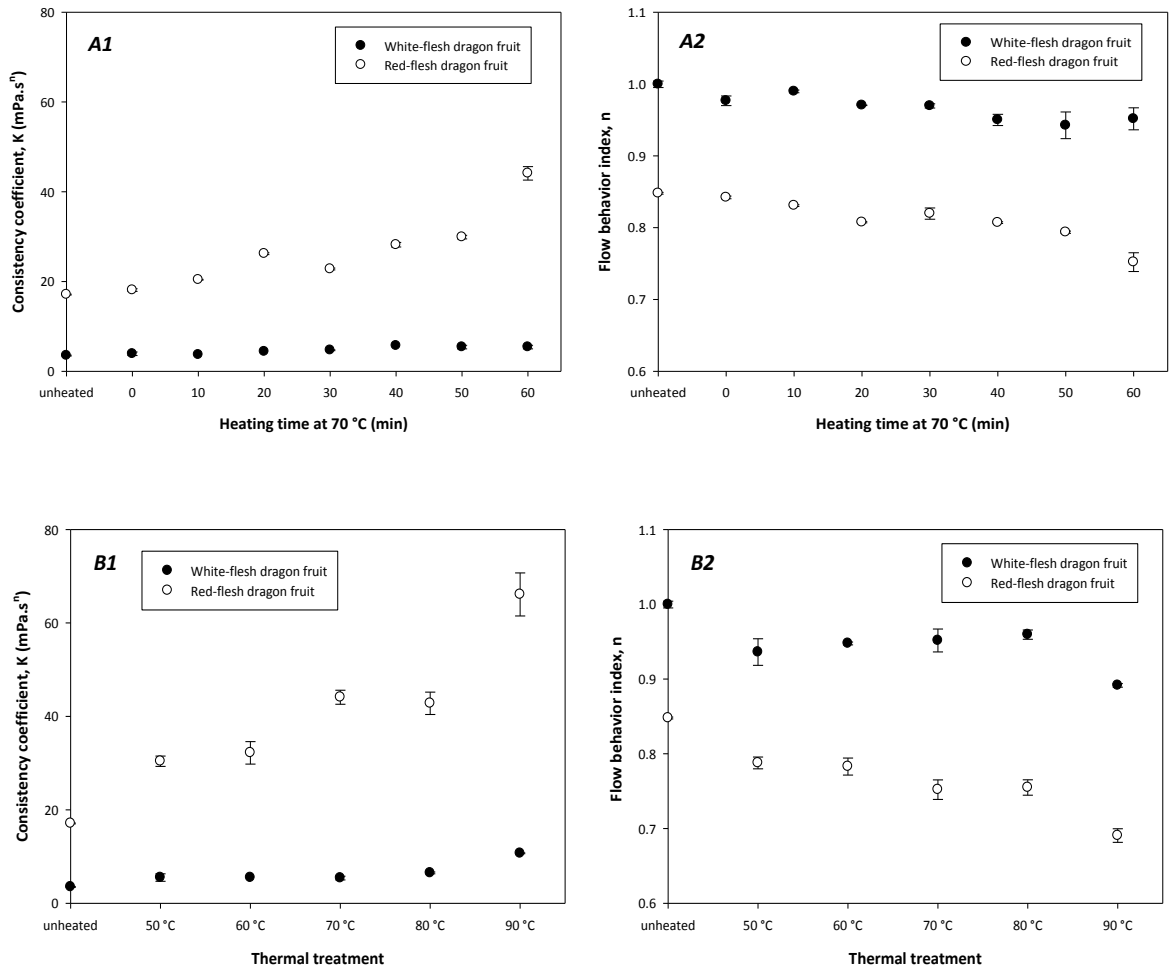


Figure 4.11 *Influence of (A) heating time at 70 °C and (B) heating temperature after 60 min on power-law parameters of the white-flesh and the red-flesh dragon fruit purees*

Based on these results, it can be concluded that the temperature and heating time affected the rheological parameters of the heated dragon fruit puree. The apparent viscosity and K

values increased, while the n values decreased with thermal treatment for all cases. It is a more pronounced effect in case of the red-flesh puree.

As it was not possible to measure the apparent viscosity of the original puree due to the large quantity of seeds, the apparent viscosity of the supernatant was analyzed and the data was recalculated to predict the apparent viscosity of the original dragon fruit purees by using **Eq. 2.4**. The results are given in **Table 4.7**. The predicted viscosity of both dragon fruit purees also gave the same trend as their supernatant. After heating at 90 °C for 60 min, they increased up to fourfold based on the unheated. Therefore, it can be suggested that the red-flesh puree can probably be used for gel-system production which undergo high temperature and long time processing because this puree provided an increasing viscosity with increasing temperature and time, while the white-flesh puree may not be used due to a relatively low viscosity.

Table 4.7 *Influence of thermal treatment on predicted viscosity of the dragon fruit puree*

Thermal treatment		White-flesh puree	Red-flesh puree
70 °C	Unheated	10.69 ± 0.1^{a}	$42.30 \pm 0.2^{a*}$
	0 min	12.02 ± 0.8^{ab}	$44.83 \pm 0.5^{a*}$
	10 min	11.42 ± 0.1^{a}	$50.53 \pm 0.2^{b*}$
	20 min	13.47 ± 0.1^{ab}	$64.82 \pm 0.3^{d*}$
	30 min	14.45 ± 0.2^{bc}	$56.41 \pm 0.4^{c*}$
	40 min	17.53 ± 0.1^{c}	$69.74 \pm 0.1^{de*}$
	50 min	16.66 ± 0.8^{c}	$73.91 \pm 0.7^{e*}$
	60 min	16.56 ± 0.9^{c}	$109.39 \pm 3.1^{f*}$
60 min	Unheated	10.69 ± 0.1^{a}	$42.30 \pm 0.2^{a*}$
	50 °C	16.92 ± 0.5^{b}	$75.34 \pm 2.2^{b*}$
	60 °C	16.82 ± 0.1^{b}	$79.72 \pm 4.8^{b*}$
	70 °C	16.56 ± 0.9^{b}	$109.39 \pm 3.1^{c*}$
	80 °C	19.98 ± 0.4^{c}	$106.12 \pm 4.8^{c*}$
	90 °C	33.00 ± 0.2^{d}	$163.81 \pm 9.2^{d*}$

Given values are apparent viscosities (mPa.s) as mean ± SD (N=3),
the superscripts within each column for each treatment are significantly different ($p < 0.05$)
*and * indicates the significance among treatments of the red-flesh dragon fruit puree*

4.5 Conclusion

The puree of two species of dragon fruit, white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), was subjected to a heat treatment for 0 to 60 min at temperatures between 50 °C and 90 °C. The influence of the different heat treatments on

the physicochemical properties, microbial destruction, antioxidative properties and rheological parameters were investigated. The outcome of this research can be used for the commercial process optimization and the different techniques can be used for online monitoring to obtain high quality products in terms of nutritional values, colour and antioxidative properties.

After exploring with heating, the heated dragon fruit purees remained relatively high in vitamin C and contained higher dietary fibre content compared to initial value. The degradation of betacyanin pigment of the red-flesh dragon fruit puree occurred during thermal treatment. After 90 °C for 60 min, the betacyanin content of the puree decreased to 32% based on the initial puree. It also results in the colour changes of the puree during thermal treatment. Hence, the L* and b* values of the dragon fruit purees can be recommended as an on-line quality control due to the strong correlation with high R^2 values (0.7733-0.8680). The TCC value of the red-flesh puree can be also used to predict the betacyanin content (R^2 = 0.9442). It is interesting that the antioxidative activities of the heated dragon fruit purees increased with heating time and temperature. This finding can be explained by the releases of some antioxidative components from the dragon fruit seeds and probably that Maillard reaction products might be occurred and may play a role as antioxidant during thermal treatment. The strong linear correlation among the antioxidative properties was also observed. After heating, the apparent viscosity of the dragon fruit purees increased, particularly the red-flesh puree. All cases of the heated purees fitted very well with the power-law model, showing R^2 values greater than 0.9988. Thus, the heated dragon fruit purees, particularly the red-flesh puree, offer possibilities to be applied in foodstuffs such as jelly, jam and yogurt due to their interesting physicochemical, nutritional and rheological attributes after thermal treatment.

CHAPTER 5

Characterization of Dragon Fruit Seed Oil

This chapter is redrafted after:

Liaotrakoon, W., De Clercq, N., Van Hoed, V. and Dewettinck, K. (2013) Dragon fruit (Hylocereus spp.) seed oils: their characterization and stability under storage conditions. ***Journal of the American Oil Chemists' Society****, 90(2): 207-215.*

5. Characterization of dragon fruit seed oil

5.1 Introduction

Dragon fruit (*Hylocereus* spp.) is generally grown in tropical climate countries, including those of Southeast Asia, e.g. Thailand, Malaysia and Vietnam. The fruit has a distinctive appearance and has the potential to be used as a source of functional ingredients (Barbeau, 1993). The dragon fruit flesh contains numerous small black seeds covered with a gelatinous carbohydrate layer. The dragon fruit seeds contain oil like most grainy seeds, e.g. grape seeds (Baydar *et al.*, 2007), linseeds (Lemcke-Norojarvi *et al.*, 2001) and berry seeds, i.e. blackberry, blueberry and red raspberry seeds (Van Hoed *et al.*, 2009). Oils of these edible seeds (Baydar *et al.*, 2007; Lemcke-Norojarvi *et al.*, 2001; Van Hoed *et al.*, 2009) exhibit a significantly high content of PUFAs, particularly linoleic acid (C18:2). The latter is an essential fatty acid which is necessary, but cannot be synthesized by the human body. Up-to-date, specialty high-value oils (e.g. grape seed and berry seed oils) are gaining much attention due to their health benefits which are linked to their relative high content of essential fatty acids and endogenous antioxidants.

Vitamin E, basically considered as a natural antioxidant, can be found in most seed oils which contain different levels of vitamin E activity. Since humans are not able to synthesize their own vitamin E, they primarily acquire vitamin E from plant sources. Vitamin E consists of eight structurally related forms, but the main forms in most plant and seed oils are found to be α- and γ-tocopherols. Amongst these components, α-tocopherol is the most active *in vivo* antioxidant and it is, moreover, an efficient biologically active compound with health benefits (Krichene *et al.*, 2010; Yoshida *et al.*, 2003) like reducing the risk of coronary heart disease (Lemcke-Norojarvi *et al.*, 2001). Also, γ-tocopherol may possess positive health effects (e.g. anti-inflammatory properties) reducing the risk of cardiovascular disease and cancer (Jiang *et al.*, 2001).

The oil composition (especially the fatty acid composition), amount of natural antioxidants and the storage conditions (e.g. temperature, light and oxygen) have a significant effect on the oil quality. Moreover, the oxidation of UFAs, particularly PUFA, is the main cause of reduced oil stability, resulting in quality deterioration such as sensory quality, functional properties and nutritional value of oils (Kamkar *et al.*, 2010; Silva *et al.*, 2010).

Recently, only very few works have been published describing a limited number of properties of dragon fruit (*Hylocereus* spp.) seed oil. In addition, there is no study available

describing the stability of dragon fruit seed oil under different storage conditions, and therefore, the characterization of dragon fruit seed oil needs to be investigated more. In the current study, the aim of the experimental work was to characterize the physicochemical properties, fatty acid profile, TAG composition, tocopherol content, and melting and crystallization properties of dragon fruit seed oil which was extracted from two different species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). In addition, the influence of different storage temperatures, more particularly cold temperature (CT) and room temperature (RT), of the extracted dragon fruit seed oil on the oxidative stability and the behaviour of endogenous antioxidative components (tocopherols) present in the oil were monitored during a 3-month storage period.

5.2 Research strategy

A schematic overview of the experimental set-up of this chapter is presented in **Figure 5.1**. Dragon fruit seeds are considered to be an excellent source of antioxidative activities (see **Section 4.4.4.1**). It is suggested that the most abundant antioxidative components present in dragon fruit seeds are tocopherols which are lipid-soluble antioxidative components. In order to gain insight into the antioxidative potential of dragon fruit seeds, oil was extracted from the seeds of white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*), by cold solvent extraction using petroleum ether. The dragon fruit seed oils were characterized by their fatty acid profile, TAG composition and tocopherol content as well as their melting and crystallization behaviours.

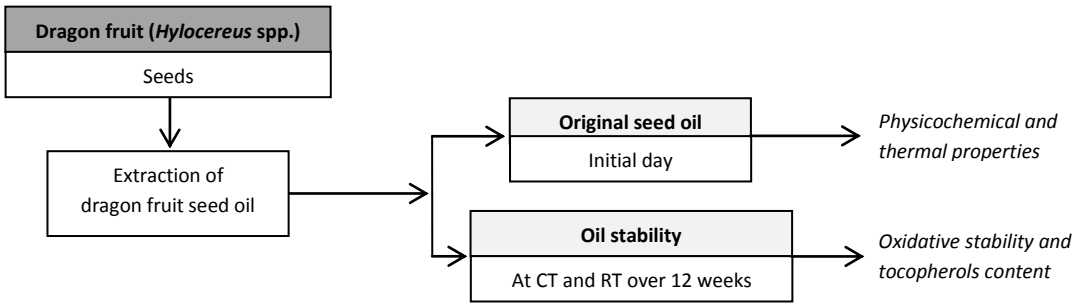


Figure 5.1 *Schematic overview of the experimental set-up to characterize the dragon fruit seed oil*

In addition, the effects of storage conditions on the oxidation progression and the behaviour of natural tocopherol present in the dragon fruit seed oil were also investigated over 3 months. So, the stability of dragon fruit seed oil was monitored at two different temperatures, i.e. CT represents a refrigerator condition (~4 °C) and RT corresponds to a kitchen condition (~20 °C). Hereto, the outcome of the research can be used to estimate the oil quality and its oxidative stability during storage. This experimental work may allow for maximum utilization of dragon fruit seeds which are generally considered as by-products.

5.3 Materials and methods

5.3.1 Dragon fruit seed oil extraction

Figure 5.2 illustrates a schematic representation of the oil extraction from dragon fruit seeds. Two species of dragon fruit, the white-flesh dragon fruit (*H. undatus*) and the red-flesh dragon fruit (*H. polyrhizus*) originating from Thailand, were used in the work. The fruit was washed under running tap water, hand-peeled and cut into pieces. Due to a mucilage layer between the flesh and the seeds that making it difficult to separate the seeds from the flesh, the mucilaginous materials were decomposed by heating process. Thus, the flesh was autoclaved at a pressure of 15 psi for 40 min and was then centrifuged at 8000 g for 15 min at room temperature to separate and recover the seeds. In the next step, the seeds were dried overnight in an oven at 60 °C. The dried dragon fruit seeds were then ground into very fine particles using a spice grinder (Seb Optimo Compact MB 4011, France).

To extract the dragon fruit seed oil by cold extraction process, five grams of ground dried seeds were mixed with 100 ml of petroleum ether (boiling point of 40-60 °C), stirred for 2 h at RT with constant stirring and then filtered using a Buchner funnel with Whatman No.1 filter paper under vacuum condition to remove the seed residue. Subsequently, the oil was recovered by evaporating the solvent using a rotary evaporator at 35 °C in a vacuum, resulting in the oil residue sample. Eventually, the extracted seed oil was purged with N_2 and stored in the dark at -18 °C until further analysis.

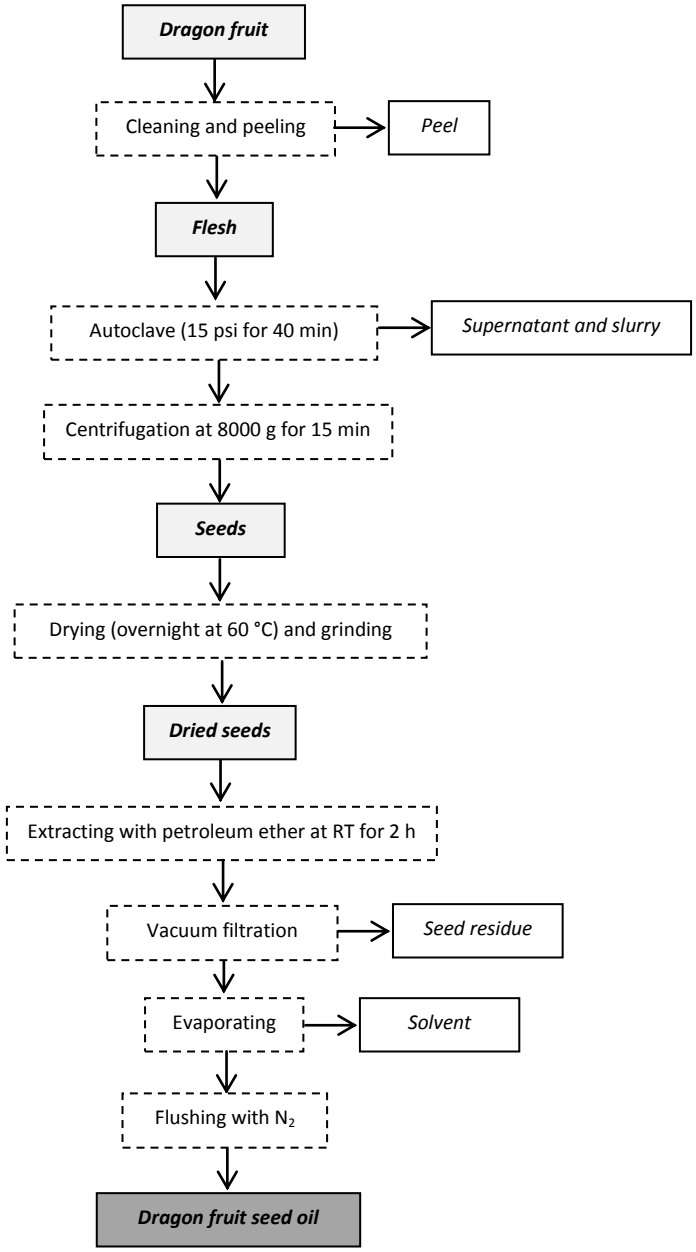


Figure 5.2 *Schematic representation of the dragon fruit seed oil extraction*

5.3.2 Storage conditions

For an experimental study of storage, the dragon fruit seed oil was placed in a brown-glass vial, closed with a stopper and stored in the dark at CT (4 ± 0.5 °C) and RT (20 ± 1 °C) over a period of 3 months. The oxidation progress was evaluated weekly by measuring the PV and the peroxide formation index as well as the amount of δ-, γ- and α-tocopherols.

5.3.3 Determination of fatty acid composition

TAG in the oil was converted into the corresponding fatty acid methyl ester (FAME) and analyzed by an interscience thermofocus GC equipped with a RTX-2330 column and a flame ionization detector in which hydrogen was used as carrier gas. The FAME was prepared by dissolving two drops of the oil sample in 9 ml hexane and reacting with 1 ml of 2 N KOH methanolic reagent. The blend was shaken for 30 sec and was then allowed to settle at room temperature. Approximately 1 ml of the hexane layer was carefully pipetted into a GC vial. FAME preparation was performed in triplicate for each sample. An aliquot of 1.0 µl was injected using an autosampler. Peak identification was based on retention time comparison with a FAME standard chromatogram. Peak quantification was expressed as percentage of FAME by taking the ratio of each peak area to the total area of all peaks in the GC chromatogram.

5.3.4 Determination of triacylglycerol composition

The separation of acylglycerols was performed using a HPLC equipped with an evaporative light scattering detector (HPLC-ELSD) (2000 ES, Alltech ELSD), as described by Rombaut *et al.* (2009). An Alltima HP C18 HL column (150 x 3.0 mm, particle size of 3 µm) was used with a mobile phase of dichloromethane and acetonitrile solution (30:70, v/v) at a flow rate of 0.72 ml/min. The dragon fruit seed oil sample was prepared in the mobile phase with a concentration of 3 mg/ml and then 25 µl was injected via a full loop injection mode using an autosampler. The temperature of the column and the detector was programmed at 20 °C and 64 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1.5 l/min for the detector.

5.3.5 Determination of tocopherols

The content of δ-, γ-, and α-tocopherols in the dragon fruit seed oil was determined in triplicate using an HPLC equipped with a UV-VIS detector (Shimadzu, Kyoto, Japan), as modified method from the procedure described by Sanchez-Machado *et al.* (2002). An Alltima HP C18 HL column (150 x 3.0 mm, particle size of 3 µm) was used with a mobile phase of methanol and acetonitrile at a flow rate of 0.7 ml/min. Prior to HPLC analysis, the oil sample was diluted in acetone and then 20 µl of the mixture (oil/acetone) solution was injected onto the column (25 °C). The tocopherols were monitored at a wavelength of 210 nm. Standard δ-, γ- and α-tocopherols at various concentrations were prepared and

analyzed following the aforementioned procedure. Their calibration curves were established accordingly.

5.3.6 Determination of colour parameters

The spectrophotometer colorimeter (CM-2500D, Minolta) was used to determine colour parameters (L*, a* and b* values) with specular component included system. This system can minimize the influences of the sample surface condition and is suitable for colour quality control. The oil colour was measured in triplicate thought the 1-cm path length of a plastic cuvette. In this coordinate system, the L*value corresponds to lightness, ranging from 0 (black) to 100 (white), the a* value ranges from -100 (greenness) to +100 (redness), and the b* value ranges from -100 (blueness) to +100 (yellowness).

5.3.7 Determination of viscosity

The viscosity measurement of the oil sample was performed in triplicate using an AR2000 Rheometer equipped with a 28-mm conical concentric cylinder. Prior to viscosity determination, the dragon fruit seed oil was put in a water bath at 25 °C, then about 20 ml of the oil was subjected to the viscosity measurement within a constant shear rate of 100 s^{-1} at 25 °C (Elleuch *et al.*, 2007). The data of the viscosity measurements were analyzed with the supporting rheometer software, TA Rheology Advantage Data analysis software.

5.3.8 Determination of oxidative stability

In the study, two different colorimetric methods: FOX and the ferric thiocyanate methods, were performed to determine the oxidative progression of the oil during storage. These methods are simple, reproducible and sensitive. They also require only a small amount of oil sample and can detect PV at a very low level.

The FOX method is based on the ability of lipid peroxides to oxidize ferrous ions (Wrolstad *et al.*, 2005). About 100 mg of the initial and stored oil sample was weighted and mixed with 9.9 ml of a chloroform and methanol solution (70:30, v/v). Then, 50 µl of 10 mM xylenol orange solution and 50 µl of 10 mM Iron (II) ferrous chloride solution were respectively added and thoroughly mixed. After exactly 5 min, absorbance was measured using a spectrophotometer at a wavelength of 560 nm with a 1-cm glass cuvette. PV was expressed

in milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg) with reference to calibration curves obtained using Iron (III) ferrous chloride standard solution with concentration between 0 and 20 μg/ml.

The peroxide formation index was also determined by the ferric thiocyanate method (Maqsood and Benjakul, 2010). A 50-μl of the oil sample was mixed with 2.35 ml of 75% ethanol, 50 μl of 30% ammonium thiocyanate and 50 μl of 20 mM ferrous chloride solution in 3.5% HCl, respectively. The mixtures were thoroughly mixed and allowed to stand for 3 min at room temperature. The absorbance at a wavelength of 500 nm for the red-coloured portion present in the solution was measured. An increase in absorbance indicates the formation of peroxide.

5.3.9 Determination of thermal properties

The melting and crystallization behaviours of the oil were determined by DSC. These experiments were performed in triplicate using a TA Q1000 DSC (TA Instrument, New Castle, Delaware, USA) with a refrigerated cooling system. The oil sample was weighted to 7 mg and hermetically sealed in an aluminium pan using a sample preparatory device. An empty pan was used as a reference. The time-temperature program applied was set as equilibrate at 50 °C for 15 min to ensure a completely liquid state, cooling at 10 °C/min to -80 °C, holding at -80 °C for 5 min and heating at 10 °C/min to 50 °C. DSC produces a thermogram representing the heat flow (watt/g) versus temperature. Thermal properties are observed from the DSC thermograms using the Universal analysis V4.5A software (TA Instruments, USA).

5.3.10 Statistical analysis

The results are shown as mean ± SD. Statistical analyses were conducted using S-PLUS 8.0 software. The significant differences among means of two dragon fruit seed oils were subjected to a two-sample *t*-test analysis. The confidence limits used in this study were based on 95% ($p < 0.05$). Linear correlations from regression analysis were also analyzed using the same software.

5.4 Results and discussion

5.4.1 Physical and oxidative properties

According to **Table 2.1**, both white-flesh and red-flesh dragon fruits contained about two-thirds of pulp and ~8% of seeds based on the weight of the whole fruit (wet basis). **Table 5.1** shows some characteristics of the dragon fruit seeds. The dry matter content of both dragon fruit seeds was comparable value (~97 g/100 g) and the yield of dried seeds was about 2% for both white-flesh and red-flesh dragon fruits compared to the initial weight of the whole fruit. After oil extraction, the seed oil content (g oil to 100 g of dried seeds) of both white-flesh and red-flesh species was determined. Both dragon fruit seed oils contained remarkable amounts of oil ranging from 32% for the red-flesh dragon fruit to 34% for the white-flesh dragon fruit. These values are comparable to the amounts found by Ariffin *et al.* (2009) and Lim *et al.* (2010a). Moreover, the oil content from the dragon fruit seeds is clearly higher than that of grape seeds (12-16%) (Baydar *et al.*, 2007) and pomegranate seeds (6-22%) (Elfalleh *et al.*, 2011).

Table 5.1 *Basic characteristics of the dragon fruit seeds*

Characteristic	White-flesh dragon fruit seeds	Red-flesh dragon fruit seeds
Dry matter (g/100 g)	97.7 ± 0.7^{a}	97.2 ± 0.4^{a}
Yield of dried seeds (%)	1.76 ± 0.5^{a}	1.74 ± 0.4^{a}
Seed oil content (%)	34.1 ± 1.2^{a}	32.0 ± 0.6^{a}

Results are expressed on the wet basis as mean ± SD (N=3)

and data within rows followed by same letters are non significantly different ($p > 0.05$)

The physical and oxidative properties of the dragon fruit seed oil were examined and the results are displayed in **Table 5.2**. The oxidative properties including PV and peroxide formation index between the white-flesh and the red-flesh dragon fruit seed oils were very comparable. The initial PVs of the dragon fruit seed oil (~3 mequiv O_2/kg of the oil) are lower than that of, e.g. the PV of olive oil (8-15 mequiv O_2/kg) (Krichene *et al.*, 2010), strawberry and red rasberry seed oils (26-43 mequiv O_2/kg) (Van Hoed *et al.*, 2009), while they are, on the other hand, higher than that of blackberry seed oil (0.6 mequiv O_2/kg) (Van Hoed *et al.*, 2009).

Table 5.2 *Physical and oxidative properties of the dragon fruit seed oil*

Characteristic	White-flesh dragon fruit seed oil	Red-flesh dragon fruit seed oil
Oxidative properties		
PV (mequiv O_2/kg)	3.04 ± 0.29^a	3.20 ± 0.16^a
Peroxide formation index	0.64 ± 0.02^a	0.65 ± 0.01^a
Physical properties		
Viscosity (mPa.s)	14.32 ± 0.36^a	14.24 ± 0.33^a
L* (lightness)	29.97 ± 0.10^a	29.33 ± 0.09^b
a* (redness)	0.80 ± 0.00^a	0.97 ± 0.01^b
b* (yellowness)	9.56 ± 0.08^a	8.26 ± 0.05^b

Results are shown as mean ± SD (N=3) and data within rows followed by different letters are significantly different ($p < 0.05$)

The apperent viscosity of the dragon fruit seed oil was relatively low (~14 mPa.s, **Table 5.2**). It is lower compared to that of sesame and coconut oils (Akhtar *et al.*, 2009) and to olive and sunflower oils (Abramovic and Klofutar, 1998) in which the range of viscosity varies from 44 to 63 mPa.s at 25 °C. This difference can be explained by the difference in saturated and unsaturated fatty acids, the degree of polymerization and the position of the hydroxyl group of the fatty acids (Abramovic and Klofutar, 1998; Akhtar *et al.*, 2009). For the colour of the dragon fruit seed oil, both seed oils showed light yellow colour like olive oil in which the L* (lightness), a* (redness) and b* (yellowness) values of the white-flesh and the red-flesh dragon fruit seed oils were comparable.

5.4.2 Fatty acids and triacylglycerol profile

In general, the fatty acid composition of the oil can be considered as the first important quality parameter. Thus, the fatty acid profile of the white-flesh and the red-flesh dragon fruit seed oils was also investigated. The GC chromatogram of the fatty acid composition of both dragon fruit seed oils is shown in **Figure 5.3**. Both dragon fruit seed oils represented the same fatty acid components, but they varied considerably in terms of the relative percentage.

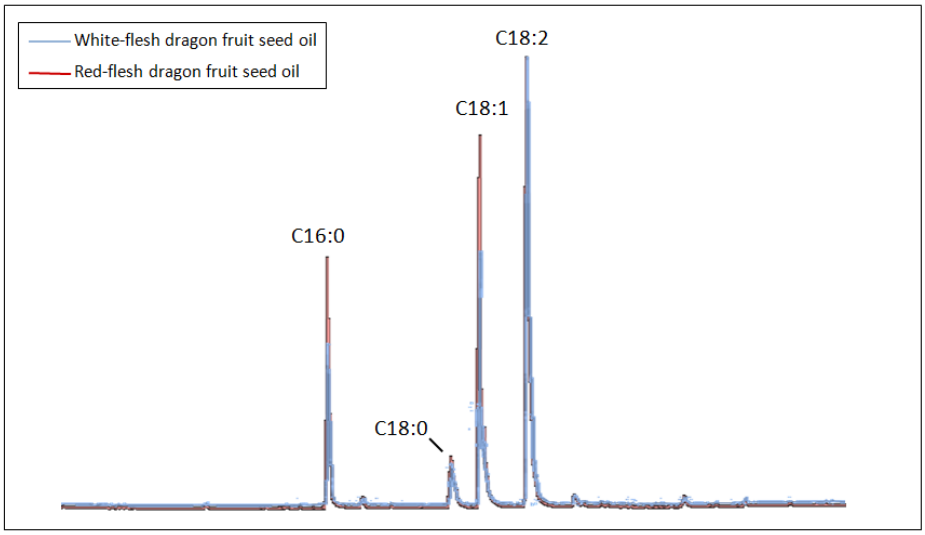


Figure 5.3 *The GC chromatogram of fatty acid composition for the dragon fruit seed oil*

Besides fatty acid composition, the TAG profile of the dragon fruit seed oil was also characterized by a reversed phase HPLC based on their chain length and degree of unsaturation of the fatty acids on the glycerol backbone. **Figure 5.4** shows the HPLC chromatogram of the TAG composition of the dragon fruit seed oil.

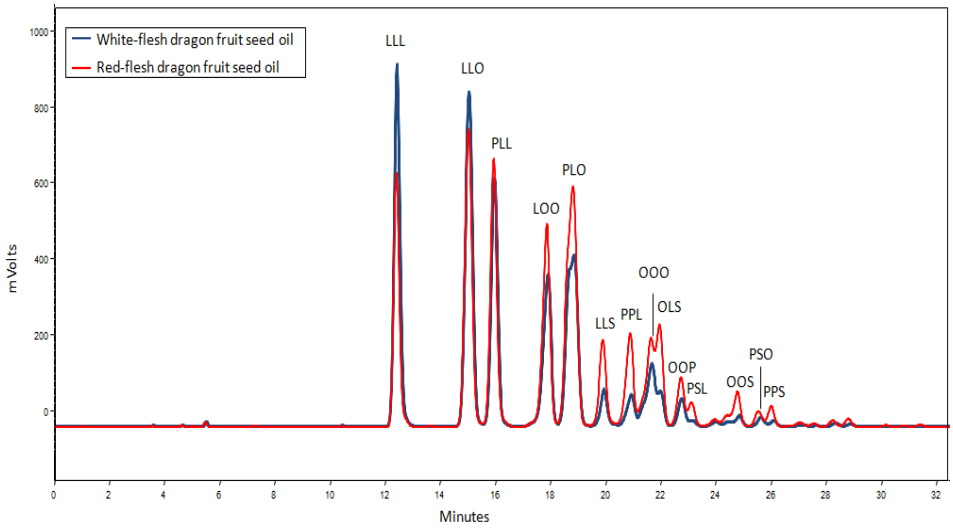


Figure 5.4 *The HPLC chromatogram of triacylglycerol composition for the dragon fruit seed oil. L: Linoleic acid, O: Oleic acid, P: Palmitic acid, S: Stearic acid*

The relative percentages of fatty acids in the dragon fruit seed oil is expressed in g/100 g of total fatty acids and the results are given in **Table 5.3**. The predominant fatty acid in both dragon fruit seed oils was linoleic acid (C18:2), showing 55.43% for the white-flesh dragon fruit seed oil and 45.21% for the red-flesh dragon fruit seed oil. The other three main fatty acids were oleic acid (C18:1), palmitic acid (C16:0) and stearic acid (C18:0) counting up to 18.67, 14.95 and 6.93% for the white-flesh dragon fruit seed oil and up to 23.61, 18.39 and 8.05% for the red-flesh dragon fruit seed oil, respectively. Thus, the dragon fruit seed oil mainly contained PUFA in which the PUFA content in the white-flesh dragon fruit seed oil was remarkably higher compared to that of the red-flesh dragon fruit seed oil (55.94% compared to 45.70%). The contents of SFA and MUFA in the white-flesh dragon fruit seed oil were significantly lower compared to those of the red-flesh dragon fruit seed oil (24.34% vs. 29.26% for SFA and 19.68% vs. 24.96% for MUFA). The total amount of UFAs in the white-flesh dragon fruit seed oil was higher than that of the red-flesh dragon fruit seed oil (75.62% compared to 70.66%).

Table 5.3 *Fatty acid composition of the dragon fruit seed oil*

Fatty acid	**Fatty acid composition g/ 100 g fatty acids**	
	White-flesh dragon fruit seed oil	**Red-flesh dragon fruit seed oil**
Myristic acid (C14:0)	0.14 ± 0.12^{a}	0.21 ± 0.01^{a}
Palmitic acid (C16:0)	14.95 ± 0.59^{a}	18.39 ± 0.47^{b}
Margaric acid (C17:0)	0.11 ± 0.01^{a}	0.10 ± 0.04^{a}
Stearic acid (C18:0)	6.93 ± 0.07^{a}	8.05 ± 0.67^{b}
Arachidic acid (C20:0)	0.95 ± 0.05^{a}	1.04 ± 0.01^{b}
Behenic acid (C22:0)	0.83 ± 0.09^{a}	1.12 ± 0.04^{b}
Lignoceric acid (C24:0)	0.44 ± 0.01^{a}	0.41 ± 0.05^{a}
ΣSaturated fatty acids	**24.34 ± 0.51^{a}**	**29.26 ± 0.50^{b}**
Palmitoleic acid (C16:1)	0.79 ± 0.01^{a}	1.04 ± 0.02^{b}
Oleic acid (C18:1)	18.67 ± 0.15^{a}	23.61 ± 0.07^{b}
Gadoleic acid (C20:1)	0.16 ± 0.03^{a}	0.24 ± 0.05^{a}
Erucic acid (C22:1)	0.05 ± 0.04^{a}	0.07 ± 0.01^{a}
ΣMonounsaturated fatty acids	**19.68 ± 0.15^{a}**	**24.96 ± 0.26^{b}**
Hexadecadienoic acid (C16:2)	0.15 ± 0.06^{a}	0.11 ± 0.08^{a}
Linoleic acid (C18:2)	55.43 ± 0.41^{a}	45.21 ± 0.61^{b}
Linolenic acid (C18:3)	0.21 ± 0.04^{a}	0.15 ± 0.09^{a}
Eicosatrienoic acid (C20:3)	0.07 ± 0.06^{a}	0.18 ± 0.02^{a}
Arachidonic acid (C20:4)	0.07 ± 0.02^{a}	0.04 ± 0.01^{a}
ΣPolyunsaturated fatty acids	**55.94 ± 0.40^{a}**	**45.70 ± 0.59^{b}**
Essential fatty acids (C18:2+C18:3)	***55.64 ± 0.40^{a}***	***45.37 ± 0.57^{b}***

Relative percentage of fatty acids is expressed as mean ± SD (N=3), data within rows followed by different letters are significantly different ($p < 0.05$) and the bold values indicate the summation of individual classes of fatty acids

In both dragon fruit seed oils, linoleic acid (C18:2) was the main PUFA (~99% of total PUFA composition), while only a small amount of linolenic acid (C18:3) was found (~0.2%). The linoleic acid (C18:2) and linolenic acid (C18:3) are essential fatty acids that need to be included in the diet because the human metabolism cannot create them from other fatty acids. Therefore, the dragon fruit seed oil is very interesting from a nutritional point of view. The amount of essential fatty acids in the dragon fruit seed oil is comparable with those of corn oil (Lemcke-Norojarvi *et al.*, 2001), but it is higher than that of olive oil (Gurdeniz *et al.*, 2010; Krichene *et al.*, 2010) and sesame oil (Elleuch *et al.*, 2007). On the other hand, higher amounts of essential fatty acids are reported for linseed oil (Lemcke-Norojarvi *et al.*, 2001), berry seed oils (Van Hoed *et al.*, 2009) and grape seed oils (Baydar *et al.*, 2007; Gomez *et al.*, 1996).

Similar to fatty acid profile, both dragon fruit seed oils represented the same TAG compositions, but they varied considerably in terms of the relative percentage, as shown in **Table 5.4**. TAG measurements showed a very good repeatability.

Table 5.4 *Triacylglycerol composition of the dragon fruit seed oil*

TAG species	Relative TAG composition (%)	
	White-flesh dragon fruit seed oil	Red-flesh dragon fruit seed oil
LLO	21.60 ± 0.11^{a}	15.66 ± 0.01^{b}
LLL	19.42 ± 0.06^{a}	10.82 ± 0.06^{b}
PLO	16.86 ± 0.01^{a}	17.89 ± 0.00^{b}
PLL	14.40 ± 0.01^{a}	13.02 ± 0.02^{b}
LOO	10.71 ± 0.03^{a}	11.29 ± 0.04^{a}
OOO	5.20 ± 0.07^{a}	5.57 ± 0.06^{a}
PPL	2.34 ± 0.02^{a}	4.56 ± 0.00^{b}
LLS	2.21 ± 0.05^{a}	5.27 ± 0.06^{b}
OLS	1.75 ± 0.05^{a}	5.24 ± 0.11^{b}
OOP	1.75 ± 0.01^{a}	2.52 ± 0.04^{b}
OOS	0.72 ± 0.01^{a}	1.82 ± 0.03^{b}
PSO	0.62 ± 0.02^{a}	0.85 ± 0.01^{b}
PSL	0.34 ± 0.00^{a}	1.22 ± 0.01^{b}
PPO	0.35 ± 0.01^{a}	0.44 ± 0.01^{b}
PPS	0.36 ± 0.00^{a}	1.08 ± 0.02^{b}
PPP	0.32 ± 0.00^{a}	0.55 ± 0.01^{b}
SSP	0.22 ± 0.00^{a}	0.37 ± 0.00^{b}

Results shown as mean ± SD (N=3) and data within rows followed by different letters are significantly different ($p < 0.05$). L: Linoleic acid (C18:2), O: Oleic acid (C18:1), P: Palmitic acid (C16:0), S: Stearic acid (C18:0)

The major TAGs in the white-flesh and red-flesh dragon fruit seed oils were found to be triunsaturated TAG (i.e. LLO, LLL, LOO, and OOO, counting up to 21.60%) or diunsaturated TAG (i.e. PLO, PLL, LLS, OLS, OOP and OOS, amounting up to 17.89%). However, a remarkable difference of the relative percentage of TAG composition between the white-flesh and the red-flesh dragon fruit seed oils was clearly observed. Among these TAGs, LLO (21.60%) and LLL (19.42%) in the white-flesh dragon fruit seed oil, and PLO (17.89%) and LLO (15.66%) in the red-flesh dragon fruit seed oil were predominant. However, both dragon fruit seed oils also contained moderate amount of monounsaturated TAG (i.e. PPL, PSO, PSL and PPO, counting up to 4.56%) with a favourable low composition of saturated TAG such as PPP, PPS and SSP (0.22-1.08%).

5.4.3 Tocopherol content

The tocopherols, endogenous fat-soluble antioxidative components, in the white-flesh and red-flesh dragon fruit seed oils were determined. The contents of δ-, γ- and α-tocopherols are given in **Table 5.5**. The most abundant tocopherol in the dragon fruit seed oils was α-tocopherol, representing about 72% of total tocopherol content, followed by γ- and δ-tocopherols, respectively. Some edible oils such as olive oil (Krichene *et al.*, 2010) and grape seed oil (Baydar *et al.*, 2007), also mainly contain α-tocopherol, while γ-tocopherol is found as a main tocopherol in some plant seed oils such as sesame oil (Aued-Pimentel *et al.*, 2006) and some berry seed oils (e.g. blackberry and red raspberry seed oils) (Van Hoed *et al.*, 2009; Van Hoed *et al.*, 2011).

Table 5.5 *Tocopherols content of the dragon fruit seed oil*

Tocopherol (mg/kg)	White-flesh dragon fruit seed oil	Red-flesh dragon fruit seed oil
δ-tocopherol	38.70 ± 0.24^{a}	34.65 ± 5.48^{a}
γ-tocopherol	75.62 ± 2.23^{a}	144.92 ± 4.76^{b}
α-tocopherol	292.94 ± 9.88^{a}	477.33 ± 6.52^{b}
Total tocopherols	407.26 ± 7.90^{a}	656.89 ± 5.80^{b}

Results are shown as mean ± SD (N=3) and data within rows followed by different letters are significantly different ($p < 0.05$)

The γ- and α-tocopherols contents in the red-flesh dragon fruit seed oil were significant higher than that of the white-flesh dragon fruit seed oil, while the δ-tocopherol content was similar for both seed oils. The total tocopherol content in the red-flesh dragon fruit seed oil

indeed was remarkably higher than that in the white-flesh dragon fruit seed oil (656 mg/kg compared to 407 mg/kg). The findings are in good agreement with the results found by Chemah *et al.* (2010) that the antioxidative properties of the seeds of *H. polyrhizus* are significantly higher than that of *H. undatus*. However, the total amount of tocopherols in the studied dragon fruit seed oil is higher compared to the values reported by Lim *et al.* (2010a). It is possible due to the different oil extraction method. Interestingly, the total tocopherol content of the dragon fruit seed oil is also higher than that of olive oil (160-378 mg/kg) (Krichene *et al.*, 2010), grape seed oil (143-358 mg/kg) (Baydar *et al.*, 2007) and some berry seed oils like strawberry seed oil (280 mg/kg) and cranberry seed oil (139 mg/kg) (Van Hoed *et al.*, 2009). The total tocopherol content of the studied dragon fruit seed oil is, however, similar to sesame oil (454-700 mg/kg) (Aued-Pimentel *et al.*, 2006), while it is much lower than blackberry and red raspberry seed oils (1369 and 2106 mg/kg, respectively) (Van Hoed *et al.*, 2009). However, the concentrations of tocopherols may vary by original source, cultivar, agronomic aspects and processing conditions.

5.4.4 Thermal properties

The dragon fruit seed oil was completely liquid at room temperature due to the presence of a large amount of low melting TAG (**Table 5.4**). The thermal properties of the dragon fruit seed oil were analyzed by DSC. The thermal melting and crystallization curves of the oil as a function of temperature are shown in **Figure 5.5**. The melting curve for the dragon fruit seed oil consisted of four endothermic peaks with a broader temperature range. These peaks were in the temperature range from -49 to 6 °C with the major melting peak (M_2, **Figure 5.5**) between the range (-30 °C for the white-flesh dragon fruit seed oil and -22 °C for the red-flesh dragon fruit seed oil). The DSC crystallization profiles of both dragon fruit seed oils had two exothermic peaks in which the main crystallization peak (C_2, **Figure 5.5**) was found in the higher temperature region. The white-flesh dragon fruit seed oil crystallized between -48 and -11 °C, whereas the red-flesh dragon fruit seed oil crystallized at slightly higher temperatures (between -42 and -8 °C).

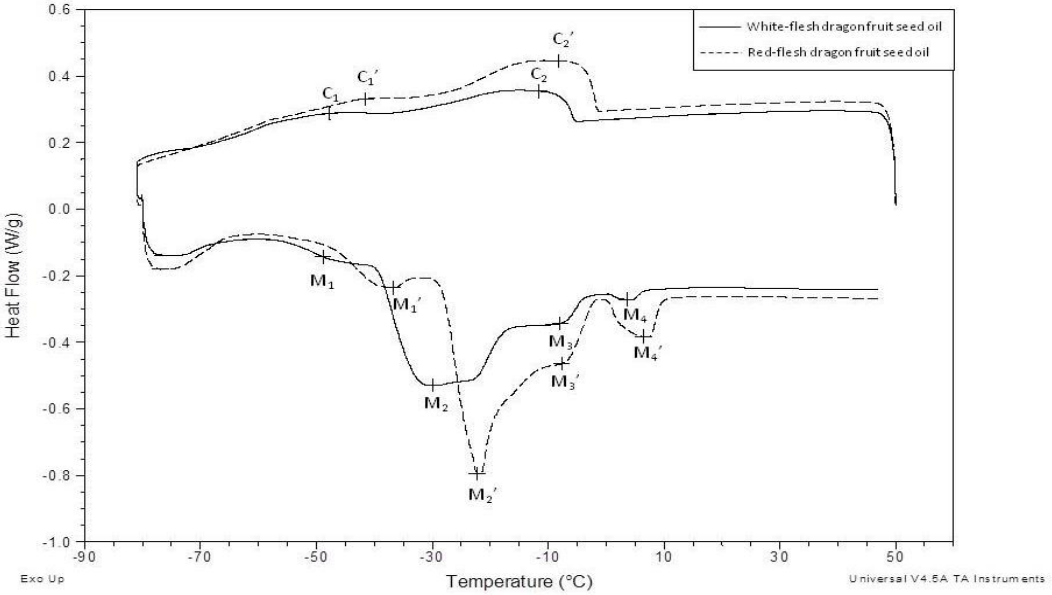


Figure 5.5 *DSC profile for melting temperature (M) and crystallization temperature (C) of the white-flesh dragon fruit seed oil (smoothed line) and the red-flesh dragon fruit seed oil (dashed line)*

Moreover, the average crystallization and the melting temperatures of triplicate analyzed of the dragon fruit seed oil are illustrated in **Table 5.6**. The melting temperatures in the red-flesh dragon fruit seed oil seemed to be higher than that in the white-flesh dragon fruit seed oil as well as a higher crystallization temperature was also found in the red-flesh dragon fruit seed oil. These results correspond well with the results of TAG (**Table 5.4**) and are in good agreement with those found by Lim *et al.* (2010a).

Table 5.6 *Crystallization and melting temperatures of the dragon fruit seed oil*

Thermal properties (°C)	White-flesh dragon fruit seed oil	Red-flesh dragon fruit seed oil
Melting temperature (M)		
M_1	-48.58 ± 0.43[a]	-36.66 ± 0.41[b]
M_2	-29.61 ± 0.82[a]	-22.01 ± 0.65[b]
M_3	-9.26 ± 1.09[a]	-7.04 ± 0.98[b]
M_4	3.41 ± 0.54[a]	6.25 ± 0.98[b]
Crystallization temperature (C)		
C_1	-47.82 ± 0.87[a]	-41.56 ± 0.28[b]
C_2	-11.38 ± 0.59[a]	-8.19 ± 0.88[b]

Results are shown as mean ± SD (N=3) and data within rows followed by different letters are significantly different ($p < 0.05$)

5.4.5 Influence of storage conditions on oxidative stability

The oxidative stability of the white-flesh and the red-flesh dragon fruit seed oils was studied under 2 different storage conditions, at CT (~4 °C) and RT (~20 °C), over 12 weeks. PV generally represents an accurate index for freshness and quality of the oil during storage. It can be determined by using different techniques. In this work, two different analytical techniques were used to monitor the oxidation progress of the oil: the FOX method expressing PV as mequiv O_2/kg and the ferric thiocyanate method representing the index of peroxide formation as the absorbance at a wavelength of 500 nm.

Figure 5.6, it can be seen that the PVs of both dragon fruit seed oils gradually increased during the storage time, suggesting the oxidation of the double bonds of UFAs in the oils occurs during the early stages of the autocatalytic free-radical chain reaction and then peroxide, a primary oxidation product, among the oxidation forms (Choe and Min, 2006). During the storage, the progress of oxidation in both seed oils was comparable. However, a slightly lower PV value was measured for the red-flesh dragon fruit seed oil at week 7-10 compared to the white-flesh dragon fruit seed oil. This can be explained by the lower UFAs content of the red-flesh dragon fruit seed oil compared to that of the white-flesh dragon fruit seed oil (**Table 5.3**) and a significant amount of tocopherols present in the red-flesh dragon fruit seed oil (**Table 5.5**). After a 3-month storage period, the influence of the storage conditions on the progress of oxidation was clearly observed for both dragon fruit seed oils. The PVs at RT were higher than that at CT (16.3 mequiv O_2/kg compared to 14.5 mequiv O_2/kg).

Additionally, the evolution of the peroxide formation index for the absorption at 500 nm during a 3-month storage period was investigated and the results are illustrated in **Figure 5.7**. Formation of primary compounds of oxidation occurred gradually until the first 8 weeks at both CT and RT conditions, but beyond week 8, a drastic increase in PV was observed at CT (from 1.42 to 1.85 for the white-flesh dragon fruit seed oil and from 1.54 to 1.71 for the red-flesh dragon fruit seed oil). It was even more pronounced at RT (from 1.66 to 2.39 for the white-flesh dragon fruit seed oil and from 1.74 to 2.51 for the red-flesh dragon fruit seed oil). From 9 weeks onwards, a remarkable change in the specific extinction at 500 nm was not observed. It can be summarized that the results of peroxide formation index are in good agreement with the results of PVs of the seed oils, showing an upward trend during storage.

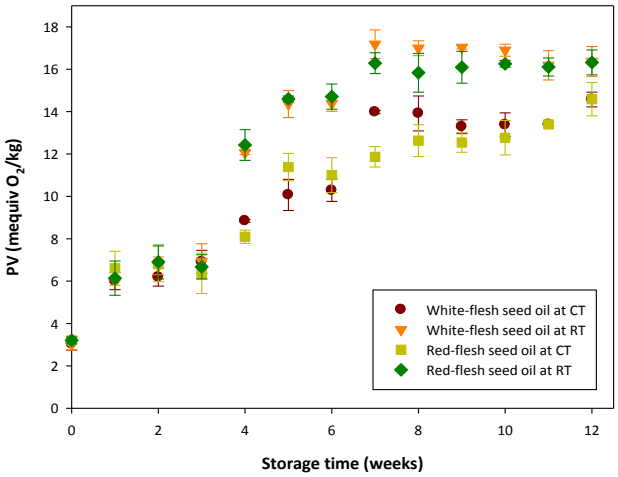


Figure 5.6 *Progress of peroxide value (PV) in the dragon fruit seed oil during storage at cold temperature (CT) and room temperature (RT)*

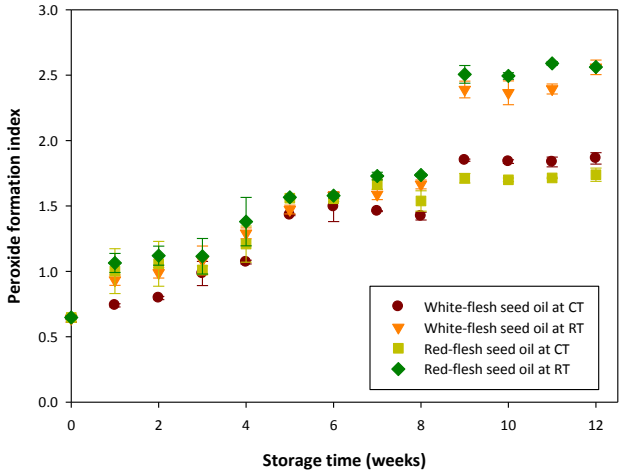


Figure 5.7 *Progress of peroxide formation index in the dragon fruit seed oil during storage at cold temperature (CT) and room temperature (RT)*

Additionally, the oxidation rate was also estimated based on the PV of the oils after storage for 12 weeks compared to that of the oils at initial day. This can be expressed in mequiv O_2/kg/week and the results are given in **Table 5.7**. Only a small increase in the oxidation rate was observed for all treatments. The oxidation rate at RT was slightly higher than that at CT (1.09-1.11 mequiv O_2/kg/week compared to 0.95-0.96 mequiv O_2/kg/week), while no significant differences between both dragon fruit seed oils were observed.

Table 5.7 *Oxidation rate (mequiv O_2/kg/week) under different storage conditions*

Seed oil	Cold temperature	Room temperature
White-flesh dragon fruit seed oil	0.96	1.11
Red-flesh dragon fruit seed oil	0.95	1.09

The oxidation rates of the dragon fruit seed oils are comparable values with the reported oxidation progress of virgin olive oil (Krichene *et al.*, 2010), which is known as an oil with a good oxidative stability (Choe and Min, 2006). Therefore, it can be claimed that the dragon fruit seed oil had a good resistance to oxidation probably due to the high presence of the endogenous antioxidants (tocopherols content, **Table 5.5**).

5.4.6 Influence of storage conditions on tocopherol content

The tocopherol present in the dragon fruit seed oil was monitored weekly over a 3-month storage period under two different conditions (at CT and RT). The results are shown in **Figure 5.8**. The tocopherol content of both dragon fruit seed oils displayed a declining behaviour with a more pronounced effect during extended storage. With storage under CT conditions, a gradual decrease in all tocopherol forms of both seed oils was observed through the storage period, particularly of the α-tocopherol. After 12 weeks of storage at CT, the α-tocopherol content in the seed oils clearly showed the highest decrease (from 293 to 241 mg/kg for the white-flesh dragon fruit seed oil and from 477 to 409 mg/kg for the red-flesh dragon fruit seed oil), followed by the δ-tocopherol (from 39 to 8 mg/kg for the white-flesh dragon fruit seed oil and from 35 to 16 mg/kg for the red-flesh dragon fruit seed oil). The γ-tocopherol content of both dragon fruit seed oils displayed the lowest decrease after a 3-month storage period (from 76 to 67 mg/kg for the white-flesh dragon fruit seed oil and from 145 to 127 mg/kg for the red-flesh dragon fruit seed oil). Thus, it can be deduced that the γ-tocopherol in the extracted dragon fruit seed oils was the most stable, while the δ-tocopherol, on the other hand, seemed to be the least stable. The decrease in the tocopherol content can be explained by the fact that the natural antioxidants are being used to protect the oil against oxidation.

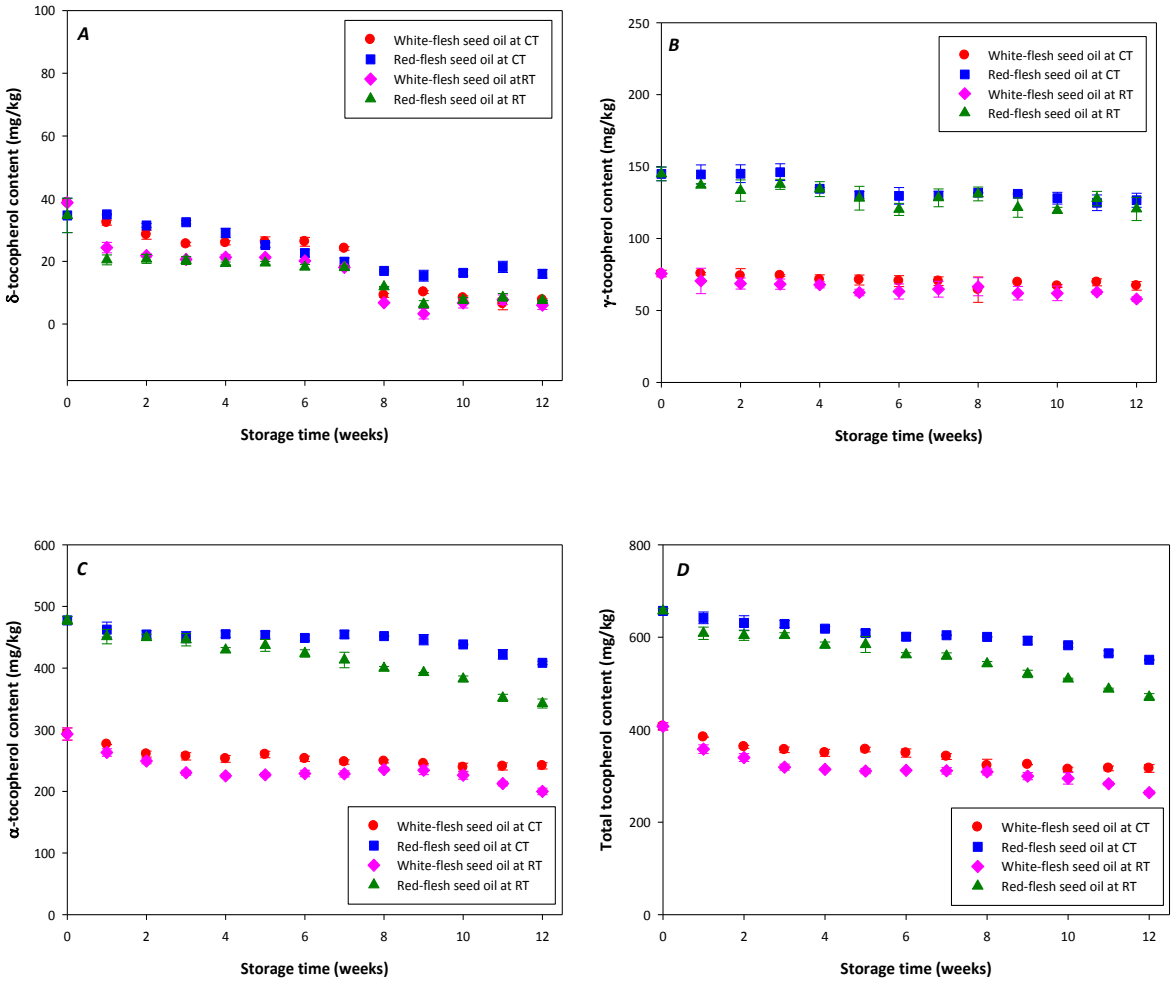


Figure 5.8 *Behaviour of tocopherols in the dragon fruit seed oil during storage at cold temperature (CT) and room temperature (RT): (A) δ-tocopherol content, (B) γ-tocopherol content, (C) α-tocopherol content and (D) total tocopherol content*

During a 3-month storage period at RT, a decrease in the tocopherol contents in the white-flesh and red-flesh dragon fruit seed oils was also observed (**Figure 5.8**). The α-tocopherol content again showed the highest decrease after 12 weeks at RT (declining to 200 and 343 mg/kg), followed by the δ-tocopherol (declining to 6 and 8 mg/kg) and the γ-tocopherol (declining to 58 and 121 mg/kg) for the white-flesh and the red-flesh dragon fruit seed oils, respectively.

From the behaviour of tocopherols at both CT and RT, it can be deduced that the decrease in total tocopherols relied mainly on the decline of α-tocopherol content. A positive correlation between total tocopherol and α-tocopherol contents was, moreover, exhibited (R^2 = 0.8721-0.9801). After 12 weeks, the total tocopherol content of the oil, however,

showed an important retention in which the total tocopherol content of the oils under CT condition was remarkably higher than that at RT (78-84% at CT compared to 65-72% at RT based on the initial content).

In addition, it was clearly shown that the apparent relationship between the reductions of tocopherols (**Figure 5.8**) corresponded well with the increase in oxidation (**Figures 5.6-5.7**) during storage. A strong negative correlation between PV and total tocopherol content was also found ($R^2 \geq 0.7173$). This indicates that tocopherols act as antioxidants. The tocopherols may act as chemical scavengers of oxygen radicals, especially singlet oxygen, as chain breakers and as physical deactivators of singlet oxygen by charge transfer mechanism (Kanner and Rosenthal, 1992). It has also been suggested that the phenolic and phytosterol compounds present in dragon fruit seed oil (Lim *et al.*, 2010a) are being used along with the tocopherols. Moreover, there are a lot more of other naturally bioactive substances present in other oils such as sesamol, sesamolin and sesamin in sesame oil (Yoshida and Takagi, 1997) and hydroxytyrosol and tyrosol derivatives in virgin olive oil (Krichene *et al.*, 2010) which may retard the lipid oxidation.

5.5 Conclusion

Currently, the consumer awareness for healthy food products is amplifying and food scientists have been searching for interesting sources of healthy components. Due to the fatty acid profile and the interesting antioxidative components of the dragon fruit (*Hylocereus* spp.) seed oil, the oil could be considered as a new potential oil. The beneficial effects of the oil are related to its fatty acid composition. Linoleic acid was the most abundant fatty acid present in both white-flesh and red-flesh dragon fruit seed oils. The dragon fruit seed oil was high in essential fatty acids, particularly in the white-flesh dragon fruit seed oil, and contained a significant amount of natural antioxidants (tocopherols), especially in the red-flesh dragon fruit seed oil. Thus, the dragon fruit seed oil can be considered as high-value oil due to its oil composition.

During a 3-month stability experiment, the progress of oxidation took place and simultaneously both the PV and the peroxide formation index increased, whilst the disappearance of all tocopherol forms was observed. This correlation indicates that tocopherols are used against the oxidation. However, after 12 weeks under CT and RT conditions, the dragon fruit seed oil still contained a relatively high amount of tocopherols and showed a low oxidation rate, demonstrating a high oxidative stability.

CHAPTER 6

Pectic Enzymes in Dragon Fruit

This chapter is based on:

Liaotrakoon, W., Van Buggenhout, S., Christiaens, S., Houben, K., De Clercq, N., Dewettinck, K. and Hendrickx, M. (2013) An explorative study on the cell wall polysaccharides in the pulp and peel of dragon fruits (Hylocereus spp.). ***European Food Research and Technology****, DOI 10.1007/s00217-013-1997-7, Published online on April 30.*

6. Pectic enzymes in dragon fruit

6.1 Introduction

Plant cell wall materials generally consist of polysaccharide components (e.g. pectic, hemicellulosic and cellulosic substances) which provide rigidity for plant cells (Cosgrove, 2005). Softening is one of the most dramatic changes accompanying the ripening of many fruits. It is associated with alterations in the cell wall and middle lamella structure and results in cell wall disassembly. This is mainly due to the actions of specific cell wall-related enzymes (Chin *et al.*, 1999a). Softening enzymes (e.g. PME, PG, PL, β-galactosidase and cellulase) hydrolyze the cell wall components according to various mechanisms which depend on the structure of the cell wall polysaccharides (Abu-Goukh and Bashir, 2003; Duvetter *et al.*, 2009). The pectin structures in cell walls can be naturally modified by pectic enzymes (i.e. PME and PG) and it clearly results in the most apparent changes that favour consumption such as appearance, flavour, viscosity and cloud stability (Buggenhout *et al.*, 2009). The general observation of fruit softening is, moreover, accompanied by solubilization and swelling of cell walls, involving mainly the action of PME and PG (Cosgrove, 2001).

PME catalyzes methyl ester from O6 of GalA in HG region, releases methanol and protons, and creates negatively charged carboxyl groups. It provides de-esterification of pectic polysaccharides, resulting in textural changes by the decrease of the intercellular adhesivity and tissue rigidity (Assis *et al.*, 2001; Duvetter *et al.*, 2009; Terefe *et al.*, 2009). On one hand, the de-esterified pectin obtained from PME catalysis can be further hydrolyzed by PG. This results in a decrease of the degree of polymerization of the pectin chains and a loss of tissue firmness. On the other hand, the de-esterified pectin chains may cross-link with bivalent ions to form pectate gels (Alonso *et al.*, 1997).

The inactivation of pectic enzymes may have beneficial effects to control either desired or undesired quality of the end products. Thermal processing is the most common technology that has been used to inactivate pectic enzymes, but it also affects the product quality attributes such as colour, viscosity, texture and nutritional values. Some authors have successfully explored the combination of novel processes with more conventional techniques to inhibit the activity of pectic enzymes with negligible food quality alterations. Examples of such are combined thermal and high intensity pulsed electric field treatments (Espachs-Barroso *et al.*, 2006), and combined thermal and high pressure processing

(Guiavarc'h *et al.*, 2005). However, conventional thermal processing remains one of the most widely used in food industry due to its simplicity and low costs.

The activity of pectic enzymes has been widely studied in various original plant sources as well as the influence of thermal treatment on enzyme inactivation. The main objective of the study was to determine the activity of pectic enzymes (i.e. PME and PG) in the peel and the pulp of two species of dragon fruit, i.e. white-flesh and red-flesh dragon fruits, and also to investigate pectic enzymes inactivation in these dragon fruits as affected by various thermal treatments.

6.2 Research strategy

A schematic overview of the experimental set-up of this chapter is presented in **Figure 6.1**. In the study, the PME and PG activities of the pulp and the peel of white-flesh and red-flesh dragon fruits were determined by using titration and colorimetric techniques, respectively. Due to heat sensitivity of the enzymes, the thermal inactivation was experimentally performed at different temperatures ranging from 30 °C to 90 °C (10 °C intervals, included 75 °C) for a constant heating time of 10 min. Then, the residual enzyme activity of heated samples was analyzed.

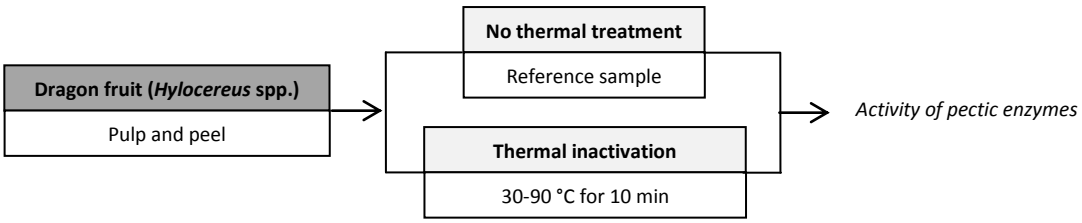


Figure 6.1 *Schematic overview of the experimental set-up to investigate the activity of pectic enzymes in the dragon fruit and to study the thermal inactivation of the enzymes*

The outcome of the study can be used for process design in dragon fruit industry such as dragon fruit juice, puree and their related-products. Moreover, the results were further applied to allow the inactivation of pectic enzymes in dragon fruits prior to isolation of cell wall polysaccharides, as explained in the following chapter.

6.3 Materials and methods

6.3.1 Preparation of dragon fruit

Two species of dragon fruit, i.e. white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*) originating from Thailand, were used in this study. Both the peel and the pulp of the two species of dragon fruit were investigated separately. Mature dragon fruit was washed, cut into pieces and peeled by hand. The dragon fruit peel was then frozen with liquid N_2, whereas the dragon fruit pulp was extracted using a juice extractor (Magicmix Le Duo, France) to remove all seeds prior to freezing with liquid N_2 and storage at -40 °C for next analysis.

6.3.2 Thermal treatment for enzyme inactivation

About 35 g of the dragon fruit sample was vacuum-packed in a polyethylene bag and subjected to a thermal treatment in a temperature-controlled water bath at 30, 40, 50, 60, 70, 75, 80 and 90 °C for a residence time of exactly 10 min. After the treatments, the heated dragon fruit sample was immediately cooled in an ice-water bath to stop the reaction, frozen with liquid N_2 and stored at -40 °C for the analysis of pectic enzymes activity.

6.3.3 Analysis of pectin methylesterase activity

6.3.3.1 Crude pectin methylesterase extraction

A procedure for crude PME extraction and PME activity assay in the dragon fruit sample is shown in **Figure 6.2**, following the procedure described by Ly-Nguyen *et al.* (2002b). In order to extract PME from the cell wall materials of the peel sample, the dragon fruit peel was twice homogenized at 7500 rpm for 20 sec in a Grindomix (GM 200, Retsch) and five grams of the crushed peel were filled in a centrifuge tube. About 10 ml (two times of the sample weight) of 0.2 M Tris-HCl buffer containing 1.0 M NaCl (pH 8.0) was then added into the tube. Afterwards, the salt extracts (supernatant phase) were collected by centrifugation at 10000 g for 60 min at 4 °C. The PME extracts were frozen with liquid N_2 and stored at -40 °C for the PME activity assay, whereas the dragon fruit pulp was subjected to the PME activity assay without extraction.

6.3.3.2 Pectin methylesterase activity assay

The PME activities of the untreated pulp and peel dragon fruit samples (see **Section 6.3.1**) and the residual PME activity of the thermally treated pulp and peel dragon fruit samples (see **Section 6.3.2**) were determined in triplicate by the following procedure. The continuous recording of the titration of the carboxyl groups released from a pectin solution with 0.01 N NaOH using an automatic pH-Stat (ΩMetrohm, Herisau, Switzerland) was preformed, as described by Ly-Nguyen *et al.* (2002b). In the reaction vessel, the pulp sample and the crude peel PME extract were added to 30 ml of a 0.35% (w/v) apple pectin solution (DM 70-75%, Sigma-Aldrich, Bornem, Belgium) containing 0.117 M NaCl at pH 7.0 and 22 °C. As the pH used in the analysis has a significant effect on the results, two pH levels (pH 4.5 and 7.0) were experimentally performed to find the best result. The results are further discussed in **Section 6.4.1**.

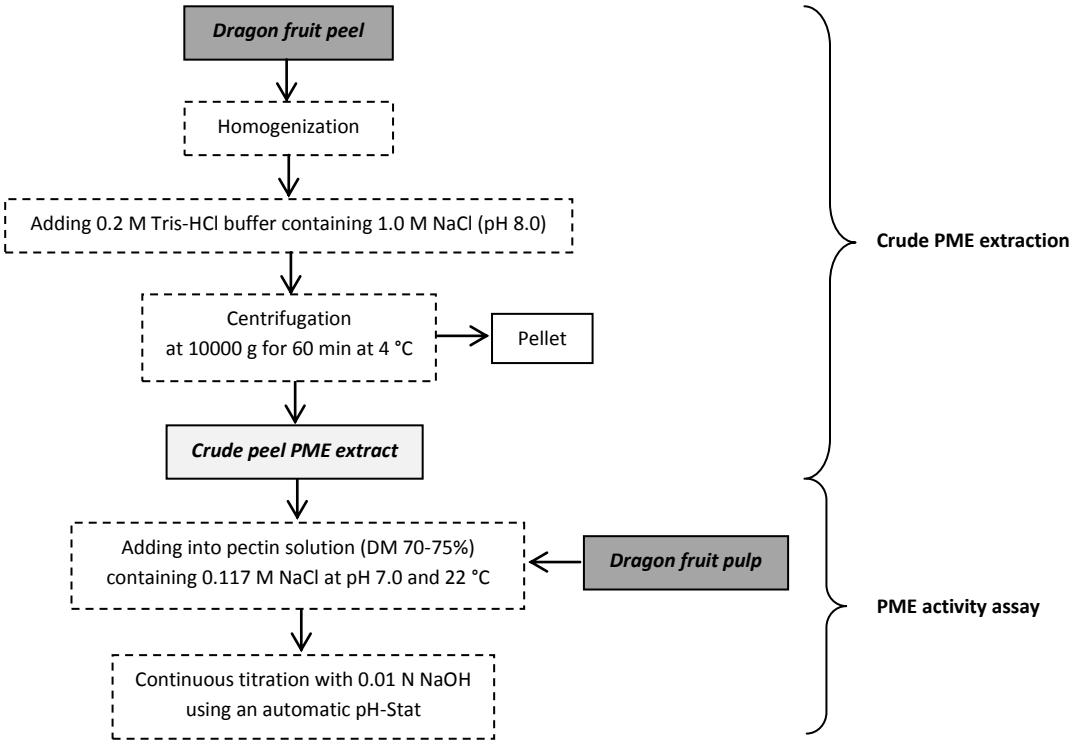


Figure 6.2 *Schematic representation of the PME activity analysis of the dragon fruit*

For PME activity calculation, one unit of PME is defined as the amount of enzyme required to release 1 µmol of carboxyl groups per min, under the aforementioned conditions. PME activity measured in units per ml (U/ml) is, therefore, computed by **Eq. 6.1**.

$$\text{PME activity (U/ml)} = \frac{\text{NaOH (ml)} \times \text{molarity of NaOH (M)} \times 10^6}{\text{time (min)} \times \text{sample (ml)} \times 10^3} \quad (\mathbf{6.1})$$

Since the titration yields the PME activity of a given sample size in ml of NaOH used per min and the molarity of NaOH is known (0.01 M), the PME activity was then recomputed by the following simplified formula (**Eq. 6.2**).

$$\text{PME activity (U/ml)} = \frac{\text{PME activity measured (ml/min)} \times 10}{\text{sample (ml)}} \quad (\mathbf{6.2})$$

Eventually, PME activity can be expressed in units per gram of the dragon fruit tissue sample (U/g) and it can be computed by **Eq. 6.3**.

$$\text{PME activity (U/g)} = \left[\frac{\text{PME activity measured (ml/min)} \times 10}{\text{injected sample (ml)}}\right] \times \left[\frac{\text{total volume of supernatant (ml)}}{\text{sample weight (g)}}\right] \quad (\mathbf{6.3})$$

The PME activity of the thermally treated (inactivated) dragon fruit samples were also expressed as a percentage of residual activity of PME as a function of temperatures.

6.3.4 Analysis of polygalacturonase activity

6.3.4.1 <u>Crude polygalacturonase extraction</u>

Figure 6.3 demonstrates a procedure for crude PG extraction and PG activity assay of the pulp and the peel of the two species of dragon fruit, following the procedure described by Pressey (1986). A 100 g of the dragon fruit peel was homogenized with 50 ml cold deionized water in a Buchi mixer (B-400, Flawil, Switzerland). Subsequently, approximately 15 g of the homogenized sample was weighed in a centrifuge tube and 10 ml of cold deionized water

was added. The mixture was adjusted to pH 3.0 with 0.1 M HCl, stirred for 15 min and centrifuged for 20 min at 9000 g. Afterwards, the pellet was again washed with 10 ml of cold deionized water, readjusted to pH 3.0 with 0.1 M HCl, stirred for 15 min and then centrifuged for 20 min at 9000 g. Next, the pellet was resuspended in 15 ml of 1.2 M NaCl and adjusted to pH 6.0 with 0.1 M NaOH. The suspension was allowed to stir for 3 h while keeping the pH constant by adding 0.1 M NaOH and was centrifuged at 10000 g for 20 min. All the crude PG extraction steps were carried out at 4 °C. The crude peel PG extracts were collected from the supernatant phase, frozen with liquid N_2 and kept at -40 °C until further analysis, while the dragon fruit pulp sample was directly applied to the PG activity assay.

6.3.4.2 Polygalacturonase activity assay

The PG activities of the pulp sample and the crude peel PG extract were measured spectrophotometrically in triplicate by the liberation of reducing groups from polyGalA, as described by Pressey (1986). The reaction mixture containing 50 µl of the sample and 350 µl of 0.3% (w/v) polyGalA in 40 mM sodium acetate buffer (pH 4.4) was incubated in a temperature-controlled water bath at 35 °C for 0, 2, 4, 6, 8 and 10 min. After reaching the incubated time, 2.0 ml borate buffer (0.1 M, pH 9.0) was immediately added to the incubated reaction mixture in an ice-water bath. Afterwards, 0.4 ml of 1% (w/v) 2-cyanoacetamide in water was also added in the mixture to react with reducing groups to give the product that absorbs at 267 nm. The reaction mixture was immersed in an oil bath at 100 °C for 10 min and then immediately cooled in an ice-water bath. After equilibration at 22 °C, the amount of reducing groups from the hydrolyzed sample was determined using a spectrophotometer at a wavelength of 276 nm at 22 °C. A blank was determined in the same way by replacing the sample with sodium acetate buffer solution. The PG activity (U/min) can be estimated from the slope of the absorbance value at 276 nm (reaction progress) against the incubated time (0-10 min) and then expressed in U/g.

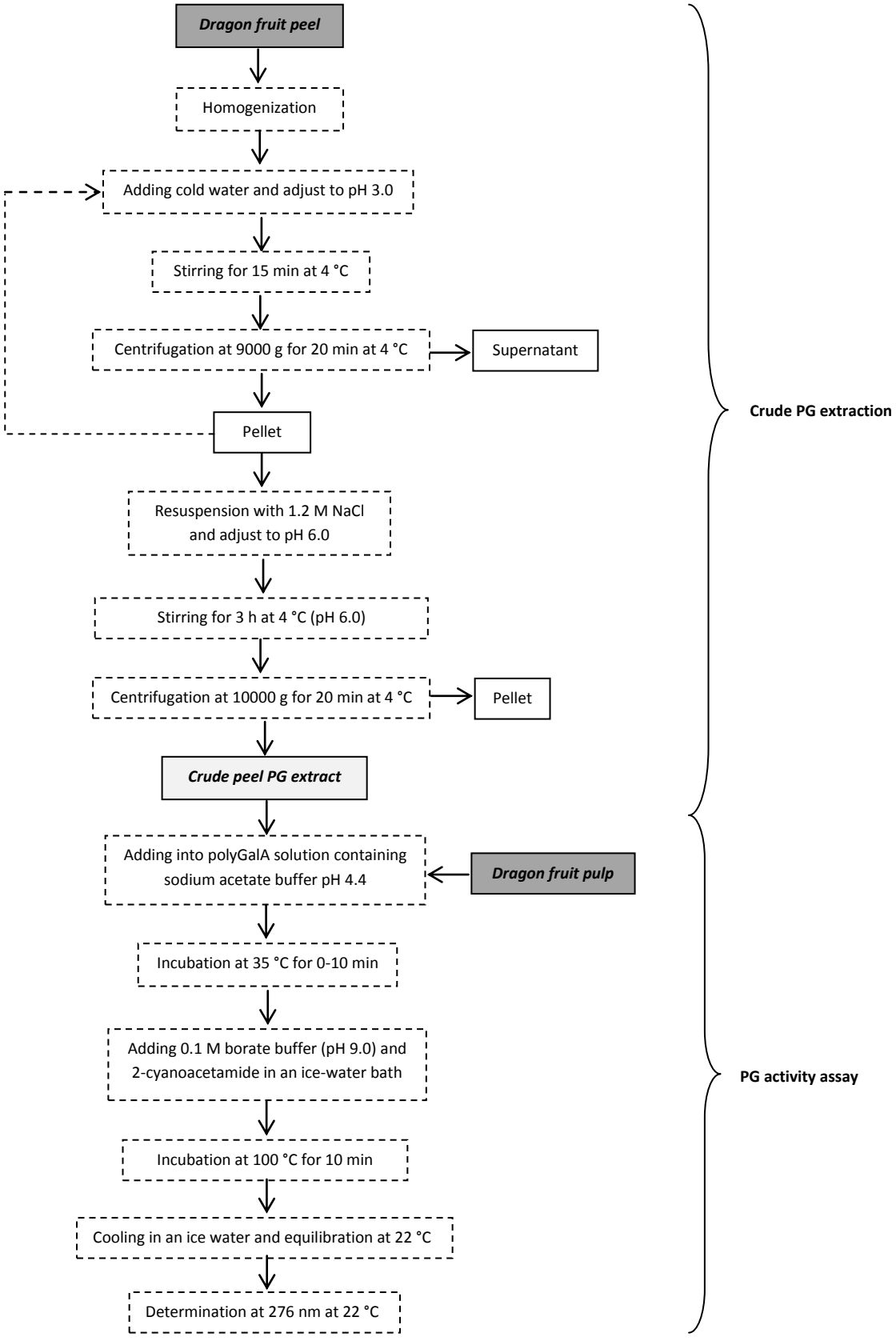


Figure 6.3 *Schematic representation of the PG activity analysis of the dragon fruit*

6.4 Results and discussion

6.4.1 Activity of pectic enzymes

As the enzymatic pectin conversion is supposed to be a consequence of PME action (pectin de-esterification) and followed by PG activity (pectin depolymerization), PME and PG are considered as important pectic enzymes in various plant cell walls. PME catalyzes pectic polysaccharides, resulting in the removal of methoxyl groups from pectin and the production of methanol and H^+ ions. Based on the measurement of PME activity by titration with alkaline solution, NaOH is used to maintain pH value at a set pH and the titration curve between volume of NaOH and time can be plotted to estimate the PME activity. In order to optimize the PME activity procedure, the assay was performed at two different pH levels: pH 4.5 which refers to the average of initial pH value of the dragon fruit sample and pH 7.0 which is usually considered as the set pH for the PME activity assay. **Figure 6.4** shows the titration curve determined at pH 4.5 and 7.0 in four dragon fruit samples, the pulp and the peel of the white-flesh and the red-flesh dragon fruits. An increase of NaOH at pH 7.0 was clearly observed compared to that at pH 4.5, indicative of the magnitude of PME activity. Thus, the PME activity assay of the dragon fruit sample was strongly recommended to be performed at pH 7.0.

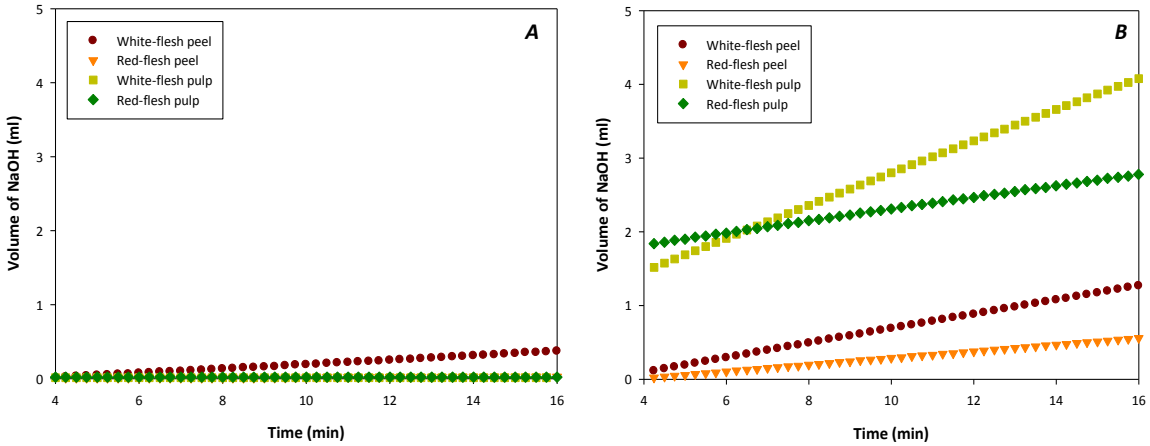


Figure 6.4 *Volume of NaOH vs. action time at (A) pH 4.5 and (B) pH 7.0 during PME measurement of the pulp and peel of the dragon fruit*

In the current work, both the pulp sample and the peel extract of the two species of dragon fruit showed measurable PME activity (**Table 6.1**), whereas no measurable amounts of PG activity in all dragon fruit samples could be determined by the selected procedure. Moreover, the PME activity in the white-flesh dragon fruit was higher than that in the red-flesh dragon fruit: in the pulp samples of, respectively, the white-flesh and the red-flesh (untreated) dragon fruit, 4.91 and 1.61 U PME activity per 1 g of fresh fruit were measured while in the peel extracts of respectively the white-flesh and the red-flesh dragon fruits, 3.18 and 2.20 U PME activity per 1 g of fresh fruit were measured.

Table 6.1 *PME activity in the pulp and peel of the dragon fruit*

Dragon fruit	PME activity (U/g)
White-flesh peel	3.18 ± 0.05
Red-flesh peel	2.20 ± 0.05
White-flesh pulp	4.91 ± 0.29
Red-flesh pulp	1.61 ± 0.03

Results are shown as mean ± SD (N = 3)

However, the PME activities of the dragon fruit are in agreement with that of immature acerola fruit (Assis *et al.*, 2001). The endogenous PME activity has a significant effect on the pectin DM due to obtaining the de-esterified pectic substances (Abu-Goukh and Bashir, 2003; Assis *et al.*, 2001; Hernandez-Pérez *et al.*, 2005) during fruit ripening as well as during fruit preparation.

6.4.2 Thermal inactivation of pectic enzymes

In the experimental work, only PME activity was monitored during thermal treatment because the thermal inactivation of PG was not possible to follow due to the absence of PG activity in the dragon fruit. In order to study the influence of heating temperature on PME activity and to identify blanching conditions that allow complete inactivation of PME present in the pulp and the peel of the dragon fruit, a thermal stability screening study was performed at 30-90 °C (interval 10 °C included 75 °C) for 10 min.

The dragon fruit pulp and peel were thermally treated at different treatments after which the residual PME activity in the peel extracts and the pulp samples was determined. In **Figure 6.5**, the residual PME activity in the different dragon fruit samples is plotted as a

function of temperature. The figure clearly shows that 10-min treatments at temperatures higher than 70 °C allow significant depletion of residual PME activity in the different dragon fruit samples. After 10-min treatments at temperatures between 30 and 70 °C, more than 79% of the PME activity in the different samples was retained. A treatment at 80 °C for 10 min resulted in an almost complete inactivation of the PME activity (6-8%). At 90 °C for 10 min, very low residual PME activity in the dragon fruit samples was measured (less than 4% compared to the initail PME activity). The PME residual of all dragon fruit samples after heating at 80 °C and 90 °C for 10 min was comparable. From an application point of view, it can be concluded that the dragon fruit PME can be inactivated by a heat treatment at 80 °C for 10 min and this condition was, moreover, selected as a blanching treatment for inactivation of the endogenous PME in the dragon fruit samples intended for pectin characterization (see **Chapter 7**).

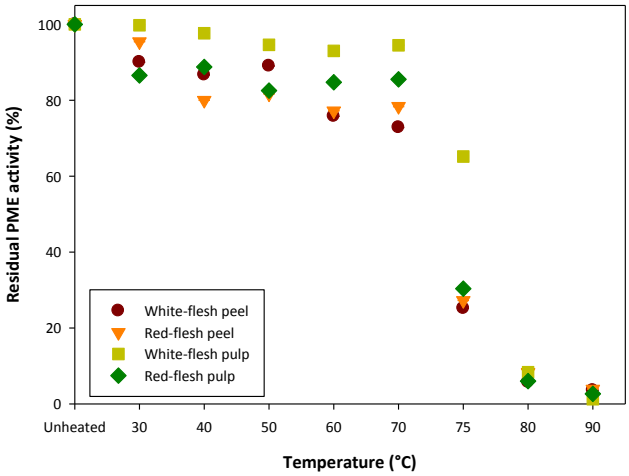


Figure 6.5 *Residual activity of PME in the dragon fruit after 10-min treatments at different temperatures*

Upon comparing the residual PME activity in the thermally treated dragon fruit pulp and peel samples with PME thermal stability data available in literature, it can be deduced that PME in the dragon fruit is rather thermostable (**Table 6.2**). The thermostability of the dragon fruit PME is comparable to banana PME (Ly-Nguyen *et al.*, 2002a), but higher than, for example, the thermostability of plum PME (Nunes *et al.*, 2006), tomato PME (Plaza *et al.*, 2007). Moreover, it is less thermally stable than that of, e.g. acerola PME (Assis *et al.*, 2007) and white grapefruit PME (Guiavarc'h *et al.*, 2005).

Table 6.2 *Thermal stability data of PME in different sources available in literature*

Purified PME Source	Thermal stability	Reference
Plum PME	Completely inactivated at 56 °C after 5 min for pH 4 and at 80 °C after 5 min for pH 7.5	Nunes *et al.* (2006)
Tomato PME	Completely inactivated at 70 °C after 5 min	Plaza *et al.* (2007)
Banana PME	Completely inactivated at 80 °C for 5 min	Ly-Nguyen *et al.* (2002a)
Acerola PME	Retained $\geq$ 66% of initial PME activity at 98 °C after 60 min	Assis *et al.* (2007)
Grapefruit PME	Retained 87% of initial PME activity at 80 °C after 10 min	Guiavarc'h *et al.* (2005)

The heating process is the most used method to inactivate enzymes, but it may cause degradation of colour, flavour and nutritional compounds as well as loss of textural attribute. The use of non-thermal technologies (e.g. high pressure and pulsed electric filed technologies) is currently gaining popularity. It would be recommended to combine with thermal treatment for inactivation of pectic enzymes and for maximizing the final product quality, e.g. textural characteristics (Duvetter *et al.*, 2009). On the other hand, some food industries need action of PME to improve the characteristic of the food items such as for fruit juice clarification (Aliaa *et al.*, 2010).

6.5 Conclusion

The untreated white-flesh dragon fruit had a higher PME activity compared to the untreated red-flesh dragon fruit, while the absence of PG activity in all untreated dragon fruit samples was found. However, the PME activity in the dragon fruit did not show a significant high amount ($\leq$ 4.91 U/g). The results also demonstrated that a mild heat processing (temperature below 70 °C) had no significant effects to inactivate PME in the pulp and the peel dragon fruits. On the other hand, a moderate heat processing (80-90 °C) allowed PME inactivation by eliminating up to 96% of the PME activity. The dragon fruit pulp is also known to contain significant amount of viscous mucilaginous polysaccharides, making the fruit difficult for juice processing and resulting in low yields during juice or pulp extraction. Therefore, the outcome of the work can be useful for dragon fruit processing. The PME activity in the dragon fruit can remain after treatment below 70 °C for 10 min and it may result in the enhancement of clear juice extraction. In contrast, a treatment at 80 °C and 90 °C for 10 min can probably contribute to a decline of destabilization and cloud loss characteristic in dragon fruit juice, while allowing pasteurization and good quality retention of the final product.

CHAPTER 7

Characterization of Cell Wall Polysaccharides of Dragon Fruit

This chapter is redrafted after:

Liaotrakoon, W., Van Buggenhout, S., Christiaens, S., Houben, K., De Clercq, N., Dewettinck, K. and Hendrickx, M. (2013) An explorative study on the cell wall polysaccharides in the pulp and peel of dragon fruits (Hylocereus spp.). ***European Food Research and Technology****, DOI 10.1007/s00217-013-1997-7, Published online on April 30.*

7. Characterization of cell wall polysaccharides of dragon fruit

7.1 Introduction

Plant cell walls consist of a complex polysaccharide network which contributes to the structural rigidity of the plant. The primary plant cell wall predominantly contains pectic substances, hemicelluloses, celluloses and water. Small amounts of glycoproteins, phenolic compounds, minerals and cell wall-related enzymes are also present (O`Neil and York, 2003). Pectin is a heterogeneous polysaccharide which is embedded in the cellulose microfibrils skeleton and comprises HG, RG-I, and RG-II. Besides GalA which is the main component of pectin, 16 different monosaccharides are present to form the pectin network (Vincken *et al.*, 2003).

HG is a linear homopolymer of 1,4-linked α-D-GalA residues (galacturonans) in which some of carboxyl groups of GalA residues are methyl-esterified at the C6 position and may also be O-acetylated at the C2 and C3 positions (Willats *et al.*, 2001). The polyGalA in HG region can be substituted with Xyl residues, refers to as XG. Some GalA residues in XG can also be methyl-esterified. RG-I, on the other hand, contains a backbone of the repeating disaccharide 1,2-linked α-L-Rha and 1,4-linked α-D-GalA in which sugar side chains (e.g. α-L-arabinofuranosyl and β-D-galactopyranosyl residues) may be substituted at the Rha residues (Ridley *et al.*, 2001; Voragen *et al.*, 2009; Willats *et al.*, 2001). GalA residues in the RG-I backbone may be O-acetylated at C3 and/or C2, but are presumably not methyl-esterified or substituted with side chains (Willats *et al.*, 2001). RG-II has, in contrast to RG-I, a backbone of GalA residues, in particular it consists of approximately nine GalA residues which are substituted with 4 hetero-oligomeric side chains. These side chains have a consistent composition containing specific sugar residues, e.g. Dha and Kdo (Vincken *et al.*, 2003; Willats *et al.*, 2001).

Besides pectic substances, hemicellulosic polysaccharides are a group of complex cell wall polysaccharides that are strongly bound to cellulose microfibrils (Brett and Waldron, 1996; Scheller *et al.*, 2007). The main sugar present in the backbone of the polymers is used to classify different hemicelluloses (Brink and Vries, 2011). For instance, xyloglucans have a backbone of 1,4-linked β-D-glucan and are extensively substituted with 1,6-linked α-D-Xyl at the C6 position of the Glc residues (Brett and Waldron, 1996). Arabinoxylans comprise a xylan backbone (1,4-linked β-D-xylan) and are decorated with Ara branches (Cosgrove, 2005). Mannans are characterized by a backbone of 1,4-linked β-D-mannose (Man) residues

which may also contain other monosaccharide residues such as 1,4-linked β-D-Glc substitution with Gal side chains (O`Neil and York, 2003).

The functional properties of pectin in food products as a gelling agent, stabilizer, thickener, texturizer and emulsifier largely depend on the structural properties of pectin (Thakur *et al.*, 1997). Depending on the DM, two main gelation mechanisms can be distinguished. High DM pectin (DM > 50%) gelates in presence of high amounts of sugar at low pH values, while the gelation of low DM pectin (DM < 50%) occurs in presence of divalent cations, particularly Ca^{2+}, and optimally at pH well above the pK_a of GalA (Sila *et al.*, 2009). Commercially, apple pomace and citrus peel are the most important sources for pectin extraction (Canteri-Schemin *et al.*, 2005). Even thought, a number of potential 'new' sources for pectin extraction such as sugar beet pulp (Yapo *et al.*, 2007), soy bean byproduct okara (Mateos-Aparicio *et al.*, 2010), passion fruit peel (Kliemann *et al.*, 2009; Kulkarni and Vijayanand, 2010) and cactus fruit (*Opuntia* spp.) (Cardenas *et al.*, 2008; Majdoub *et al.*, 2001), have been explored recently.

Dragon fruit (*Hylocereus* spp.) is commonly consumed fresh and processed in the forms of juice and puree products. The dragon fruit products have a large market potential as they can be applied into a whole range of food products. During processing of dragon fruit, a considerable amount of the dragon fruit peel (by-products) is discarded. As both the peel (Jamilah *et al.*, 2011) and the fruit pulp (Mahattanatawee *et al.*, 2006) are known to contain significant amounts of mucilaginous polysaccharide substances, in particular pectin, they might be considered as potential sources of functional pectin.

In this context, it is important to have detailed information on the structure of pectin that largely determines its functionality. Information regarding the cell wall polysaccharides in the peel of dragon fruit and in dragon fruit pulp (and fruits of different species) is, however, currently missing. Therefore, the main objective of the present study was to isolate and explore the different fractions of cell wall polysaccharides in the pulp and peel of white-flesh and red-flesh dragon fruits. These polysaccharide fractions were characterized in terms of GalA content, DM, neutral sugar composition, MM distribution and affinity towards some specific anti-pectin antibodies.

7.2 Research strategy

Figure 7.1 presents a schematic overview of the experimental set-up of this chapter. Prior to isolation the polysaccharides, the blanching at 80 °C for 10 min (see **Section 6.4.2**) was

applied to allow PME inhibition in the dragon fruit sample. After that, the pulp and the peel of the two species of dragon fruit were then subjected to an AIR extraction. The DM and GalA content of the AIR of the dragon fruit sample was determined. Subsequently, the cell wall polysaccharides of the pulp and the peel of the dragon fruit were fractionated by using various solutions to obtain five polysaccharide fractions, i.e. WSF, CSF, NSF, HF and remaining residue.

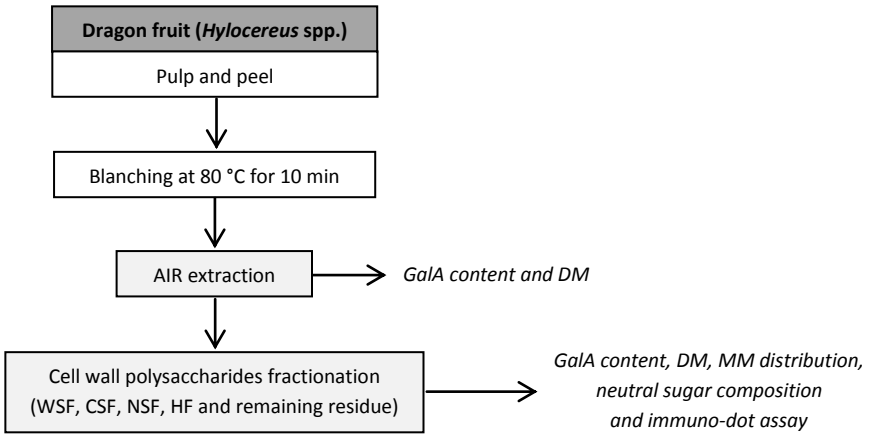


Figure 7.1 *Schematic overview of the experimental set-up to characterize the cell wall polysaccharides of dragon fruit*

To elucidate the structure of pectins and hemicelluloses in the pulp and the peel of the two dragon fruit species, the cell wall polysaccharide fractions were structurally characterized as follows: the DM of the WSF and the CSF and the GalA content of all polysaccharide fractions were determined using colorimetric methods. The neutral sugar composition of the WSF, the CSF, the NSF and the HF was determined using HPAEC for quantitation of Fuc, Rha, Ara, Gal, Glc, Xyl and Man. The MM patterns of the lyophilized WSF and CSF were investigated by HPSEC. Also, immuno-dot assays with various anti-pectin antibodies (i.e. JIM5, JIM7, LM18, LM19, LM20 and PAM1) of the WSF and the CSF were performed. The experimental work may allow for characterization the possible valuable compounds from the cell wall materials of dragon fruit and for maximal usage of dragon fruit peel (by-products).

7.3 Materials and methods

7.3.1 Preparation of dragon fruit

Mature white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*) from Thai origin were used in the work. The fruit was washed with water, cut into pieces and hand-peeled. The pulp of the dragon fruit was extracted using a juice extractor (Magicmix Le Duo, France) to remove all seeds. Then, the pulp and the peel samples were blanched at 80 °C for 10 min to inactivate pectic enzymes, frozen with liquid N_2 and stored at -40 °C until further experiments.

Additionally, the blanched pulp and the peel of the two species of dragon fruit were subjected to determine pH value and dry matter by using standard method (AOAC, 1995). The results showed that the average pH value for the pulp and the peel of the dragon fruits (irrespectively of the species) was 4.6 $\pm$ 0.01 and 4.9 $\pm$ 0.02, respectively. The dry matter content of the pulp sample was 13.1% $\pm$ 0.05 for the white-flesh dragon fruit and 11.1% $\pm$ 0.01 for the red-flesh dragon fruit, whereas that of the peel sample was 9.5% $\pm$ 0.08 for the white-flesh dragon fruit and 8.8% $\pm$ 0.01 for the red-flesh dragon fruit.

7.3.2 Extraction of alcohol-insoluble residue

The AIR was extracted following the procedure described by McFeeters and Armstrong (1984). A 10 g of the dragon fruit samples was homogenized with 63.3 ml of 95% ethanol using a mixer (Buchi mixer B-400, Flawil, Switzerland). The suspension was vacuum filtered over a filter paper (Machery-Nagel MN615, 90 mm diameter) and the residue was rehomogenized with 31.7 ml of 95% ethanol and filtered again. Subsequently, 31.6 ml of acetone was added to the residue. After stirring this solution for 10 min, the final filtration was performed. The residue, further referred to as AIR, was dried overnight at 40 °C, grounded and stored in a desiccator at room temperature.

7.3.3 Fractionation of alcohol-insoluble residue

The cell wall polysaccharides present in the AIR were sequentially fractionated in duplicate using different extraction solvents. A schematic representation of this sequential extraction procedure is shown in **Figure 7.2**.

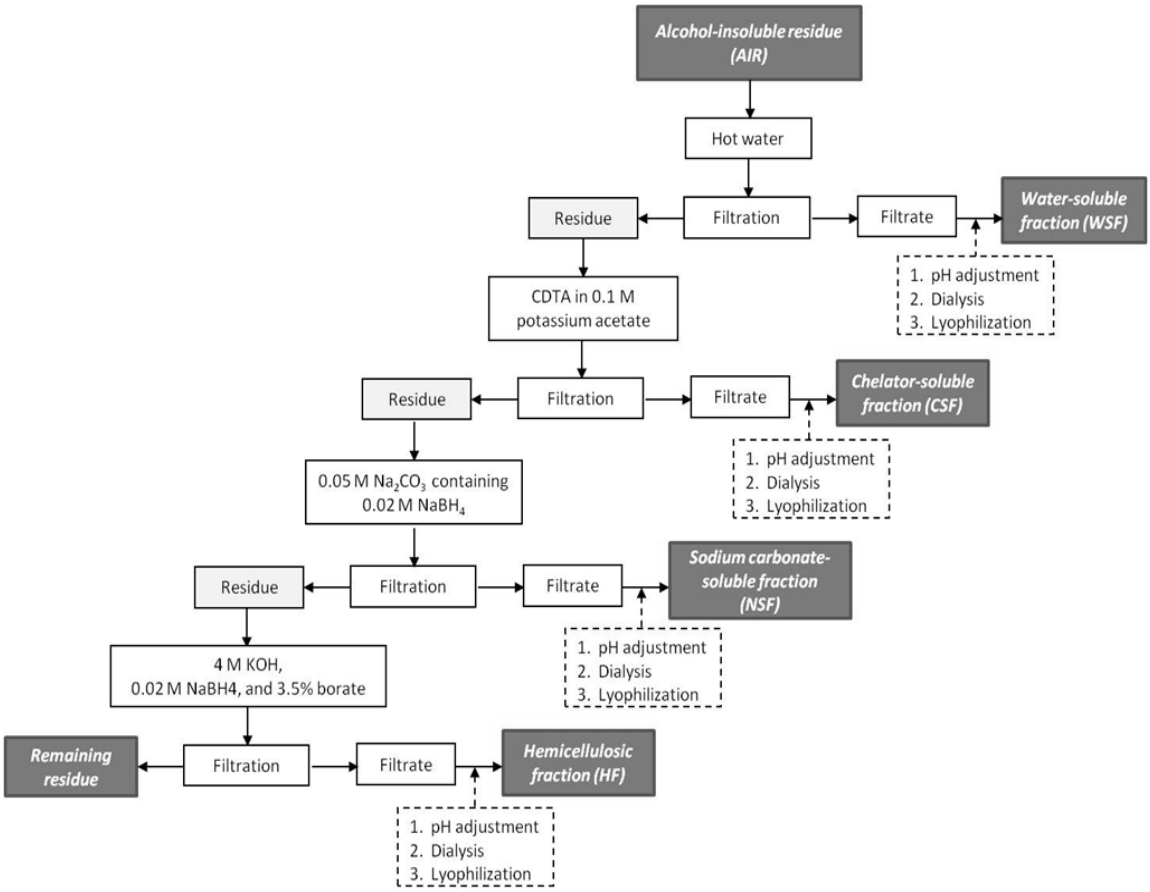


Figure 7.2 *Schematic representation of the procedure for sequential fractionation of cell wall polysaccharides from AIR of the dragon fruit*

The extraction of the WSF was preformed as following described (Braga *et al.*, 1998). The AIR (1 g) was stirred in 100 ml boiling demineralized water (pH 4) for 5 min. This mixture was cooled down and vacuum filtrated over a filter paper (Machery-Nagel MN615, 90 mm diameter). The pH of the filtrate was adjusted to pH 5 by 0.1 M HCl to obtain the WSF. The residue was subsequently incubated in 100 ml 0.05 M CDTA in 0.1 M potassium acetate at pH 5 for 6 h in a shaking water bath at 28 °C. The suspension was filtered and the filtrate was adjusted to pH 5 to provide the CSF (Chin *et al.*, 1999b). In a next step, the residue was reincubated in 100 ml 0.05 M Na_2CO_3 containing 0.02 M $NaBH_4$ for 16 h at 4 °C and for another 6 h at 28 °C in a shaking water bath. The mixture was filtered and the filtrate was adjusted to pH 5 to obtain the NSF (Chin *et al.*, 1999b). The final extraction step was performed to isolate hemicelluloses and some pectins strongly bound to cellulose or hemicelluloses by strong alkali. The residue was reincubated in 100 ml 4 M KOH, 0.02 M $NaBH_4$ and 3.5% borate for 22 h at room temperature, but under N_2 atmosphere. The suspension was filtered over a glass sinter filter and the filtrate was adjusted to pH 5 to

obtain the HF (Renard and Ginies, 2009). Next to the HF, the remaining residue was collected and dried overnight at 40 °C.

Finally, the polysaccharide fractions were extensively dialyzed (molecular weight cut-off of 3500 Da, Spectra/Por®, Spectrum Laboratories Inc., Rancho Dominguez, USA) for 48 h at 4 °C against demineralized water for the WSF, the NSF and the HF, whereas the CSF was dialyzed against 0.1 M NaCl for 24 h and demineralized water for another 24 h. The dialysis liquids were regularly changed. The dialyzed fractions were then lyophilized using a freeze-dryer (Christ Alpha 2-4 LSC, Osterode, Germany), grounded and stored in a desiccator until further characterization.

7.3.4 Determination of degree of methoxylation

The DM of the AIR and the pectin in the WSF and CSF were determined using spectrophotometric methods and calculated as the molar ratio (%) of the methyl-ester content to the GalA content of the samples. The respective hydrolyzes were done in duplicate for both determinations of methyl-ester groups and GalA. Three colorimetric analyses were performed for each hydrolyzate.

7.3.4.1 Determination of methyl-ester groups

The AIR and polysaccharide samples were saponified hydrolyzed with 2 M NaOH for 1 h at 20 °C for saponification of the ester bonds. The saponified samples were subsequently neutralized by 2 M HCl for 15 min at 25 °C and diluted in phosphate buffer pH 7.5 (Ng and Waldron, 1997). The amount of methanol released was determined by colorimetry (Klavons and Bennett, 1986). The saponified samples were allowed to react with alcohol oxidase (from *Pichia pastoris*, Sigma-Aldrich, Bornem, Belgium) containing 1 U/ml at pH 7.5 and 25 °C for 15 min. After addition of a pentanedione solution (2 M ammonium acetate, 0.05 M acetic acid and 0.02 M 2,4-pentanedione) and incubation at 58 °C for 15 min, a yellow-coloured product was produced which was measured with a spectrophotometer at a wavelength of 412 nm (Ultraspec 1100 pro, GE Healthcare, Sweden). For making a calibration curve, various concentrations of methanol standard solutions between 0 and 20 µg methanol per ml phosphate buffer were prepared.

7.3.4.2 Determination of galacturonic acid content

Prior to the GalA analysis, the sample was hydrolyzed with concentrated sulfuric acid (98% H_2SO_4) for 1 h and water (stepwise added to a final concentration of 50%) (Ahmed and Labavitch, 1977). The GalA content was estimated quantitatively by a spectrophotometric method according to the procedure described by Blumenkrantz and Asboe-Hansen (1973). The hydrolyzed sample was thoroughly mixed with 0.0125 M sodium tetraborate in 98% H_2SO_4 solution, incubated for 5 min at 100 °C, immediately cooled in an ice-water bath. The mixed solution was then allowed to react with *m*-hydroxydiphenyl solution (0.15% 3-phenylphenol in 0.5% NaOH). The absorbance of the produced hydroxyl-phenyl phenol product was measured with a UV-VIS spectrophotometer (Ultraspec 500, GE Healthcare, Sweden) at a wavelength of 520 nm at 25 °C. In the assay, GalA monohydrate standard solutions containing 0-110 µg GalA per ml water were used to perform a standard curve.

7.3.5 Analysis of neutral sugar composition

Lyophilized polysaccharide fractions were hydrolyzed with 4 M trifluoroacetic acid at 110 °C for 1.5 h (Roeck *et al.*, 2008). The digested samples were cooled, dried under N_2 at 45 °C, neutralized with 1 M $NH_3.H_20$ and dried again under N_2 at 45 °C. Before analysis of neutral sugars, the samples were resolved with demineralized water (organic free, 18 MΩ cm resistance), supplied by a Simplicity™ water purification system (Millipore, Billerica, USA). The monosaccharides were analyzed using HPAEC with a Dionex system (DX600), equipped with a GS50 gradient pump, a CarboPac™ PA20 column (150 x 3 mm), a CarboPac™ PA20 guard column (30 x 3 mm). An ED50 electrochemical detector (Dionex, Sunnyvale, USA) equipped with a reference pH electrode (Ag/AgCl) and a gold electrode was used in the pulsed amperometric detection mode, performing a quadruple potential waveform. Potentials E_1 = 0.1 V, E_2 = -2.0 V, E_3 = 0.6 V and E_4 = -0.1 V were applied for duration times t_1 = 400 ms, $t_2 + t_3$ = 40 ms and t_4 = 60 ms.

Samples (10 µl) were injected and eluted at a flow rate of 0.5 ml/min at 30 °C. Two separate runs for each sample enabled a complete chromatographic separation of the targeted monosaccharides (Fuc, Rha, Ara, Gal, Glc, Xyl and Man). After equilibration with 100 mM NaOH for 5 min and another 5 min with 15 mM/0.5 mM NaOH (for run 1/2), samples were isocratically eluted using 15 mM/0.5 mM NaOH (for run 1/2) during 20 min. Afterwards, the column was regenerated using 500 mM NaOH for 10 min. Mixtures of commercial sugar standards (Fuc, Rha, Ara, Gal, Glc, Xyl and Man) at concentrations of 1 to 10 ppm were used daily as external standards for identification and quantitation.

To correct for degradation of the monosaccharides during the acid hydrolysis step, recovery values were estimated. Hereto, mixtures of the sugar standards were subjected to the aforementioned hydrolysis conditions and the peak areas were compared to those of untreated standard mixtures.

7.3.6 Analysis of molar mass distribution

The MM distribution of the polysaccharides fractions of the dragon fruit pulp and peel was studied using HPSEC. More specifically, an ÄKTA purifier equipped with a mixed-bed column of TSK-gel ($GMPW_{XL}$, 300 mm x 7.8 mm, pore size of 100-1000 Å, particle size of 13 µm, theoretical plates/column $\geq$ 70000, Tosch Bioscience, Stuttgart, Germany) in combination with a TSK guard column (PW_{XL}) was used. Data were analyzed with UNICORN software. After dissolving the lyophilized polysaccharide fractions in 0.05 M $NaNO_3$ and filtration (Millex-HV, Millipore filter, Carrigwohill, Co. Cork, Ireland), samples (20 µl) were injected in the system. Elution was performed at 35 °C with a 0.05 M $NaNO_3$ buffer at a flow rate of 0.7 ml/min. The eluent was monitored using a Shodex R101 refractive index detector (Showa Denko, K.K., Tokyo, Japan). Pullulan standards with a molecular weight ranging from 188 to 788000 Da were eluted to allow a rough estimation of the MM of the polysaccharide fractions.

7.3.7 Immuno-dot assays on polysaccharide fractions

Immuno-dot assays with anti-pectin antibodies, as described by the procedure of Christiaens *et al.* (2011c), were performed using following anti-pectin antibodies: JIM5, JIM7, LM18, LM19, LM20 and PAM1 (PlantProbes, Leeds, United Kingdom). The specificity of these antibodies is given in **Table 7.1**. Lyophilized pectic fractions were diluted to a concentration of 5 µg/µl, 0.5 µg/µl, 50 ng/µl and 5 ng/µl in demineralized water. One microlitre aliquots of each dilution were dotted onto a piece of nitrocellulose (Amersham Biosciences, 0.45 µm pore size) which was allowed to dry for 30 min. After drying, the nitrocellulose membrane was blocked for 1 h with phosphate-buffered saline (PBS, pH 7.4) containing 5% milk powder (MPBS) while shaking. After washing with demineralized water and PBS, an incubation step (1.5 h while shaking) with the primary antibody (diluted 1/20 in 1% MPBS) was performed for 1.5 h.

Table 7.1 *Anti-pectin antibodies used for immuno-dot assays*

Monoclonal antibody	Antigen	Specific action	Original reference
JIM5	HG	Binds to pectin with a medium DM	VandenBosch *et al.* (1989): Knox *et al.* (1990)
JIM7	HG	A general anti-HG probe	Knox *et al.* (1990)
LM18	HG	Preference for low-esterified pectin	Verhertbruggen *et al.* (2009)
LM19	HG	Preference for low-esterified pectin	Verhertbruggen *et al.* (2009)
LM20	HG	Preference for high-esterified pectin	Verhertbruggen *et al.* (2009)
PAM1	De-esterified HG blocks	Binds to long blocks of unesterified GalAs	Willats *et al.* (1999): Manfield *et al.* (2005)

After another extensive washing step, the membranes were incubated with secondary antibody for 1 h. An anti-rat Ig horseradish peroxidase conjugate (Nordic Immunology, Tilburg, The Netherlands) was diluted 1/1000 in 1% MPBS and used as secondary antibody for primary antibodies JIM5, JIM7, LM18, LM19, and LM20. In the case of the primary antibodies PAM1, an anti-polyhistidine peroxidase antibody (Sigma-Aldrich, St. Louis, Missouri), diluted 1/2000 in 1% MPBS, was applied as secondary antibody. Finally, a colour reagent (a mixture of chloronaphthol solution (3% chloronaphthol in absolute ethanol, w/v) and 27% H_2O_2 in PBS) was added. After 40 min incubation in a dark room, the development of positive (purple stained) blots was evaluated. All steps were carried out at room temperature.

7.4 Results and discussion

7.4.1 Degree of methoxylation

The resulting values of the DM, an essential parameter for pectin functionality, are given in **Table 7.2**. The DM of the pectic substances in the NSF, the HF and the remaining residue could not be determined due to the saponification of the methyl-ester groups of the GalAs during the fractionation procedure. In general, the DM of the AIR and of pectin in the WSF and CSF of all dragon fruit samples, and particularly of the white-flesh pulp samples, is low (5-39%). Hereby, it must also be emphasized that intact peel samples were blanched prior to the preparation of AIR. In this way, the obtained DM values reflect the DM values of pectins in intact dragon fruit peels. On the contrary, pulp samples were extracted from fresh fruits (after which a blanching step was performed prior to AIR extraction). As a consequence, PME might have lowered the DM of the pectin during this preparation step and the obtained DM values might no longer reflect the DM values of the intact fruit flesh.

Practically, it is, however, very difficult to determine the *in situ* DM of liquid-like fruit flesh such as dragon fruit flesh, using extracted cell wall material. The observed DM values for both the pulp and the peel samples were, however, lower for the white-flesh dragon fruit compared to the respective red-flesh dragon fruit samples which is probably linked to the higher endogenous PME activity in the red-flesh dragon fruit (as previously discussed in **Section 6.4.2**).

Table 7.2 *GalA content of the AIR and degree of methoxylation of the dragon fruit*

Dragon fruit	GalA content (mg/g AIR)	Degree of methoxylation (%)		
	AIR	AIR	WSF	CSF
White-flesh peel	252.3 ± 8.7	23.5 ± 0.6	24.4 ± 0.8	21.7 ± 1.1
Red-flesh peel	268.2 ± 9.6	37.9 ± 0.4	39.4 ± 0.2	24.9 ± 1.2
White-flesh pulp	108.2 ± 4.6	18.3 ± 0.8	6.2 ± 0.4	5.0 ± 0.9
Red-flesh pulp	111.6 ± 5.6	20.9 ± 0.3	16.5 ± 0.4	16.1 ± 0.7

Results show as mean ± SD (N=2 hydrolyzates x 3 measurements).

AIR: Alcohol-insoluble residue, WSF: Water-soluble fraction, CSF: Chelator-soluble fraction

The DM values in the pectin fractions of the red-flesh dragon fruit peel can be compared with values in literature: they seem to be in good agreement with the DM values obtained by other authors (31-47% (Ismail *et al.*, 2012) and 16% (Tang *et al.*, 2011)). On the other hand, the DM value of pectin in red-flesh dragon fruit pulp (extracted using an extractor) found by Ramírez-Truque *et al.* (2011) was remarkably higher (80%) than that the value obtained in the current work. In this case, pectic material was isolated using an acid extraction procedure. The observed difference between this result (80%) and the low DM values obtained in the current study reflect the fact that acid-extracted pectins and pectin in the AIR can be quite different. Also, in other tropical fruits (and by-products) like, for example, yellow passion fruit rind (26-32% DM (Yapo and Koffi, 2006) and 5-38% DM (Yapo and Koffi, 2008)), prickly pear peel and nopal pulp (*Opuntia* spp.) (9-32% DM (Majdoub *et al.*, 2001)), low-esterified pectin seems to be present.

The higher DM values of the AIR from dragon fruit peel samples compared to those of the pulp are reflected in the DM values obtained for the WSF and CSF. Also, the peel of white-flesh and red-flesh exhibits a similar pattern for the DM: a high value for the WSF (24.4-39.4%), a lower value for the CSF (21.7-24.9%) and the DM of the AIR in between (23.5-37.9%). The low DM value of the AIR of the pulp samples (18.3-20.9%) is, in contrast to the peel samples, not reflected in the DM values of the WSF (6.2% for the white-flesh pulp and

16.5% for the red-flesh pulp) and CSF (5.0% for the white-flesh pulp and 16.1% for the red-flesh pulp). This discrepancy may be ascribed to high-esterified *in situ* NSF.

7.4.2 Neutral sugar composition and galacturonic acid content

The GalA content of the AIR of the dragon fruit pulp and peel is given in **Table 7.2**. A remarkably higher GalA content in the AIR of the peel was found in comparison with the GalA content in the AIR of the pulp. This difference in GalA content will particularly be related to the difference in GalA content in the WSF of pulp and peel samples. **Table 7.3** shows the neutral sugar and GalA content of the different polysaccharide fractions of the dragon fruit peel and pulp. GalA is the main monomers in all the pectic fractions (WSF, CSF and NSF) of both the peel and the pulp samples, but the amount of GalAs is especially high in the WSF of the peel samples, indicating the importance of pectic HG in this fraction. The lower GalA content in the WSF in the pulp samples compared to the WSF in the peel samples can be ascribed to the preparation of the dragon fruit pulp samples in which some pectin, and particularly water-soluble (and thus loosely bound) pectin, is removed from the pulp upon juice extracting.

In the CSF and the NSF of the peel samples and in all the pectin fractions of the pulp samples, GalAs are also the main monomers, but the contribution of neutral sugars, and especially of Rha, Ara and Gal, to these fractions is significant, suggesting that next to HG, RG-I pectic components are of relative importance within these fractions. In the WSF of the pulp samples, a relative high amount of Ara and Gal, both sugar monomers in the side chains of RG-I (arabinan, galactan, and/or arabinogalactan side chains), are present. On the other hand, especially Ara seems to be present within the CSF of the pulp samples and especially Rha, the sugar monomer of the RG-I backbone, seems to be present in the CSF and the NSF of the peel samples.

Also, a small amount of GalAs seems to be present in the HF of all samples, indicating that part of the pectic substances within the cell wall are strongly attached to the other polysaccharides, hemicellulose and cellulose. The monomer composition of the HF was, however, shown to be largely different from the monomer composition of the pectin-rich fractions (WSF, CSF and NSF). The most important sugars in all HFs were Glc and Xyl, revealing the presence of xyloglucans and xylans. Man is also present in significant amounts in the HFs of the samples, and especially in the HF of the peel samples, indicative for the presence of mannans in the hemicellulosic fraction. Also, a remarkable high content of Fuc in the HF was observed in comparison with the pectin-rich polysaccharide fractions.

Table 7.3 *Neutral sugar and GalA contents (mmol/g AIR) of the polysaccharide fractions of the dragon fruit peel and pulp*

Dragon fruit		Sugar content (mmol/g AIR)							
		Fuc	**Rha**	**Ara**	**Gal**	**Glc**	**Xyl**	**Man**	**GalA**
White-flesh peel	WSF	0.0009 ± 0.0000	0.0749 ± 0.0039	0.0219 ± 0.0001	0.0351 ± 0.0030	0.0191 ± 0.0025	0.0270 ± 0.0005	0.0101 ± 0.0007	0.6989 ± 0.0052
	CSF	0.0001 ± 0.0001	0.0108 ± 0.0004	0.0081 ± 0.0000	0.0088 ± 0.0001	0.0008 ± 0.0001	0.0020 ± 0.0004	0.0008 ± 0.0000	0.1890 ± 0.0018
	NSF	0.0003 ± 0.0001	0.0145 ± 0.0001	0.0092 ± 0.0009	0.0184 ± 0.0015	0.0005 ± 0.0000	0.0049 ± 0.0012	0.0006 ± 0.0000	0.1158 ± 0.0109
	HF	0.0033 ± 0.0005	0.0013 ± 0.0003	0.0076 ± 0.0007	0.0187 ± 0.0004	0.0578 ± 0.0010	0.0626 ± 0.0057	0.0349 ± 0.0051	0.0218 ± 0.0030
Red-flesh peel	WSF	0.0008 ± 0.0002	0.0874 ± 0.0039	0.0158 ± 0.0040	0.0277 ± 0.0010	0.0186 ± 0.0018	0.0239 ± 0.0022	0.0109 ± 0.0013	0.6770 ± 0.0009
	CSF	0.0001 ± 0.0001	0.0066 ± 0.0004	0.0063 ± 0.0010	0.0063 ± 0.0016	0.0008 ± 0.0005	0.0018 ± 0.0006	0.0007 ± 0.0001	0.1085 ± 0.0045
	NSF	0.0004 ± 0.0002	0.0217 ± 0.0021	0.0088 ± 0.0021	0.0200 ± 0.0002	0.0004 ± 0.0004	0.0031 ± 0.0002	0.0005 ± 0.0001	0.2064 ± 0.0334
	HF	0.0050 ± 0.0018	0.0017 ± 0.0004	0.0119 ± 0.0011	0.0193 ± 0.0015	0.0539 ± 0.0037	0.0875 ± 0.0051	0.0424 ± 0.0020	0.0264 ± 0.0113
White-flesh pulp	WSF	0.0018 ± 0.0003	0.0388 ± 0.0030	0.0943 ± 0.0050	0.1014 ± 0.0045	0.0374 ± 0.0037	0.0211 ± 0.0001	0.0129 ± 0.0002	0.1102 ± 0.0052
	CSF	0.0002 ± 0.0001	0.0092 ± 0.0031	0.0498 ± 0.0058	0.0149 ± 0.0055	0.0011 ± 0.0006	0.0026 ± 0.0004	0.0015 ± 0.0002	0.1028 ± 0.0036
	NSF	0.0005 ± 0.0000	0.0107 ± 0.0011	0.0501 ± 0.0064	0.0192 ± 0.0023	0.0021 ± 0.0004	0.0036 ± 0.0001	0.0035 ± 0.0002	0.0761 ± 0.0001
	HF	0.0031 ± 0.0010	0.0107 ± 0.0006	0.0399 ± 0.0016	0.0301 ± 0.0002	0.0614 ± 0.0033	0.0830 ± 0.0026	0.0162 ± 0.0010	0.0365 ± 0.0050
Red-flesh pulp	WSF	0.0016 ± 0.0003	0.0418 ± 0.0010	0.1101 ± 0.0055	0.0953 ± 0.0018	0.0415 ± 0.0066	0.0194 ± 0.0013	0.0153 ± 0.0009	0.0888 ± 0.0027
	CSF	0.0002 ± 0.0000	0.0113 ± 0.0002	0.0329 ± 0.0003	0.0107 ± 0.0021	0.0008 ± 0.0001	0.0016 ± 0.0003	0.0014 ± 0.0003	0.0868 ± 0.0047
	NSF	0.0002 ± 0.0001	0.0111 ± 0.0006	0.0394 ± 0.0045	0.0097 ± 0.0011	0.0011 ± 0.0001	0.0023 ± 0.0004	0.0016 ± 0.0003	0.0742 ± 0.0021
	HF	0.0020 ± 0.0003	0.0059 ± 0.0013	0.0229 ± 0.0038	0.0187 ± 0.0019	0.0602 ± 0.0048	0.0623 ± 0.0009	0.0119 ± 0.0002	0.0292 ± 0.0039

Given values of neutral sugar composition obtained from HPAEC and GalA content obtained from spectrophotometric method show as mean ± SD (N=3).

AIR: Alcohol-insoluble residue, WSF: Water-soluble fraction, CSF: Chelator-soluble fraction, NSF: Sodium carbonate-soluble fraction, HF: Hemicellulosic fraction

Additionally, molar sugar ratios were calculated to interpret the sugar composition of the different dragon fruit samples (Houben *et al.*, 2011). The sugar ratios for the pectin-rich fractions (WSF, CSF and NSF) were estimated as follows: sugar ratio 1 is the ratio of GalA (indicative for the presence of the pectin backbone) to the sum of Fuc, Rha, Ara, Gal and Xyl (indicative for the presence of pectin side chains) (**Eq. 7.1**). This sugar ratio can be used as an estimation of the degree of linearity of pectin.

$$\text{Sugar ratio 1 (linearity of pectin)} = \frac{\text{GalA}}{\text{Fuc+Rha+Ara+Gal+Xyl}} \quad \textbf{(7.1)}$$

Sugar ratio 2 defined as the ratio of Rha to GalA, indicatively expresses the contribution of RG-I to the entire pectin population (**Eq. 7.2**), and sugar ratio 3 defined as the proportion of the sum of Ara and Gal to Rha, indicates the extent of RG-I branching (**Eq. 7.3**).

$$\text{Sugar ratio 2 (contribution of RG to pectin population)} = \frac{\text{Rha}}{\text{GalA}} \quad \textbf{(7.2)}$$

$$\text{Sugar ratio 3 (branching of RG-I)} = \frac{\text{Ara+Gal}}{\text{Rha}} \quad \textbf{(7.3)}$$

Although these three molar sugar ratios can be used to indicatively express the occurrence of certain pectic polysaccharides (HG and RG-I) under the assumption that pectin has a linear backbone in which RG-I and RG-II form a continuous backbone with the linear HG part (Willats *et al.*, 2006), it must be emphasized that these number do not provide structural information. To determine the molecular structure of pectin, structural analysis should be performed (Ralet *et al.*, 2009).

In the HF, sugar ratio 4 defined as the ratio of Man to Xyl, gives an indication of the contribution of mannans to the hemicellulose population (**Eq. 7.4**). Although the sugar ratios are an expression for importance of structural information of cell wall polysaccharides, it should be noted that they do not provide information related to the exact fine chemical structure of polymers.

Sugar ratio 4 (contribution of mannans to hemicelluloses) = $\frac{\text{Man}}{\text{Xyl}}$ **(7.4)**

The sugar ratios of the polysaccharide fractions of the pulp and the peel of dragon fruit are given in **Table 7.4**. Based on the resulting molar sugar ratios, further information on the level of the polymeric pectin structure was obtained. The resulting sugar ratios illustrate substantial differences between the pectic fractions of the dragon fruit pulp and the dragon fruit peel. For the peel of the dragon fruits, it can be concluded that the WSF, the CSF as well as the NSF are fairly linear (sugar ratio 1 between 2.45 and 6.34) and have a low contribution of RG to pectin population (sugar ratio 2 between 0.06 and 0.13). The resulting values for relatively low sugar ratio 3 (0.50-1.91), moreover, learn that the pectic RG-I parts are not branched extensively, confirming the relative high amount of Rha compared to Ara and Gal in these samples (**Table 7.3**). However, the NSF and CSF of the peel samples seem to contain more RG-I type compared to the WSF of the peel (1.3-1.9 compared to 0.5-0.8, sugar ratio 3).

Table 7.4 *Molar sugar ratios in relation to functional properties of polysaccharide fractions of the dragon fruit*

Dragon fruit		**Sugar ratio**			
		1	**2**	**3**	**4**
White-flesh peel	WSF	4.38	0.11	0.76	-
	CSF	6.34	0.06	1.55	-
	NSF	2.45	0.13	1.90	-
	HF	-	-	-	0.56
Red-flesh peel	WSF	4.35	0.13	0.50	-
	CSF	5.16	0.06	1.91	-
	NSF	3.78	0.11	1.33	-
	HF	-	-	-	0.48
White-flesh pulp	WSF	0.43	0.35	5.05	-
	CSF	1.27	0.09	7.03	-
	NSF	0.94	0.14	6.45	-
	HF	-	-	-	0.20
Red-flesh pulp	WSF	0.33	0.47	4.92	-
	CSF	1.53	0.13	3.87	-
	NSF	1.18	0.15	4.43	-
	HF	-	-	-	0.19

Upon comparing the sugar ratios for the different pectic fraction of the dragon fruit peel, it becomes also clear that the pectin linearity is the highest for the WSF, followed by the CSF and NSF, respectively. Interestingly, the sugar ratios of pectin in the dragon fruit pulp were shown remarkably different from the sugar ratios of the peel samples. Based on the values, it can be concluded that pectin in the dragon fruit pulp samples seem to be a lower linearity (sugar ratio 1 between 0.33 and 1.53), a higher RG content (sugar ratio 2 between 0.09 and 0.47) and higher branched RG-I (sugar ratio 3 between 3.87 and 7.03) compared to pectin in the peel samples. Especially the pectin in the WSF of the pulp samples seems to contain pectic material relatively rich in neutral sugars and thus has a high contribution of RG to the total pectin population. In the HF, it can be concluded that the HF in the peel of dragon fruit possesses a higher contribution of mannans to the hemicellulosic substances compared to the pulp samples (~0.5 compared to 0.2 based on sugar ratio 4).

Based on the amount of neutral sugars and GalA present in each fraction and the weight of each fraction, the extraction yield of pectin and hemicelluloses from AIR into the respective fractions was evaluated and the results are shown in **Figure 7.3**. The relative amount of fraction was calculated based on the amount of neutral sugars and GalA in the different fractions relative to the sum of the amount of neutral sugars and GlaA in the different fractions. The WSF appeared to be the larger fraction (38-47%) for all samples, followed by the HF (30-35%) and the NSF (11-14%) and the CSF (6-13%).

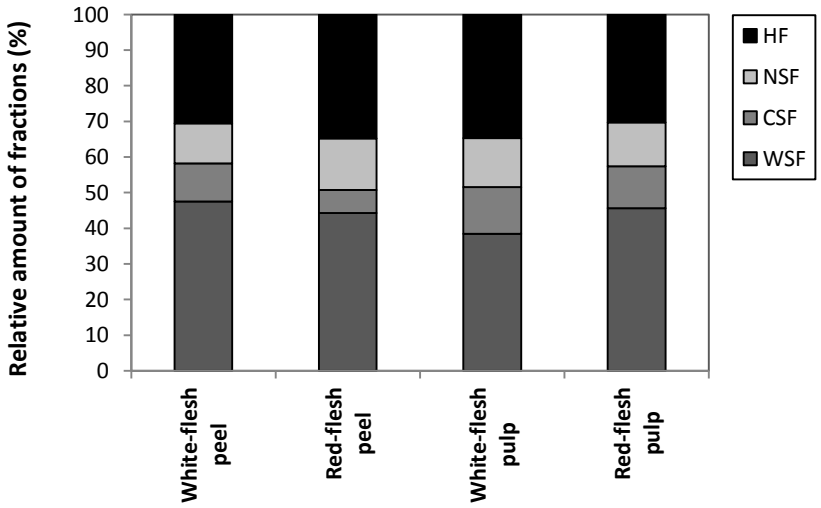


Figure 7.3 *Relative amount of the pectic fractions in the different dragon fruit samples. WSF: Water-soluble fraction, CSF: Chelator-soluble fraction, NSF: Sodium carbonate-soluble fraction, HF: Hemicellulosic fraction*

No remarkable differences in extraction yield of the different fractions between, on the one hand, pulp and peel and, on the other hand, between the white-flesh and the red-flesh species were observed. This means that both the peel and pulp of dragon fruits of the two different species contain a high amount of weakly bound pectic substances (WSF) and small amounts of ionically bound pectin (CSF) and ester-bound pectin (NSF). Hereby, it must, however, be emphasized that the WSF of the pulp samples has a lower DM and a much lower amount of GalA compared to the peel samples. These properties might have changed due to the pulp extraction procedure.

7.4.3 Molar mass distribution

The MM profiles of the isolated cell wall polysaccharides (WSF and CSF) were investigated to obtain an estimation of the dimensions of these polysaccharide fractions. The results are represented in **Figure 7.4**. Some minor differences in the MM distributions between the white-flesh and the red-flesh dragon fruits were observed, but a remarkable difference between the pulp and the peel samples was found. The pectic polymers in the WSF and the CSF of the pulp samples display a broad MM distribution, whereby the main average MM values can be estimated around 94-99 kDa and from 673 kDa to larger than 788 kDa. The relative abundance of the pectic polymers with a high MM (from 673 kDa up to larger than 788 kDa) is higher for the CSF compared to the WSF. On the contrary, the WSF of the peel samples displays a very broad MM distribution, whereby the presence of two unresolved peaks (with a maximum at elution times of approximccately at 10.8-10.9 min and 9.0-9.4 min) reveals the (very) high MM (equivalent MM 306-373 kDa and larger than 788 kDa) of the pectic polymers in this fraction.

The pectins in the CSF of the peel samples, on the other hand, seem to have a lower MM (equivalent MM 39-170 kDa at elution time of 11.3-12.3 min). For the CSF, a small unresolved peak around an elution time of 9.3 min is showing up, indicating also the presence of very high MM pectic substances in this fraction. The *in situ* presence of pectic molecules with a MM much larger than 788 kDa is rather unexpected. Therefore, the peak around an elution time of 9.3-9.4 min present in both the elution profile of the WSF and the CSF is the result of entangled pectic polymers excluded from the column which is highly believable because of the low DM of these pectic molecules.

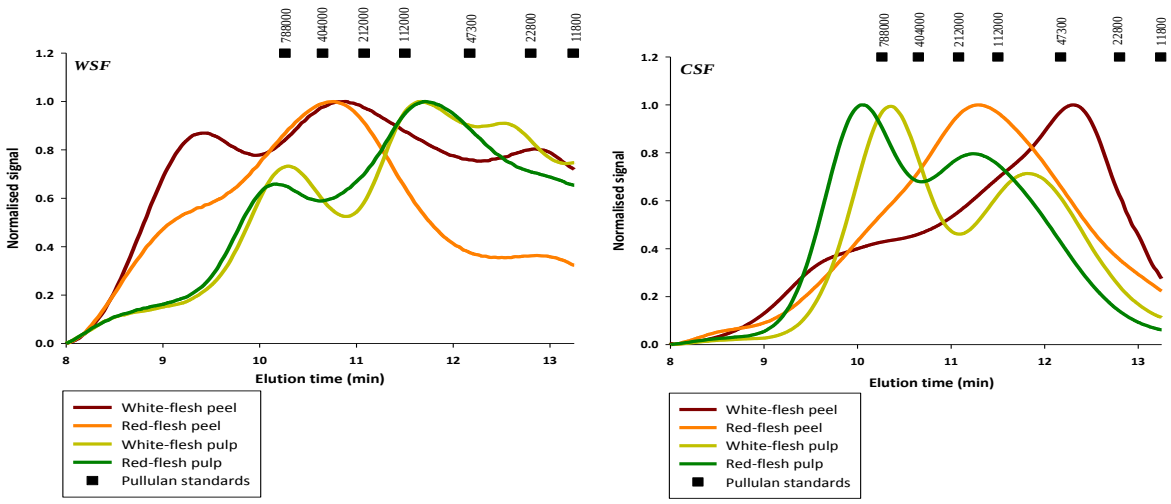


Figure 7.4 *Molar mass distribution of WSF and CSF of the dragon fruit and pullulan standards are used to indicate the estimation of molar mass (Da)*

Large differences in conformation between the extracted polysaccharide of the peel and pulp dragon fruits possibly mainly due to different of pectin and hemicellulose structures, linearity and RG-I branching that relevance to the neutral sugar composition. Upon combining the MM distribution results with the sugar composition results, it thus becomes clear that the loosely-bound pectin (WSF) in the peel is less linear and longer than the ionically bound pectic substances (CSF) in the peel. For the pectic molecules in the dragon fruit pulp, on the contrary, the ionically bound pectin seems to be less linear and longer than the loosely bound pectin.

7.4.4 Immuno-dot analysis with anti-pectin antibodies

The pectic substances in the WSF and the CSF of the pulp and peel dragon fruit samples were analyzed using various anti-pectin antibodies (JIM5, JIM7, LM18, LM19, LM20 and PAM1) in immuno-dot assays. The positive immuno-dot present on a nitrocellulose is shown in **Figure 7.5**. The immuno-dot analyses of the NSF and HF were not performed because these fractions were completely de-esterified during the extraction procedure.

All tested antibodies bound, to a larger or lesser extent, to the WSF as well as to the CSF of the four different samples. This observation suggests that in both fractions of the dragon fruit peel as well as of the pulp, a wide range of epitopes, including long blocks of unesterified GalA residues as well as (short) blocks of esterified GalA residues, is present.

This result indicates that average DM values determined based on chemical analysis should be interpreted carefully.

JIM7, known as a general anti-HG probe binding to pectic substances with a very broad range of DM (Christiaens *et al.*, 2011c), perfectly bound to both pectic fractions of all dragon fruit samples. Differences in binding intensities between the pectin fractions of, on the one hand, the peel samples and, on the other hand, the pulp samples correspond with the higher GalA content measured for the peel compared to the pulp (**Table 7.3**).

Both antibodies JIM5 and LM19, recognizing medium- and low-esterified pectin (Christiaens *et al.*, 2011c), both positively reacted with all dragon fruit samples. Interestingly, the overall labeling intensity for JIM5 was lower than the labeling intensity observed for LM19. As the epitope of JIM5 contains both methyl-esterified and non-methyl-esterified GalA residues, while a stretch of unesterified GalA residues is necessary for the binding of LM19. This observation points at the presence of long blocks of unesterified GalA residues in the pectic substances in both the pulp and peel of dragon fruits. On its turn, this might suggest a blockwise action pattern of the PME present in dragon fruits.

As antibodies PAM1 and LM18 are also known to bind to long block of unesterified HG (Christiaens *et al.*, 2011c), the respectively strong and weak binding of PAM1 and LM18 to the dragon fruits samples confirms the presence of these blocks of unesterified GalA in the samples. The reason why labeling with LM18 is much fainter compared with the labeling intensity observed for PAM1 is not clear although this has also been observed for, for example, pectic fractions isolated from fresh and processed tomato fruit tissues (Christiaens *et al.*, 2012).

Based on the immuno-dot results for LM20, a probe for highly-methoxylated pectic substances (Christiaens *et al.*, 2011c), it becomes, moreover, clear that both the WSF and the CSF of dragon fruit pulp and in particular of the peel contain also highly-methoxylated pectic epitopes despite their low average DM value (**Table 7.2**).

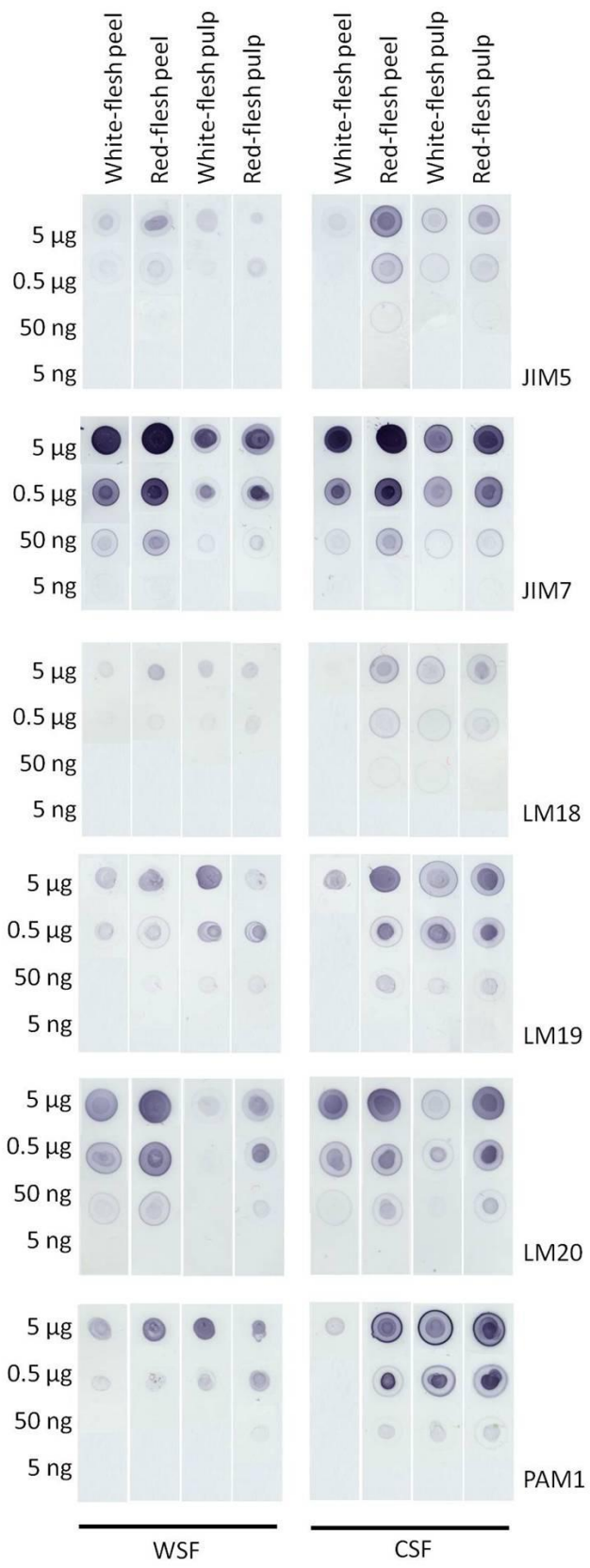


Figure 7.5 *Immuno-dot assays of JIM5, JIM7, LM18, LM19, LM20 and PAM1 binding to WSF and CSF of the dragon fruit. Samples were performed in dilution series at the levels indicated*

No remarkable differences between the immuno-dot results of the pectic substances of the white-flesh and the red-flesh dragon fruits were observed. Differences in labeling intensities between the pulp and the peel samples, most remarkably the higher labeling intensity for the peel samples with LM20 and the lower labeling intensity for the peel samples with LM19, correspond well with the higher DM values observed for the WSF and the CSF of the peel samples when compared to the respective fractions of the pulp samples. Also, the lower average DM values of the fraction of the white-flesh pulp sample compared to the red-flesh sample fraction are reflected in the immuno-dot results. This is particularly obvious for the antibodies LM19, for which the white-flesh pulp WSF is more intensely labeled, and for LM20 that bound more intensely to the red-flesh pulp WSF.

7.5 Conclusion

The results of the current study clearly show that cell wall polysaccharide of the pulp, and particularly of the peel, from the white-flesh dragon fruit (*H. undatus*) as well as from the red-flesh dragon fruit (*H. polyrhizus*) contained significant amounts of pectic substances. The pectic substances in the peel as well as in the pulp were shown to be lowly methyl-esterified and highly water-soluble (between 38 and 47%). The monomer composition of the WSF of the pulp and peel samples was, however, very different revealing a higher contribution of pectic HG in the peel samples compared to the pulp samples. In the ionically-bound (CSF) and ester-bound pectic fractions (NSF) of both pulp and peel samples, the RG-I type neutral sugars, rich in neutral sugars (Rha, Ara and Gal), were also present in relative high amounts compared to GalA.

The DM values of pectin in the dragon fruit pulp and in the WSF and CSF of the pulp were relatively low. The chemically-determined DM values corresponded well with the immuno-dot assay results using a range of specific anti-pectin antibodies. Despite the low average DM value obtained for the pulp as well as for the peel samples, anti-pectin antibodies revealed, however, the presence of a wide range of pectic epitopes, including long blocks of unesterified GalA residues as well as (short) blocks of esterified GalA residues in both dragon fruit samples. The presence of long blocks of unesterified GalA residues as shown by using the anti-pectin antibody PAM1 corresponds well with a blockwise action pattern which can be hypothesized for plant PMEs like dragon fruit PME. The antibody-based results clearly show the added-value of using anti-pectin antibodies to elucidate the pectin structure in detail. No major differences between the species were observed, except for the higher PME activity and the lower DM of the WSF and the CSF in the white-flesh dragon fruit

compared to the red-flesh dragon fruit. Moreover, the structural features of the pectic substances in the white-flesh and the red-flesh dragon fruit pulp and peel, revealed in this study, suggest that the dragon fruit (and by-products) could possibly be used as a source of low-esterified pectin.

CHAPTER 8

GENERAL CONCLUSIONS

8. General conclusions

The food industry is in search of profitable alternatives to increase revenue from tropical fruits' production and processing, develop innovative fruit products and find more meaningful applications for high-value fruit components. One promising tropical fruit is dragon fruit (*Hylocereus* spp.) because of its spectacular appearance, high levels of nutrients and antioxidants, and its increasing popularity with consumers. In light of its great popularity, there is now an increasing need for more research to be undertaken to understand the exact characteristics of dragon fruit and its related components. The findings of this doctoral research will allow for greater insights into possibilities of improving the quality of dragon fruit products, and developing valuable components as well as their applicability in food products.

8.1 Summary of major findings

This chapter recapitulates the main findings obtained from all investigations of this doctoral study. The results provide a better understanding of the overall characteristics of both fresh and processed products of white-flesh dragon fruit (*H. undatus*) and red-flesh dragon fruit (*H. polyrhizus*). The interesting components of dragon fruit, including pigment, seed oil and cell wall polysaccharides, are also successfully characterized as valuable components. With respect to fresh (untreated) dragon fruit, significant levels of antioxidative properties are present in the fruit, particularly red-flesh dragon fruit. Red-flesh pulp and both dragon fruit peels are also excellent sources of betacyanin. Dragon fruit puree, especially red-flesh dragon fruit, is a source material of mucilage, making it behave as a shear-thinning fluid.

An overview of the utilization of dragon fruit based on the findings of this doctoral research is suggested in **Figure 8.1**. Many valuable components from dragon fruit and its by-products were satisfactorily valorized and characterized by using several techniques and strategies. These strategies may also be applied to other interesting and related fruits to further expand their potentials for utilization. Dragon fruits are normally produced in the following forms: dragon fruit juice and pulp as well as seeds-containing dragon fruit puree. Dragon fruit pulp may further be freeze-dried to explore its potential applications as an instant juice powder and a promising betacyanin source. Freeze-dried dragon fruits have good quality attributes due to the efficient preservation of valuable components, especially their nutrients, colour and pigments. Freeze-dried dragon fruit pulp was well soluble in water,

indicating that it is convenient and easy-to-use. High amounts of betacyanin were also found in freeze-dried red-flesh pulp with high colour stability over a pH range of 1-11. This might serve as a suitable natural colourant and may also be considered as a powerful antioxidative pigment. Hence, average yields of freeze-dried dragon fruit pulp were estimated at 83 kg per ton of the whole dragon fruit and betacyanin in freeze-dried red-flesh pulp accounted for about 0.2 kg. Recent interest in the colouring agents from plant-based foods has accordingly intensified because of their possible health benefits as bioactive compounds and dietary antioxidants, and consumers' concern for healthy products. However, natural food dyes may induce higher costs compared to synthetic ones.

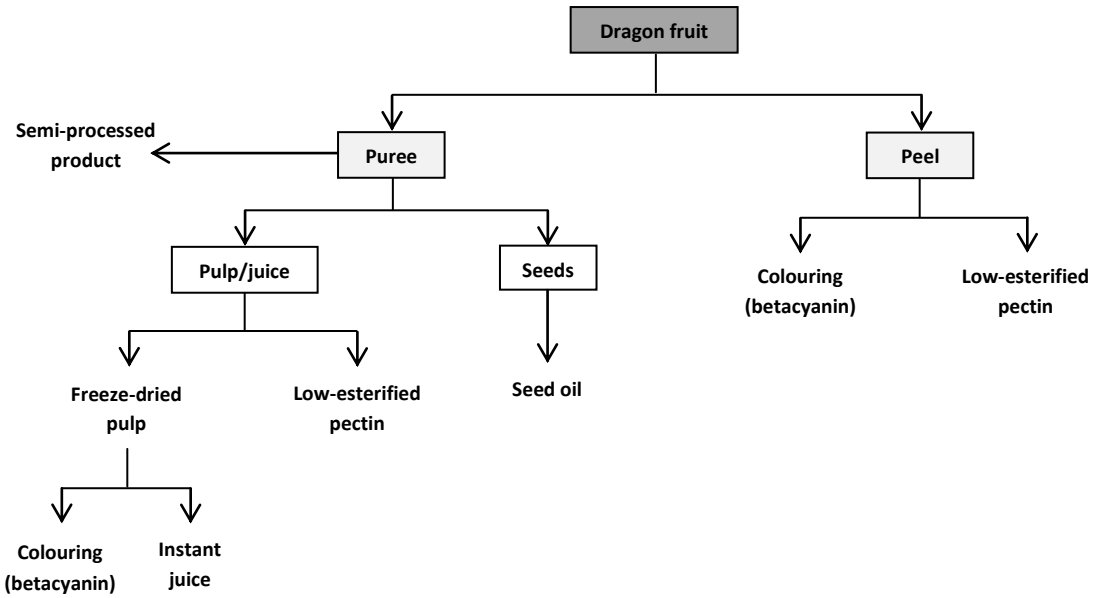


Figure 8.1 *Schematic flow chart of the dragon fruit production processes*

Dragon fruit puree (seed containing), which is usually used as a semi-processed product, is interesting from a nutritional point of view because it contains significant levels of antioxidative activities. When heating was applied to dragon fruit puree, the antioxidative properties increased with increasing heating time and temperature. This can be explained by the release of some antioxidative components from dragon fruit seeds and the formation of Maillard reaction products (represented as browning formation pigment values). These products may serve as antioxidants. The degradation of betacyanin in dragon fruit puree occurred when heating-up, resulting in a colour shift. Colour parameters like L* and b* values can be recommended as quality control indicators for online monitoring to obtain high-quality products after heat processing. The apparent viscosity of dragon fruit puree

also increased with thermal treatment. This was especially the case for red-flesh dragon fruit. Moreover, the outcome of this work can be used in the commercial process optimization to meet consumer and manufacturer needs.

Dragon fruit seeds and peel are abundant by-products of dragon fruit processing, accounting for about one-third of the whole dragon fruit (w/w). Therefore, their potential utilization could be favourable to food technologists. The processing of dragon fruit seeds into valuable products like dragon fruit seed oil is proposed. Dragon fruit seed oil can be extracted from dragon fruit seeds by using an uncomplicated technique where oil is extracted with petroleum ether without any heating. It is suggested that the experimental procedure of seed oil extraction might be suitable and available for scaling-up to commercial production towards enhancing the value of the fruit due to its simple procedure and high oil output. Average seed yields of dragon fruit were evaluated to be 80 kg per ton of the whole dragon fruit (wet basis) and the oil yield from this amount of dragon fruit seeds was estimated at about 6 litres which contained around 4 g of tocopherols content. The estimated yield of dragon fruit seed oil is comparable with grape seed oil (Nerantzis and Tataridis, 2006). Since production of dragon fruit in Thailand can reach as high as 65 tons per ha per year, average wet seed and extracted seed oil yields were estimated at 5.2 tons and 390 litres per ha, respectively. These estimated values are comparable with rapeseed oil which is a high yielding oil (360 to 600 litres per ha) (Wiemer and Altes, 1989). However, the cost of seed oil is difficult to estimate and can be negotiated depending on various factors such as agricultural, logistic, energy and water supply, location and scale of manufacture, oil composition (essential fatty acids and antioxidants) and purpose of use.

Dragon fruit seed oil could be considered as innovative high-value oil due to its fatty acid profile and interesting antioxidative components. The health benefits of dragon fruit seed oil could be promoted and are related to its fatty acid composition. Linoleic acid (C18:2) was the most abundant fatty acid in dragon fruit seed oil, particularly in white-flesh dragon fruit seed oil, resulting in high dietary essential fatty acids which play a critical role in human health. A significant amount of endogenous tocopherol, especially in red-flesh dragon fruit seed oil, was also observed. During a 3-month storage period under cold and room temperatures which could affect oil deterioration, dragon fruit seed oil still contained a relatively high amount of total tocopherols and showed a low oxidation rate, demonstrating the high oxidative stability of dragon fruit seed oil.

Research regarding the activity and thermal inactivation of pectic enzymes (i.e. PME and PG) in white-flesh and red-flesh dragon fruits was carried out. The activity of PME in fresh (untreated) white-flesh dragon fruit was higher than that in red-flesh species, whilst the

activity of PG in the fruit could not be detected. Dragon fruit PME is a rather thermostable enzyme. It was still active at temperatures below 70 °C for 10 min, while heating at higher temperatures (80 and 90 °C) for 10 min led to PME inactivation. The results may be useful for dragon fruit processing to control the quality of the product such as texture and cloud stability which are influenced by pectic enzymes' activity.

The conversion of dragon fruit pulp and peel into other valuable products, i.e. pectin, is also possible with an acceptable yield. In dragon fruit pectin isolation, average pectin yield of dragon fruit peel was estimated at 14 kg of freeze-dried pectin per ton of the whole dragon fruit, whereas of dragon fruit pulp the yield was estimated much lower at 2 kg/ton. It is clearly shown that the conversion of dragon fruit peel into extracted pectin is preferable. This is due to its higher pectin yield as well as pectin being a by-product of dragon fruit peel. Additionally, the cell wall polysaccharides of dragon fruit peel contain higher amounts of pectic substances (based on GalA content as a backbone of HG) compared to dragon fruit pulp, whilst pectin in both dragon fruit pulp and peel has low average DM values. The lowest DM was observed in white-flesh pulp due to its high endogenous PME activity. The chemical structure of pectin obtained from dragon fruit revealed that the pectic substances in dragon fruit peel and pulp are lowly methyl-esterified and highly water-soluble. The pectic polysaccharides present in dragon fruit pulp seem to contain more branched pectic substances next to HG when compared to that of dragon fruit peel. Long blocks of unesterified GalA residues were present in the pectic substances of both dragon fruit pulp and peel, indicating a blockwise action pattern of dragon fruit PME during fruit ripening. Dragon fruit pectin may offer possibilities to be used as a source of low-esterified pectin, having some specific functional properties that might be suitable for food applications.

8.2 Future perspectives in dragon fruit research

This section further describes the implications, limitations and recommendations for further research relevant to dragon fruit and other tropical fruits.

The further characterization of freeze-dried dragon fruit products, more particularly the impact of freeze-drying and subsequent storage conditions on various attributes such as moisture content, water activity, water solubility, colour parameters, antioxidative properties, nutritional values and microbiological stability, is worthwhile to examine. In the specific case of freeze-dried products exhibiting low T_g values (e.g. freeze-dried sugar/acid-rich fruit juice), the addition of high molecular compounds (e.g. maltodextrin) could be a strategy to prevent caking during processing and storage.

Thermally processed dragon fruit puree, especially red-flesh dragon fruit puree, high in antioxidative activities should be promoted as a functional food product. In this case, the biological activities and health effects of individual bioactive compounds, e.g. tocopherols and phenolic compounds, in dragon fruit puree during thermal processing would have been more studied. It is also suggested that the application of dragon fruit puree, particularly red-flesh dragon fruit puree, in some gel-network foodstuff (e.g. jellies and jams) can be further investigated. It seems that the apparent viscosity of puree during heating increases, which is possibly due to the presence of cell wall polysaccharides like pectic substances in dragon fruit.

Since oil can be extracted from fruit seeds, seed flour basically remains as by-products following seed oil extraction. Further research into the evaluation of dragon fruit seed flour regarding its chemical composition and bioactive compounds like antioxidants could be of consideration. So, the potential utilization of dragon fruit seed flour in food products as an alternative for other seed flours is also of great interest.

Screening of PME inactivation as affected by thermal processing could be performed at different heating times to elucidate the complete kinetic PME inactivation mechanism. A study of PME activity (probably together with other pectic enzymes) would be very useful to improve yield and productivity of juice production and to control the clear juice or cloud loss attributes during juice and puree processing. The use of innovative techniques such as high-pressure processing, is gaining popularity to produce food products. The combination of high-pressure processing with conventional heating could be applied to provide better results for enzyme inactivation and appearance (e.g. texture modification and viscosity) of plant-based food. Therefore, the combined thermal treatment with high-pressure processing is another area requiring further study, specifically focusing on the influence of PME activity on quality attributes of fruit organs.

Considering the structural features of pectic substances present in dragon fruit, it is recommended that dragon fruit pulp and its by-products (dragon fruit peel) could possibly be used as a source of low-esterified pectin. In this context, further research to elucidate the functional properties of dragon fruit pectin is required. Low-esterified pectin has been used as a fat or sugar replacer in low-sugar or low-calorie foods due to an increasing demand by calorie conscious consumers and partly to fill the need for sugar-free products by diabetics. However, pectin production today seems to be limited to citrus and apple pectins. This is probably due to historical, industrial and sociological reasons.

Additionally, further research is required to investigate the interactions of dragon fruit components and products (e.g. colouring agent, instant juice, processed puree, seed oil and low-esterified pectin) with other food components in real food systems. Effects of these interactions on physicochemical, antioxidative and rheological properties as well as sensory attribute could be studied. The main focus is on beneficial components to explore their utilization in food (and pharmaceutical) products for new products development and health promotion of dragon fruit. Pilot scale experiments could also be considered to offer possibilities for scaling-up laboratory experiments to industrial level. In this case, the commercialization of dragon fruit seed oil could be suggested due to the needs of health-conscious consumers and the big market share of edible and cosmetic oils.

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CURRICULUM VITAE

Wijitra Liaotrakoon was born in Nakhon Pathom, Thailand on January 13, 1979. She completed "Bachelor of Science: Biotechnology" in 2001 from Mahidol University, Thailand. Immediately after her bachelor study, she continued her Master degree, and two years later, she graduated from Chiang Mai University (Thailand) in 2003, as "Master of Science: Food Science and Technology". During a year later, she worked as an assistance researcher at National Research Council of Thailand and also as a research and development supervisor at Kewpie (Thailand) Co., Ltd., Thailand. Since 2004, she has been a lecturer at Rajamangala University of Technology Suvarnabhumi, Thailand, where her role has been focused on "Food Processing and Food Engineering". After about 4 years of being a lecturer, the University gave her the scholarship for PhD studying abroad.

In August 2008, she began her PhD research in "Applied Biological Sciences: Food Technology and Engineering" program, in the Laboratory of Food Technology and Engineering, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Belgium. Her research led to a PhD dissertation entitled "Characterization of dragon fruit (*Hylocereus* spp.) components with valorization potential" under supervision of Prof. dr. ir. Koen Dewettinck. In 2011, she collaborated with other researchers of the Laboratory of Food Technology, Department of Microbial and Molecular Systems, Katholieke Universiteit Leuven, Belgium, to work on cell wall polysaccharides of dragon fruit for a year. During her stay at the Laboratory of Food Technology and Engineering, she participated in different research and academic activities as well as social activities. She is the author of several international scientific publications and has also attended many international and national scientific conferences.

Publications in international peer-reviewed journals

Liaotrakoon, W., De Clercq, N., Lewille, B. and Dewettinck, K. (2012) Physicochemical properties, glass transition state diagram and colour stability of pulp and peel of two dragon fruit varieties (*Hylocereus* spp.) as affected by freeze-drying, *International Food Research Journal*, 19(2): 743-750.

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Liaotrakoon, W., De Clercq, N., Van Hoed, V. and Dewettinck, K. (2010) Characterisation of the physicochemical properties of two species of dragon fruit seed oil (*Hylocereus undatus* and *Hylocereus polyrhizus*), *16th PhD Symposium on Applied Biological Sciences*, December 20, Ghent University, Belgium.

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