Processing of Orange (*Citrus unshiu*) Powder by Micro Wet Milling and Vacuum Spray Drying

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ABBREVIATION

BET	Brunauer-Emmett-Teller
CD	Degree of Caking
CI	Carr Index
COJ	Concentrated Orange Juice
DE	Dextrose Equivalent
DPPH	2, 2-diphenyl 1-picrylhydrazyl
DSC	Differential Scanning Calorimetry
EMC	Equilibrium Moisture Content
FD	Freeze Drying
FRAP	Ferric Reducing Power Assay
GAB	Guggenheim-Anderson-de Bore
GAE	Gallic Acid Equivalent
HR	Hausner Ratio
MD	Maltodextrin
MWM	Micro Wet Milling
OJ	Orange Juice
QE	Quercetin Equivalent
SD	Spray Drying
SEM	Scanning Electron Microscopy
TE	Trolox Equivalent
TFC	Total Flavonoid Content
TPC	Total Polyphenol Content
TS	Total Solids
TSS	Total Soluble Solids
VSD	Vacuum Spray Drying
WSI	Water Solubility Index

Chapter 1. Introduction

1.1 Background

Orange is one of the important citrus fruits grown all over the world. The consumption of fruit juice specially citrus increasing worldwide, due to the public perception of juice to favor a healthy quality of life. Citrus fruits are the major sources of health-promoting constituents such as flavanones, polyphenols, ascorbic acid and antioxidants (Gil-Izquierdo et al., 2002; Stinco et al., 2012). Particularly intake of orange juice shows positive effects on cardiovascular diseases (Elzbieta M. Kurowska et al., 2000). Narirtutin, hesperdin and didymin belong to the flavanone glycoside group and the most available flavonoid present in the orange juice. According to So et al. (1996), hisperdin in citrus juice can inhibit the chemically induced breast cancer. On the other hand, orange juice is the most popular fruit juice in the world market due its attractive color, appealing sensory properties. Moreover, orange juice contains a higher amount of ascorbic acid around 30-50 mg/100 mL (Johnston and Hale, 2005; Meléndez-Martínez et al., 2007) and the health-related properties of polyphenol contained in orange juice (Meyer et al., 1998) are based on their antioxidant activity. The physicochemical, functional and antioxidant properties of orange juice were studied by Kelebek et al. (2009) as shown in Table 1-1.

Orange juice (OJ) also a good source of carotenoid which is responsible for the attractive color of the juice (Meléndez-Martínez et al., 2007; Stinco et al., 2012). The fruits are consumed either fresh and juice. Due to the presence of higher amount of water (85-90%) (Kelebek et al., 2009), the fruits are easily deteriorated. The fruits are usually preserved as juice or powders for extending the shelf life and ensure the availability through the year. There are different methods used to process and preserve the juice or as a powder. During the processing and preservation, OJ undergoes changes in their chemical and functional components. According to Johnston and Hale (2005) and REKHA et al. (2012), it was found that 30-40% of initial ascorbic acid in some

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fruit juice could be lost on dehydration. It is very important, therefore, to preserve or enhance the functional components of the processed OJ or OJ products, particularly high level of ascorbic acid, total polyphenol, flavonoid, carotenoid and the associated antioxidant activity.

Table 1-1. General composition of Kozan orange juice

Juice composition	Amount
Yield (%)	40.0±0.71
Density (g/mL)	1.052±0.01
Total acidity (g/L)	9.11±0.01
pH	3.35±0.01
Brix	11.8±0.00
Ash (g/L)	3.7±0.08
Sugar (g/L)	120.19±3.84
Citric acid (g/L)	12.66±0.16
Ascorbic acid (g/L)	0.49±0.01
Malic acid (g/L)	1.06±0.01
Hydroxybenzoic acid (mg/L)	4.28±0.29
Hydroxycinnamic acid (mg/L)	60.38±3.87
Flavanones (mg/L)	252.7±12.38

Source: (Kelebek et al., 2009)

In addition, OJ with pulp containing high nutritional content, because of the higher flavonoid content in OJ pulp. Flavonoid neutralizes the oxidative and inflammatory stress generated by the unhealthy food and helps to prevent blood vessel damage (So et al., 1996). OJ with pulp is an important source of dietary fiber to favor a healthy digestive system (Uichard, 2004). During the industrial processing of OJ or OJ powders, the pulp and seed are usually discarded or use another purpose. The percentages of different parts varied depend on species. Usually Japanese orange (*citrus unshiu*) doesn't contain any seed and skin are thin comparatively other oranges.

Therefore, developments of suitable processing methods for utilization of orange pulp and necessary to reduce the wastes, to increase the functional constituents and also enhance the economic value of the fruit.

1.2 Processing of orange juice

Fresh OJ has limited shelf life and the most common method for extending the shelf life of fresh OJ is inactivation of microorganisms and enzymes by thermal processing (Fellers, 1988). Orange juice is subjected to a process by industrially or in domestic. Orange juice is commonly marketed in three forms: Fresh juice or single strength juice or ready to drink juice, as a frozen concentrated OJ, which is diluted with water after purchases, as a reconstituted liquid, which has been concentrated and diluted before sale (Gil-Izquierdo et al., 2002; Stinco et al., 2012). There are only very few studies which reported the effects of processing technique that affects the compounds of orange juice (Tomás-Barberán and Clifford, 2000). According to Gil-Izquierdo et al. (2002) reported the steps of processing technique at an industrial scale on orange juice as shown in Figure 1-2. During the industrial processing of orange juice, pulp, seed, and skin are commonly discarded then undergoes some thermal treatment like pasteurization or evaporation, that extend the shelf-life but deteriorate the color, flavor, ascorbic acid or other heat-sensitive functional constituents (Escudero-gilete et al., 2012).

3

Orange

↓

Washing and Cleaning

↓

Commercial Squeezing- FMC

↓

Centrifuging ⟶ **Pulp**

↓   ↓

Juice   Pasteurization (95℃/30sec)

Pasteurization (75℃/30sec)	Pasteurization (95℃/30sec)	Pasteurized Pulp

Freezing   Concentration

+ Pasteurized   Thawing   + H$_2$O   + Pasteurized Pulp

Fresh   From Frozen   From Concentrated Juice   Standard Juice
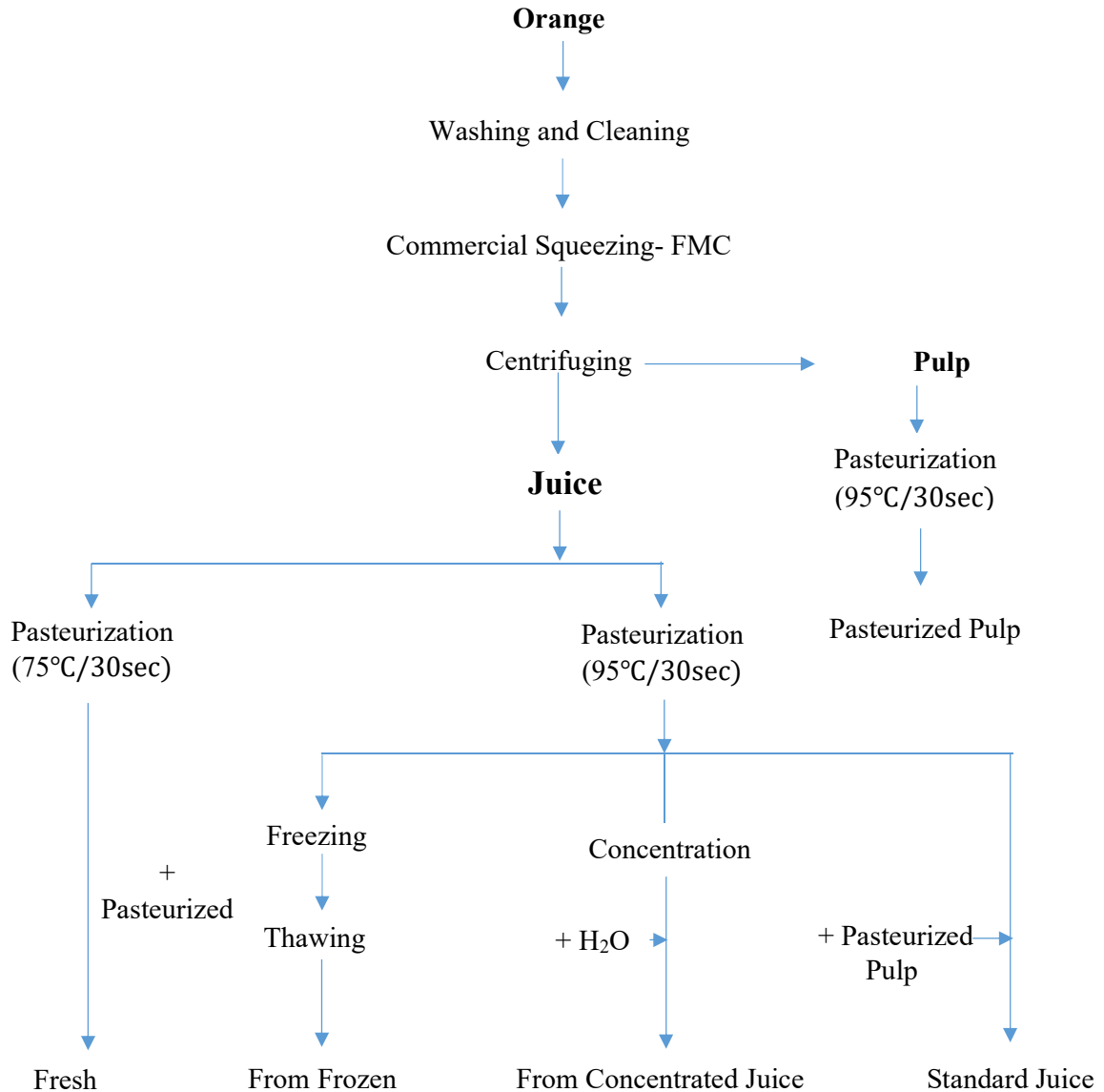
Figure 1-1. Scheme of the commercial orange juice processing (Gil-Izquierdo et al., 2002)

Pasteurization of OJ caused a loss of 58% of the initial ascorbic acid, 20-35% of total phenol, 25-30% of total flavanones, and OJ processed from concentrated and frozen also responsible for the loss of total phenols and flavanones (Gil-Izquierdo et al., 2002). Nonthermal processing such as High pressure and plus electric field are the new technique can minimize the loss of nutrient during processing of fruit juice. High-pressure treatment led to an increased the carotenoid

release (53.88%) and vitamin A value of 38.74% during OJ processing (Sánchez-Moreno et al., 2005), but their industrial use quite limited (Gil-Izquierdo et al., 2002).

A new technique of processing of high dietary fiber containing juice with minimum particle size is wet milling. Processing of juice pulp or peel in micro level particle size is known as Miro Wet Milling. This technique is quite known for processing of cereal milling or developing new beverage from rice (Koyama and Kitamura, 2014). The application of MWW for processing of concentrated juice from whole fruit will minimize the loss of fruit pulp and enhanced the nutritional content of the juice. Thus in a study of OJ processing, it is necessary to investigate the feasibility of MWM and its effects on the processing of OJ in relation to the retention of color and nutrient composition.

1.2.1 Current situation and limitation of drying of concentrated orange juice

The better preservation of fruit juice might be in powder form due to the fruit juice powder have many benefits and economic potential over their liquid counterparts such as reduced volume or weight, reduced packaging, easier handling and transportation and also enhanced the shelf-life of the product. Fruit juice powder is convenient for instant use in liquid preparation and also other uses like seasoning blends, confectionery, and pharmaceutical.

Drying is the unique method for producing fruit juice powder. There are different drying techniques widely used for producing fruit juice powder namely air, freeze, vacuum and spray drying. However, dehydration of fruit juice is complex, the technique of drying fruit juice is very specific for each drying method (Brennan et al., 2007). However, air drying is the simplest method for producing fruit powder, but the quality of the product is fairly poor, particularly as the product from the heat sensitive material.

Freeze-drying (FD) is the best method for producing high-quality fruit juice powder. Freeze drying is the process of free water separation by ice formation or the total solidification of the original solution and the ice sublimation at a very low temperature (-40 to -85℃) and very low

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total gas pressure (high vacuum). The limitations of FD are the batch process, long processing time, low productivity, as a result, the operational cost is higher. According to Kitamura and Yanase (2011) the product cost of FD is 10 times higher than spray dried products.

Spray drying (SD) is widely used to the transformation of feed from a liquid or slurry to form a dry powder. During SD the liquid feed is atomized into a chamber where spray mixes with hot air which removes the moisture resulting in mostly amorphous (glassy) solid or powder produced (Shrestha et al., 2007a). The schematic diagram of spray drying process as shown in Figure 1-3. One of the main indices of spray dryer efficiency is the product recovery. Low productivity of SD is due to the adhesion of dried material into the wall. Long time retention of dried powder on the wall or frequent cleaning or collection is not cost effective or not able to get good quality of powder (Goula and Adamopoulos, 2010). A sticky product like fruit juice that contains higher sugar and acid is difficult to spray dry. During drying all the amorphous material change from the glassy to rubbery state at a glass transition temperature, that is specific for each material. The stickiness of the product occurred due to the low glass transition temperature or consequently higher drying temperature (Goula and Adamopoulos, 2010). To overcome the stickiness problems, various drying aids used for producing free-flowing powders such as maltodextrin, gum Arabic, soybean protein, dextrin (Chegini and Ghobadian, 2007, 2005a). The addition of carrier agent before atomizing is widely used to increase the glass transition temperature (Tonon et al., 2008). Another drawback of spray drying of orange juice is the loss of heat-sensitive components like ascorbic acid and antioxidant constituents, due to the high heat treatment (150-250℃) (Goula and Adamopoulos, 2006; Johnston and Hale, 2005; VILLOTA and KAREL, 1980). As a result of such investigations, depending on the quality of the final product and other considerations, a suitable and effective drying technique can be chosen.
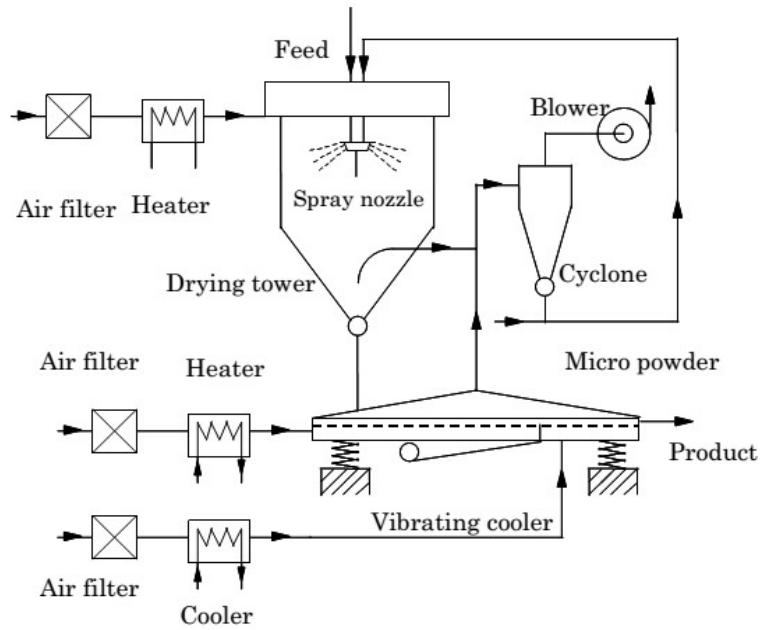
Figure 1-2. Schematic of spray drying process (Matsuno et. al. 1991)

Spray drying under low pressure or in a vacuum condition is a new technique. In addition, the superheated steam as a heating medium instead of hot air is another new approach. Superheated steam drying has been known for over 100 years (Pronyk et al., 2010), but it's application in spray drying under vacuum condition i.e. vacuum spray drying with superheated steam is unknown. The superheated steam that has additional sensible heat added so that its temperature is above the saturation temperature at a given pressure. The higher enthalpy of the superheated steam can transfer heat very quickly from the product to be processed. The benefits of superheated steam drying (SSD) are: superheated steam drying can lead to saving energy as high as 50 to 80% over the use of hot air (F., 2010). According to Alfy et al. (2016) the major advantages of superheated steam drying can produce a product with better quality in terms of color, flavor, shrinkage and rehydration characteristics. SSD can also reduce the oxidation, deodorization of the product, and higher energy efficiency.

Vacuum spray drying (VSD) is a new technique applied to powderization of liquid food containing thermosensitive ingredients under low temperature (40-60°C) and low pressure (3 to 5 kPa) as compared to the existing methods as shown in Figure 1-4.
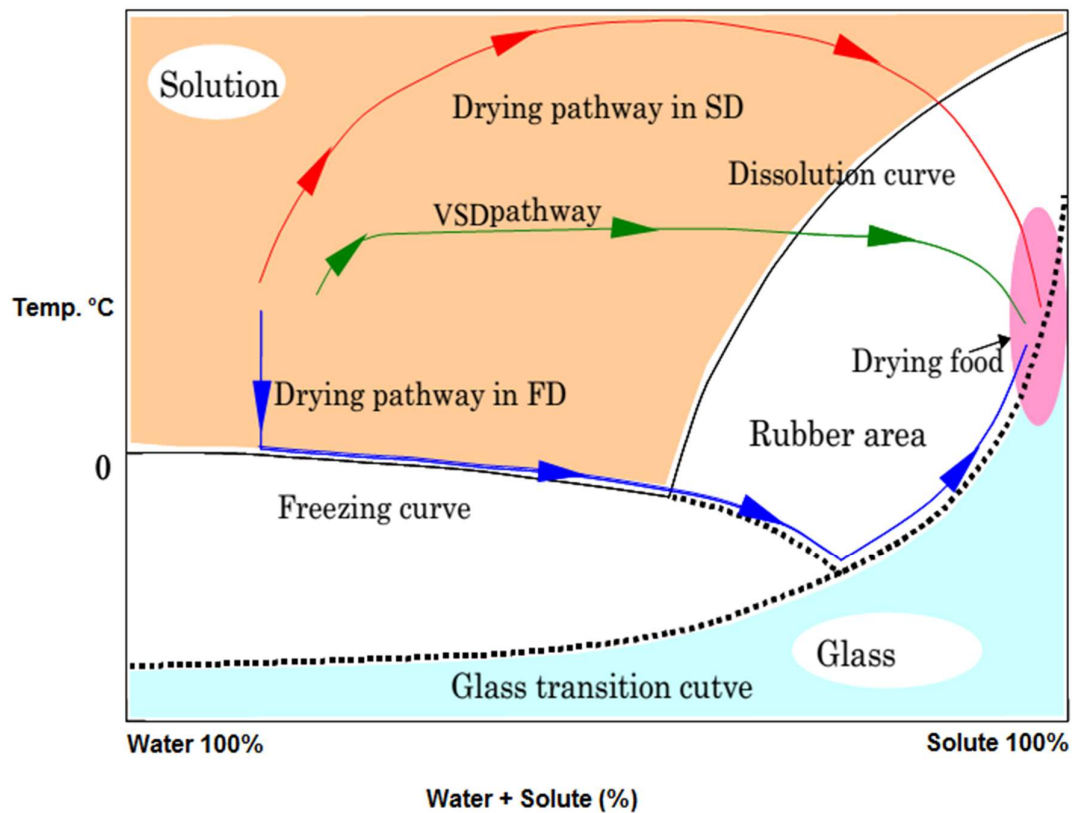
Figure 1-3. Model food state diagram (Takai, 2000)

The major food friendly drying methods with their features are shown in Table 1-2. Previously Kitamura and Yanase (2011) developed vacuum spray drying system for drying of probiotic powders, where the drying temperature was controlled by a far-infrared heater and a heating system with an outside jacket. The same year, Semyonov et al. (2011), conducted ultrasonic vacuum spray drying of probiotic powders, But the there is no literature of the application of superheated steam as a drying medium and producing heat-sensitive fruit juice powders.

Table 1-2. Features of the major food friendly dryer

	VSD	SD	FD
Heat Source	Superheated steam	Hot air	Freezing (cold air)
Temp. of heat source (℃)	150~250	200-400	-40 ~ -50
Product Temp. (℃)	33-48	<100	-40
Operating pressure	3~5 kPa	below atmospheric pressure	4 Pa
Driving method	continuous operation	continuous operation	batch operation
Thermal alteration	less	more	Less
Oxidation	less	more	Less
Processing volume	less	more	Less
Milling process	none	none	necessary
Energy efficiency	high	high	Low
Equipment price	minimum	cheaper	High

Source (Kitamura and Yanase, 2011)

Pre-treatment before drying of food powder is well known to reduce the color, flavor and higher retention of antioxidants. Similarly, the stability study of the product during storage plays an important role in the food processing industry. The quality of the fruit juice powders changes over time due to the shrinkage, caking or degradation of functional components when stored in undesirable conditions. As a result, the need for a stability study of OJ powder is important in establishing the effects of storage conditions.

1.3 Research objectives

The experiments designed for this study aims to process the concentrated orange juice powder by the application of micro wet milling and vacuum spray drying as a new technique.

In chapter 2, Moisture sorption isotherm and glass transition phenomenon of vacuum spray dried concentrated orange juice powder in detail. The feasibility investigation of vacuum spray drying of concentrated commercial orange juice with maltodextrin as a carrier agent.

In chapter 3, will discuss the process development for Micro Wet Milling (MWM) of orange juice with pulp and quality evaluation. Finally, a brief comparison with commercial orange juice is carried out.

In chapter 4, Analysis of physicochemical and antioxidant properties of MWM orange juice powder by vacuum spray drying and spray drying. A comparative study will be conducted in details. This chapter also discusses the suitable drying conditions for MWM orange juice with maltodextrin as drying aid.

In chapter 5, the investigation of the storage stability of the vacuum spray dried orange juice powders, in terms of degradation kinetics of ascorbic acid, color characteristics, and total antioxidant activity. Finally, the stability of MWM orange powders will be discussed based on the water activity and glass transition temperature.

In chapter 6, the conclusion and recommendation of our study will be summarized.

Chapter 2. Moisture sorption isotherm and glass transition phenomenon of vacuum spray dried concentrated orange juice powder

Vacuum spray drying is a new technique used to produce concentrated orange juice powder using maltodextrin as a drying agent. The dryer was developed for the low-temperature (40-50°C) drying powderization of liquefied food using superheated steam (200°C) as a heating medium. The physical properties of orange juice powder with four different combinations of juice solids to maltodextrin solids at 60:40, 50:50, 40:60, and 30:70 were determined. The moisture content, hygroscopicity, water activity, particle size, particle morphology, were significantly affected by the maltodextrin concentration and drying conditions. Highest product recovery as cyclone recovery was obtained from OJ/MD 30:70 powders. Moisture sorption isothers were constructed by isopiestic method, while thermal transition of the powder at different water activity levels ($a_w = 0.11 - 0.86$) was predicted by DSC. The data obtained were well fitted to both BET and GAB models. A strong plasticizing effect of water on Tg was predicted by the Gordon-Taylor model, where Tg was greatly reduced by the increasing moisture content of the powder.

2.1 Introduction

For many years, water activity is a widely-accepted concept and important to evaluate the product quality and stability than the total amount of water. The available or the free water presence in food product influences the microbial growth and enzymatic/or non-enzymatic activities (Rahman and Labuza, 1999). Expressing the relation between water activity and equilibrium moisture in a graphical form is known as sorption isotherm. The sorption isotherm is an important thermodynamic tool, specially for low moisture foods (Goula et al., 2008) and provides useful information for design, calculation of the food processing operations such as drying, packaging, and storage. Sorption isotherm commonly expressed based on empirical and/or theoretical models, those models parameters determine how water strongly bound to the solids or the amount of water that a material hold if it is exposed to air a certain relative humidity and temperature (Edrisi Sormoli and Langrish, 2015). The glass transition temperature (Tg) is the temperature at which an amorphous system changes from glassy to rubbery state. For a given substance, the fraction of water content, molecular weight, and nature of dry matter can describe by the glass transition temperature (Roos, 1995; Slade and Levine, 1991).

Spray drying is widely used to transform liquids into shelf-stable products. Spray dried powder have many benefits and economic potential over the liquid counterparts. Fruit juice powder can easily handle and transfer due to its reduced weight or volume as well as a reduced packaging system and have a much longer shelf life (Shrestha et al., 2007a). Spray drying parameters affect the quality of spray dried powder and drying condition they were the best way to describe the quality change factors of orange products (Brennan et al., 2007). However, the major drawbacks and complex in spray drying of orange juices is the matter of stickiness and flow problems of the powder (Tonon et al., 2008;Goula and Adamopoulos, 2010). Fructose, glucose, and citric acid are the main components of orange juice with very low Tg values (5, 31 and 16°C, respectively) in a pure, dry state, which decreases drastically when moisture is absorbed. During spray drying, fruit juice forms an amorphous solid or a syrupy/sticky powder; sticky products

are typically produced because the low glass transition temperature (Tg), high viscosity, low melting point and high hygroscopicity (Adhikari et al., 2003) lead the juice components to adhere to the drying chamber and agglomerate on the conveying system (Bhandari and Howes, 2005).

To overcome the stickiness problem, various methods that are able to produce free-flowing fruit juice powder have been suggested: using an adjunct or a carrier agent (maltodextrin, gum, starch or gelatin) as an additive in the feed material during spray drying (Saénz et al., 2009), scrapping the drying surfaces, spray freezing or cooling the drying chamber wall (Chegini and Ghobadian, 2005b; Chegini et al., 2008). Carrier agents with higher molecular weights increase the glass transition temperature and reduce the hygroscopicity and stickiness of the powder, which may, in turn, increase the final product yield and efficiency (Lee et al., 2013). Goula and Adamopoulos (2010) produced spray dried concentrated orange juice powder by using dehumidified air as drying medium and maltodextrin DE 6, DE 12, DE 21 as a carrier agent. Shrestha et al. (2007a) conducted spray drying of orange juice with various levels of maltodextrin solids as a carrier agent. For this reason, maltodextrin is commonly used as a carrier agent because it is relatively inexpensive and highly soluble in water with low viscosity and a bland flavor (Carolina et al., 2007; Saénz et al., 2009); it is also more efficient at protecting bioactive compounds from adverse conditions (Ferrari et al., 2012).

In the present study, a vacuum spray drying (VSD) method is proposed as a new technique for vacuum pressure spray drying of concentrated orange juice. The dryer was developed to turn liquefied food into a powder at a low temperature (40-60°C) drying using superheated steam as the heat source. Ultrasonic vacuum spray drying has been used to produce dried probiotic powder (Semyonov et al., 2011). Kitamura et al. (2009) and Kitamura and Yanase (2011) who were developed and conducted vacuum spray drying of probiotic foods at 40-60°C. However, very few studies on the sorption isotherm and glass transition phenomenon of orange juice powder produced with other technique such as spray drying associated higher temperature, freeze drying

(Edrisi Sormoli and Langrish, 2015; Goula and Adamopoulos, 2010) are available but no data exists regarding this vacuum spray drying technique at a low-temperature treatment for orange juice powder.

Therefore, the objectives of this study were to produce orange juice powder by VSD with different combinations of maltodextrin; to physical properties of orange juice powders and to provide experimental data on water sorption isotherms and glass transition temperatures of the concentrated orange juice powder with a carrier agent for insight on powder stability. In order to predict the sorption characteristics by widely used BET and GAB models, and the Gordon-Taylor model used to determine the glass transition temperature and water plasticizing effect on the concentrated orange juice powder.

2.2 Materials and Methods

2.2.1 Raw materials

Concentrated orange juice was obtained from Ehime beverage Ltd., Japan. The basic composition of the concentrated orange juice was total soluble solid (TSS) 62±0.45%, citric acid 4.8-5.7%. Maltodextrin (MD) 12DE (Showa Sangyo Co. Ltd., Japan) with a moisture content of 4.15±0.02% was used as a carrier agent. Considering the TSS contents of concentrated orange juice (COJ) and maltodextrin solids at 60:40, 50:50, 40:60, and 30:70 (concentrated orange juice: maltodextrin) by weight were chosen for vacuum spray drying. A total of 1000 g of COJ and MD were prepared with 33 Brix% solutions for VSD. Moreover, in a previous work, it was found that the proportions of orange juice : maltodextrin by weight at 40:60, 35:65, 30:70 and 25:75 were used during spray drying at 160°C Shrestha et al. (2007a). and Goula and Adamopoulos (2010) conducted spray drying of concentrated orange juice with matodextrin as carrier agent at a solid ratio of 4, 2, 1, and 0.25. The present study considered these conditions and also other higher concentration of orange OJ/MD 60:40, 50:50 by weight for vacuum spray drying.

2.2.2 Vacuum Spray Drying (VSD) System

The experimental VSD (Tanabe Engineering Corporation, Japan) diagram with its working principles is shown in Fig 2-1. The COJ/MD mixture is sprayed at 300 mL/h through a metering pump with an upward flow and atomized into the drying chamber by two fluid nozzles. The nozzles use compressed air at a rate of 40 N-L/min to atomize the mixture into droplets with small diameters of 10-50 μm. Heat exchange occurs as the atomized droplets come into contact with steam from another steam nozzle that has been superheated to 200°C by an electric heater. The latent heat required to evaporate water in the droplet is supplied by the sensible heat of the superheated steam. Therefore, superheated steam, whose thermal capacity is higher than that of dry air, can supply more heat capacity per unit area. However, an average droplet is not completely dried instantly, so the product temperature does not reach the saturated steam

temperature, which is 40°C for a vacuum of approximately 5 kPa in the evaporator. In addition, the main body of the drying chamber is decompressed by a vacuum pump, and the evaporator jacket temperature is maintained at 50°C by a supply of hot water. The dry powder it is completely captured by the first or second cyclone. To prevent the formation of wall deposits and classification in the cyclone, a hot water-2, and a return hot water-2 at the end of the first cyclone were supplied. The low-pressure dry air was used to collect the powder in a receiver at 45 °C. However, superheated steam can greatly reduce the capacity by cooling with a condenser by supplying cool water; the steam can then be collected as water in a water reservoir, and the vacuum pump can be downsized to more easily maintain the pressure.
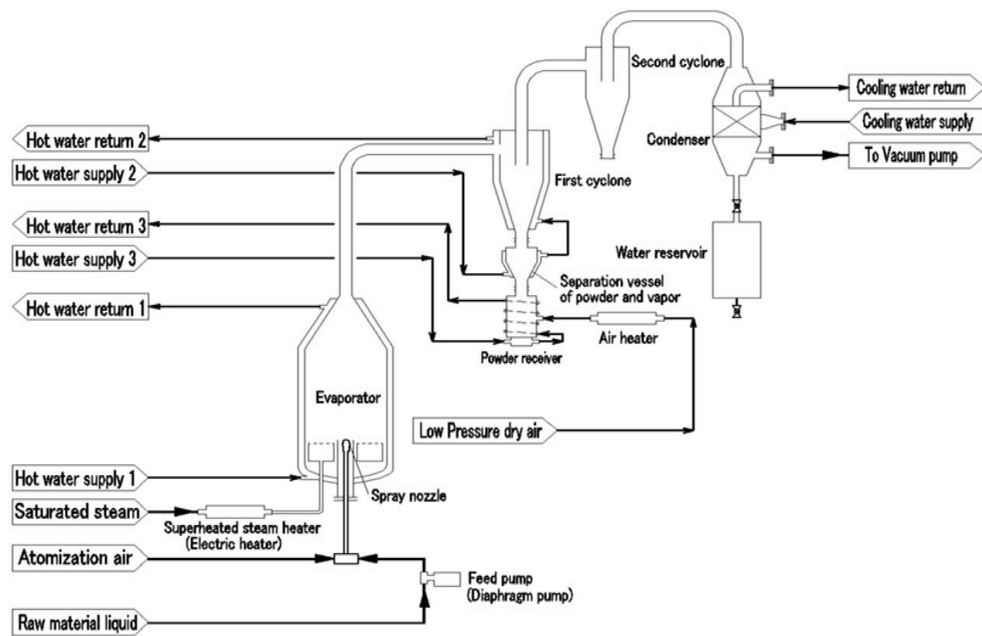
Figure 2-1. Schematic flow diagram of the vacuum spray drying
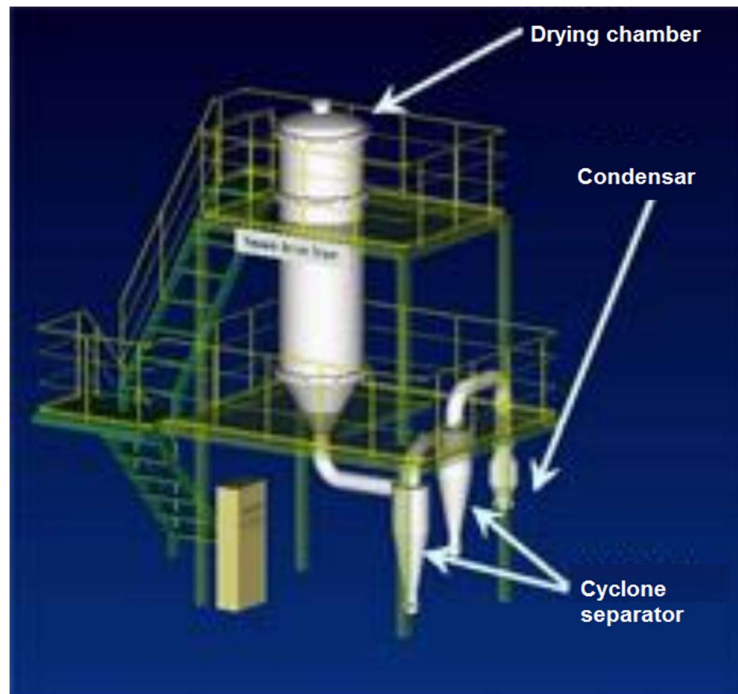
Figure 2-2. Vacuum spray dryer

Source: https://www.tanabe-ind.co.jp/en/VSD-development-en?cat=215 (2016)
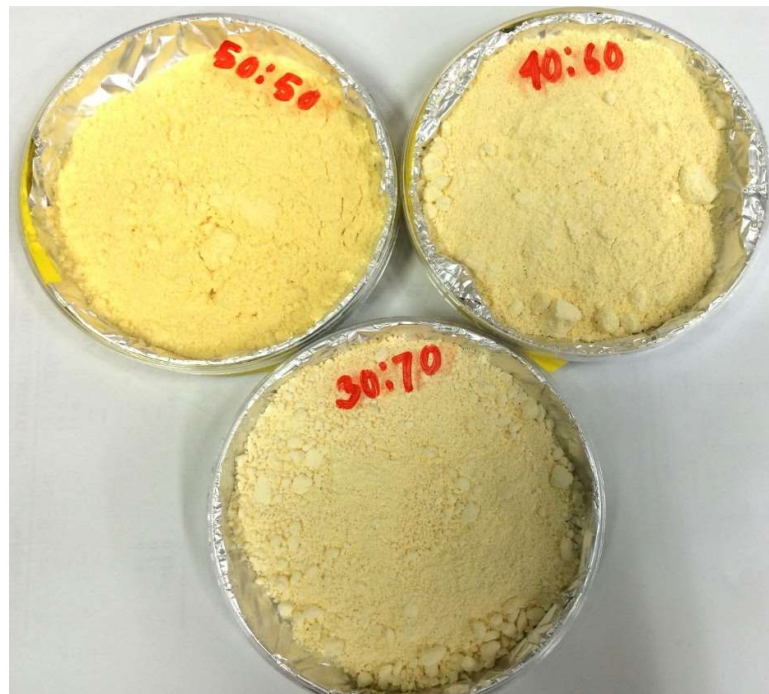
Image 1. Vacuum Spray Dried Commercial Orange Juice powders

2.2.3 Assessment of the physical properties of orange juice powder

2.2.3.1 Moisture content

The moisture contents of the orange juice powder were determined by drying in an oven at 70°C until consecutive constant weights were obtained, followed by 2 h intervals and which gave variation less than 0.3%. Moisture content expressed as % of moisture in wet basis (Goula and Adamopoulos, 2010).

2.2.3.2 Bulk density

The bulk density of the orange powder was measured by gently adding 2 g of powder to an empty 10 mL graduated cylinder and holding the cylinder in a vibrator for one minute (Goula et al., 2004). The volume was then recorded and used to calculate the bulk density in g/mL.

2.2.3.3 Rehydration

Rehydration of orange powder was determined according to the method described by Goula and Adamopoulos (2010) with slight modifications. For measurement 2 g of powder were added in a 50-mL distilled water at 26°C in a 100 mL low form glass beaker. The mixture was agitated in a digital hot plate/stirrer at 900 rpm, using a magnetic stirrer bar with a size of 2 mm × 7 mm time required in a sec for the powder to be completely rehydrated was recorded.

Figure 2-3. Hot plate with magnetic stirrer for determination of rehydration time

2.2.3.4 Hygroscopicity

For hygroscopicity, 1.5g of the orange juice powder was kept in Conway unit (Model: 060310-02A, Shibata Co. Ltd., Tokyo, Japan) containing saturated salt solution of NaCl (75.29% RH). Samples were weighed after one week and hygroscopicity was expressed as the weight of the hygroscopic moisture per 100 g of dry solid (Cai and Corke, 2000).
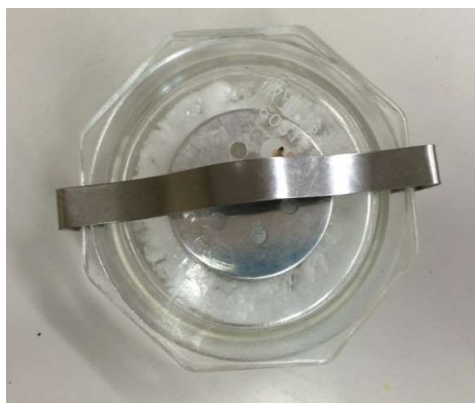
Figure 2-4. Conway unit (SHIBATA Co. Ltd, Japan)

2.2.3.5 Water activity

The water activity of the orange powder was determined with the standard water activity of the saturated salt solution such as Lithium Chloride (PubChem CID: 433294), Potassium acetate (PubChem CID:517044), Magnesium Chloride (PubChem CID: 5360315), Magnesium nitrate hexahydrate (PubChem CID: 202877), Strontium chloride hexahydrate (PubChem CID: 6101868), Sodium chloride (PubChem CID: 5234), and Potassium Chloride (PubChem CID: 4873) of 0.11 0.22, 0.33, 0.53, 0.71, 0.75 and 0.84water activities respectively at 25°C (A. ArabHosseini, 2005); Greenspan, 1977; (Young, 1967) by Conway unit (Model: 060310-02A, Shibata Co. Ltd., Tokyo, Japan) and the graph intersection method described by Kitamura and Yanase (2011).

One gram of powder was kept in an aluminium case with aluminium foil and weighed. Then 3 g of each salt placed into the Conway unit. After that, a few drops of water were added in the

salt and the sample containing aluminum case kept inside of the Conway unit and conditioned with the saturated salt solution. Finally, the lid of Conway unit was covered and kept in 25°C for 2h conditioned with the saturated salt solution. After 2 h, the weight of the sample containing aluminum case was measured. Then the differences of sample weight (Y-axis) were plotted against the known water activity of the saturated salt (X-axis). A straight line was drawn from each data set and intersection with the X-axis, which corresponds to zero sample weight variation, and which was defined as the water activity of the sample as shown in Figure 2-5. For each of the sample, triplicate measurements were conducted.
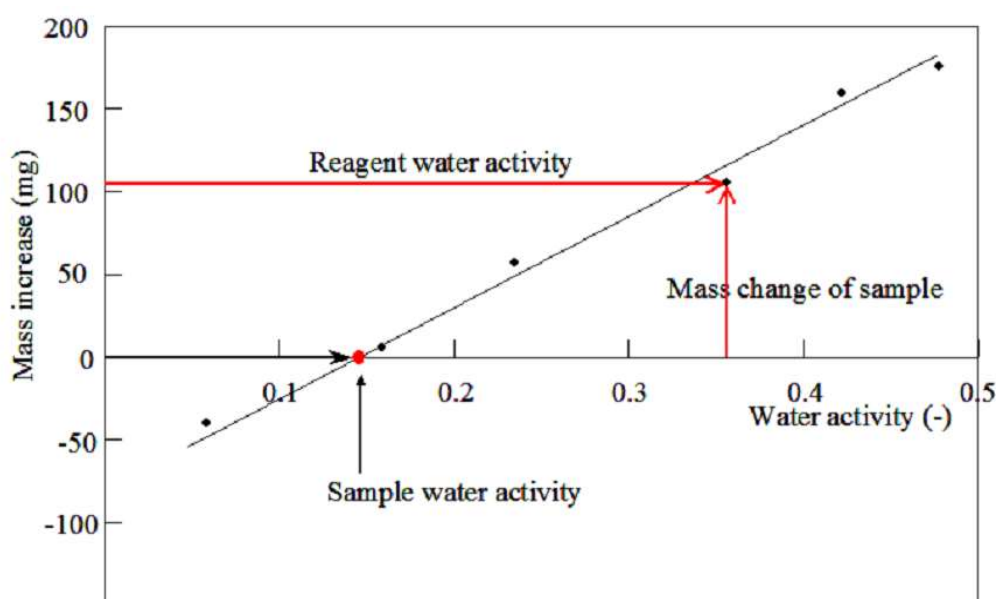
Figure 2-5. Extraction of water activity (Kitamura and Yanase, 2011)

2.2.3.6 Particle size and particle size distribution

Particle size (D50 and D75) and its size distribution were determined by a laser diffraction particle size analyzer (SALD-2200, Shimadzu Corporation, Japan) in dry measurement mode and according to (Koyama and Kitamura, 2014). Working conditions are as follows: sample suction type is hand shot; Pressure is at 0.6-0.8 kPa, filtering rate is 5μm or larger; Operating

temperature and the humidity are 25°C and 60% respectively, and particle size was taken as D50 and D75 values expressed as µm. D50 is defined as the median diameter, and D75 is the particle size corresponding to 75% of the particles being under size by mass. The particle size distribution profile was constructed by the particle size and frequency of the distribution.

2.2.3.7 Particle morphology

Particle morphology was evaluated by field emission scanning electron microscopy (FE-SEM) according to (Mishra et al., 2014a) and slight modifications. The powders were mounted onto stubs with double-sided adhesive tape, and the samples were coated with a thin layer of gold under vacuum. Then, the samples were examined on an FE scanning electron microscope (SU8020, HITACHI, Japan) at 5 kV and 5000-fold magnification. The taking microphotographs were carried out with a camera coupled to the microscopic (Cano-Chauca et al., 2005)

2.2.3.8 Water sorption isotherm

The equilibrium moisture contents of the orange juice powder were evaluated at different values of water activity by isopiestic method Rockland (1987). Seven saturated salt solutions were prepared as mentioned before. One gram of each of the three orange powders were weighed in aluminum vials and equilibrated in the saturated salt solutions in a desiccator at 25°C with H_2SO_4 to maintain proper relative humidity. Sample equilibration took 3-4 weeks based on the change in weight, which did not exceed 0.01%. The equilibrium moisture content was determined by oven drying at 70°C unit a constant weight was obtained (AOAC, 1990).

Sorption isotherms are generally explained by the Brunauer–Emmett–Teller (BET) (Mrad et al., 2012) and Guggenheim–Anderson–de Boer (GAB) (Bizot, 1983) mathematical models based on the following empirical and theoretical parameters:

BET Model: $X_e = \dfrac{X_m\, C_{BET}\, a_w}{(1-a_w)(1-a_w + C_{BET}\, a_w)}$ \hfill (1)

GAB Model: $X_e = \dfrac{X_m\, C_{GAB}\, K_{GAB}\, a_w}{(1-K_{GAB}\, a_w)(1-K_{GAB}\, a_w + C_{GAB}\, K_{GAB}\, a_w)}$ \hfill (2)

In the present study, sorption isotherm data were fitted to the BET and GAB models and analyzed by Origin Pro 8.5 software. The goodness of fit was evaluated by determining the coefficient R^2, and mean deviation modulus was calculated by the following formula (Goula et al., 2008):

$$M_e = \frac{100}{N} \sum_{i=1}^{N} \left| \frac{V_e - V_p}{V_e} \right| \qquad (3)$$

2.2.3.9 Glass Transition Temperature

The glass transition temperatures, Tg, of the orange powder were determined by differential scanning calorimetry (DSC) (DSC-60, Shimadzu Corporation, Japan) according to Shrestha et al. (2007a) . Five to ten grams of the orange powder were scanned in a hermetically sealed 20 µL DSC aluminum pan. All samples had previously been equilibrated over a saturated salt solution with different relative humidities at 25°C. An empty aluminum pan was used as a reference. The rate of thermal scanning was carried out in the following order: (1) isothermal at -30°C for 1 min, (2) heating at a rate of 10°C/min from -30°C to a temperature just over the apparent or predetermined Tg, (3) rapidly cooling at 50°C/min to -30°C and (4) heat scanning at a rate of 10°C/min from -30°C to 200°C. All analyses were conducted in triplicate. The midpoint temperature of the DSC thermogram was considered the transition temperature (Goula et al., 2008).

The glass transition temperature of a binary water-solid mixture depends on the plasticizing effects of the water and is described by the Gordon-Taylor model (Gordon and Taylor, 1952).

$$Tg = \frac{(1 - x_w) \cdot Tg_s + k \cdot x_w \cdot Tg_w}{(1 - x_w) + k \cdot x_w} \qquad (4)$$

Where Tg, Tg_s, and Tg_w are the glass transition temperatures of the mixture, solids, and water, respectively, x_w is the mass fraction of water, and k is the Gordon-Taylor parameter.

2.3 Results and discussion

2.3.1 Physical properties of orange juice powder

To evaluate the physical conditions of the powder, water activity (a_w), moisture content, glass transition temperature (Tg) and product recovery were determined as shown in Table 2-1. The moisture content of the powder had a great impact on the flowability, stickiness and storage stability because of its plasticizing effects and crystallization behavior. The availability of free water in a food component is expressed as water activity, which is responsible for microbiological and biochemical reactions. All of the powder samples had water activity (a_w) values of 0.25 ± 0.00-0.25 ± 0.01 and moisture contents of 2.29 to 3.35%. whereas (Shrestha et al., 2007a) found the wetness of the powder was high as indicated the moisture contents and water activity of 4.3 - 4.5% and 0.30 to 0.40. The present research had the lower moisture content and water activity than spray dried of concentrated orange juice by Goula and Adamopoulos (2010). This was due to the higher moisture removal during vacuum spray drying, and where a greater the heat gradient occurred in VSD between the steam and the particles than hot air so that there was a higher heat transfer into the particles, which supplied the driving forces of the moisture removal. The moisture contents and other parameters of powder produced by a COJ/MD ratio of 60:40 were not evaluated because of the stickiness, low glass transition temperature of this powder. The moisture content and water activity (a_w) of powder decreased with increasing maltodextrin concentration likely because the sugar and acid in juice-rich powder are highly hygroscopic and maltodextrin increases the total solid of the feed and reduces the amount of water evaporation. This result agrees with the findings of Shrestha et al. (2007a) in spray-dried orange juice powder. The water activity values of all powder samples were below 0.30, which improves powder stability. The glass transition temperature Tg has a great impact on the surface stickiness of the amorphous powder during spray drying (Lloyd et al., 1996). The Tg values of orange juice/maltodextrin powder were investigated by DSC. The characteristic parameters Tg_{onset} and $Tg_{midpoint}$ (°C) were observed from the DSC thermogram as shown in Table 2-1. The

glass transition temperature of the powder increased with increasing the amounts of maltodextrin solids because of the higher molecular weight of maltodextrin. Roos and Karel (1996) stated that the similar phenomenon, Tg value of binary mixture (sucrose:maltodextrin) increases with increasing the maltodextrin concentration. Bhandari and Howes (2005) mentioned that the recovery of spray dried sugar rich product was the direct functions of the temperature and moisture of the outlet air. The increases in the maltodextrin solids also increased the cyclone recovery because of low Tg sticks or collapses occur during drying, that's why in the present study OJ/MD 60:40 was failed due the low Tg (39.76°C) and higher moisture content ($\geq$ 4%) . In general, several authors stated that the recovery of feed solid in the final product increases with increasing the maltodextrin (Bhandari and Howes, 2005; Papadakis and Bahu, 1992). The pattern of the findings was similar to Shrestha et al. (2007a). The highest cyclone recovery, 63.3%, was obtained with 30:70 COJ/MD powders, which had the highest Tg of approximately 86.63°C, and the lowest recovery of 53.0% was observed with 50:50 powder at 61.78°C. These results differed from Shrestha et al. (2007a), who found that 50:50 orange juice/maltodextrin powder had Tg values of 66.4°C and no powder output in the cyclone. These differences are caused because the present study was conducted at a low temperature in a vacuum condition and superheated heated steam, as the drying medium, which has a higher heat capacity than dry air, as a result the COJ/MD 50:50 powders were produced at a low Tg (61.78°C).

Table 2-1. Physical properties of an orange juice powder

Types of Powder COJ/MD	Moisture %	Water activity	Glass Transition Temperature Tg °C		Cyclone recovery, %
			Tg_{Oneset}	$Tg_{midpoint}$	
50:50	3.35±0.28[a]	0.25±0.00[a]	56.42±1.35[c]	61.78±0.97[c]	53
40:60	2.57±0.12[b]	0.17±0.02[b]	68.01±1.52[b]	75.94±0.92[b]	60.5
30:70	2.29±0.16[bc]	0.15±0.01[b]	79.35±1.28[a]	86.63±0.97[a]	63.3

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly (p≤ 0.05)

Table 2-2 shows the hygroscopicity of the VSD powder in relations with the maltodextrin concentration. Hygroscopicity of powder decreased from 0.195 ± 0.02 to 0.143 ± 0.01 gH_2O/g with increasing maltodextrin concentration. This was in an agreement with Tonon et al. (2008). This trend was also similar to that of Cai and Corke (2000) and Rodríguez-Hernández et al. (2005) for spray-dried Amaranthus and cactus pear juice powder. According to Table 2-2, it is indicated that by increasing the maltodextrin, the solubility of the powder increases and rehydration index were greatly reduced. Because the maltodextrin has superior solubility in water (Cano-Chauca et al., 2005), the fast rehydration may be due to the low moisture contents of the powder (Goula et al., 2008).The solubility index of the potato flour was increased with increasing the maltodextrin solids (Grabowski et al., 2006). The bulk densities of the powder were not significantly different at $p\leq 0.05$. The determined bulk density of VSD orange juice powder agreed with that of Chegini and Ghobadian (2005b).

Table 2-2. Physical properties of orange juice powder (ii)

Types of Powder COJ/MD	Hygroscopicity, gH_2O/g	Rehydration, Sec	Bulk density g/mL	Particle size, μm	
				D50	D75
50:50	0.195 ± 0.02^a	253.65 ± 1.05^a	0.70 ± 0.03^a	7.75 ± 0.25^a	$12.84\pm.62^a$
40:60	0.188 ± 0.03^b	237.40 ± 1.25^b	0.72 ± 0.02^a	6.36 ± 0.45^b	8.69 ± 0.21^b
30:70	0.143 ± 0.01^c	122.34 ± 1.56^c	0.73 ± 0.02^a	6.02 ± 0.16^c	7.68 ± 0.26^b

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly ($p\leq$ 0.05)

Table 2-2 shows that the VSD orange juice powder particle diameters were significantly different at $p\leq 0.05$. The median (D50) values of COJ/MD 30:70 powders had the lowest peak with a smaller volume distribution and smaller particle diameter as shown in Figure 2-6. Powder containing a high amount of orange juice are rich in acid and sugar, which imparts a high hygroscopicity and stickiness to the liquid feed; as a result, larger particles are produced during spray drying ((Shrestha et al., 2007a). Powders with 50:50 COJ/MD had significantly (at $p\leq$

0.05) higher particle size and size distributions. These trends are similar to the findings described by (Shrestha et al., 2007a); however, powder particle sizes in the present study were much smaller because of the difference atomization at a lower temperature. Tonon et al. (2011) stated that when the inlet temperature is low, the particle remains shrunk and maintains a smaller diameter, whereas at higher temperatures, faster-drying rates, and higher swelling form larger particles. D75 powder particles with OJ/CMD ratios of 40:60 and 30:70 were not significantly different at p≤ 0.05.
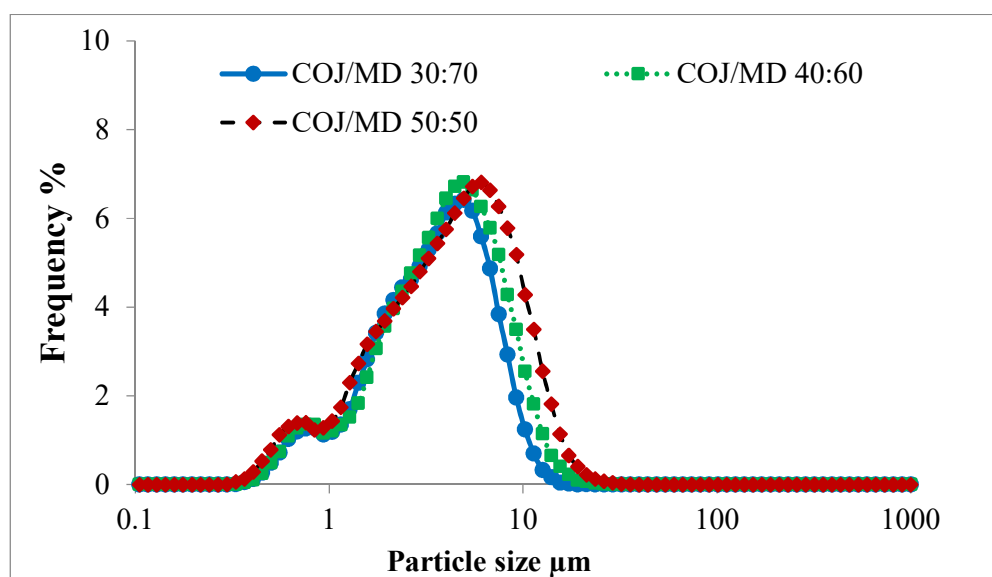
Figure 2-6. Particle size distributions of VSD orange juice powder.

The micrograph shows that the morphology of the particles depends on the concentration of the carrier agent and the drying conditions Figure 2-7. The 30:70 COJ/MD powder produced with higher amounts of maltodextrin had the smoothest surface with smaller spherical shapes and no shrinkage. This agrees with the results published by Ravindra et al. 2013, who stated that a higher maltodextrin concentration was more susceptible to shrinkage during the spray drying of white-flowered *O. stamineus* plant extract and observed that the particle surface becomes smoother when the maltodextrin concentration increases from 0.53% to 10.67%. The 40:60 COJ/MD

powder had smoother spherical shapes, but some smaller particles adhered to the larger ones, making the particle size larger than that of the 30:70 COJ/MD powder. The VSD powder with 50:50 COJ/MD had dented surfaces with wrinkles and deformation compared to the other powder.

COJ/MD 50:50

COJ/MD 40:60

COJ/MD 30:70
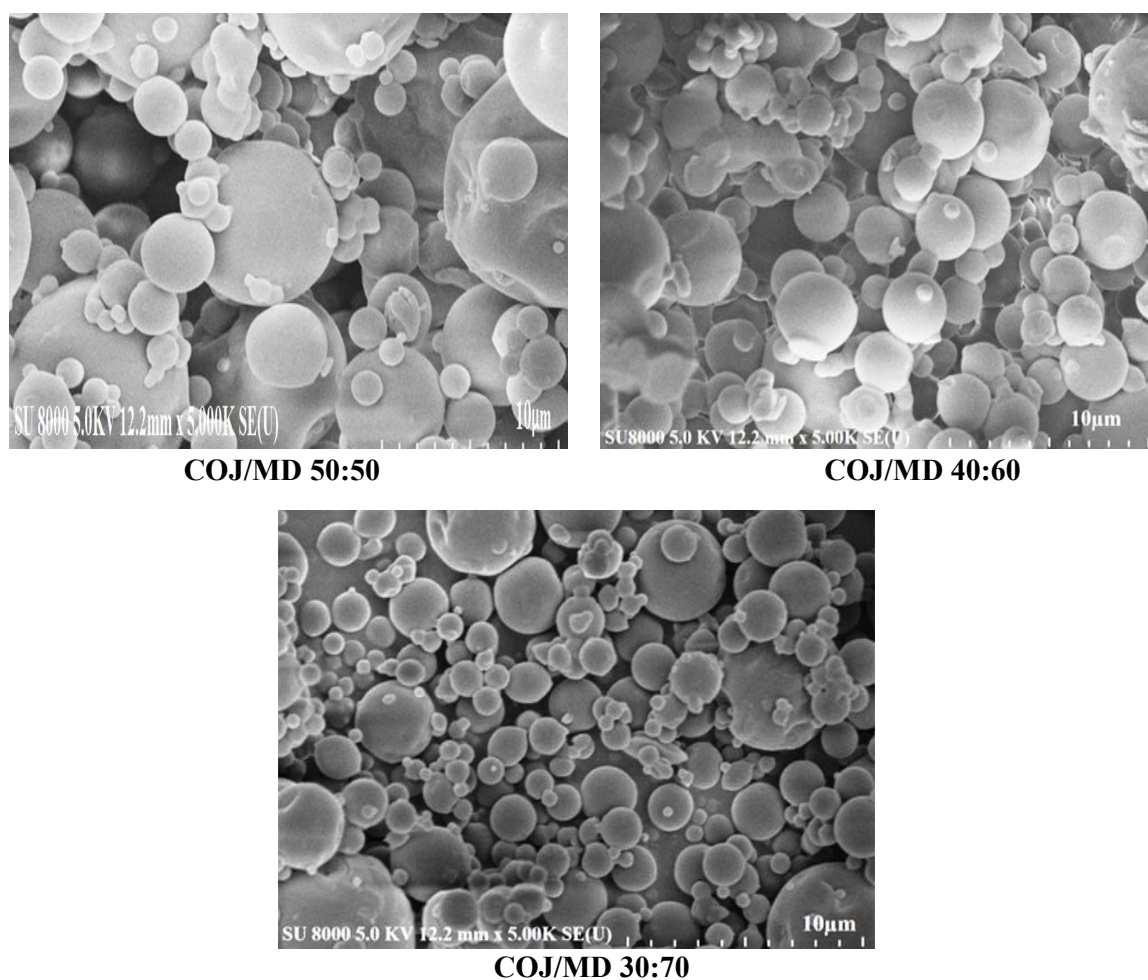
Figure 2-7. SEM micrograph of VSD orange juice powders

2.3.2 Moisture sorption isotherm of orange juice powder

Water sorption isotherms were constructed for the vacuum spray-dried orange juice powder, and the experimental moisture sorption data for the equilibrium moisture contents (EMC, X_e g H_2O/g of dry solid) are given in Table 2-3. With increases in water activity (a_w), the equilibrium moisture contents (X_e) of the powder also increased at a constant temperature. The multilayer

sorption region at low and intermediate water activity, the moisture content increase linearly, whereas at capillary condensation region at higher water activity levels, the moisture content increases rapidly with water activity. The nature of the sorption isotherm can be described by the phenomenon that the physical sorption of the active sites occurs by proteins, whereas the water can be sorbed only to surface OH sites of crystalline sugar (Falade and Aworh, 2004).

Table 2-3. Equilibrium moisture content (EMC) of orange juice powder at different water activity level.

Water activity (a_w)	Equilibrium moisture content, EMC (gH_2O/g of dry solid)		
	COJ/MD 50:50	COJ/MD 40:60	COJ/MD 30:70
0.11	0.0236 ±0.0002	0.0251±0.0003	0.0195±0.0008
0.22	0.0389±0.0003	0.0393±0.0001	0.0378±0.0011
0.33	0.0575±0.0001	0.0421±0.0012	0.0398±0.0017
0.53	0.0786±0.0011	0.0773±0.0001	0.0685±0.0019
0.71	0.1325±0.0013	0.1256±0.0015	0.1194±0.0004
0.75	0.178±0.0004	0.1585±0.00019	0.1507±0.0020
0.84	0.290±0.0012	0.2388±0.0010	0.2104±0.0013

The values are mean ± S.D of three independent determinations

Experimental data were also fitted to the GAB and BET models. A sharp increase in the equilibrium moisture content of orange powders observed in Figure 2-8 at higher water activity levels $a_w > 0.52$ at 25°C. The similar phenemonen reported by Kammoun Bejar et al. (2012) and Maroulis et al. (1988) for high sugar containing fruits such as orange peels and leaves, and raisins, figs, appricots, prunes, this was due to the dissolution of fruit sugar in the sorbed water vapour. The other authors mentioned that, the orange juice powder with high solid content consists more that 80% sugar with sugar content ratio of 2:1:1 sucrose:glusoce:fructose respectively showed the similar sorption behavior (Kelebek et al., 2009).
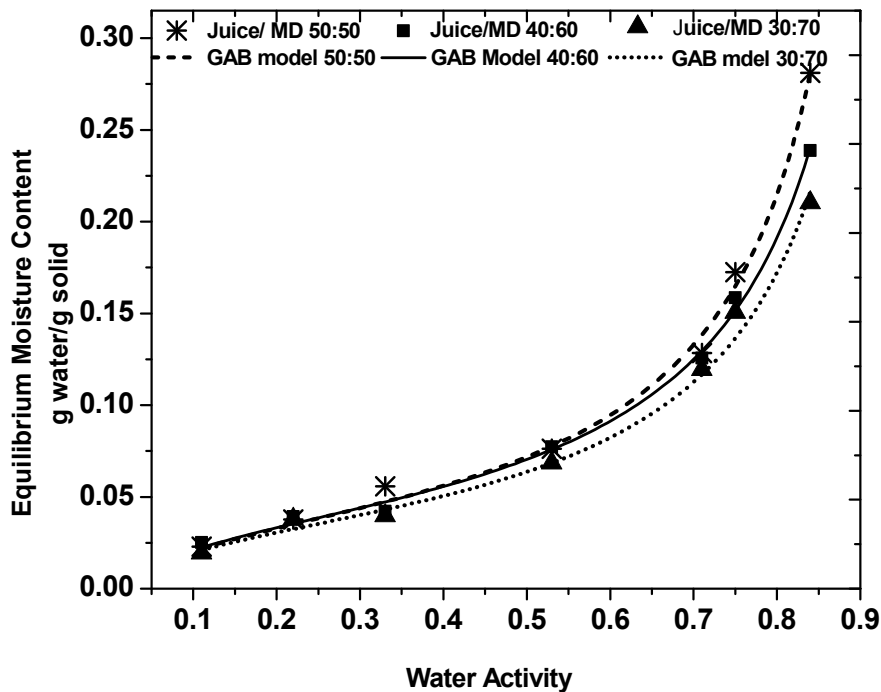
Figure 2-8. Sorption isotherm of VSD orange juice powder and the fitted GAB model

The regression data for the GAB and BET models are presented in Table 2-4. The coefficients

of determination (R^2) and mean deviation modulus (M_e) were calculated to find the best fit model.

The other model parameters such as X_m is known as the monolayer moisture content, is defined

as the safest moisture content that strongly absorbs to active sites on the food surface at a given

temperature. The strength of the binding for the water molecules to the primary binding sites on

the product surface can be explained by the parameter c. The larger value of c represents the

stronger bonds between the water molecules in the monolayer. The parameter k represents the

values which determine the correction factors for multilayer molecules relative to the bulk liquid,

such as when k =1, which describe the same characteristics of the molecules beyond monolayer

and pure water (Quirijns et al., 2005). The GAB models resulted in X_m values of 3.54-4.03%.

These results agree with (Tonon et al., 2009a) and (Caparino et al., 2013) for spray-dried Acai

juice powder and freeze-dried mango powder, respectively. Whereas Edrisi Sormoli and

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Langrish (2015) reported the molonayer moisture content of spary dried orange juice powders were in the ranges of 9.8 to 12.6 % at 20 to 50℃ temperature range. COJ/MD 50:50 powder exhibited the highest monolayer moisture water (4.03%) and also demonstrated the higher adsorption as shown in Figure 2-8. In respect to GAB parametes, COJ/MD 30:70 showed the highest c value (8.96) and lowest k value (0.98) whereas the powder 50:50 showed the higher k values and higher adsorption, this was due to the high hygroscopic nature of powder rich in orange juice solids. Conversely, powder with higher maltodextrin concentrations are less hydrolyzed and have fewer hydrophilic groups, causing them to absorb less moisture and which explains its lower hygroscopicity (Cai and Corke, 2000).

Table 2-4. Estimated BET and GAB model parameters for VSD orange juice powders

Model	Model Parameters	COJ/ MD 50:50	COJ/MD 40:60	COJ/MD 30:70
BET	X_m	0.0478	0.0402	0.0360
	C_{BET}	4.31	5.50	7.56
	R^2	0.99	0.99	0.98
	% Me	9.85	9.28	5.39
GAB	X_m	0.0403	0.0395	0.0354
	C_{GAB}	7.73	8.24	8.96
	K_{GAB}	1.02	0.99	0.98
	R^2	0.98	0.99	0.99
	% Me	6.63	6.92	8.41

The sorption data of the orange juice powders were also fitted to the BET model and typical curve as shown in Figure 2-11. The fitted parameters were presented in Table 2-11. The BET model consists of two parameters such as monolayer moisture X_m and the parameter c. The fitnes of the model was determined based on the parameter coefficients of determination (R^2) and mean deviation modulus (M_e). The X_m values of orange juice/maltodextrin powder range from 3.60 to 4.78% and c values showed in the ranges of 4.32 to 7.56. Whereas BET models don't have the third parameter and it's difficult to explain the sorption behavior of the product in the larger water activity levels (0.05 $<a_w<$0.9) (Jonquières and Fane, 1998).
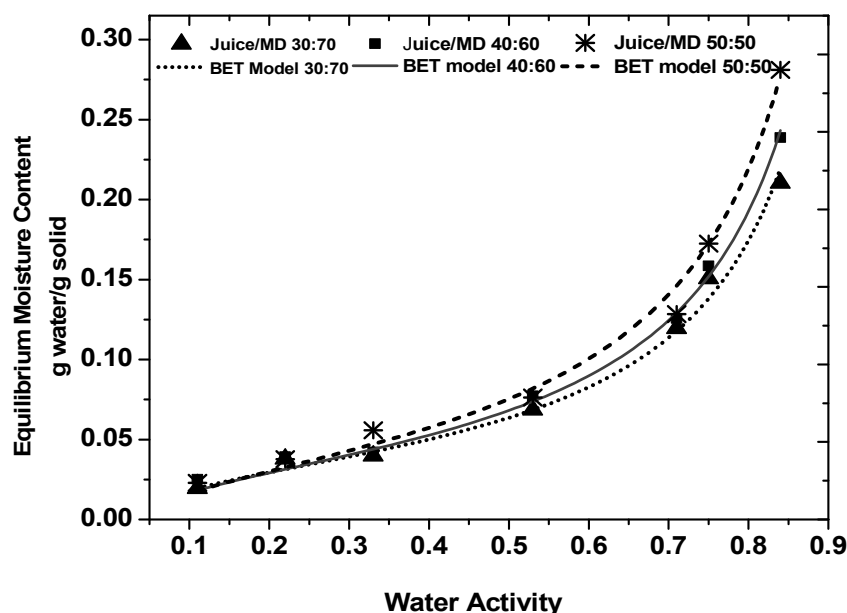
Figure 2-9. Sorption isotherm of VSD orange juice powder and the fitted BET model

The shape of the sorption isotherm Figure 2-10 and Figure 2-11, of the orange juice powder predicted by the BET and GAB models, showed a typical sigmoid curve and a type III (J shape) and typical of sugar-rich products according to Brunauer's classification. According to (Gabas et al. (2007) and Edrisi Sormoli and Langrish (2015) observed similar types of the curve for vacuum-dried pineapple juice powder with maltodextrin or gum Arabic as carrier agents and spray dried orange juice powder.

GAB models resulted in the highest R^2 and lowest M_e values, similar to Goula et al. (2008) and Tonon et al., (2009a) for spray-dried Acai juice and tomato pulp powder, respectively. Both the BET and GAB models had satisfactorily fitted values for powder produced by COJ/MD 30:70 powders. According to Table 6, GAB model was highly fitted to the experimental data of the orange juice powder, because this model has the third parameter k and it can predict over range of water activity (0.05 ~ 0.9) than BET model fails over water activity 0.5 (Jonquières and Fane,

1998); (Goula et al., 2008). The similar results reported for the sorption behavior of the spray dried orange juice powder (Edrisi Sormoli and Langrish, 2015).

The physical characteristics of orange juice powder were investigated during storage at 25°C with different water activities. According to Labuza and Altunakar (2007) the safest monolayer moisture content in the ranges of 0.2 to 0.3, the dehydrated products within this range having the maximum shelf life. The VSD orange powders showed the monolayer moisture within the reported ranges. The free-flowing powder had water activities below 0.53, and stickiness or agglomeration was observed above a_w of 0.53. At a relative humidity above 75%, collapse and liquefaction were observed in the powder.

2.3.3 Glass transition temperature

Several studies have shown that stickiness problems of amorphous solids during spray drying occurs as a result of particle plasticization, and this phenomenon can be explained by the glass transition temperature (Lloyd et al., 1996). The glass transition temperature of VSD orange powders was investigated by DSC. The onset, midpoint, and end-set temperature were determined from the characteristic DSC thermograph. However, some authors have used the midpoint transition as a glass transition temperature (Goula and Adamopoulos, 2010, 2006). Based on the transitions occur at Tg_{onset}, but the present study considered midpoint temperature of the change in heat capacity was taken as the safest the glass transition temperature of the orange juice powder, furthermore it was used to establish the relationship between Tg, water activity and water content of the orange juice powder. The figure 2-10 shows the glass transition temperature of the vacuum spray dried orange juice powders. The results show that the addition of maltodextrin in orange juice significantly increased the glass transition temperature.
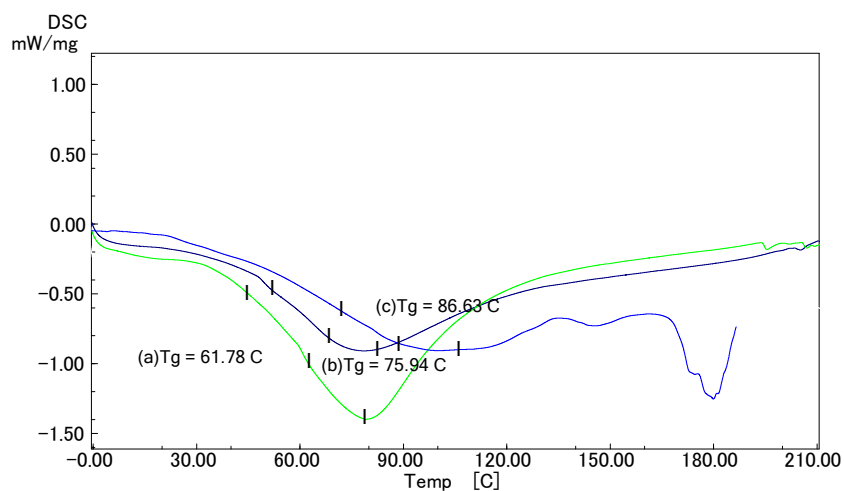
Figure 2-10. DSC profile of orange juice powders and conditioned at water activity (a_w = 0.33). where (a) COJ/MD 50:50, (b) COJ/MD 40:60 and (c) COJ/MD 30:70

Previously mentioned that OJ/MD 60:40 powder were unsuccessful, this was due to the stickiness of the powder. The OJ/MD 60:40 powders had the lower glass transition temperature (37.15 ℃) . The DSC thermograph of OJ/MD 60:40 powders as shown in Fig. 2-10.
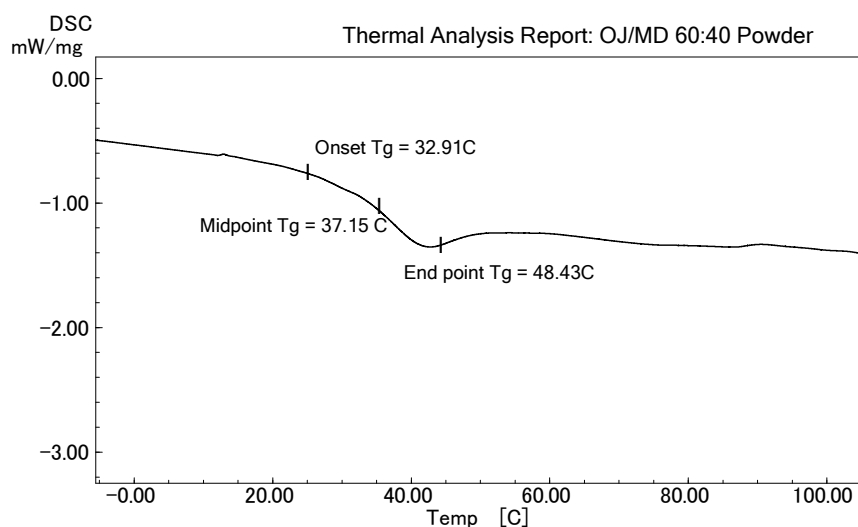
Figure 2-11. DSC profile for VSD orange juice powder (COJ/MD 60:40) and conditioned at water activity (a_w = 0.33)

Similar curves were also obtained for all other powders as presented in Figure 2-11. The DSC profile shows that with an increase of carrier agent, Tg of the powders increased. The similar phenomenon obtained by Goula et al. (2008); Shrestha et al. (2007a) and Tonon et al. (2009b) for spray dried orange juice, tomato pulp, and acai juice powders. The results show that there was a huge jump in Tg from 61.78 to 86.63 due to the increases of maltodextrin as 20% solids. Product with higher juice solid had lower glass transition temperature. The glass transition temperature of VSD orange juice powders was also determined at different relative humidity. The results are shown in Table 2-5. Glass transition temperature of VSD orange juice powders decreased with increased of moisture content, this behavior due to the plasticizing effect of water. The similar results were obtained for spray dried orange juice, tomato pulp, acai juice powders (Goula et al., 2008; Goula and Adamopoulos, 2010; Tonon et al., 2009b).

Table 2-5. Glass transition temperature of VSD orange juice powders at different water activity levels

Water activity (a_w)	Glass Transition Temperature, Tg, (°C)		
	COJ/MD 50:50	COJ/MD 40:60	COJ/MD 30:70
0.11	61.78±1.23	77.94±2.15	88.63±2.12
0.22	53.12±2.11	67.76±1.19	74.85±1.17
0.33	45.16±0.05	63.23±1.13	68.13±1.12
0.53	32.23±1.13	45.67±1.23	55.13±2.13
0.71	15.15±1.19	17.21±1.11	30.12±1.13
0.75	-2.32±0.03	4.14±0.92	15.19±1.11
0.84	-30.25±0.95	-23.96±1.21	-12.67±1.23

The values are mean ± S.D of three independent determinations.

The predicted values are given in Table 7, and the fitted curve is shown in Fig 5; satisfactory values for R^2 and M_e were obtained During storage and also in the processing orange juice powder becomes agglomerated and caking occur due to the plasticizing effects of water on the particle surface. These physical phenomena were successfully explained and predicted by the

glass transition concept (Chuy and Labuza, 1994). The predicted values are given in Table 2-6, and the fitted curve is shown in Figure 2-11; satisfactory values for R^2 and M_e were obtained.

Table 2-6. Estimated Gordon Tailor Parameters for VSD concentrated orange powders

Parameter	COJ/MD 50:50	COJ/MD 40:60	COJ/MD 30:70
Tgs (°C)	71.89	95.18	103.82
K	2.498	3.194	3.52
R^2	0.99	0.98	0.99
E (%)	6.70	3.84	3.47

The Gordon Taylor parameters Tgs represents the glass transition temperature of the binary solid mixture, lower the Tgs mean higher plasticizing effects of water into solids. Due to the plasticizing effects of water, the Tg of the powder decreased with increasing water content.

The present study shows that the Tg_s values changed from 71.89 to 103.82°C with an increasing maltodextrin solid content from 50 to 70%. The other parameters k presents the heat capacity of water around its glass transition to the heat capacity of the dry solids.The parameter k also known as the adjustable parameter, which determined the degree of curvature of the Tg-moisture curve. Incorporating of maltodextrin could increase the k value of the VSD orange juice powders from 2.50 to 3.35. The similar results reported by (Wang and Zhou, 2012) for spray dried soy sauce powder with maltodextrin as a carrier. In contrast, higher k values (0.83-0.85) obtained by (Goula and Adamopoulos, 2010) for spray-dried concentrated orange juice with maltodextrin. These differences in the Gordon-Taylor parameter may result from the different feed compositions and drying conditions of the orange juice/maltodextrin powder. Another possible reason reported by Wang and Zhou (2012), the predicted results of Tgs and k showed higher due to the lower DE value of maltodextrin.

COJ/MD 30:70

COJ/MD 40:60

COJ/MD 50:50
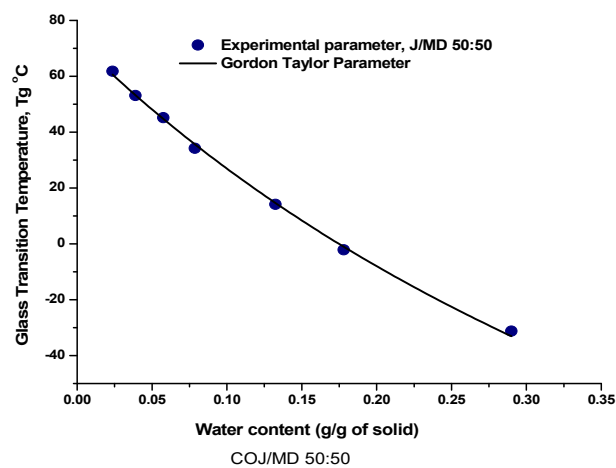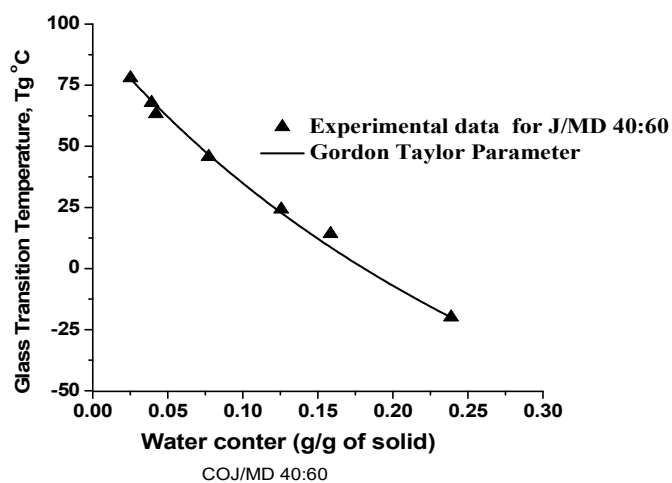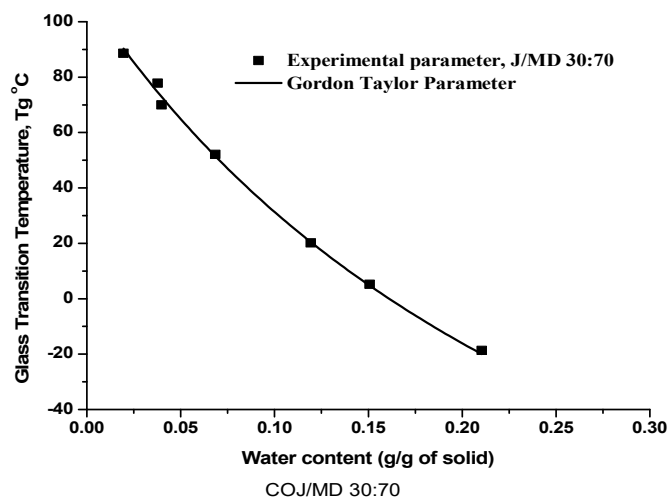
Figure 2-12. Relationship between the glass transition temperature and water content of VSD orange juice powders with curves fitted to the Gordon-Taylor model

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Our present study used maltodextrin DE 12 whereas Goula and Adamopoulos (2010) used DE 6 as a carrier for spray drying. Lower the molecular weight of maltodextrin showed the higher glass transition temperature (Goula and Adamopoulos, 2010; Tonon et al., 2008). The higher R^2 values and lower percent modulus values indicated that Gordon Taylor model was satisfactorily fitted to the experimental data. Truong et al. (2005) stated that glass transition temperature is normally 10-23℃ lower than the sticky point temperature and in spray drying, particles which are above this temperature stick to the dryer wall and adversely affecting the free-flowing property. In the case of VSD orange juice powder COJ/MD 60:40, a sticky temperature much lower than outlet temperature of the dryer (<60℃) and that would result in high degree of stickiness and thus in an insignificant powder yield, whereas other combinations had successfully produced a powder.

2.4 Conclusions

Vacuum spray drying was successfully produced a concentrated orange juice powder with maltodextrin as a carrier. The produced VSD orange powders were stable due to their low moisture content (2.29-3.49%), low water activity (0.15-0.25) and higher glass transition temperature. An optimum concentration of drying aid as maltodextrin to VSD of concentrated orange juice and their relationship with glass transition temperature was established. Due to the stickiness problem, a COJ /MD 60:40 combinations was unsuccessful. Cyclone recovery increased up to 63.3% with increasing as Tg and maltodextrin. The particle sizes of the VSD orange powder were smaller, smooth and spherical in morphology. Overall, the sorption behavior of the orange juice powder exhibited a type III sigmoid curve, and the highest and lowest water adsorption occurred at a_w values above and below 0.53, respectively. The experimental water adsorption data were satisfactorily correlated by both the BET and GAB models. Based on the stability and product recovery the present study concluded that COJ/MD 30:70 by weight can be used in industrially to produce orange juice powder.

Chapter 3. Process development for Micro Wet Milling of orange juice with pulp and quality evaluation

In the present chapter, the aim was to produce concentrated orange juice with pulp by micro wet milling system. Micro wet milling refers to the milling of wet material into particle sizes of a micrometer. The suitable milling conditions were achieved by varying the feeding rate and rotational speed of the mill. Feeding rate of 15 mL/min and rotational speed of 50 rpm can able to produce orange juice with smaller particle sizes. The physicochemical and thermal properties of MWM OJ and commercial OJ were determined. SEM of MWM OJ revealed that MWM disintegrated the cell of the fibrous material in the OJ pulp and consequently produces OJ with better color than the commercial orange juice. The bioactive compounds such as ascorbic acid, total polyphenol and total flavonoid were also measured. The MWM OJ contained higher bioactive compounds than the commercial orange juice. The antioxidant activity DPPH and FRAP of the orange juice demonstrated that MWM can produce OJ with higher antioxidant activity than the industrially processed orange juice.

3.1 Introduction

Orange juice (OJ) is the most appreciated juice beverage due to its pleasant aroma and healthful properties. Moreover, it is a good source of vitamin C, polyphenol, carotenoid, flavonoid and other antioxidant constituents (Kelebek et al., 2009). The quality of the juice depends on the consumer demand and greatly influenced by its characteristic fresh orange flavor (Nisperoscarriedo and Shaw, 1990). In the industrial processing, orange juice (OJ) is passed through the finisher for separating juice from pulp and seeds and undergoes some thermal treatments that extend its shelf life but may deteriorate the color and flavor, and substantially decrease vitamins and functional compounds (Farnworth et al., 2001). Orange juice is marketed in different forms: Fresh juice or single strength juice or ready to drink juice, as a frozen concentrated OJ, which is diluted with water after purchases, as a reconstituted liquid, which has been concentrated and diluted before sale (Gil-Izquierdo et al., 2002; Stinco et al., 2012). The drawback of de-pulping of orange juice can lead to an enormous loss of aroma compounds (Rega et al., 2004). The flavor and nutrient contents of process juice dramatically differ from the juice containing pulp. Orange juice consists of two heterogeneous phase such as serum that contained the water-insoluble particles and another is the clear aqueous phase containing soluble compounds. The insoluble particle enhances the attractive color, flavor, aroma and antioxidant potential of the juice, which are highly desirable for commercial product and also consumer demand (Rega et al., 2004). The solid contents of the pulp contribute to the mouthfeel and sensory appealing properties, may be desirable or not, depends on the consumer choice (Parish, 1998). The larger the particle sizes of the juice can sediment easily and two distinguished phase can easily observe. This attributes may influence the acceptability of the juice, although orange juice with pulp contained desirable dietary fiber. According to Garau et al. (2007), oranges have high fiber and vitamin contents as well as other associated bioactive compounds such as flavonoids and terpenes which exhibit interesting antioxidant properties. Bioaccessibility of bioactive compounds from plant materials was found to increase during mechanical or thermal

processing due to the disruption of the natural matrix (Parada and Aguilera, 2007). Stinco et al. (2012) reported that reduction of particle size increased the surface area of OJ particles and enhanced the carotenoid accessibility and the higher pulp amount and smaller particle size gave a more attractive color than that of single juice. There are only very few studies which reported the effects of processing technique that affects the compounds of orange juice (Tomás-Barberán and Clifford, 2000). So it is necessary to apply a technique to reduce the particle sizes and increase the solubility of the juice.

In the drug processing industry, introduced wet milling process to improve the solubility and ultimately, the bioavailability of poorly water-soluble drugs (Loh et al., 2014). The term milling, size reductions, communication, grinding and pulverization are often used interchangeably. Milling refers to the process to break down of the coarse particle to the finer one by the application of mechanical energy and its regarded as the top-down approach in the production of smaller particles (Rabinow, 2004). The milling also alters the shape and surface roughness of the particles that improve the dissolution property of drug (Loh et al., 2014).

In general, dry milling cannot able to make a product in particle size as micro or nanometer, whereas wet milling is a more intensive process to make a particle size as micro level (Loh et al., 2014). Wet milling also called wet media milling or Micro wet milling in a process in which particles are dispersed in the fluid by shearing, by impact or crushing or by attrition (Loh et al., 2014). The application of wet milling for minimization of particle size during fruit juice processing is quite new. Some mechanical process like homogenization, extrusion, blending and other chemicals or enzymatic treatments were known to process the fruit juice. But there is no literature was found for processing of fruit juice with pulp by the wet milling process. The present study shows interest to process the orange juice with pulp by Micro Wet Milling (MWM) process.

The aim of the present study was to develop the milling conditions of orange juice with pulp, in order to achieve fine juice particle and also to determine the effects of MWM on color, particle sizes, and antioxidant properties of OJ. Finally, to compare the physicochemical and nutritional components of MWM orange juice to the mechanical squeezed commercial orange juice.

3.2 Materials and Methods

3.2.1 Raw materials

Fresh and matured ripe oranges (*Citrus unshiu*) were collected from the National Agricultural and Food Research Organization, Western Region Agricultural Research Center, Kagawa Prefecture, Japan. Another sample of commercial orange juice was collected from Ehime beverage, Japan.

All chemicals were analytical grade used in this study.

3.2.2 Mechanical blending of orange juice

The whole fruits were washed with distilled water, the peels were removed, and the whole fruits were blended to make juice by a blender (Cuisinart SPB 600J, USA). The blending time was determined based on the constant particle size obtained. The blended juice was stored at -20℃ for further study.

3.2.3 Micro wet milling of orange juice with pulp

Micro wet milling refers to the milling of wet material into particle sizes of a micrometer. The experimental MWM system is shown in Figure 3-1. The blended juice was kept in a tank and the tubing pump simultaneously fed the sample to the stone mill. The mill consists of two stone. Only the lower stone mill was rotated by an electric motor. The rotational speed varied from 20 to 50 rpm. The gap size of the stone mill is maintained from 250μm at the beginning of the operation and then reduced to 60 μm by controlling screw. The processed juice was obtained simultaneously in the collecting tank. Grooves were cut into the stone mill, which had an exterior

surface area of 207 cm^2 and radius of 12 cm. The larger the contact surface of the mill particles with sizes on the order of micrometers.

3.2.3.1 Milling conditions of orange juice with pulp

Initially, the feeding rate of orange juice was set to 5, 10, 15, 20, and 25 mL/min and rotational speed was varied from 20 to 50 rpm. The gap size was maintained between 60 to 200 μm. During operation, the overflow occurred at higher feed rate. Similarly, higher rotational speed and larger gap size produced concentrated juice with larger particle sizes. The optimum conditions were determined based on the smaller particle size and avoiding overflow of the juice during milling.
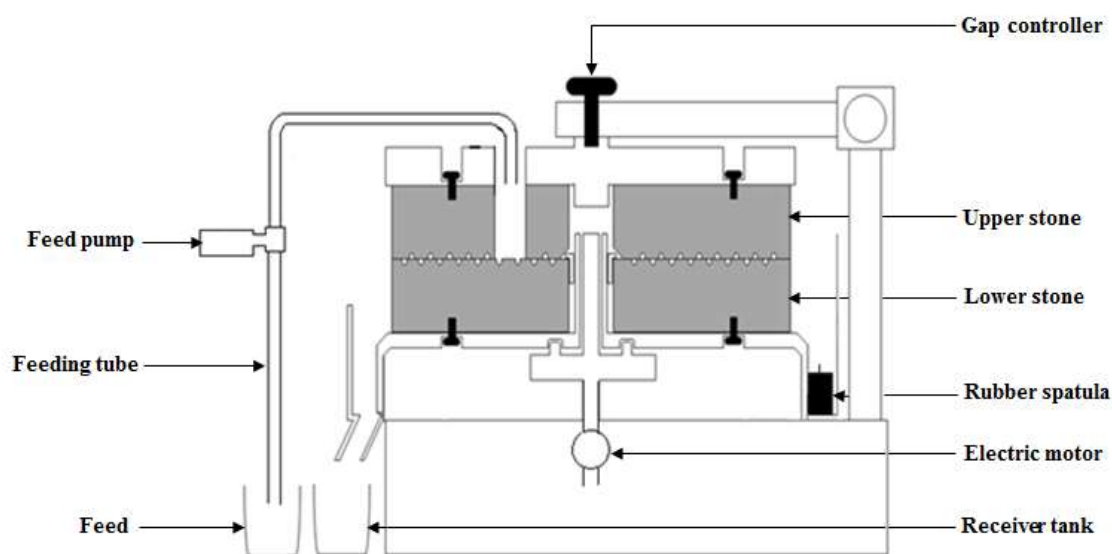
Figure 3-1. Schematic flow diagram of the MWM system

Figure 3-2. Micro wet milling system and upper and lower stone of the mill

3.2.4 Physicochemical properties of orange juice

3.2.4.1 Moisture

The moisture contents of the orange juice were determined by drying in an oven at 70℃ until consecutive constant mass were obtained, followed by 2 h intervals and which gave variation less than 0.3%. Moisture content expressed as % of moisture in wet basis (Goula and Adamopoulos, 2010).

$$\% \text{ Moisture} = \frac{\text{Loss in mass (g)}}{\text{mass of samples (g)}} \times 100 \qquad (1)$$

3.2.4.2 Titratable acidity

The total titratable acidity was determined by titration with standard sodium hydroxide (0.1 N) solution and values were expressed as citric acid/100 mL of juice.

3.2.4.3 Total soluble solids (Brix)

The total soluble solids content of OJ as % Brix content was determined by refractometer (ATAGO PAL-1, Japan)

3.2.4.4 2.4.4. pH of orange juice

pH of orange juice was assessed by pH meter (F-51, Horiba, Japan)

3.2.4.5 Viscosity

The viscosity of the orange juice was determined by a viscometer (DV-E Brookfield LV viscometer, USA) with spindle No. 62 at 25℃ and 12 rpm.

3.2.4.6 Density

The density of orange juice was determined by pycnometer method. At first, the weight of the empty pycnometer (50 mL) was taken. Then the pycnometer was filled with distilled water at 20 ℃ and stopped. Outside water was removed and kept in an oven to dry the outside moisture

completely. The weight of the pycnometer with water was taken and mass of water determined by subtracting the empty weight of the pycnometer. The similar procedure was repeated for determining the mass of the orange juice. Finally, density was determined by using the following formula.

$$\text{Density (g/mL)} = \frac{\text{mass of orange juice } m_o}{\text{mass of water } m_h} \times \text{density of water at } 20°C \qquad (2)$$

3.2.4.7 Glass transition temperature and specific heat capacity

The glass transition temperature, Tg, of the orange juice was determined by differential scanning calorimetry (DSC) (DSC-60, Shimadzu Corporation, Japan) according to Shrestha et al. (2007b); Tonon et al. (2009a) and Zainal et al. (2000) with some modifications. Orange juice (5 mg) was scanned in a hermetically sealed DSC aluminum pan of 20 µL. The equipment was calibrated with indium ($T_{melting}$ = 156.6°C). Samples were heated at 10°C/min from -150 to 200 °C. Preliminary runs with 2 and 5 °C/min heating rates gave virtually the same results, so the 10 °C/min was chosen for the convenience. An empty pan was used as a reference, liquid nitrogen was used for sample cooling before the runs. The midpoint of the glass transition was considered as the characteristic temperature of the transition (Goula et al., 2008; Goula and Adamopoulos, 2010). All measurements were done in triplicate.

For determination of specific heat capacity, 2.5 mg water used as external standard and 3 mg of orange juice was taken in an aluminum pan and heat scan was carried out from 20 to 100 °C with a heating rate of 10 °C/min. Specific heat capacity was calculated using the following equation.

$$C_{PS} = W_w D_s C_{PW} / W_S D_W \qquad (3)$$

Where C_P, W, and D are the specific heat capacity, weight, and recorded displacement or the heat flow for the sample (S) and water (W) respectively.

Figure 3-3. Differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Japan)

3.2.4.8   Thermal conductivity

Thermal conductivity (k) was determined by oven drying method and it was calculated by the following formula described by Sweat (1986).

$$k = 0.14 + 0.42 \text{ w J/m} \cdot \text{s} \cdot ℃ \qquad (4)$$

Where w is the moisture content of the samples.

3.2.4.9   Thermal diffusivity

The thermal diffusivity of orange juice was determined based on the three thermos-physical properties and calculation was carried out from Laplace equation of conduction.

$$α = k/ρC_p \qquad (5)$$

Where, $α$ = thermal diffusivity ($×10^{-7} \text{m}^2/\text{s}$), k is the thermal conductivity (J/m.s. ℃), $ρ$ = density and C_p is the specific heat (kJ/kg℃) of orange juice.

3.2.4.10 Particle size and size distribution

Particle size and size distributions of orange juice were determined by a laser diffraction particle size analyzer (SALD-2200, Shimadzu Corporation, Japan) according to Koyama and Kitamura (2014) when using the wet measurement modes. Operating temperature and the humidities were

25℃ and 60% respectively, and particles size was taken as D50 and D75. D50 is defined as the median diameter; D75 is the particle size corresponding to 75% of the particles being under size by mass. The particle size distribution profile was constructed by the particle size and frequency of the distribution.

Figure 3-4. Laser diffraction particle size analyzer (SALD-2200, Shimadzu Corporation, Japan)

3.2.4.11 Morphology of the particles

Particle morphology of orange juice was evaluated by Field Emission Scanning Electron Microscope FE-SEM (JSM-6330F, JEOL, Tokyo, Japan). For juice sample, a small drop of sample was put on a glass slide and dried in an oven under vacuum at 40℃ for 24 h to ensure the complete dry. The sample containing glass slide was then put into the stub with a binding material and dried again at 40℃ for 24 h. The both samples were coated with a platinum-palladium in a sputter-coating unit (E-1045, Hitachi, Tokyo, Japan) under vacuum. The observations were done at 5 kV and 4000-fold magnifications.

Figure 3-5. Scanning Electron Microscope FE-SEM (JSM-6330F, JEOL, Tokyo, Japan)

3.2.4.12 Color characteristics of orange juice

The color parameters of the orange juice were determined by a Colorimeter (CR-200, Minolta Co., Japan). The results were expressed by the Hunter color values, L, a and b where large and small L denote lightness and darkness, +a and –a denote redness and greenness, and +b and –b denote yellowness and blueness. The color intensity was expressed as the Chroma value, calculated by the formula as Chroma = $(a^2+b^2)^{1/2}$, and the H^o hue angle, calculated by $H^o = \tan^{-1}(b/a)$. The pure colors of red, yellow, green, and blue are denoted by hue angle values as 0°, 90°, 180° and 270°, respectively (Quek et al., 2007; Santhalakshmy et al., 2015).

Figure 3-6. Colorimeter (CR-200, Minolta Co., Japan)

3.2.5 Antioxidant properties of orange juice

3.2.5.1 Determination of ascorbic acid

Preparation of sample extract: 5-10 g of orange juice was homogenized with 60-80 mL of 3% HPO_3 (PubChem CID: 3084658) for 1 min. The obtained extract was filtered through Whatman No.542 paper into a 100 mL volumetric flask, and 3% HPO_3 was added until the total volume equaled 100 mL. Prior to HPLC, the sample was filtered through a 0.25 μm membrane filter. All measurements were run in triplicates.

Procedure for determination of ascorbic acid: Total ascorbic acid was determined by liquid chromatography according to Romero Rodriguez et al. (1992). The analysis was carried out by HPLC (LC 20AD Shimadzu Corporation, Japan) with UV detection at 254 nm and a Shim-Pack-ODS (75 x 4.6 mm) analytical column. A mobile phase of methanol/water (5:95, v/v) and pH = 3 (adjusted with H_3PO_4) was used. The flow rate was 0.75 mL/min and the injection volume was 20 μL.

A standard solution of L-ascorbic acid (Wako, Japan) in 3% HPO_3 was used at concentrations of 10, 30, 60, 90, 120, and 150 mg/100 mL.
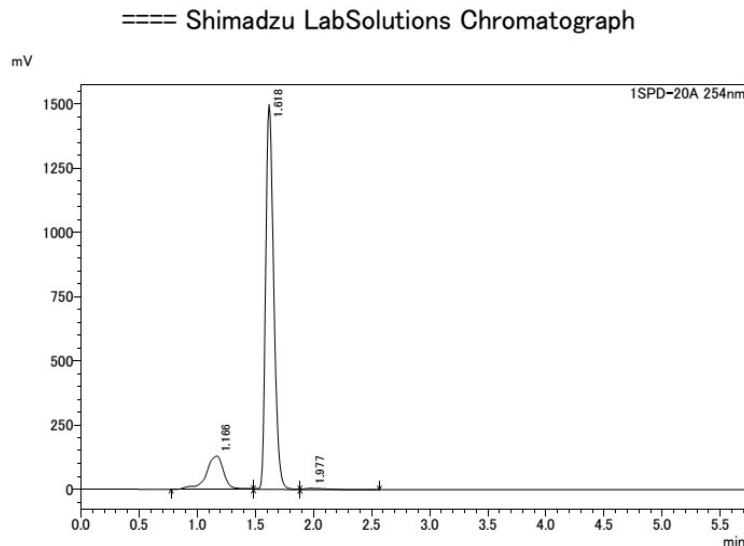
Figure 3-7. Standard curve for ascorbic acid

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3.2.5.2 Determination of total polyphenol content (TPC)

Preparation of sample extract

The orange juice samples were extracted based on the method explained by Asami et al. (2003) with slight modifications. The samples were extracted with 80% methanol with a ratio of 1:10 (sample: solvent), vortexed for 1 min and kept in an ultrasonic bath for 15 min. Then crude extracts were centrifuged (HSIANGTAI CN-1050) at 3000 rpm for 15 min at 20℃. The supernatant was then filtered through a Whatman #1 filter and the extracts were stored at -20℃ for further analysis.

Procedure

TPC was determined according to the Folin–Ciocalteu Spectrophotometric method (Rapisarda et al., 1999). Absorbance was measured at 765 nm in a UV-Vis spectrophotometer (HITACHI U-1900, Japan). TPC was expressed as Gallic acid equivalents (mgGAE/100 mL of samples). Gallic acid (Wako- 071-06095) standard solutions were prepared at a concentration ranging from 0 to 1000 mg/L.
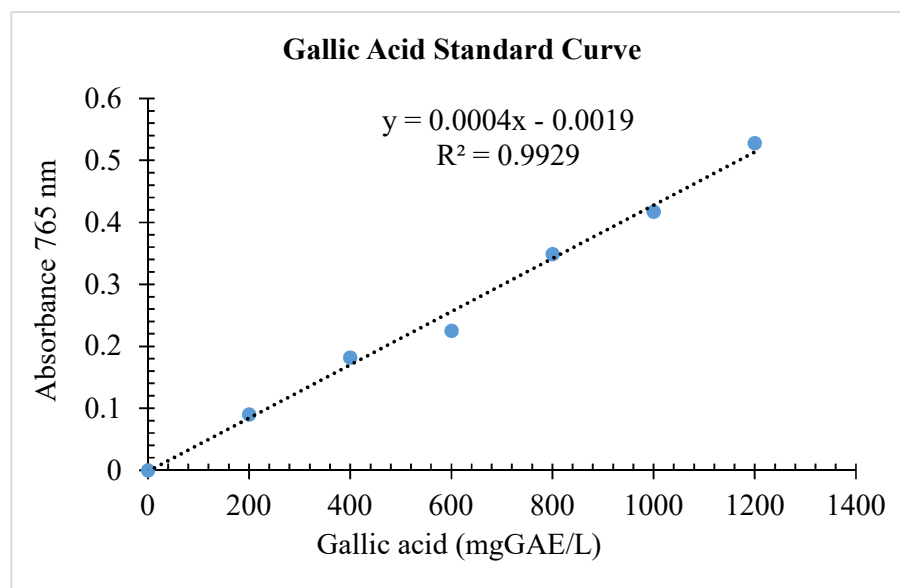
Figure 3-8. Gallic acid standard curve for TPC

3.2.5.3 Determination of Total Flavonoid content (TFC)

Preparation of sample extract

The orange juice samples were extracted based on the method explained by Asami et al. (2003) with slight modifications. The samples were extracted with 80% methanol with a ratio of 1:10 (sample: solvent), vortexed for 1 min and kept in an ultrasonic bath for 15 min. Then crude extracts were centrifuged (HSIANGTAI CN-1050) at 3000 rpm for 15 min at 20℃. The supernatant was then filtered through a Whatman #1 filter and the extracts were stored at -20℃ for further analysis.

Procedure

TFC was evaluated by the aluminum trichloride method using a spectrophotometer (HITACHI U-1900, Japan) as described by Saikia et al. (2014). Briefly, 0.5 mL of orange juice extract was taken into 10 mL glass tube, then 1.5 mL of 95% ethanol, 0.1 mL of aluminum trichloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of deionized water were mixed properly. The sample was then kept in an incubator for 40 min, after incubation, the reaction mixture absorbance was measured at 415 nm against deionized water blank in a spectrophotometer (HITACHI U-1900, Japan). The results were expressed as quercetin equivalent (mgQE/100 mL) of the sample.

Standard Quercetin curve

$y = 0.0007x - 0.0028$
$R^2 = 0.9927$

Absorbance at 415 nm
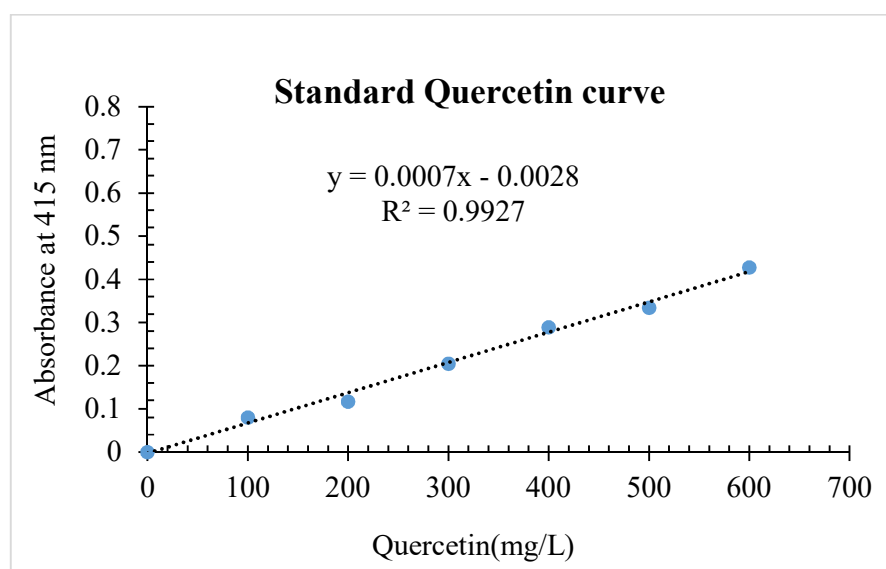
Quercetin(mg/L)

Figure 3-9. Quercetin standard curve for TFC

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3.2.6 Determination of antioxidant activity

3.2.6.1 DPPH radical scavenging activity

Preparation of sample extract: The orange juice samples were extracted based on the method explained by Asami et al. (2003) with slight modifications. The samples were extracted with 80% methanol with a ratio of 1:10 (sample: solvent), vortexed for 1 min and kept in an ultrasonic bath for 15 min. Then crude extracts were centrifuged (HSIANGTAI CN-1050) at 3000 rpm for 15 min at 20℃. The supernatant was then filtered through a Whatman #1 filter and the extracts were stored at -20℃ for further analysis.

Antioxidant activity was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method described by Shimada et al. (1992). Briefly, 0.5 mL of extract and ascorbic acid was taken at different concentration (25, 50, 75, 100 and 150 mg/mL), then mixed with 2.9 mL of 100 μM of DPPH (dissolved in 80% ethanol). The mixture was vigorously shaken and kept at in a dark place at room temperature for 30 min. The 80% ethanol was used as blank. The absorbance was taken at 515 nm by using UV-vis spectrophotometer (HITACHI U-1900, Japan). The scavenging effects was calculated by using the following formula

$$\text{Radical scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \qquad (6)$$

Where A_0 and A_s correspond to the absorbance of control blank and sample, respectively.

3.2.6.2 Ferric reducing powder (FRAP) assay

Preparation of sample extract: The orange juice samples were extracted based on the method explained by Asami et al. (2003) with slight modifications. The samples were extracted with 80% methanol with a ratio of 1:10 (sample: solvent), vortexed for 1 min and kept in an ultrasonic bath for 15 min. Then crude extracts were centrifuged (HSIANGTAI CN-1050) at 3000 rpm for 15 min at 20℃. The supernatant was then filtered through a Whatman #1 filter and the extracts were stored at -20℃ for further analysis.

The ferric reducing antioxidant activity of the OJ sample was measured by the method described by Benzie and Strain (1996). Briefly, 1.8 mL FRAP reagent was taken in a test tube and incubated in a water bath for 10 min at 30 ℃. Then the absorbance was taken at 593 nm in a UV-Vis spectrophotometer (HITACHI U-1900, Japan) and its recorded as 0 min absorbance. Next, 100 μL of extract sample or standard solution and 100 μL of distilled water was added to the test tube and mixed vigorously, then incubated the test tube at 30 ℃ for 4 min. After incubation, absorbance of the sample mixture was recorded. The standard curve was made from ferrous salt and concentration ranged from 50 – 200 μM. The antioxidant potential of the orange juice was expressed as μM Fe^{2+} equivalent per 100 mL of juice. FRAP value was calculated from the following equation.

$$\text{FRAP value} = \text{Absorbance}_{(sample+FRAP\ reagent)} - \text{Absorbance}_{(FRAP\ reagent)} \qquad (7)$$

3.2.7 Statistical analysis

The analysis of variance was carried out by the statistical software Origin pro 8.5. The mean significant differences ($p \leq 0.05$) were determined by Duncan's test. The mean difference of two samples was evaluated by student t-test. All data were presented mean ± S.D of three independent determinations.

3.3 Results and discussion

3.3.1 Effect of milling conditions on the particle size

Micro wet milling was performed to produce OJ with minimum particle size. Before MWM, fresh orange was blended for a certain period to achieve a minimum particle size of the juice. Figure 3-10 shows the effect of blending time on the particle size of the orange juice with pulp. The particle size of the juice was measured from one to ten minutes and it shows that with the increasing the blending time, particle size was decreased. After blending six minutes it showed the constant median particle size of 139.45 µm. Furthermore, the blending time was also increased to 10 minutes but the median particle size was not changed. Finally, the results showed non-significant values of particle size as (139.45 µm $\pm$ 1.5) during six minutes to ten minutes blending. In the present study, the blending of OJ with pulp was carried 6 minutes to get minimum particle size and to reduce the time and electrical energy consumption.
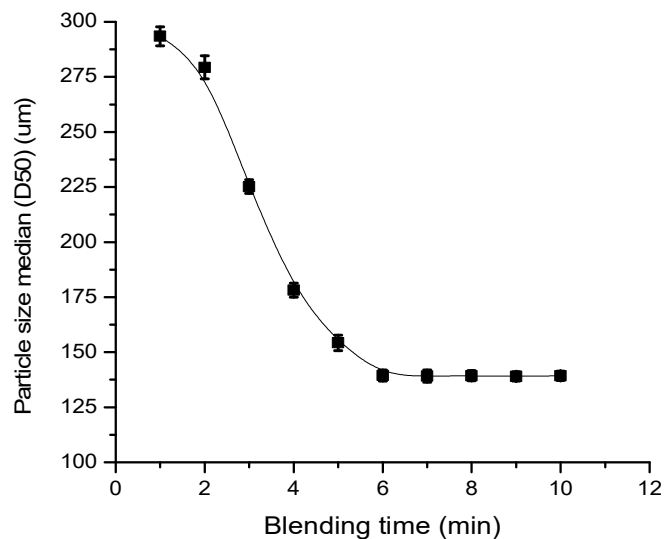
Figure 3-10. Effects of blending time on particle size of orange juice with pulp

After blending six minutes, the sample was micro wet milled at various milling conditions. The suitable milling conditions were also determined based on the minimum particle size of the orange juice. Figure 3-11, shows that the effect of different milling conditions on the particle size (median diameter as μm) of the orange juice with pulp. The particles diameter was measured with respect to the different feeding rate and different milling rotation. The milling rotation had greatly influenced the particle size. Its shows that with increasing milling rotation from 20 to 50 rpm, the particle sizes were decreased from 137μm to 45 μm for 5 mL/min feeding rate. The particle sizes were also varied with the different feeding rate and milling rotation as shown in Figure 3-11. When the milling rotational speed is increased, then the milling time of the sample is shortened (Larrauri, 1999). But according to Koyama and Kitamura (2014), suggested that shear stress had more significant effects on wet milling than the milling time. Based on the fluid classifications, orange juice with pulp is a Non-Newtonian and Bingham plastic fluid. The relationship between viscosity and shearing stress can be explained by the following equations.

$$\tau = \tau_o + \eta \frac{\partial u}{\partial y} \qquad\qquad (8)$$

Where τ = the shearing stress (N/m^2), τ_o is the yield strength (N/m^2), η is the Non-Newtonian viscosity (Pa·s), u is the share rate (m/s) and y is the distance (m). Considering the above equations for orange juice during wet milling, if the share rate u replaced by the milling speed (rpm) and the distance y by the gap size of the upper and lower stone mill, it is suggested that increasing milling speed and decreasing gap size produced higher shearing stress, as a result minimum particle size will be achieved. Our data was in accordance with this assumption.

The suitable milling conditions were also determined by varying the feeding rate from 5 mL/min to 20 mL/min. When the feeding rate above 20 mL/min overflow occurred during wet milling of orange juice at a constant gap size 60 μm. The results showed the linear relationship between the particle sizes and feeding rate and it was demonstrated that with decreasing the feeding rate there was decreased in particle sizes of the wet milled orange juice. According to Koyama and

Kitamura (2014), increasing the feeding rate that influenced to discharge the particle quickly from milling stone, hence produced a larger particle size of rice slurry. The present study showed the similar trend and the particle size of 20 mL/min feeding rate as 81.14 µm of mean diameter. On the other hand, although the lower feeding of 5 mL/min can produce a smaller particle size of 43.17 µm median but the output was very little and it's time-consuming and high energy consumption compared to the other feeding rate. In case of 10 mL/min and 15 mL/min feeding rate, the particle sizes were not significantly differed and the particle sizes showed 55±1.5 µm as mean diameter. So that for the effective milling, feeding rate 15 mL/min and wet milling speed 50 rpm and 60 µm gap size can produce minimum particle sizes orange juice with pulp.
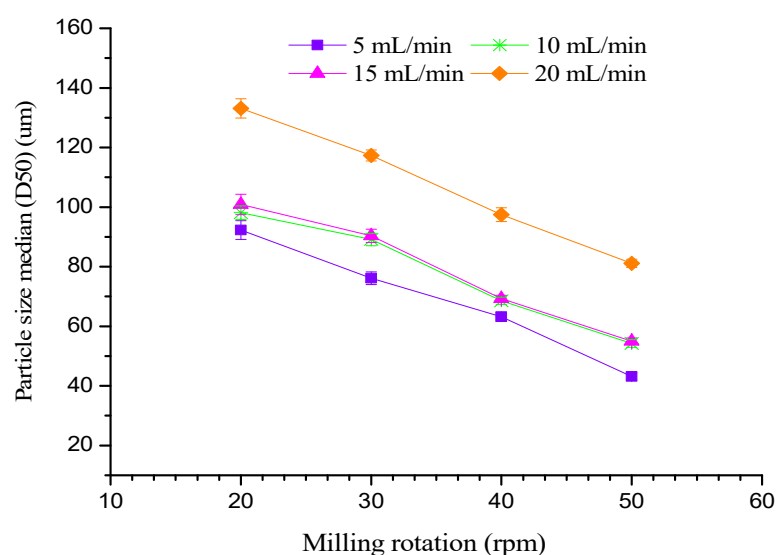
Figure 3-11. Effect of milling conditions on the particle size of the orange juice with pulp

3.3.2 Effects of MWM on the physicochemical properties of OJ

3.3.2.1 Physicochemical properties of OJ

Physicochemical properties are important due to their potential impact on sensory evaluation and further processing. The physicochemical properties of micro wet milled OJ are presented in Table 3-1. The MWM OJ physicochemical properties were also compared with commercial orange juice, data shown in Table 3-1. The water content in a sample is very important because higher water content allows to grow organisms and decrease the shelf life of the product. MWM OJ contained water content as 79.95 % whereas commercial OJ contained 87 %. MWM contained higher total solid % due to the presence of pulp and it also influenced to decrease the water content. In the previous study, Kelebek et al. (2009) reported the Brix 11.6 % of the Kozan orange juice. TSS of MWM OJ was higher than values also reported by Stinco et al. (2012), who found 11-13% Brix of Valencia OJ produced by mechanical extraction. The pH and acidity values of the two samples were more or less similar, and also similar to values reported by Stinco et al. (2012). The pH content of MWM OJ was a little bit higher than the commercial orange juice but significant difference was not found. Similarly, acidity was not significantly differed, although commercial orange juice had the higher value. This may be due to the presence of higher pulp in MWM OJ.

Table 3-1. Physicochemical properties of orange juice

Parameters	MWM Orange Juice	Commercial orange juice
Moisture (%)	79.95± 1.05	87±1.51*
Acidity (Citric acid g/100 mL)	0.71±0.01	0.77±.02
Brix (%)	20.05± 0.51*	13±0.11
pH	3.68±.03	3.65±0.13
Viscosity (mPa· s)	6.25± 0.015*	3.10± 0.015
Density (g/mL)	1.05± 0.10	1.02± 0.51

The values are mean ± S.D of three independent determinations. * $p \leq 0.05$; significantly differed by student t-test.

The density of a liquid can be defined as mass per unit volume. Density values of liquid can play an important role in heat transfer and mass transfer mechanism. The flow properties and consistency of OJ influenced by the density values. The nature of the solvents, solute (presence of sugar, total solids, and other macromolecules) and their interaction with water affects the density of the liquid sample (Manjunatha et al., 2012). The density of MWM OJ was increased (1.05 g/mL > 1.02 g/mL) as compared to the commercial orange juice. Manjunatha et al. (2012) reported that the density of enzyme clarified lime juice was decreased with increasing of water content. The present study showed the similar phenomenon, due to the presence of higher juice solids and lower water content, MWM OJ showed the higher density. MWM OJ density value was lower than the values reported by Ramos and Ibarz (1998) for concentrated juice (20% TSS) of 1.08 g/mL. This behavior may be due to the presence of small particle sizes of the MWM had a great interaction between water. The interaction between water and solute influenced the density parameter (Manjunatha et al., 2012).

The viscosity of a liquid sample can be defined as the interaction of a liquid or its tendency to resist flow. The viscosity content of MWM OJ was 6.25 mPas whereas commercial OJ contained 3.10 mPas. The viscosity of MWM OJ was significantly higher than the commercial OJ and its was near to double. The magnitude of the viscosity of MWM OJ increases due to the decreases in the water content of the juice. The viscosity of OJ depended on the interaction between the soluble solid to the solvent and inter-molecular forces, as the water content decreased, the viscosity was increased due to increases of hydrate molecules and bonding with solute molecules (Manjunatha et al., 2012). On the other hand, the increases in moisture content lead to decreases the intermolecular forces, hydration state and solute aggregation mechanism. The present study commercial OJ showed the similar behavior to the presence of higher water content in the juice.

3.3.2.2 Thermo-physical properties of orange juice

The glass transition temperature of a liquid sample is the transition from liquid stage to the solid or rubbery stage and it is a very important parameter for characterizing and designing the effective processing system. The higher the glass transition temperature better the structure relaxation. The glass transition temperature greatly influenced by the water content, higher the water content had greater plasticizing effects and finally lowering the glass transition temperature (Kurozawa et al., 2012). The MWM juice showed a higher T_g value than the commercial OJ, which may be due to the higher pulp and lower water content of the juice as shown in Table 3-2.

The specific heat of a liquid sample plays a vital role in heat transfer behavior. It is necessary to know the specific heat capacity of food materials to determine the amount of heat energy required to increase the temperature at unit mass by unit degree at a given temperature during heating or cooling process. The water and solid contents of food affect the specific heat capacity. The MWM OJ had the lower specific heat capacity (3.461±0.31 kJ/kg℃) than the commercial orange juice (3.638±0.03 kJ/kg℃). According to Manjunatha et al. (2012) and Zainal et al. (2000), who were reported that increases of moisture content and decreases of solids lead to increases of specific heat capacity of lime and guava juice respectively, this was due to the magnitude of specific heat of water was high as compared to the fruit juice.

Thermal conductivity and thermal diffusivity of MWM OJ was slightly decreased than the commercial orange juice, due to the lower water content of the MWM orange juice as shown in Table 3-2. It is well known that water is a good heat conductor due to the higher heat conductivity. Fruit juice showed poor conductors of heat, that why heat energy diffusion or transfer during freezing, drying or pasteurization are likely to be slow as compared to the water reported by O.J. Ikegwu and F.C. Ekwu (2009). Zainal et al. (2000) reported that thermal conductivity of guava

juice slightly decreased with increases of soluble solids. The present study showed similar behavior due to the presence of higher solid content in the juice. The thermal diffusivity of the liquid sample is related to the thermal conductivity of that sample, the movement or diffusion of heat energy from one point to another depends on the heat conductivity, higher conductivity showed higher heat diffusion. The thermal diffusion of MWM OJ showed lower than the commercial orange juice due to a higher soluble solids content in the juice.

Table 3-2. Thermo-physical properties of orange juice

Parameters	MWM orange juice	Commercial orange juice
Glass transition temperature (℃)	-21±1.03*	-42±1.03
Specific heat capacity (kJ/kg℃)	3.461±0.31	3.638±0.03
Thermal conductivity (J/m · s · ℃)	0.568±0.01	0.585±0.02
Thermal diffusivity ($\times 10^{-7}$ m^2/s)	1.563 ±0.12	1.578±0.01

The values are mean ± S.D of three independent determinations. * p≤ 0.05; significantly differed by student t-test.

3.3.2.3 Particle size and distributions, and SEM of MWM orange juice

The particle size is a key parameter that influences mouth feel, color and processing properties of fruit juices. The particle sizes of orange juice (D50 and D75) is shown in Figure 3-12. The particle sizes of MWM orange juice significantly lower than the mechanical extracted commercial orange juice. The MWM system produced concentrated OJ with particle sizes of 55±1.05 µm, whereas the commercial orange juice particles sizes of 115.39 µm. MWM OJ particle sizes were much lower than the results reported by Stinco et al. (2012), which were 406.62±46.06 µm in industrially extracted OJ. In case of D75 values, commercial orange juice also showed the higher particle sizes compared to the MWM orange juice. Figure 3-13, shows that the distributions of MWM orange juice and commercial orange juice.
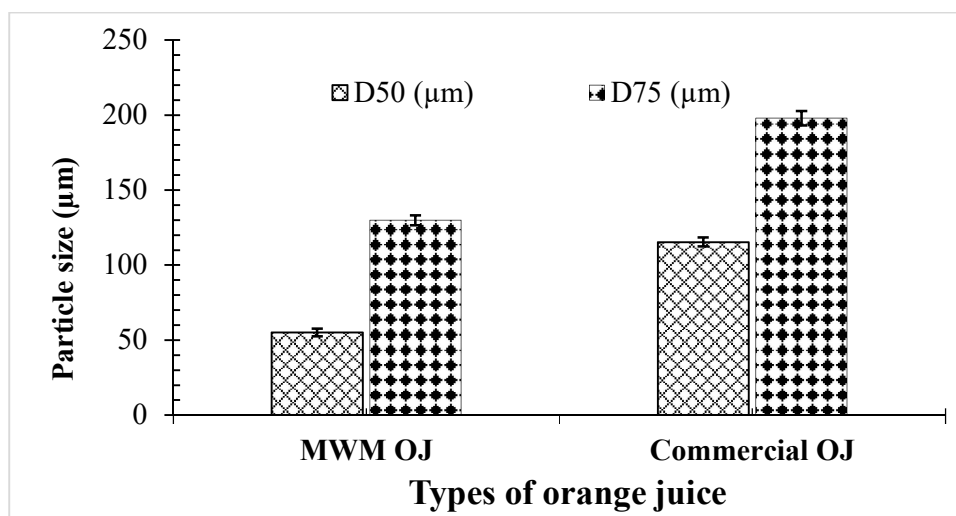
Figure 3-12. Particle sizes of orange juice expressed as D50 and D75 value

It's demonstrated that both distributions were unimodal and one distinct peak with frequency as 4.27 and 4.40 for MWM and commercial orange juice respectively. The curve of the commercial orange juice shifted to the right in a larger particle size region and represented the larger particle sizes.
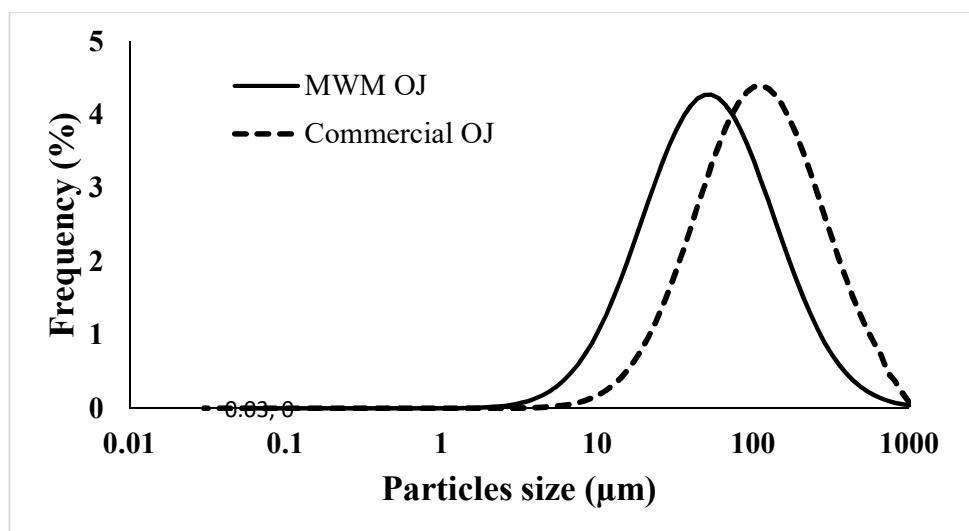
Figure 3-13. Particle size distributions of orange juice

Wet milling of orange juice particles was broken down due to the grinding of stone. Figure 3-14 shows that the SEM of the orange juice before and after micro wet milling. Before MWM, the blended orange juice contained large numbers particles with large and long fibrous material

but after micro wet milling the cells walls in the fiber were disrupted and no fibrous materials observed. The MWM has successfully disintegrated the cell wall of the fibrous materials of orange pulp and finally produced orange juice with minimum particle sizes.
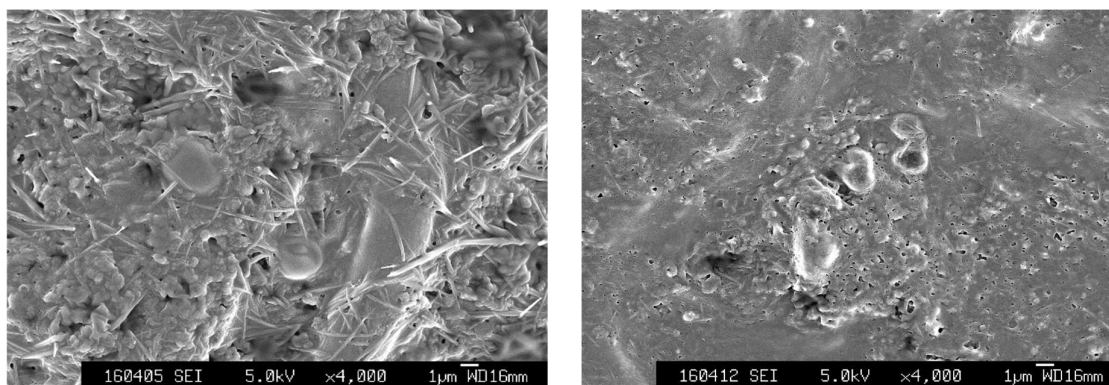
Figure 3-14. SEM micrograph of orange juice before and after micro wet milling

3.3.2.4 Color characteristics of orange juice

Table 3-3 shows the color parameters of orange juices treated my micro wet milling and industrial processed orange juice. The uses of a color parameter such as L, a, b chroma and H^o to classify the different orange juice by industrially processed has been reported by Arena et al. (2000) and Fernández-vázquez et al. (2010). Table 3-3, shows that the color attributes of the MWM OJ are higher in terms of qualitative (L, Chroma) and quantitative (Hue angle, H^o) parameters than commercial juice. The MWM OJ retained higher Chroma and hue values than industrially squeezed orange juice Stinco et al. (2012), who determined the color parameters of different processed OJ. This may be due to the higher pulp content and lower particle sizes of the OJ. Another reason explained by Arena et al. (2000) is that the color modifications of concentrated juices are affected by the changes of pulp structure by mechanical disruption. MWM reduced the particle size and increased the total surface area of the particles, making the juice brighter, yellowish and colorful.

Table 3-3. Color characteristics of orange juice

Color parameters	MWM orange juice	Commercial orange juice
L	76.73±1.32	74.65±1.31
a	12.76±1.04	15.00±1.04
b	76.23±1.21	69.78±1.12
H°	80.49±0.05*	77.86±0.03
Chroma	77.29±0.12*	71.37±0.02

The values are mean ± S.D of three independent determinations. * $p \leq 0.05$; significantly differed by student t-test.

The color parameter chroma indicated the color intensity combined with the four-basic color combination like red to green and yellow to blue of the juice. MWM orange juice had significantly higher chroma value of than the commercial orange juice. According to Escudero-gilete et al. (2012), the color intensity of orange juice increased due to the higher pulp and consequently lower particle sizes of the juice. The color properties highly depends on the extraction than the thermal or another process, the mechanical disrupted pulp containing orange juice showed better color property due to the higher extraction of carotenoid content of the juice, which responsible for the orange color of the juice (Escudero-gilete et al., 2012; Lemmens et al., 2010; Maas et al., 2011; Rajha et al., 2014).

3.3.3 Antioxidant properties of orange juice

3.3.3.1 Total polyphenol and total flavonoid

Citrus is the important sources of major groups of antioxidants such as phenolic, flavonoid, ascorbic acid and vitamin E. The phenolics presents in fruit and vegetables have received considerable attentions due to their antioxidant activity. The phenolic content as total polyphenol was determined by Folin-Ciocalteu reagent and expressed equivalent to gallic acid. The total polyphenol content of orange juice is shown in Table 3-4 and it's demonstrated that MWM

orange juice contained significantly higher TPC than commercial orange juice. The MWM OJ showed higher TPC than commercial OJ and to the findings reported by Stinco et al. (2012) and Vijaya Kumar Reddy et al. (2010) for industrially processed OJ. But the results were in accordance with the phenomenon explained by Stinco et al. (2012), who reported that the accessibility of functional compounds was increased due to the mechanical disruption of the cells and minimization of the particle size. In addition, Uichard (2004) found that an increase in pulp content increased the aroma compounds of OJ. Brat et al. (2003) and Guo et al. (2003), who were reported that the peel, seed, and pulp contained higher antioxidant potential than the single juice and pulp content in fruit juice increased the bioactive compounds.

Flavonoids are the most diverse and widespread natural phenolics. The major groups of flavonoid compounds in citrus juices are flavanones glycosides, narirutine, quercetin, hesperidin, naringin and neohesperidin. The flavonoids showed the free radical scavenging properties due their natural compounds and biological activities (Weng et al., 2010). The flavonoid in orange juice combined with aluminum to form a complex flavonoid-aluminium that could measure by UV-Vis- Spectrophotometer with 415 nm (Saikia et al., 2014). The values were expressed as total flavonoid content equivalent to equivalent mg quercetin/100 mL. Table 3-4 shows the total flavonoid contents of MWM OJ and commercial OJ TFC and results show that TFC content of commercial OJ was lower than the MWM OJ. Robards, Xia, Antolovich, & Boyd (1997) reported that the flavonoid contents in orange juices depend on the extraction method, thermal treatment, and storage and it was suggested that TFC contents of fresh squeezed orange juice much higher than the concentrated juice, processed by heat treatment. On the other hand, Escudero-gilete et al. (2012) found that mechanical disruptions can enhance the TFC content of orange juice. The present study showed the similar behavior, due to the disintegration of pulp fiber to minimum particles and as a result, the extracted bioactive compounds were increased. According to Loh et al. (2014) and Merisko-Liversidge, Liversidge, & Cooper (2003) found that

wet milling can able to minimize the particle sizes and enhanced the solubility of poorly soluble drugs in water. It was suggested that MWM decreased the particles and increase the solubility of the particles in the water and finally improved the extraction of bioactive compounds like TFC and TPC.

Table 3-4. Antioxidant properties of orange juice

Parameters	MWM Orange Juice	Commercial orange juice
Ascorbic acid (mg/100 mL)	63.45±2.01*	44.8±1.12
Total polyphenol (mgGAE/100 mL)	92.3±1.31*	62.45±1.12
Total flavonoid (mgQE/100 mL)	42.34±1.02*	15.76±1.05

The values are mean ± S.D of three independent determinations. * $p \leq 0.05$; significantly differed by student t-test.

3.3.3.2 Ascorbic acid

Ascorbic acid is the most important heat sensitive antioxidant in citrus fruit juices (Babashahi-Kouhanestani et al., 2014). Table 3-4 shows that ascorbic acid contents of MWM OJ higher amount than commercial juice because it contained more pulp, whose high flavonoids content reactivate and strengthen the ascorbic acid and bioactive compounds of fruit juice Guan et al. (2015). MWM also able to disrupt the cell wall of the fibrous pulp and minimizes the particle sizes and consequently increased the solubility of the pulp into the juice. Ascorbic acid is the water soluble vitamin, as a result MWM enhanced the ascorbic acid content of the juice and also minimize the loss. In the industrial processing, the pulp is separated and then juice goes to some thermal treatments, that destroy the major portions of ascorbic acid and other pigments (Berlinet et al., 2007; Gil-Izquierdo et al., 2002). Ascorbic acid is the natural antioxidants, which is easily degraded during heat treatment and if a compound contains higher ascorbic acid, its shows the higher antioxidant potentiality.

3.3.4 Antioxidant activity

3.3.4.1 DPPH inhibition scavenging activity

The antioxidant activity of MWM OJ was evaluated by DPPH scavenging activity. DPPH is a commercial oxidizing radical which can be reduced by samples antioxidants. During scavenging activity, DPPH reduced to pale yellow color from violet color because of the abstractions of a hydrogen atom from antioxidant compounds. Higher the DPPH reactions in the extract represent the most antioxidant potential. Similarly, a higher scavenging activity of samples means the higher reductions of DPPH (Blois, 1958). Our results demonstrated that DPPH scavenging activity of MWM OJ was higher (67.13%) than the commercial OJ (58.65%) as shown in Figure 3-15. This was because of the higher ascorbic acid and phenolic compound content of the MWM juice, which exhibited extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents (Rice-Evans and Burdon, 1993). MWM contained higher natural antioxidant (ascorbic acids) and total phenols than the commercial orange juice and as a result high phenolic contents could be the main reason for its high antioxidant activity.

3.3.4.2 Ferric reducing antioxidant power (FRAP) assay

There are many antioxidants compounds in fruits and it's difficult to measure separately. It is also noted that many methods used to measure the antioxidants namely DPPH, FRAP, ABTS and ORAC methods. We selected FRAP methods based on some reasons such as FRAP assay treats the samples antioxidants as reductants in a redox-linked colorimetric reaction (Guo et al., 2003), FRAP can easily standardize and procedure is relatively simple. The reducing potential of a samples antioxidants can be measured by FRAP assay, during measurements antioxidants reacts with ferric tripyridyltriazine complex and form ferrous with blue color. The higher FRAP values indicated that the higher antioxidants potential of samples, which can be obtained only when the higher absorbance of a sample. Our results showed that commercial orange juice had

lower FRAP value than the MWM OJ in Figure 3-15. Several authors reported that there is a close relationship between total phenolic compounds to the antioxidant activity, higher the phenolic compounds showed greater antioxidants activity (Benzie and Strain, 1996; Guo et al., 2003; Kalt et al., 1999). The another reason for higher antioxidants activity of MWM OJ, this could be the higher extraction of antioxidants from MWM OJ, due to the smaller particle, larger surface area and disrupted cells in the juice particle. Guo et al. (2003) and Huang, Xia, Hu, Lu, & Wang (2007) found that antioxidants potentials of fruit pulp much higher than the single juice. Similarly industrially processed juice had lower antioxidant compounds due to some thermal treatments than the fresh squeezed juice (Klimczak et al., 2007).
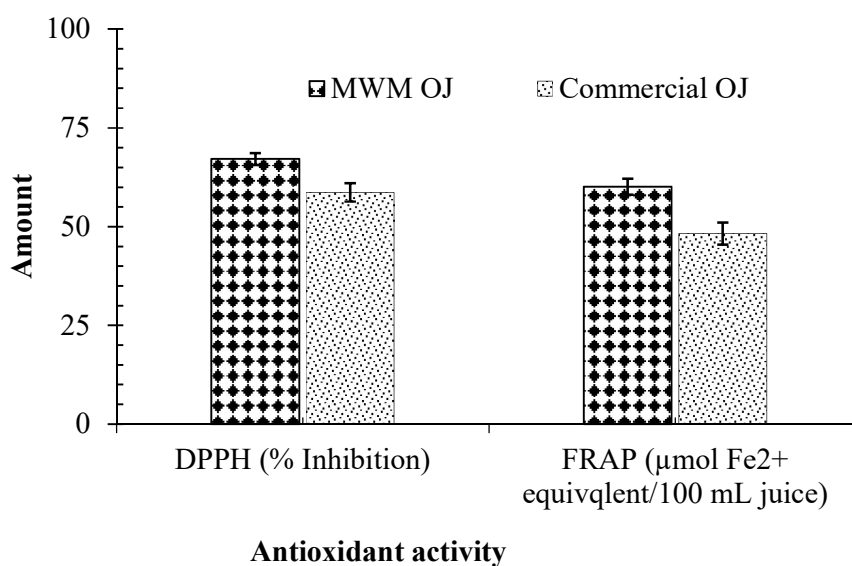
Figure 3-15. Antioxidant activity of orange juice

3.4 Conclusion

A new technique for producing concentrated orange juice with pulp by micro wet milling (MWM) was developed. The feeding rate and rotational speed significantly affect the particle sizes of the juice. The optimum feeding rate 15 mL/min and rotational speed 50 rpm can produce orange juice with minimum particle sizes and better color than the industrially extracted orange juice. The physiochemical properties and thermophysical properties of MWM OJ and commercial orange juice were determined and the properties were significantly varied due to the presences of higher pulp contents in the MWM OJ. In case of antioxidants, MWM OJ contained higher ascorbic acid, total polyphenol, and total flavonoid contents than the commercial OJ. The estimated DPPH scavenging activity and Ferric reducing antioxidant power (FRAP) assay of MWM OJ demonstrated higher value than the commercial orange juice. We concluded that MWM can be used to minimize the losses in fruit juice processing and can also able to provide fiber enriched fruits juice with higher nutrient values.

Chapter 4. Analysis of physicochemical and antioxidant properties of MWM orange juice powder by VSD and SD: A Comparative Study

The aim of this study was to produce concentrated micro wet milled (MWM) orange juice (OJ) powders by the application vacuum spray drying (VSD) and spray drying (SD)process. VSD process was conducted at low temperature (40-60℃) using superheated steam (200°C) as a heating medium whereas spray drying was conducted at 120 ℃ with hot air as a heating medium. In both cases, maltodextrin (10DE) used as a carrier. The effects of VSD and SD on physicochemical and antioxidant properties of MWM OJ powders produced with four different weight ratios of juice solids to maltodextrin solids; 60:40, 50:50, 40:60 and 30:70 were investigated. VSD can able to produce powders with the higher juice solid ratio (60:40) where SD was not successful. The cyclone recovery of SD at higher juice solid combinations was much lower than the VSD. The obtained powders were analyzed for moisture content, water activity, bulk density, tapped density, particle density, porosity, and particle size and distributions and microstructure of the particles. The quality in respect to the physical properties of VSD OJ powders was improved except color parameter with increases of maltodextrin solids. The VSD powders retained a higher amount of ascorbic acid than spray drying. VSD orange powder with overall good quality in terms of color, yield, ascorbic acid, TFC, TPC and antioxidant activity as compared with the SD powders.

4.1 Introduction

Spray drying is widely used for commercial production of fruit juice powders because fruit juice powders have many benefits and economic potentials over their liquid counterparts such as reduced volume or mass, reduced packaging, easier handling and transportation, and a much longer shelf life (Shrestha et al., 2007b). The largest disadvantage of spray drying is the deterioration of heat sensitive functional ingredients, color, and flavor during drying at a high temperature of 150-250℃. Goula and Adamopoulos (2006) studied that ascorbic acid loss in tomato pulps occurred in spray drying due to the thermal degradation and oxidation. On the other hand, freeze drying is suitable for maximum retention of bioactive compounds but takes longer time and higher cost than the spray drying. According to Kitamura and Yanase (2011), freeze dried products can be ten times more expensive than spray dried products.

The present study shows interest in the processing of OJ powder by the application of two new techniques, namely micro-wet milling (MWM) of fresh orange with pulp and vacuum spray drying (VSD) process. MWM produces juice with smaller particle size, which increased its nutritional content and makes the juice more concentrated and suitable for spray drying. Vacuum spray drying method is proposed as a new drying technique for producing OJ powder that will suppress the damage or loss of thermosensitive functional ingredients. The main feature of this method is the low-temperature (40-60℃) at which drying and powderization of liquefied foods take place, by the use of high superheated steam as a heat source. Vacuuming the drying tower of the VSD results in lower drying temperatures compared to other existing drying systems, and food powders that are rich in high heat sensitive ingredients are expected to be obtained continuously. The special features of VSD as compared with the existing spray drying system is the heating medium. VSD uses superheated steam instead of hot air. The benefits of superheated steam drying (SSD) instead of hot air drying are: superheated steam drying can lead to saving energy as high as 50 to 80% over the use of hot air (F., 2010). According to Alfy et al. (2016) the major advantages of superheated steam drying can produce a product with better quality in

terms of color, flavor, shrinkage and rehydration characteristics. SSD can also reduce the oxidation, deodorization of the product, and higher energy efficiency. It is also expected to produce orange juice powders at a lower glass transition temperature, as because of the lower drying temperature. To overcome the stickiness problems, maltodextrin with lower dextrose equivalent were used as a carrier agent, due to its higher solubility in water with lower viscosity, inexpensive, increase the glass transition temperature and it is also more efficient at protecting bioactive compounds from adverse conditions (Ferrari, Germer, & de Aguirre, 2012; Saénz et al., 2009; Lee, Wu, & Siow, 2013). Our previous study on VSD evaluated the water sorption and glass transitions behavior of commercial OJ powder (Islam et al., 2016). However, the effects of MWM and VSD on the physicochemical and antioxidant properties of OJ with pulp powder and comparison with the spray drying have not yet been studied.

The aims of this study were to produce micro wet milled orange juice powders the application of VSD and SD respectively with different solid ratios of maltodextrin (MD) as a carrier agent and to compare the physicochemical and antioxidant properties of OJ powders.

4.2 Materials and Methods

4.2.1 Materials

Micro wet milled orange juice with 20.05 % Brix and carrier agent MD 10DE (Showa Sangyo Co. Ltd., Japan) with a moisture content of 4.35±0.02% was used in the present study. All chemicals were analytical grade used from laboratory stocks. Samples were prepared with different proportions of OJ concentrate (based on total solids) to MD solids at 60:40, 50:50, 40:60, 30:70 (OJ: MD) by weight were chosen for VSD. A total of 1000 g of OJ and MD mixture were prepared with 30 Brix% solutions for VSD

4.2.2 Vacuum spray drying process

As discussed in chapter 2

4.2.3 Spray drying process

Spray drying process was performed in a laboratory scale spray dryer, spray dryer SD-100 (EYELA, Japan) with a 0.71 mm diameter two-way nozzle and main spray chamber of 300W × 300D × 350H mm, 30 L as shown in Figure 4-1. The mixture of OJ/MD was fed into the main drying chamber by a pump at a feed rate of 300 mL/h, spray air pressure of 30 kPa and heated air volumetric flow rate of 0.70 m^3/min. The inlet and outlet temperature was maintained at 120 ± 2.0 ℃ and 78±2.0 ℃ respectively.
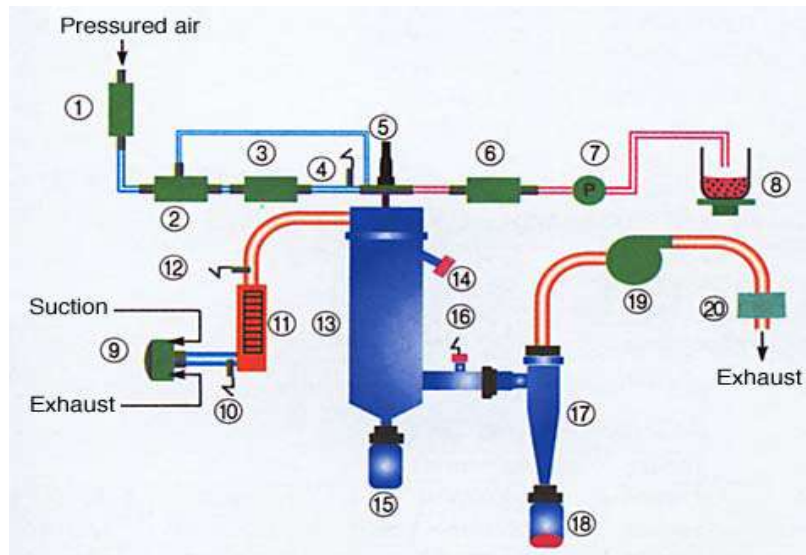
1. Spray airline valve
2. Magnetic valve
3. Regulator
4. Pressure sensor (spraying air)
5. Spray nozzle
6. Pressure sensor (feed)
7. Circulating Pump
8. Sample with stirrer
9. Suction filter
10. Sensor
11. Heater
12. Sensor (inlet temp.)
13. Evaporation tube
14. Cleaning port
15. Separator
16. Sensor (outlet temp.)
17. Cyclone
18. Recovery flask
19. Suction blower
20. Exhaust filter

Figure 4-1. Schematic diagram of Spray drying process

(Excerpt from http://www.eyelaworld.com/product_view.php?id=204&type=5, 2016)

| OJ/MD 60:40 | OJ/MD 50:50 | OJ/MD 40:60 | OJ/MD 30:70 |

Image 2. Vacuum Spray Dried Micro Wet Milled Orange Juice powders
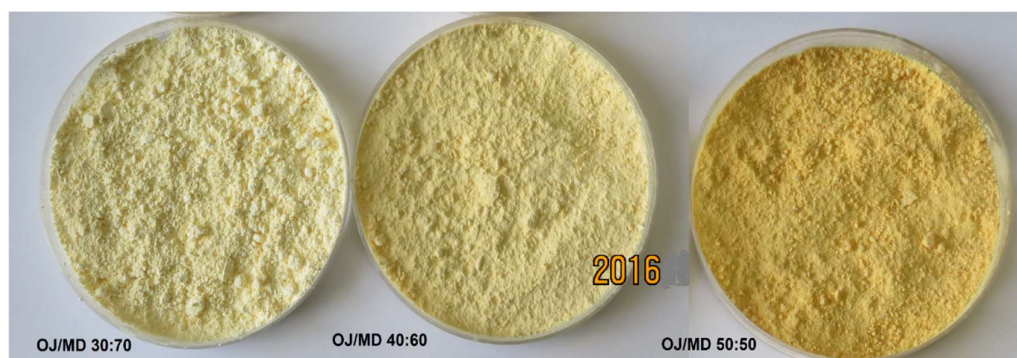
Image 3. Spray Dried Micro Wet Milled Orange Juice powders

4.2.4 Assessment of physicochemical properties

4.2.4.1 Moisture

The moisture content of the orange samples was determined according to Goula and

Adamopoulos (2010) by drying in an oven at 70℃ until constant mass was obtained.

4.2.4.2 Water activity

A water activity meter (Novasina Labmaster-a_w, Switzerland) was used to measure water

activity of the OJ powders.

4.2.4.3 Glass transition temperature

The glass transition temperature, Tg, of the orange juice and powders were determined by

differential scanning calorimetry (DSC) (DSC-60, Shimadzu Corporation, Japan) according to

Shrestha et al. (2007b) and Tonon et al. (2009a). As discussed in details in Chapter 2.

4.2.4.4 Determination of bulk tapped and particle density

The bulk density (ρ_B) of orange powders was measured by gently adding 2 g of powder in a 10

mL empty graduated cylinder. The volume was then recorded and used to calculate the bulk

density as g/mL.

The tapped density (ρ_T) was measured by tapping the cylinder for 2.5 min (60 taps per minute)

using powder characteristics measuring instruments (A.B.D-72, Tsutsui physics and, chemistry

instrument Co., Ltd. Japan). Then the final volume was recorded and tapped density was

expressed as g/mL.

Particle density was measured according to the method described by Jinapong et al. (2008). One

gram of powder was measured into a 10 mL measuring cylinder with a glass stopper. Then, 5

mL of petroleum ether was gently added, and the mixture was shaken for a few minutes until all

the powder particles were suspended. Finally, the wall of the cylinder was rinsed with 1 mL

petroleum ether and the total volume of petroleum ether and suspended particles were recorded.

Particle density was calculated as follows.

$$\text{Particle density} = \frac{\text{Mass of the powder (g)}}{\text{[Total volume of petroleum ether and suspended particles-6] (mL)}} \qquad (1)$$

4.2.4.5 Hausner ratio and Carr index

The cohesiveness of powders was determined in terms of Hausner ratio (HR) calculated from bulk density (ρ_B) and tapped density (ρ_T) (Jinapong et al., 2008).

$$HR = \rho_T / \rho_B \qquad (2)$$

The flowability of powders expressed in relation to the Carr index (CI) was calculated from Hausner ratio as shown below.

$$CI = 100 - \frac{100}{HR} \qquad (3)$$

CI (%)	Flowability		HR	Cohesiveness
<15	Very good		<1.2	Low
15-20	Good			
20-35	Fair		1.2-1.4	Intermediate
35-45	Bad			
>45	Very bad		>1.4	High

4.2.4.6 Porosity

Porosity (ε) was calculated using powders particle density (ρ_p) and tapped density (ρ_T) as follows (Jinapong et al., 2008). $\varepsilon = \frac{\rho_P - \rho_T}{\rho_P} \qquad (4)$

4.2.4.7 Hygroscopicity and degree of caking (CD)

For measurement of hygroscopicity, 1.5 g of the OJ powder was kept in a Conway unit (060310-02A, Shibata Co. Ltd., Japan) containing saturated salt solution of NaCl (75.29% RH). Samples were weighed after one week and hygroscopicity was expressed as the mass of the hygroscopic moisture per 100 g of dry solid (Islam et al., 2016).

The wet sample after measuring the hygroscopicity was dried in a vacuum oven at 70°C until constant mass obtained. Then the dried sample was cooled and transferred into a sieve of 500 μm.

The sieve was then shaken for 5 min in a shaking apparatus. The amount of the powder remaining in the sieve was measured. The degree of caking (CD) was calculated as (Goula and Adamopoulos, 2010).

$$CD = \frac{100 \cdot a}{b} \qquad (5)$$

Where a is the amount of powder remaining on the sieve after sieving and b is the initial amount of powder.

4.2.4.8 Water solubility index (WSI)

The WSI of VSD orange powders were evaluated according to the method described by Anderson et. al. (1969). The WSI was determined based on the percentage of dried supernatant with respect to the original amount of OJ powder.

4.2.4.9 Particle size and distribution

Particle size and size distributions of MWM OJ and powders were determined by a laser diffraction particle size analyzer (SALD-2200, Shimadzu Corporation, Japan). The methodology was discussed in chapter 2.

4.2.4.10 Morphology of the particles

Particle morphology of OJ and powders was evaluated by field emission scanning electron microscopy (FE-SEM) (JSM-6330F, JEOL, Tokyo, Japan). For powder, the samples were mounted onto stubs with double adhesive tape, and dried in an oven under vacuum at 40℃. The samples were coated with a platinum-palladium in a sputter-coating unit (E-1045, Hitachi, Tokyo, Japan) under vacuum. The observations were done at 5 kV and 1800 fold magnifications.

4.2.4.11 Color parameters

The color parameters of the OJ and powders were determined by a Colorimeter (CR-200, Minolta Co., Japan). Procedure for the measurement was discussed in chapter 3.

4.2.5 Assessment of antioxidant properties of orange juice powders

Sample extraction: Briefly discussed in chapter 3.

4.2.5.1   Ascorbic acid

Ascorbic acid of VSD and SD orange juice powders were determined by liquid chromatography (HPLC AD 20, Shimadzu, Japan). The method was discussed in details in chapter 3.

4.2.5.2   Total polyphenol

TPC was determined according to the Folin–Ciocalteu Spectrophotometric method (Rapisarda et al., 1999). Absorbance was measured at 765 nm in a UV-Vis spectrophotometer (HITACHI U-1900, Japan). The measurement procedure was discussed in chapter 3.

4.2.5.3   Total Flavonoid content

TFC was evaluated by the aluminum trichloride method using a spectrophotometer (HITACHI U-1900, Japan) as described by Saikia et al. (2014). The methodology was briefly explained in chapter 3.

4.2.6 Assessment of antioxidant activity of orange juice powders

4.2.6.1   DPPH radical scavenging activity

Antioxidant activity was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method described by Brand-Williams et al. (1995) with slight modifications. Briefly, the sample extraction was done using an acetone solution (70%). About 0.25 g of orange powder

and 25 mL of acetone vortexed for 1 min and kept in an ultrasonic bath for 15 min. Then crude extracts were centrifuged (HSIANGTAI CN-1050) at 3000 rpm for 15 min at 20℃. The supernatant was then filtered through a Whatman #1 filter and the extracts were stored at -20℃ for further analysis.

Precisely, 100 μL of extract was added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). After 30 min, the absorbance at 515 nm was measured against a blank (100μL methanol in 1.4 mL of DPPH radical solution) using a UV-Vis spectrophotometer (HITACHI U-1900, Japan). For the construction of a standard curve, the same procedure was performed for Trolox methanolic solutions and values were expressed as Trolox equivalents mg TE/g of dry solids (excluding the carrier agent).

4.2.6.2   Ferric reducing Power (FRAP) assay

The ferric reducing antioxidant activity of the OJ powders was measured by the method described by Benzie and Strain (1996). The measurement method was discussed in details in chapter 3.

4.3 Results and discussions

4.3.1 Physical properties of MWM orange juice powders

4.3.1.1 Moisture content and water activity

Moisture content is a key factor related to the drying performance of the fruit juice powders. It has a large impact on the quality parameters of the powders such as flowability, caking, stickiness and storage stability. The moisture content of VSD & SD OJ powders varied from 3.06 to 4.18 % and 4.24 to 4.45% respectively as shown in Table 4-1, and decreased with the increase of MD solids. SD OJ/MD 60:40 was not successful due to the stickiness of the powders. A higher ratio of OJ lead to a higher sugar and acid content, and as a result, powders became more hygroscopic and absorbed more water (Shrestha et al., 2007b). The results showed a similar trend with our previous findings of VSD commercial OJ (Islam et al., 2016). However, this trend was in discordance with Goula and Adamopoulos (2010) for spray dried concentrated OJ powders with MD as a carrier. The moisture content of VSD was lower than the findings of spray dried concentrated OJ powders and also to the previous study conducted by Goula and Adamopoulos (2010) and Shrestha et al. (2007). This was due to the higher moisture removal during vacuum spray drying, and where a greater the heat gradient occurred in VSD between the superheated steam and the particles than hot air so that there was a higher heat transfer into the particles, which supplied the driving forces of the moisture removal. Alfy et al. (2016) reported that the higher enthalpy of the superheated steam can transfer heat very quickly from the product to be processed and the superheated steam that has additional sensible heat added so that its temperature is above the saturation temperature at a given pressure.

The availability of free water in food components is defined as water activity. Many biochemical and microbiological reactions occur due to higher water activity and hence shorten the shelf life of food materials. All the VSD OJ powders showed water activity below 0.28 whereas the SD powders contained higher water activity in the ranges from 0.28 to 0.38 as shown in Table 4-1. The water activity of MWM &VSD orange powders were much lower than those of the spray

dried OJ powders and also the water activity of concentrated orange juice powders ($a_w = 0.30$-0.40) reported by Shrestha et al. (2007).

Table 4-1. Moisture content and water activity of orange juice powders

Powder	Moisture content (%)		Water activity	
OJ/MD	VSD	SD	VSD	SD
60:40	4.18±0.03[a]	NA	0.27±0.01[a]	NA
50:50	3.73±0.02[b]	4.45±0.04[a]	0.23±0.01[b]	0.38±0.02[b]
40:60	3.45±0.02[c]	4.32±0.12[b]	0.22±0.01[bc]	0.30±0.02[c]
30:70	3.06±0.01[d]	4.25±0.01[c]	0.20±0.01[c]	0.28±0.02[c]

The values are mean ± S.D of three independent determinations. The means with different superscripts in a row differs significantly (p≤ 0.05).

4.3.1.2 Glass transition temperature and cyclone recovery

The glass transition temperature has a great impact on the stability of fruit juice powders and the surface stickiness of amorphous powders are closely related to the glass transition temperature (Abbas et al., 2010; Lloyd et al., 1996). The DSC profiles for glass transition temperature of VSD & SD orange powders are shown in Figure 4-2 and Figure 4-3 respectively. Low glass transition temperatures have adverse effects on powder stickiness, hygroscopicity, caking and free flowing properties. The Tg values (midpoint Tg) of the VSD OJ powders significantly (p≤ 0.05) varied between each other.
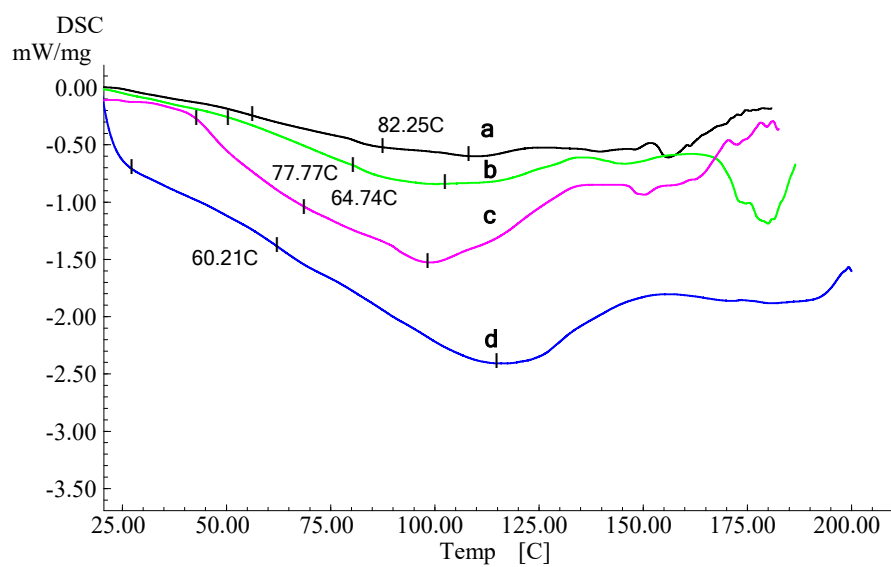
Figure 4-2. DSC thermograph of VSD & MWM orange juice powders
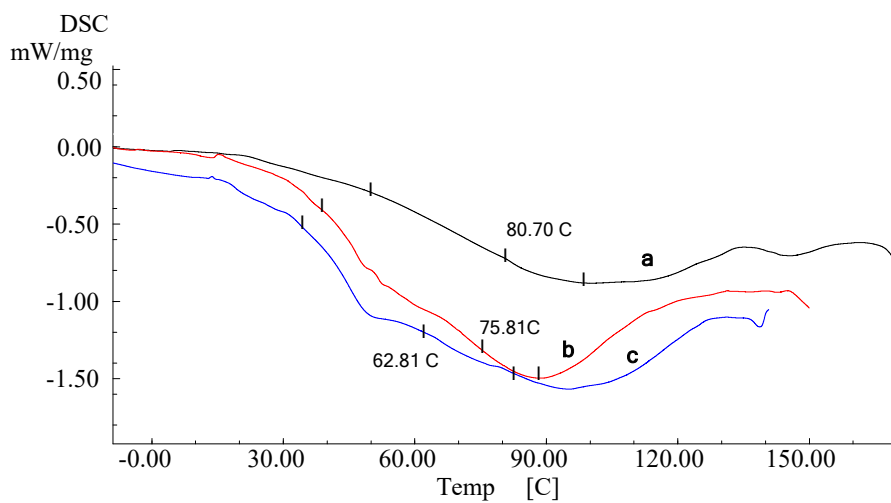
Figure 4-3. DSC thermograph of SD & MWM orange juice powders

The highest Tg was found in OJ/MD 30:70 powders in both VSD and SD powers as denoted 'a' in Figure 3 & 4. OJ/MD 50:50 (c) and OJ/MD 60:40 (d) for VSD showed the sharply decreased trend on the DSC diagram and represents the lower glass transition temperature. This was due to the presence of higher juice solids, water activity and also higher moisture contents of the powders that influenced to the decrease in glass transition temperature.

In this study, we investigated the effects of glass transition temperature and maltodextrin on the cyclone recovery of OJ powders as shown in Figure 4-4. In the case of SD, OJ/MD 60:40 was not successful due to the lower glass transition temperature. Most of the OJ/MD 60:40 powders were stuck on the wall and no powders came into the cyclone. Whereas in VSD operation, cyclone recovery of OJ/MD 60:40 was 58.76 %, and rest of the powders were recovered from inside of the dryer wall by manual sweeping (sweeping recovery 32.45%). For VSD, when maltodextrin solids were increased from 40 to 70%, the cyclone recoveries were increased from 58.78 to 68.78 % and the manual sweeping recovery decreases from 32.45 to 24.10 %. The similar trend was obtained by Shrestha et al. (2007) for spray dried concentrated orange juice powders, but the cyclone recoveries were a little bit higher than VSD. This may be due to the higher glass transition temperature produced by lower dextrose equivalent (DE6) of maltodextrin than the present study (DE 10). In the case of total recovery, there was no significant differences for OJ/MD 30:70 and 40:60. For SD of MWM orange juice powders OJ/MD 50:50, the product cyclone recovery was very lower (40.02 %) than the VSD recovery. This was because of the lower glass transition temperature with respect to the drying temperature of the hot air. According to Brostow et al. (2008); Feng et al. (2013) and Sablani et al. (2007), stickiness was produced due to lower glass transition temperature of the amorphous solids when drying associated with a higher temperature and consequently lower recovery of the product. In Figure 4, shows that the increases of OJ solids there was a decreased of glass transition temperature and product recovery as well. More interestingly it is also demonstrated that there were no significant differences of glass transition temperature of VSD and SD for maltodextrin 50% solids, but the

cyclone recovery significantly differed. This was due to the lower drying temperature (45℃ < 120℃) of VSD can able to produce MWM orange juice powders with higher juice solids combinations. It is also noted that Goula and Adamopoulos (2010) and Shrestha et al. (2007) were also failed to produce orange juice powders with 50% or more orange juice solid combinations of maltodextrin. This was due to the lower glass transition temperature of orange juice solids with respect to the drying temperature.
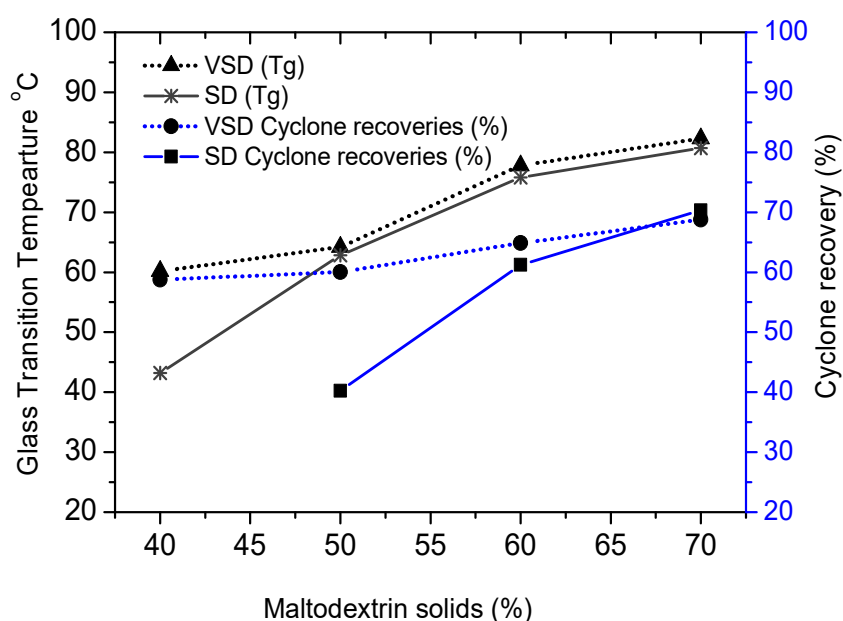
Figure 4-4. Cyclone recoveries and glass transition temperatures (Tg) of MWM orange juice powders with various combinations of maltodextrin solids.

4.3.1.3 Density, Hausner ratio, and Carr Index

The bulk density of the VSD OJ powders significantly ($p \leq 0.05$) varied from 0.04 g/mL to 0.58 g/mL (Table 4-2) with different OJ to MD ratios. The powders OJ/MD (60:40) showed the higher bulk density of the powders. The findings were in accordance with Chegini and Ghobadian (2005), who reported that powders with higher moisture content had a higher bulking mass due to the presence of water, which is considerably denser than dry solids. This behavior was

observed in our study because the orange powders associated with higher juice solids showed higher moisture content. Whereas Goula and Adamopoulos (2010) stated that the bulk density of OJ powders decreased with increased with maltodextrin concentration. The bulk density of VSD oranges powders was higher than the SD powders as shown in Table 2. This was due to the larger particle sizes of the SD powders had bigger volume occupied and consequently obtained smaller density. According to Santhalakshmy et al. (2015), lower bulk density implies larger volume of packaging and also the more occluded air within the powders and therefore, the greater possibility of oxidation and finally reduced the storage life of the powders.

The particle density is a well-defined quantity which represents the true density of the particles that make up the powder. The particle densities related to the porosity of the dried materials. VSD orange powders particle density were varied from 3.52 to 5.24 g/mL, whereas SD powders had the higher particle density value in the ranged from 5.47 to 6.75 g/mL. Both OJ/MD 30:70 had the lowest particle density and the powders were significantly different from each other (p≤ 0.05).

With respect to the tapped densities, the VSD OJ powders significantly varied from 0.48 g/mL to 0.71 g/ mL and SD powders were ranged from 0.41 to 0.61g/mL The VSD powders showed the higher tapped density than the SD powders because of the lower particle sizes and the intermolecular space was reduced after tapping. VSD and SD both OJ/MD 60:40 and 50:50 had the higher tapped density due to the higher moisture content that increased the bulking weight compared with the reduced volume after tapping. This study was in accordance with the findings of Abadio et al. (2004), who reported that the increase of encapsulation concentration resulted in the decreased of true density of pineapple juice powder.

Table 4-2. Types of density of VSD & SD orange juice powders

Types of powder (OJ/MD)	Bulk density (g/mL)		Particle density (g/mL)		Tapped density (g/mL)	
	VSD	SD	VSD	VSD	SD	VSD
60:40	0.54 ± 0.01^d	NA	5.24 ± 0.16^a	NA	0.71 ± 0.01^d	NA
50:50	0.49 ± 0.01^c	0.45 ± 0.04^a	4.84 ± 0.06^b	6.75 ± 0.05^a	0.64 ± 0.01^c	0.61 ± 0.07^a
40:60	0.46 ± 0.01^b	0.39 ± 0.02^a	4.19 ± 0.30^c	5.86 ± 0.03^b	0.57 ± 0.02^b	0.52 ± 0.03^b
30:70	0.40 ± 0.01^a	0.32 ± 0.01^b	3.52 ± 0.11^d	5.47 ± 0.02^c	0.48 ± 0.01^a	0.41 ± 0.08^b

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly ($p \leq 0.05$).

Quality parameters of powders such as the Carr Index and Hausner ratio were also determined. As mentioned in the methods section, a high Carr Index and Hausner ratio indicates poor flowability and high cohesiveness of the powders. The VSD OJ/MD (30:70) powder showed the lowest CI = 15.76% and also demonstrated lower Hausner ratio of 1.19 compared with other powders as shown in Table 4-3. The powders with OJ/MD 40:60 showed CI value of 18.70 which indicates the good flowability of the powders. The other powders showed fair flowability (CI = 22.50- 23.59%< 35) and intermediate cohesiveness in relation to the Hausner ratio = 1.23-1.30< 1.4 values. Powders with higher hygroscopicity showed higher HR and CI values. Finally, we can conclude that Hausner ratios of 1.2-1.3 were obtained which indicate a non-cohesive, free flowing powder, which was also observed when handling the powder. For SD & MWM orange juice powders, both Carr Index (flowability) and Hausner ratio (cohesiveness) were larger than the VSD powders as shown in Table 3. SD all powders showed the intermediate cohesiveness (HR > 1.2) and Carr index (20<CI<30) indicated that the fair flow property, whereas VSD powders OJ/MD 40:60 and 30:70 showed the better flow property and less cohesiveness due to the lower moisture content and water activity of the powders.

Table 4-3. Hausner ratio and Carr index of orange juice powders

Types of powder (OJ/MD)	Hausner ratio (Cohesiveness)		Carr Index (Flowability)	
	VSD	SD	VSD	SD
60:40	1.30 ± 0.01^a	NA	23.07 ± 0.32^a	NA
50:50	1.29 ± 0.02^a	1.35 ± 0.02^a	22.48 ± 1.31^a	26.22 ± 1.05^a
40:60	1.23 ± 0.02^b	1.33 ± 0.01^a	18.69 ± 1.21^b	25.00 ± 0.05^a
30:70	1.19 ± 0.01^b	1.28 ± 0.01^b	15.96 ± 1.15^c	21.95 ± 0.03^b

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly ($p \leq$ 0.05).

4.3.1.4 Porosity

The porosity of the dried powders plays an important role in dissolution by controlling the rehydration speed. The porosity of the VSD OJ powders was not significantly deferred from each other ($p \leq 0.05$) as shown in Table 4-4, but lower than the spray dried MWM orange juice powders. The VSD and SD OJ/MD powders had the porosity value ranged from 86.30 to 86.69%, and 90.96 to 92.50 % respectively. The larger porosity indicates that much space remained between the particles and the lower porosity values of powder, which has the least chance of degradation during storage among the other powders. Santhalakshmy et al. (2015) reported that spray dried Jumon powders with maltodextrin showed higher porosity values ranged from 90.77 to 92.60 % than VSD but similar with the SD powders. VSD MWM orange juice powders with smaller particle size reduced the intermolecular spaces and lead to a smaller porosity value.

Table 4-4. Porosity, Hygroscopicity, and Degree of caking of orange powders

Types of powder (OJ/MD)	Porosity (%)		Hygroscopicity (gH_2O/solid)		Degree of caking (%)	
	VSD	SD	VSD	SD	VSD	SD
60:40	86.34±0.45[a]	NA	0.071±0.02[a]	NA	35.2±1.23[a]	NA
50:50	86.69±0.35[a]	90.96±1.05[a]	0.067±0.02[ab]	0.068±0.01[a]	32.4±1.05[b]	35.6±0.15[a]
40:60	86.30±0.99[a]	91.12±0.51[a]	0.062±0.02[c]	0.064±0.03[b]	29.1±1.51[c]	30.6±1.31[b]
30:70	86.36±0.53[a]	92.50±0.71[a]	0.059±0.01[cd]	0.062±0.01[c]	27.2±1.31[c]	28.1±1.05[c]

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly (p≤ 0.05).

4.3.1.5 Hygroscopicity and degree of caking (CD)

Table 4-4, shows that the hygroscopicity of the VSD and SD OJ powders decreased with the increase of MD solids, and ranged from 0.059 to 0.071gH_2O/g of solid and 0.062 to 0.068 gH_2O/g respectively. Powders with low glass transition temperature and high moisture content were highly hygroscopic. These findings were in accordance with our previous study Islam et al. (2016), and with other findings of Cai and Corke (2000) and Rodríguez-Hernández et al. (2005). The most desirable CD values of food powders range between 5 and 34% (Jaya and Das, 2004). The CD values of the VSD OJ powders ranged from 27.21 to 35.21% and SD OJ powders varied from 28.1 to 35.6 %. The CD values of MWM orange juice powders were higher than those of spray dried OJ powders (5.9 to 24.9%) conducted by Goula and Adamopoulos (2010), due to the presence of higher pulp contents that imparted the stickiness of the powders.

4.3.1.6 Water solubility index (WSI)

The WSI indicated that how the powders rehydrated in water. Higher the WSI indicated the better solubility of the powders. Figure 4-5 shows the water solubility index of VSD and SD powders. The WSI of VSD OJ powders were not significantly different from each other as shown in Figure 4-5. WSI of powders increased with the increase of MD solid ratio, and the

VSD OJ/MD 30:70 showed the highest WSI (95.70 %) among the four powders. This may be attributed to the superior solubility properties of MD (Cano-Chauca et al., 2005; Goula and Adamopoulos, 2010). In the case of SD OJ powders, WSI was lower than the VSD powders, due to the larger particle and higher moisture content influenced to soluble in delay as compared to the dried powders. Papadakis, el al. (1998) reported that lower moisture content and smaller particle sizes of powders influenced to increases the solubility of the powders. The similar behavior was obtained by Saikia et al. (2014) for spray dried Khasi mandarin and other fruit juice powders.
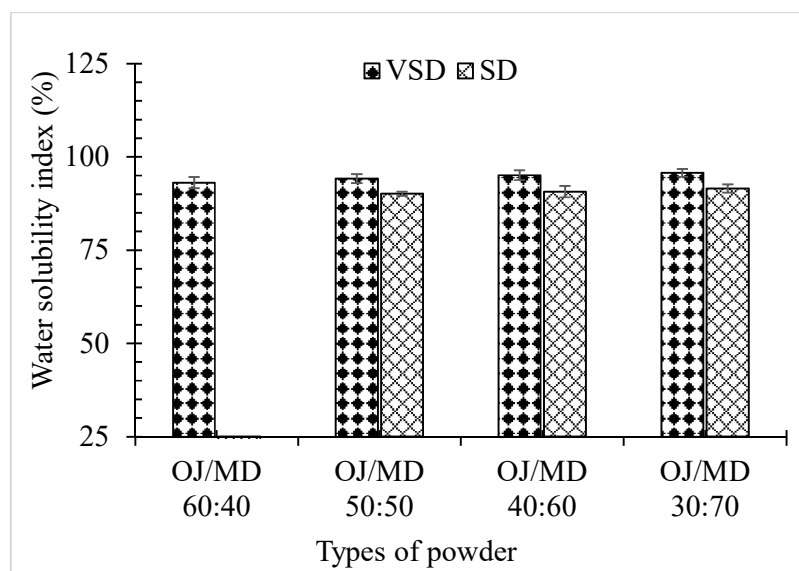
Figure 4-5. Water solubility index of orange juice powders

4.3.1.7 Particle size and size distributions

The particle sizes of different VSD and SD MWM orange juice powders are shown in Figure 4-6 as the median diameter (D50 value). Particle sizes (median D50) of VSD orange juice powders differed among the four samples. OJ/MD 60:40 showed the higher particle sizes of 13.82 μm. The particle sizes of SD powders were much larger than the VSD powders as shown in Figure 4-6.
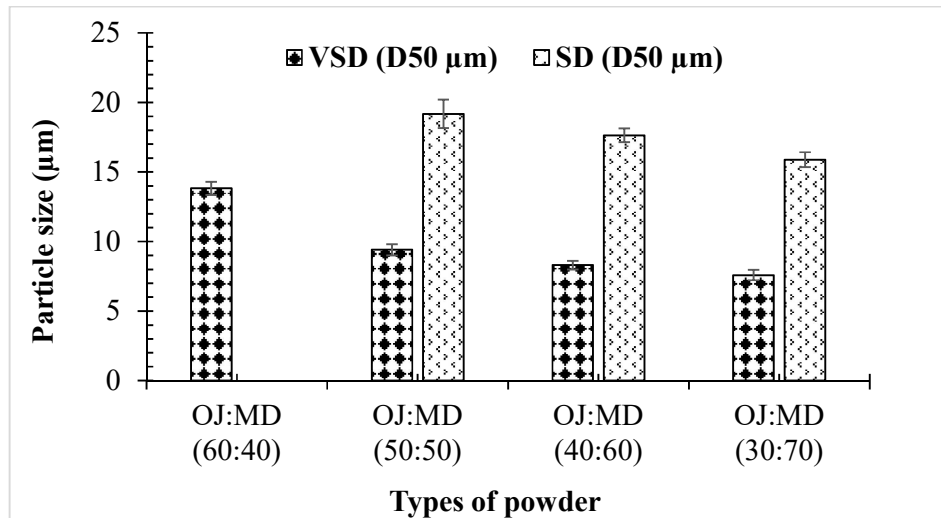
Figure 4-6. Particle sizes median (D50) of MWM orange juice powders

Figure 4-7 shows the particle size distributions of VSD & MWM orange juice powders. All VSD powders showed the bimodal particle size distribution with two distinct peaks. For VSD one with a lower frequency (<2%) and smaller particle diameter (predominant sizes of $0.9 - 1.08$ μm). The second peak with larger frequency (around 5-7%) and the larger particle sizes varied according to increases of OJ solids for VSD orange juice powders. The particle size distributions of SD & MWM orange juice powders as shown in Figure 4-8 and it is represented that the multimodal distributions with three distinct peak. The first peak showed the frequency of (<2%) with smaller particle sizes of 0.3 to 0.8 μm. The major peak (2nd peak) showed the lower frequency of >5.5% for OJ/MD 50:50 and > 4.0% than the VSD powder but the peak shifted to the larger particle size region with highest particle sizes of median diameter 19.18 μm for OJ/MD 50:50 powders. The 3rd peak showed in higher particle sizes region of 40 to 100 μm with the frequency of below 2% all of the powders. The bimodal distribution is important for powder products, the smaller particles can penetrate into the void space between the large particle, which can impact both bulk density and flowability.
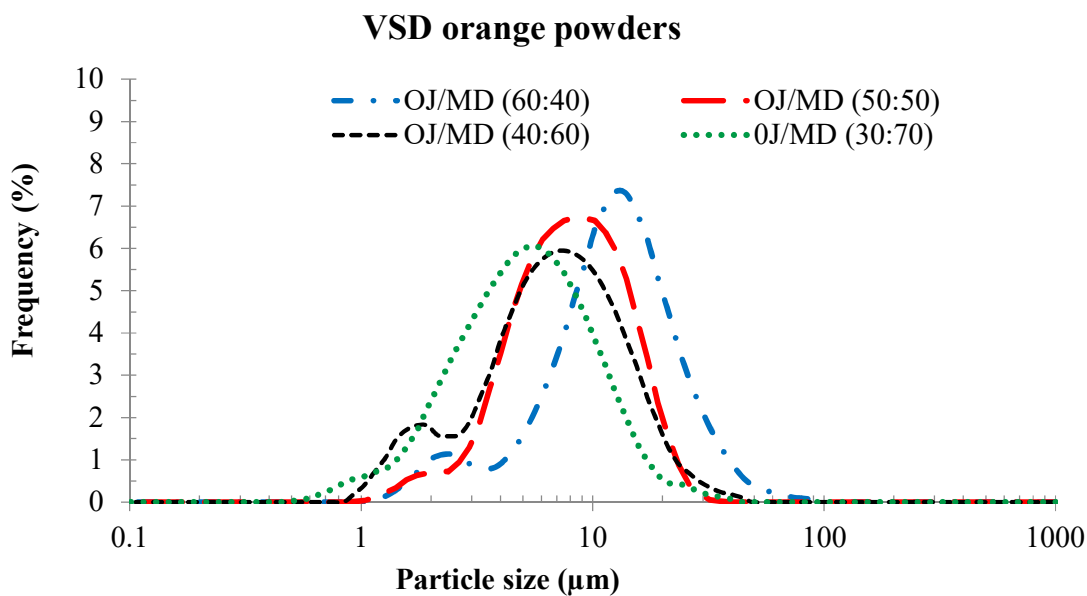
Figure 4-7. Particle size distributions of VSD orange juice powders
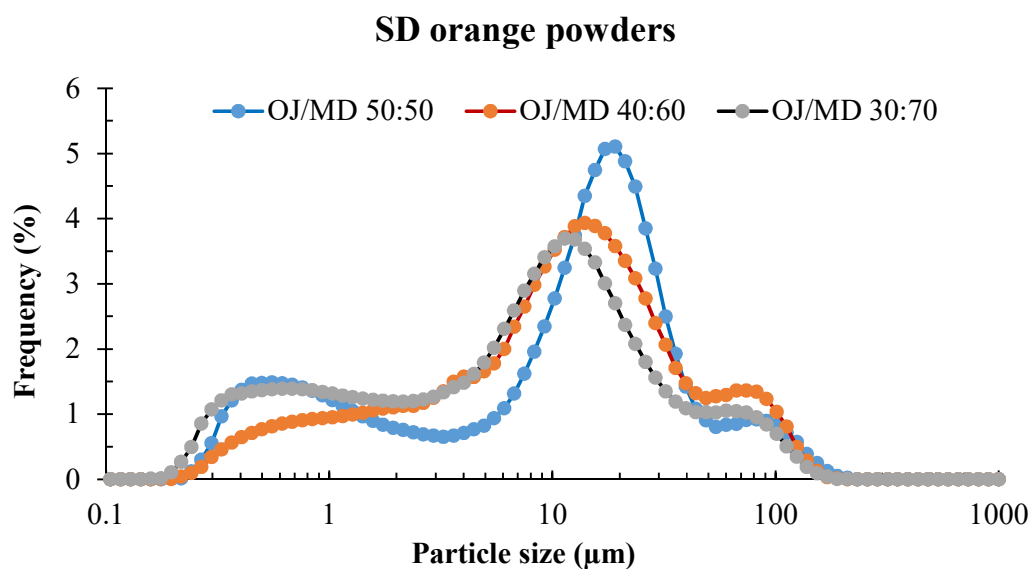
Figure 4-8. Particle size distributions of SD & MWM orange juice powders

4.3.1.8 Scanning electron micrograph of orange juice powders

The SEM of the VSD and SD orange powders is shown in Figure 4-9. SEM study revealed that OJ/MD 60:40 powders were found to be fused and clumped together to one another for VSD but in the case of SD OJ/MD 60:40 showed the agglomerated large particles, due to the higher stickiness of the powders. For VSD OJ/MD 50:50 showed smooth surface and agglomerated but SD OJ/MD 50:50 showed the large agglomeration with small particles, this was due to the stickiness of the OJ solids during drying at a higher temperature. Another possible reason was similar to the phenomenon described by Shrestha et al. (2007), who stated that powders with higher OJ solid contain more sugar and acid, which increase the stickiness of the feed mixture during spraying in the drying chamber, and agglomeration occurred, consequently larger particles are produced. The agglomerations were not found in the VSD powders produced with higher maltodextrin (OJ/MD 40:60 AND OJ/MD 30:70). The surface of the particles was smooth and spherical shape with some shrinkage occurred in the large particles. According to Kurozawa et al. (2012), minimum shrinkage of the particles occurred when drying was conducted at a lower inlet temperature. The higher inlet temperature and lower maltodextrin concentration during spray drying resulted in strong adherence of the smaller particle surface to the larger particles to form many agglomerations and which lead to increase particle sizes. The particle sizes of the MWM & VSD OJ powders were smaller than Shrestha et al. (2007) for spray dried OJ powders. The best possible reason is in accordance with the previous study reported by Tonon et al. (2011) for spray drying with higher temperatures imparts a faster drying rate which causes the particles to swell, whereas a low temperature at the dryer inlet prevents the particles from swelling, resulting in particles with a smaller diameter.

No Powder
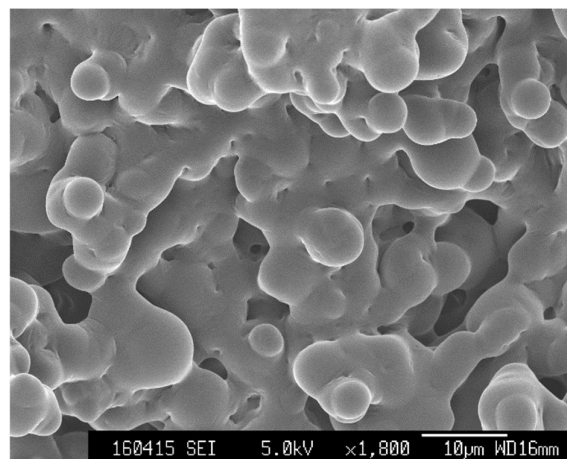
SD (OJ/MD 60:40)

VSD (OJ/MD 60:40)
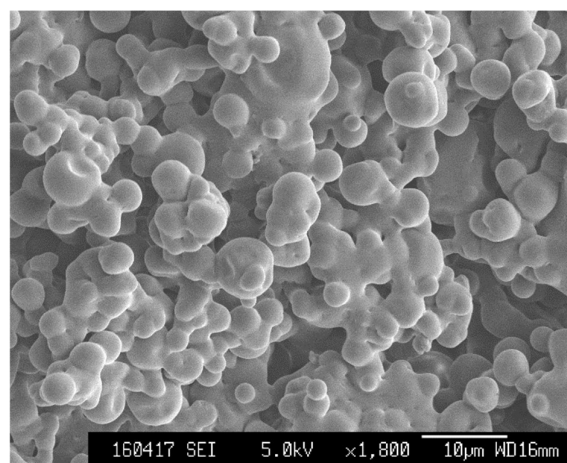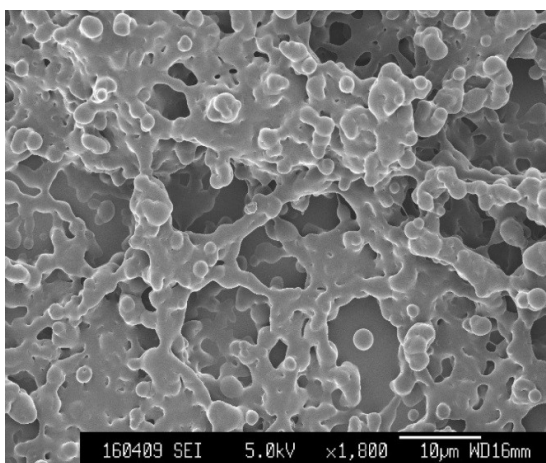
SD (OJ/MD 50:50)

VSD (OJ/MD 50:50)
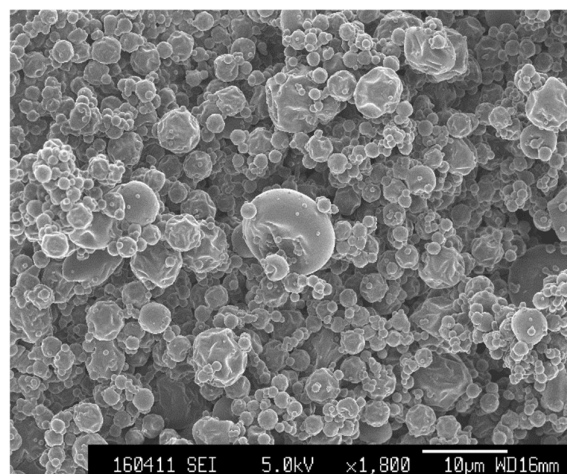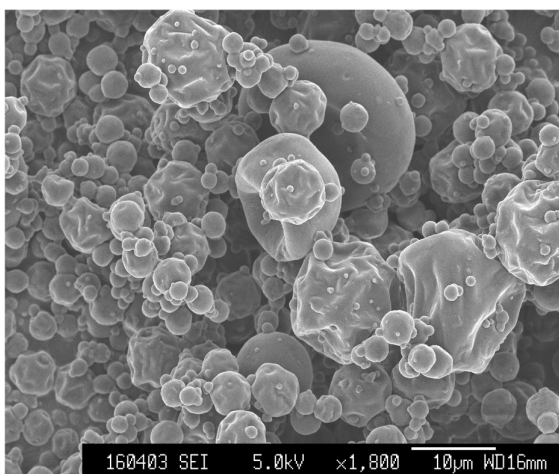
SD (OJ/MD 40:60)

VSD (OJ/MD 40:60)

| SD (OJ/MD 30:70) | VSD (OJ/MD 30:70) |
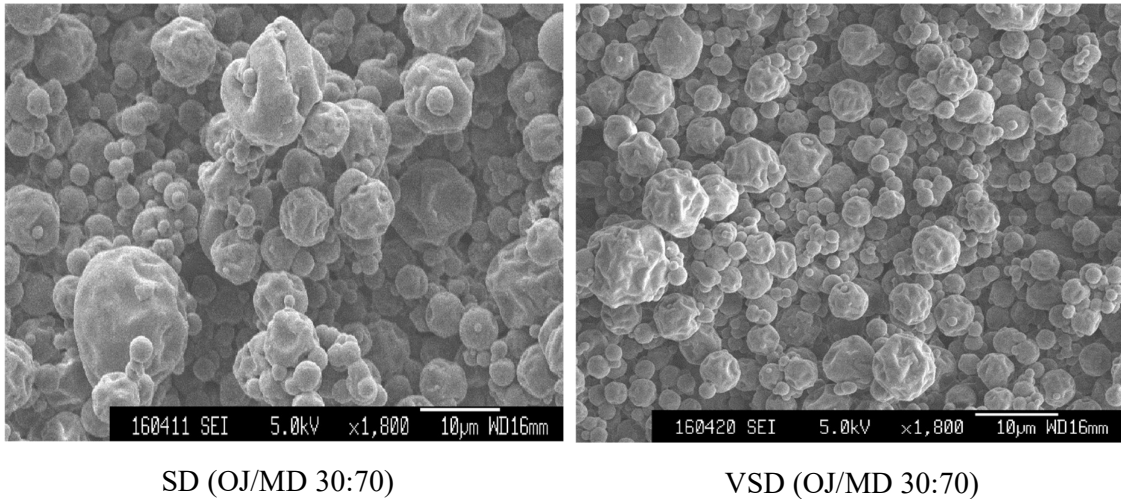
Figure 4-9. Microstructure of VSD & SD orange juice powders

4.3.1.9 Color properties

Color characteristics of spray dried powders were greatly influenced by the MD concentration. The color parameters of VSD and SD OJ powders are shown in Table 4-5. The present study demonstrated that with an increase in MD solid, the color of the powders became lighter (+L), but the redness (+a) and yellowness (+b) values decreased. Grabowski et al. (2006) found that the redness of sweet potatoes decreased and hue angle increased when MD concentration increased, and Quek et al. (2007) mentioned that spray dried powders lost their attractive red-orange color when the powders were produced by 10% or more MD solids. The MWM OJ powders lost their attractive red-yellow color compared to fresh MWM OJ. The VSD OJ powders retained more qualitative (+L,Chroma) and quantitative (Hue angle, H°) color parameters as shown in Figure 4-10 than MWM SD orange powders and also the results reported by Shrestha et al. (2007) and Saikia et al. (2014) for spray dried OJ powders at 160℃ with MD as a carrier agent. Another study confirmed that with an increase in the inlet temperature, the red color of carrot significantly decreased (Chen et al., 1995). By drying in vacuum drying conditions with lower temperatures of 50-60℃, powders could be produced with less color loss.

Table 4-5. Color parameters of orange juice powders

Types of powder (OJ/MD)	L		a		b	
	VSD	SD	VSD	SD	VSD	SD
60:40	88.34±0.83[a]	NA	-1.39±0.17[d]	NA	40.37±0.91[a]	NA
50:50	90.48±0.63[a]	92.87±0.51[c]	-2.34±0.10[bc]	-2.52±0.05[c]	36.97±1.02[b]	28.96±0.52[a]
40:60	90.77±0.88[a]	94.31±0.05[b]	-2.25±0.13[a]	-2.60±0.31[b]	33.03±0.38[c]	23.64±0.05[b]
30:70	90.84±1.50[a]	95.31±1.18[b]	-2.30±0.34[b]	-2.74±0.02[a]	29.05±0.97[d]	22.52±1.21[b]

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly ($p \leq$ 0.05).
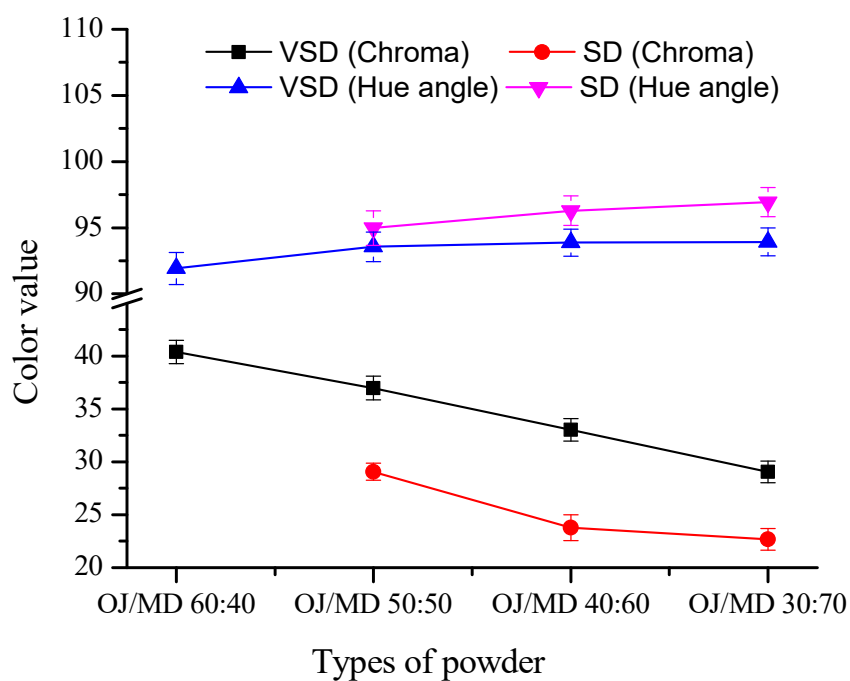
Figure 4-10. Chroma and Hue angle of orange juice powders

4.3.2 Effects of VSD and SD on antioxidant properties of MWM OJ powders

4.3.2.1   Ascorbic acid

Ascorbic acid not only has a great impact on human nutrition but is also used as a quality indicator for food processes. The ascorbic acid amounts and percentages of retention of MWM & VSD OJ powders are shown in Table 4-6. The ascorbic acid contents of OJ/MD mixture before drying ranged from 9.57 to 19.03 mg/dry solid. The results show that a higher ratio of MD had a positive impact on the retention of ascorbic acid during drying, and powders with OJ/MD 30:70 retained the maximum percentage of ascorbic acid content of 73.97%). The amount of retained ascorbic acid was higher than the results investigated by Islam et al. (2016) for vacuum spray drying of commercial orange juice, this may be due to the higher amount of pulp which contains much fiber that acts as a carrier of ascorbic acid.

The present study also demonstrated that spray drying with higher temperature can retain maximum 39.72% of ascorbic acid when the powder associated with higher maltodextrin ratio. Several authors reported that ascorbic acid degradation was dependent on moisture and temperature (Villota and KAREL 1980). As conventional spray drying of gooseberries had a 62.1% loss of vitamin C at 140°C (Thankitsunthorn et al., 2009) and the percentage of vitamin C retention varied from 28 to 51% during spray drying of cactus pear juice powder at 205°C and 225°C (Rodríguez-Hernández et al., 2005). (Kaya et al., 2010) also reported that 27.47% of vitamin C was retained during drying of kiwifruits without carrier agent at 60°C. During spray drying of passion fruit at 180°C and 190°C with MD as a carrier, maximum 50% of ascorbic acid was retained reported by Angel et al. (2009), whereas, in our present study carried out by low-pressure superheated steam drying at 50-60°C, the retention percentage was higher. It means that vitamin C is highly sensitive to heat and oxidation. That is why low-temperature treatment and carrier agent retain maximum vitamin C in the present study. Our findings were in accordance with Kongsoontornkijkul et al. (2006) for low-pressure superheated steam drying of Indian gooseberry.

Table 4-6. Effects of VSD & SD on ascorbic acid of MWM orange juice powders

Types of powder (OJ/MD)	Before drying (mg/g of solid)	After drying (mg/g of solid)		Retention %	
		VSD	SD	VSD	SD
60:40	18.96±2.51[aA]	13.18±1.02[bA]	NA	69.56	NA
50:50	15.80±1.31[aB]	11.06±1.11[bB]	5.12±1.21[cA]	70.05	27.00
40:60	12.64±1.12[aC]	9.14±0.05[bC]	5.39±1.13[cA]	72.35	34.12
30:70	9.48±1.05[aD]	7.01±0.03[bD]	5.02±0.05[cA]	73.97	39.72

The values are mean ± S.D of three independent determinations. The means with different superscripts with uppercase in a column differs significantly (p≤ 0.05) and means with different lower case superscripts in a row differs significantly (p≤ 0.05)

4.3.2.2 Total polyphenol (TPC) and Total flavonoid content (TFC)

Phenolic compounds are potent antioxidants in citrus fruits. TPC and TFC of vacuum spray dried OJ powders are shown in Table 4-7 and Table 4-8. The TPC and TFC contents of OJ/MD mixture before drying ranged from 31.56 to 36.92 mgGAE/g dry solid and 6.36 to 12.72 mgQE/g dry solid, respectively. The maximum retention percentages of total polyphenol content of 93.5% and total flavonoid content of 93.91% found in VSD OJ/MD 30:70 powders whereas SD retain maximum polyphenol content ranged from 88.23 to 91.12 but the total flavonoid content was less the VSD of OJ powders as mention in Table 4-8. The TPC contents of VSD and SD were not significantly differed due to the heat liable properties of phenolic compounds (Saikia et al., 2014).

Table 4-6. Effects of VSD & SD on ascorbic acid of MWM orange juice powders

Types of powder (OJ/MD)	Before drying (mg/g of solid)	After drying (mg/g of solid)		Retention %	
		VSD	SD	VSD	SD
60:40	18.96±2.51[aA]	13.18±1.02[bA]	NA	69.56	NA
50:50	15.80±1.31[aB]	11.06±1.11[bB]	5.12±1.21[cA]	70.05	27.00
40:60	12.64±1.12[aC]	9.14±0.05[bC]	5.39±1.13[cA]	72.35	34.12
30:70	9.48±1.05[aD]	7.01±0.03[bD]	5.02±0.05[cA]	73.97	39.72

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Phenolic compounds are potent antioxidants in citrus fruits. TPC and TFC of vacuum spray dried OJ powders are shown in Table 4-7 and Table 4-8. The TPC and TFC contents of OJ/MD mixture before drying ranged from 31.56 to 36.92 mgGAE/g dry solid and 6.36 to 12.72 mgQE/g dry solid, respectively. The maximum retention percentages of total polyphenol content of 93.5% and total flavonoid content of 93.91% found in VSD OJ/MD 30:70 powders whereas SD retain maximum polyphenol content ranged from 88.23 to 91.12 but the total flavonoid content was less the VSD of OJ powders as mention in Table 4-8. The TPC contents of VSD and SD were not significantly differed due to the heat liable properties of phenolic compounds (Saikia et al., 2014).

In the present study, TPC contents of SD orange powders increased at higher temperatures, although there was a significant loss of ascorbic acid which is a natural antioxidant. Similar findings were obtained by Saikia et al. (2014) who mentioned that with spray drying of OJ, TPC and TFC increased because of the reactions or structural degradations of various phenolic compounds that occurred at a higher temperature (185℃). However TPC and TFC values obtained in the present study were higher than those reported by Saikia et al. (2014) which may

be due to 1) the MWM process that produced orange pulp with smaller particles, enhancing the extraction of phenolic compounds, 2) drying in vacuum conditions with superheated steam in contrast to hot air drying, or 3) differences in fruit maturity or horticultural practices. Several authors reported that the similar findings with the VSD were also obtained by Sormoli and Langrish (2016) for spray drying orange peel extract. The results demonstrated that the increase of MD solids, which showed efficiency in protecting bioactive compounds from adverse conditions. This was in accordance with the findings of Ferrari et al. (2012) for spray drying Blackberry powders.

The TPC contents of VSD orange juice powders were significantly varied. The powders contained with higher maltodextrin retained maximum polyphenol contents but in the case of total amounts of TPC and TFC were decreased with the increased of maltodextrin solids. This phenomenon was similar to the findings reported by Fang and Bhandari (2011) and Mishra et al. (2014) for spray dried Amla juice powders.

Table 4-7. Effects of VSD & SD on total polyphenol content of orange juice powders

Types of powder (OJ/MD)	Before drying (mg GAE/g of solid)	After drying (mg GAE/g dry solid)		Retention %	
		VSD	SD	VSD	SD
60:40	37.00 ± 1.15^{aA}	32.19 ± 2.11^{bA}	NA	87	NA
50:50	34.77 ± 1.17^{aB}	31.12 ± 1.31^{bA}	34.12 ± 1.51^{aA}	89.5	92.23
40:60	32.97 ± 0.05^{aC}	29.81 ± 1.05^{bB}	32.51 ± 1.21^{aA}	90.4	93.53
30:70	31.56 ± 1.10^{aD}	29.52 ± 1.05^{aB}	31.03 ± 1.51^{aB}	93.5	94.12

The values are mean ± S.D of three independent determinations. The means with different superscripts with uppercase in a column differs significantly ($p\leq 0.05$) and means with different lower case superscripts in a row differs significantly ($p\leq 0.05$)

Table 4-8. Effects of VSD & SD on total flavonoid content of orange juice powders

Types of powder (OJ/MD)	Before drying (mg QE/g of solid)	After drying (mg QE/g dry solid)		Retention %	
		VSD	SD	VSD	SD
60:40	12.75±1.41[aA]	11.76±2.11[aA]	NA	92.18	NA
50:50	10.65±1.81[aB]	9.87±1.01[aA]	9.21±0.05[aA]	92.60	86.34
40:60	8.51±1.03[aB]	7.98±0.01[aB]	7.49±1.03[aB]	93.76	88.12
30:70	6.36±0.67[aC]	5.98±1.02[aC]	5.75±1.51[aC]	93.90	90.32

The values are mean ± S.D of three independent determinations. The means with different superscripts with uppercase in a column differs significantly (p≤ 0.05) and means with different lower case superscripts in a row differs significantly (p≤ 0.05)

4.3.3 Effects of VSD and SD on antioxidant activity

4.3.3.1   DPPH Scavenging activity

DPPH radical model is widely used to evaluate the free radical scavenging activity of different samples. The effects of antioxidants on DPPH radical scavenging is presumed to be due to their hydrogen donating ability. Blois (1958) stated that samples related to the higher reduction of DPPH showed higher scavenging activity. The DPPH scavenging activity of VSD and SD OJ powders were expressed as Trolox equivalent mg/g of dry solids. The results are presented in Table 4-10. Antioxidant activity of VSD OJ powders was varied from 68.23 to 83.23 mgTE/g of dry solids whereas SD powders contained from 38.89 to 43.67 mg TE/g dry solids. The MWM & VSD OJ powders showed higher antioxidant activity than the SD OJ powders due to the presences of higher natural antioxidants (ascorbic acid contents) in the powders than the SD powders also results reported by Saikia et al. (2014) for spray dried OJ powders.

The antioxidant activity of OJ/MD 30:70 powder was reduced due to the lower ratio of OJ solids, hence, lower ascorbic acid and phenolic compound amounts. Rice-Evans and Burdon (1993) found that phenolic compounds showed free radical scavenging activity due to their hydrogen or electron-donating agents and metal ion chelating properties. Another study also mentioned

that DPPH radical scavenging activity of citrus fractions is attributable to their hydrogen donating ability (Jayaprakasha et al., 2008).

Table 4-9. Effects of VSD & SD on antioxidant activity of orange juice powders

Types of powder (OJ/MD)	DPPH activity (mg TE/g of dry solids)		FRAP (µM/ 100 g of dry solid)	
	VSD	SD	VSD	SD
60:40	83.23±1.05[a]	NA	1894.32±3.21[a]	NA
50:50	77.54±1.12[b]	43.67±1.31[a]	1802.43±2.45[b]	1777.34±1.02[a]
40:60	72.12±1.02[c]	39.33±1.05[b]	1693.78±2.03[c]	1678.23±1.10[b]
30:70	68.23±0.05[d]	38.89±1.32[c]	1611.03±1.02[d]	1594.12±1.03[c]

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column differs significantly (p≤ 0.05).

4.3.3.2 Ferric reducing antioxidant powder (FRAP) assay

FRAP assay was used to evaluate the antioxidant potential of the VSD and SD orange juice powders. The higher FRAP values indicated the more antioxidant potentials of the products than those having the lower FRAP values. VSD orange juice powders represent the higher FRAP values in the ranged from 1611.03 to 1894.32 µM/100 g of dry solid whereas SD powders showed lower FRAP values and varied from 1594.12 to 1777.34 µM/100 g of dry solid as reported in Table 4-9. In both cases, the FRAP values were higher than the results reported by Saikia et al. (2014) for spray dried OJ powders. As mentioned earlier VSD MWM OJ powders retained maximum natural antioxidants those showed the higher antioxidant activity than the SD powders. Several authors reported that there is a close relationship between total phenolic compounds to the antioxidant activity, higher the phenolic compounds showed greater antioxidants activity (Benzie and Strain, 1996; Guo et al., 2003; Kalt et al., 1999). The VSD and SD MWM OJ orange juice powders contained more bioactive compounds than the spray dried single strength orange juice. Guo et al. (2003) and Huang, Xia, Hu, Lu, & Wang (2007) found

that antioxidants potentials of fruit pulp much higher than the single juice. The FRAP values of VSD powders were varied significantly due to the presences of higher maltodextrin solids doesn't show the antioxidant potential. Similar findings were achieved for SD orange juice powders.

4.4 Conclusion

A new technique for producing MWM OJ powders using VSD and SD was developed. VSD and SD can able to produce orange juice powders with different combinations of maltodextrins solids. The physicochemical, bioactive and antioxidant activity of the VSD orange juice powders were compared with SD orange juice powders. VSD successfully produced heat resistant OJ powders with higher juice solids combinations whereas SD was failed to produced powders higher than 50% juice solids combinations. The addition of MD was effective in the preservation of physicochemical and antioxidant properties of powders, producing powders with lower water activity, higher glass transition temperature, lower hygroscopicity, and higher ascorbic acid, TFC retention than the SD powders. Powders produced at OJ/MD 30:70 ratios showed better physical properties with the exception of color parameters. With respect to the particle sizes, powder recovery, free flowing properties and color characteristics, and antioxidant properties, VSD with a low temperature of 50-60℃ using superheated steam as a heating medium was able to produce powders with high quality. We conclude that the combination of MWM and VSD showed a great potential over the SD in the food industry for the production of powders such as those demonstrated in this study.

Chapter 5. Degradation kinetics and stability of vacuum spray dried micro wet milled orange juice (*Citrus unshiu*) powder

The aim of the study was to determine the ascorbic acid degradation, color and antioxidant activity of powders throughout the storage of 90 days. Powders were stored at 10, 25, 35℃ and relative humidity of 33%. Temperature and storage time negatively influenced the stability of ascorbic acid and color, whereas antioxidant activity increased at the beginning of storage at a higher temperature then decreased slightly after 60 days. For stability study, powders were stored at different water activities (0.11 to 0.84) in order to determine the plasticizing effects of water on glass transition temperature. Both water activity and glass transition temperatures were used to predict the critical conditions for storage. Vacuum spray dried orange juice solids/maltodextrin solids (30:70) ratio powder considered as the most stable, once it showed the higher critical water activity ($a_w = 0.61$) and moisture content of 0.10 g water/g of dry solid. The combination of maltodextrin additions and use of vacuum spray drying was proven to be an effective way of producing heat resistant fruit juice powders.

5.1 Introduction

One of the trends in modern food technology is to maximize nutrient retention during both processing and storage. The loss of nutrients such as ascorbic acid is a critical factor for the shelf life of products such as orange juice concentrates and powders (Lee and Coates, 1999), which are the excellent sources of vitamin C. During processing and storage of food, Vitamin C is considered as a nutrient quality indicator since it is known that if ascorbic acid is well retained, other nutrients are also well retained (Uddin et al., 2002). This is because ascorbic acid is easily degraded during storage when exposed to light and heat (Burdurlu et al., 2006). The degradation of ascorbic acid is susceptible to environmental conditions such as temperature and water activity (Uddin et al., 2002). This fact is of great importance to the manufacturers to properly process and store the fruit juice powders so that consumers can obtain powders with high nutrient quality.

Our previous study showed that the novel technique, vacuum spray drying (VSD), is efficient in producing orange juice powders with higher (approximately 30-40%) ascorbic acid content than the conventional process (Islam et al., 2016). It is necessary to understand the degradation reaction of ascorbic acids by investigating the kinetics of ascorbic acid losses during storage of powders. However, no studies were found for producing of orange juice with pulp powders by the application of micro wet milling (MWM) and VSD. Furthermore, no literature was found on kinetic parameters of ascorbic acid loss in orange powders during storage.

During the processing and storage of orange powders, a number of changes occur including changes in physical characteristics such as color and structure, and degradation of nutritional components. Structural collapse of the powders occurs when drying is performed under inadequate operating conditions (Slade and Levine, 1991). Sorption isotherms are used to predict physical, chemical and microbiological stability during processing and storage of food products (Caparino et al., 2013). However, many authors have been reported the limitations of using water activity alone for predicting the stability of food products. These limitations are (i) water activity

is defined at equilibrium, whereas foods may not be in a state of equilibrium, (ii) the critical limits of water activity may be shifted to higher or lower levels by other factors such as pH, salt, heat treatment and temperature and (iii) water activity does not indicate the state of the water present and how much it is bound to the substrate (Rahman and Labuza, 1999). The glass transition concept was put forwarded considering the limitations of water activity. Glass transition temperature (Tg) is one of the product properties found to be linked to structural changes during thermal processing and storage (Khalloufi et al., 2000a). Recent studies have shown that glass transition temperature is an indicator of the onset of product deterioration i.e. when storage temperature is higher than Tg, the product quality is seriously altered (Peleg, 1996; Slade and Levine, 1991). To predict deterioration, stability and shelf-life of food powders it has been proposed to apply both water activity and glass transition concepts, because in many cases it is insufficient to use glass transition alone (Roos, 1995). State diagrams can be constructed using glass transition temperature as a function of water activity or water content for a particular food system for determining the stability of food products (Sablani et al., 2010). Several mathematical expressions have been established to find out the glass transitions temperature as a functions of molecular weight, weight fractions, moisture content and water activity (Gordon and Taylor, 1952; Khalloufi et al., 2000b; Roos, 1987). However, there is no published information about the applications of water sorption (water activity) and glass transition concepts to find out the stability of the VSD orange powders.

The aim of the study was to evaluate the degradation kinetics of ascorbic acid in VSD MWM orange powders during a three-month storage at three different temperatures, 10℃, 25℃ and 35℃. Changes of color properties and antioxidant activity were also investigated. Finally, stability of powders was determined based on the water adsorption and the glass transition concept.

5.2 Materials and Methods

5.2.1 Materials

Vacuum spray dried four types of micro wet milled orange juice powders were subjected to determine the degradation kinetics and stability study during storage.

5.2.2 Methods

5.2.2.1   Storage conditions

Storage studies were conducted according to the method described by Uddin et al. (2002a) with slight modifications. Orange powders were placed in a Conway (Model: 060310-02A, Shibata Co. Ltd., Tokyo, Japan) unit and kept in desiccators over saturated salt solution ($MgCl_2 \cdot 6H_2O$) to create atmospheric relative humidity of 33±2.0%. The temperature was maintained by placing the desiccator in a refrigerator at 10°C and for circulation oven set at the desired temperature (25, and 35°C) with maximum variation of ±1°C. The storage period was about three months.

5.2.2.2   Determination of total color change

The Hunter color parameters of L, a and b, where large and small values of L denote lightness and darkness, +a and –a denote redness and greenness, and +b and –b denote yellowness and blueness were estimated by a Colorimeter (CR-200, Minolta Co., Japan). The total color change was determined by using the following formula:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \qquad\qquad (1)$$

where Δ indicates the differences between the L, a and b parameters on the first and 90th day.

5.2.2.3   Determination of ascorbic acid

Ascorbic acid was determined by HPLC according to (Romero Rodriguez et al., 1992) as described on the Chapter 3.

5.2.2.4 Kinetic data analysis for ascorbic acid

The loss of nutrients is explained using the reaction rate and the dependence of reaction rate on temperature. The degradation of ascorbic acid in orange powders was evaluated by first order kinetics (Burdurlu et al., 2006).

$$lnC = lnC_o - kt \qquad (2)$$

where C is the concentration at time t and C_o is the concentration at time zero. For a first order reaction, ln C is plotted against t and the rate constant k is represented by the slope. Half-life ($t_{1/2}$) corresponds to the time at which ascorbic acid content is reduced by 50% with respect to zero time, and is calculated by using the following equation (Burdurlu et al., 2006).

$$t_{1/2} = \frac{ln\,2}{k} \qquad (3)$$

The temperature dependent ascorbic acid degradation was evaluated by the Arrhenius equation:

$$k = k_o exp(-E_a/RT) \qquad (4)$$

where, k_o is the frequency factor or the Arrhenius constant (1/time), E_a is the activation energy, R is the universal gas constant (8.3145 J/m K), and T is the absolute temperature (^{o}K). Substituting k from equation (3) in (1)

$$ln\,C/C_0 = k_o(Exp - E_a/RT) \cdot t \qquad (5)$$

The decimal reduction time (D value) which is the time required to reduce the ascorbic acid concentration of 90%. Alternatively, the D-value is the time required for one log cycle reduction of ascorbic acid. The first order rate constant k and it's inversely related to the decimal reduction time D and following equations would be described as

$$lnC_o - lnC = t/D \qquad (6)$$

Based on the equations (2) and (6), the decimal reduction time D can be defined as

$$D = 2.303/k \qquad (7)$$

5.2.2.5 Antioxidant activity

Antioxidant activity was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method described by Brand-Williams et al. (1995). The methodology was reported on the chapter 4. Antioxidant activity of different powders at different storage temperature during storage of 0 days to 90 days were evaluated. Antioxidant activity was expressed as expressed as Trolox equivalents mg TE/g of dry solids.

5.2.2.6 Sorption isotherm

The detailed methodology was discussed on the chapter 2.

5.2.2.7 Glass Transition temperature

The procedure for determination of glass transition temperature was reported on the chapter 2.

5.2.2.8 Stability study based on sorption isotherm and glass transition temperature

Both water activity and glass transition temperature have been used extensively to predict the stability of dried food powders. The experimental sorption data were analyzed by Guggenheim–Anderson–de Boer (GAB) Bizot (1983) mathematical models based on the following empirical and theoretical parameters.

$$X = \frac{C_{GAB} \cdot K_{GAB} \cdot X_m \cdot a_w}{(1 - K_{GAB} \cdot a_w)(1 - K_{GAB} \cdot a_w + K_{GAB} \cdot C_{GAB} \cdot a_w)} \qquad (8)$$

Where, X is the equilibrium moisture content (g water/g of dry solid), C_{GAB} and K_{GAB} is the GAB model constant, X_m is the monolayer moisture content (g water/g of dry solids), and a_w is the water activity.

The plasticizing effects of the water content were predicted by the glass transition model. The widely used glass transition model is described by Gordon and Taylor (1952) as follows:

$$T_g = \frac{T_{gs} \cdot W_1 + k \cdot T_{gw} \cdot W_2}{W_1 + k \cdot W_2} \qquad (9)$$

where, T_g is the glass transition temperature (°C), w_1 and w_2 are the mass fractions of solid and water, T_{g_s} is the glass transition temperature of solid fraction (°C), T_{g_w} is the glass transition temperature of water (-135 °C), and k is the Gordon Taylor model parameter.

GAB model was used to predict the water content as functions of water activity and Gordon Taylor model was used to determine the glass transition temperature as functions of water content. Both models were combined and developed a new empirical model by (Khalloufi et al., 2000b) for predicting glass transition temperature as function of water activity (a_w) for fruit juice powders.

$$Tg = \frac{A \cdot a_w^2 + B \cdot a_w + C}{\alpha \cdot a_w^2 + \beta \cdot a_w + 1} \qquad (10)$$

Where Tg is the glass transition temperature (°C) and A, B, C, α, and β are the model constants. The goodness of fit was evaluated by determining the coefficient R^2, and mean relative percentage error was calculated by the following formula (Goula et al., 2008).

$$\%E = \frac{100}{N} \sum_{i=1}^{N} \frac{V_e - V_p}{V_e} \quad\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (11)$$

Where, % E is the mean relative percentage error, N is the number of observations, V_e is the experimental value, and V_p is the predicted value.

5.2.2.9 Statistical analysis

Results were given as mean and standard deviation of three independent measurements. One-way ANOVA was used to compare the means. Sorption isotherm GAB models (equations 7) equilibrium moisture content vs water activity data were fitted by non-linear regression analysis with Origin pro 8.5 statistical software (Light-Stone Corporation, Tokyo, Japan). Similarly, Gordon Taylor model's (equation 8) and Khalloufi model's (equation 9) were fitted by nonlinear regression analysis by plotting glass transition temperature of powders to the water content and water activity respectively.

5.3 Results and Discussion

5.3.1 Moisture content, water activity, and glass transition temperature

The moisture content of VSD MWM orange juice powders significantly varied between 3.42 and 4.45% (Table 5-1), and more or less similar water content obtained for spray dried orange juice powders with maltodextrin as a carrier (Goula and Adamopoulos, 2010; Shrestha et al., 2007b). VSD orange powders showed water activity values in the range of 0.30 to 0.37. Water activity denotes the availability of free water in food components, and values below 0.40 are positive for powder stability due to the low presences of free water available for biochemical reactions and microorganism growth (Rahman and Labuza, 1999). The present study showed similar water activity values obtained by Shrestha et al. (2007b) for spray drying of orange powders. There are many factors associated with deteriorating mechanisms, and recently it has been shown the glass transition temperature as one of them. Peleg (1996) reported that when the drying and/or storing temperature of food is higher than glass transition temperature, the quality of foodstuffs is seriously altered.

According to Lloyd et al., (1996), glass transition temperature has a great impact on the surface stickiness and flowability during spray drying. The sticky powder is produced when the glass transition temperature is lower than the drying temperature. Tg values of VSD MWM orange were investigated by DSC and the characteristic Tg (midpoint) (℃) temperature determined from the DSC thermogram as shown in Table 5-1. Tg of orange powders increased with the increasing of maltodextrin solids. VSD OJ/MD 30:70 powders showed a significantly higher Tg value (80℃), whereas OJ/MD 60:40 powders showed a much lower Tg (59℃). This was due to the presence of higher orange juice solid and higher moisture content, which lowers Tg. Our previous study showed that higher Tg had positive effects on product recovery (Islam et al., 2016). All of the OJ/MD powders were free flowing and non-sticky before and after storage. According to Truong et al., (2005), sticky powders are produced when exposed to a temperature

20-30℃ above the glass transition temperature. In the present study, VSD successfully produced free-flowing orange juice powders with maltodextrin. In the case of pure orange juice, glass transition temperature (-31℃) is much lower than 60℃ (drying temperature), which would cause a high degree of stickiness and be unable to produce a powder.

Table 5-1. Moisture content, water activity, glass transition temperature, ascorbic acid and antioxidant activity of VSD orange juice powders at the beginning of storage

Types of Powder OJ/MD	Moisture content (%)	Water activity	Glass transition temperature Tg ℃	Ascorbic acid (mg/g of solid)	Antioxidant activity (mg TE/g of dry solids)
30:70	3.4±0.05 [d]	0.30±0.01[d]	80.0 ± 2.1[a]	13.18±1.02[a]	83.23±1.05[a]
40:60	3.7±0.03[c]	0.33±0.01[c]	75.8±1.5[b]	11.06±1.11[b]	77.54±1.12[b]
50:50	4.1±0.01[b]	0.35±0.01[b]	63.4±1.2[c]	9.14±0.05[c]	72.12±1.02[c]
60:40	4.5±0.05[a]	0.37±0.01[a]	59.0 ± 1.1[d]	7.01±0.03[d]	68.23±0.05[d]

The values are mean ± S.D of three independent determinations. The means with different lowercase superscripts in a column differs significantly (p≤ 0.05).

5.3.2 Color stability during storage

The color is an important feature for the acceptance and reflecting the sensory quality of food products. The color of food powders may change due to the oxidation and hydrolysis reactions during storage (Cortés-Rojas et al., 2016). VSD orange powders OJ/MD 60:40 showed a bright yellow color at the beginning of storage indicated as L = 88.4, a = -1.4 and b = 40.4, whereas the powders containing higher maltodextrin solids (OJ/MD 50:50) showed L= 89.0, a = -2.3, and b = 36.9; OJ/MD 40:6 represents L = 90.7, a = -2.3 and b = 33.2 and OJ/MD 30:70 powders color parameters were L= 90.5, a = -2.3, and b = 29.1 respectively. Powders with higher maltodextrin solids showed the greater lightness value. These findings were similar with (Quek et al., 2007) for spray dried watermelon powders with maltodextrin. Storage study showed that powder containing higher juice solid (60:40) had a greater color difference as 7.5 at 35℃ (Table 5-2)

whereas OJ/MD 30:70 indicated smaller color changes (1.5) during storage at 10℃. The greater

colors changes mean that OJ/MD 60:40 powders lost their original colors with respect to the

color of the powders at beginning of the storages and finally produced larger color differences,

similarly powders with higher maltodextrin lower (ΔE) represents the powders lost their original

color with respect to the beginning of the storage at minimum. This may be because the higher

amount of carrier agent prevented the color loss. According to Kha et al. (2010), maltodextrin

acts as a carrier or as an encapsulating agent that prevents the loss of color pigments such as

carotenoids during heat treatment. Table 5-2 shows that significant ($p \leq 0.05$) color changes

occurred during storage at higher temperatures, whereas during storage at a lower temperature,

there were no significant color differences. This is in accordance with the results published by

(Kha et al., 2010), who stated that increase of temperature significantly resulted in an increase

in color differences of Gac fruit aril powders. Gvozdenović et al., (2000) observed that packed

orange powders become darker in color during a 12-month long storage, and also mentioned that

the possible reasons may be oxidation reactions or non-enzymatic browning reactions during

storage.

Table 5-2. Color differences of VSD & MWM orange juice powders during storage period of 0 day to 90 days at different temperature

Types of Powder OJ/MD	Color differences at different temperature		
	10°C	25°C	35°C
30:70	1.5±0.2[aC]	3.1±0.53[bB]	3.6±0.01[dA]
40:60	1.9±0.3[aC]	3.6±0.05[bB]	5.4±0.05[cA]
50:50	2.1±0.3[aC]	5.4±0.13[aB]	7.0 ±0.11[bA]
60:40	2.0±0.1[aC]	6.1±1.21[aB]	7.5±0.51[aA]

The values are mean ± S.D of three independent determinations. The means with different lowercase superscripts in a column and uppercase superscripts in a row differs significantly ($p \leq 0.05$).

5.3.3 Degradation kinetics of Ascorbic acid

The degradation of ascorbic acid in VSD MWM orange juice powders was studied in terms of ascorbic acid concentration. Initial ascorbic acid contents of orange powders (OJ/MD 60:40, 50:50, 40:60 and 30:70) were 13.18, 11.12, 9.16 and 7.01 mg/100 g of dry solids respectively shown in Table 5-1. It was observed that during storage, ascorbic acid concentrations in all orange powders gradually decreased with time, and the rate increased as the storage temperature increased. The degradation of ascorbic acids in all orange powders was fitted by first order kinetics as depicted in Fig 5-1. The estimated rate constant (k) at each storage temperatures (10, 25, 35 ℃) of each powder and their standard errors are reported in Table 5-3. The quality of the models was assessed by the magnitude of the coefficients of determinations R^2. The higher R^2 value (0.95-0.99) indicated that ascorbic acid contents of VSD powders follow first order kinetics. This is in agreement with the other studies investigated by (Lee and Coates, 1999), who reported that the loss of ascorbic acid content in orange juice follows first-order kinetics when stored below 50℃. The values recorded for k (day^{-1}) obtained from the first order kinetics 0.0092, 0.013 and 0.017 at 10, 25 and 35 ℃ respectively for OJ/MD 30:70 powders. The degradation rate of ascorbic acids significantly varied (p ≤ 0.05) and increased with increasing the storage temperature. On the other hand, at the same storage temperature (like 25℃) k values of different types of powders were also increased from 1.3 to 1.6 with the increasing of orange juice solids. The higher degradations of powders due the lower encapsulation materials and exposure to the light, oxygen (Tonon et al., 2010). The deterioration rate of ascorbic acid was highest in OJ/MD 60:40 powders at 35℃, followed by OJ/MD 50:50, 40:60 and 30:70 and lowering temperatures 25 to 10℃. The degradation rate of ascorbic acid was higher at the beginning of storage, which may be due to the presence of free oxygen (Polydera et al., 2003). Kinetic parameters such as the rate of ascorbic acid loss together with their R^2 correlations, half destruction time and thermal resistance are reported in Table 5-3.
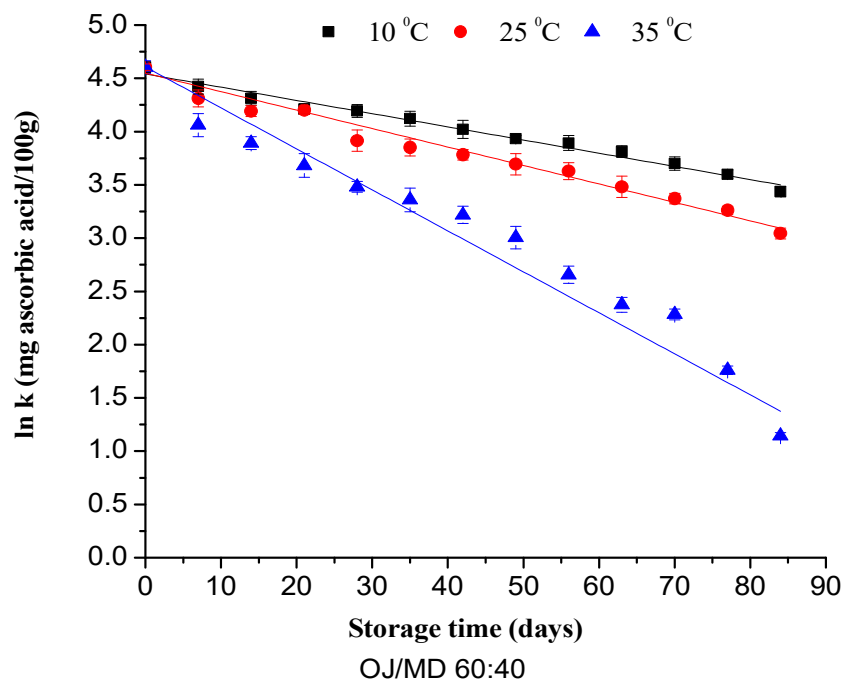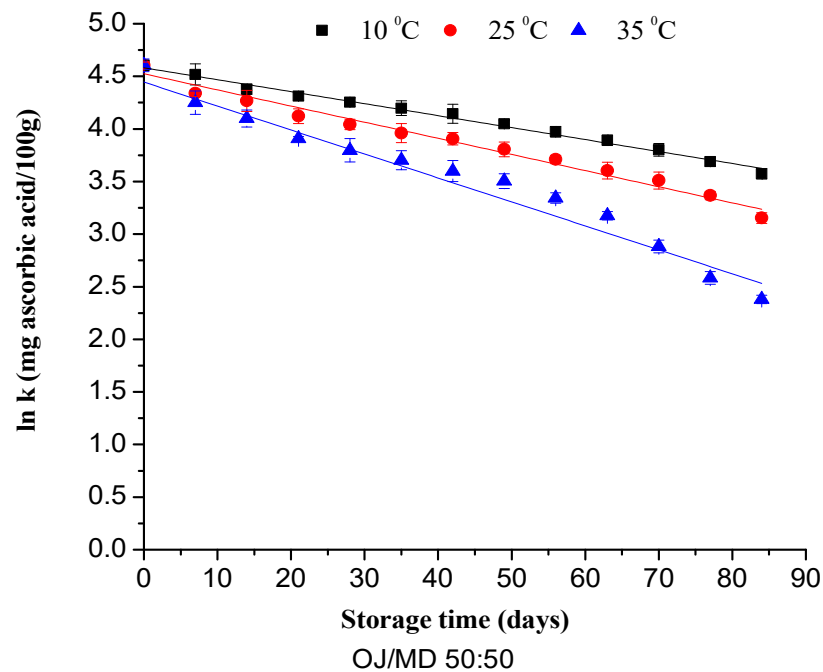
OJ/MD 30:70

OJ/MD 40:60

OJ/MD 50:50

OJ/MD 60:40
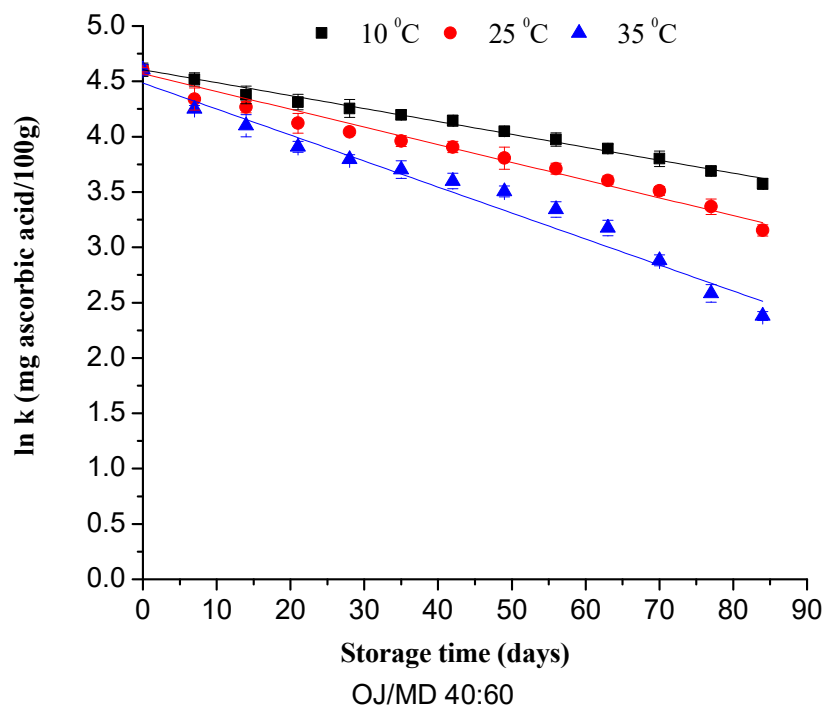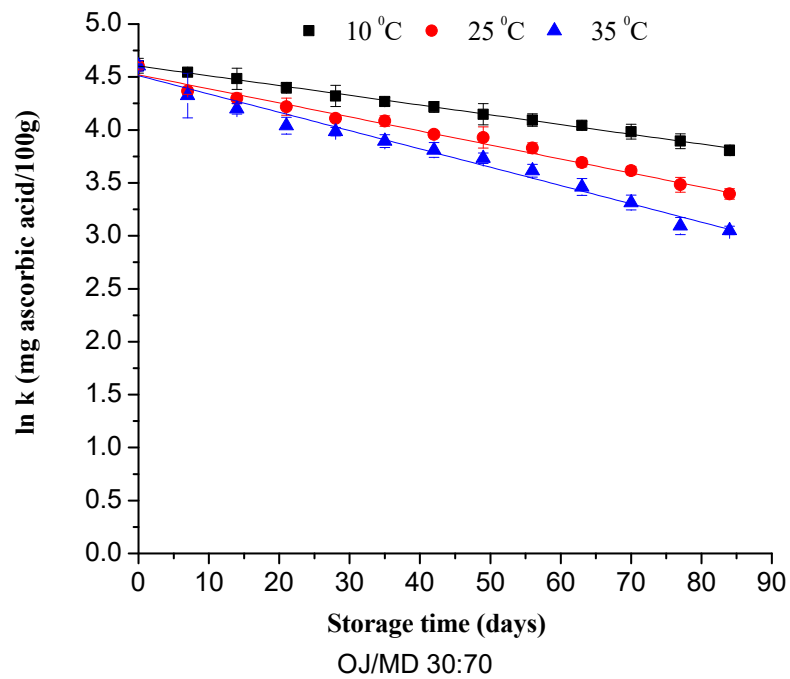
Figure 5-1. Ascorbic acid degradation during 90 days long storage of VSD MWM orange juice powders at 10, 25 and 35°C respectively

Table 5-3. Activation energy, rate constant, half-life and thermal resistance parameters for ascorbic acid degradation

Types of powders	Storage Temperature (°C)	Times of half destruction, D-value and determination coefficients of first order ascorbic acid degradation				Activation Energy kJ/m	
		$t_{1/2}$ (day)	$k \times 10^{-2}$ (day^{-1})	R^2	D (day)	$E_a \pm$ SD	R^2
OJ/MD 30:70	10	75.7±1.8[a]	0.92±3.5E-4[c]	0.99	250.4±4.4[a]		
	25	53.0±2.1[b]	1.3±4.0E-4[b]	0.98	175.8±2.1[b]	18.3±1.1[d]	0.98
	35	40.7±2.4[c]	1.7±2.0E-4[a]	0.97	135.5±5.5[c]		
OJ/MD 40:60	10	60.2±1.9[a]	1.2±4.5E-4[c]	0.99	200.5±4.5[a]		
	25	46.5±2.6[b]	1.5±3.6E-4[b]	0.98	154.5±3.5[b]	22.7±1.6[c]	0.98
	35	29.7±2.8[c]	2.3±5.0E-4[a]	0.98	98.5±3.5[c]		
OJ/MD 50:50	10	58.7±2.1[a]	1.2±5.1E-4[c]	0.99	195.2±1.9[a]		
	25	45.3±2.7[b]	1.5±2.5E-4[b]	0.98	150.5±4.5[b]	25.2±1.5[b]	0.97
	35	24.7±3.1[c]	2.9±8.1E-4[a]	0.98	81.5±2.5[c]		
0J/MD 60:40	10	56.8±3.9[a]	1.2±1.6E-4[c]	0.98	188.8±2.0[a]		
	25	42.6±2.6[b]	1.6±4.5E-4[b]	0.97	141.3±5.0[b]	30.1±2.3[a]	0.97
	35	19.8±4.9[c]	3.3±8.7E-4[a]	0.95	65.8±5.1[c]		

The values are mean ± S.D of three independent determinations. The means with different superscripts in a column of each individual powder and superscripts for activation energy in a column differs significantly (p≤ 0.05).

The $t_{1/2}$, the time required for the ascorbic acid to degrade 50% of its original and results demonstrated that lower $t_{1/2}$ values were obtained from higher storage temperature and the values of each powder significantly varied among the storage temperatures. (Burdurlu et al., 2006) reported that half-life destruction ascorbic acid of the orange juice concentrated significantly decreased with increased of the storage temperatures (28 to 45℃). Half destruction time of ascorbic acid was shortest in OJ/MD 60:40 in all storage temperatures than the others powders, due to the higher juice solids and lower encapsulating materials had the highest exposure to the light and oxygen during storage. In order to investigate the effects of different storage temperatures on ascorbic acid degradation the decimal reduction time (*D* value) was calculated at 10, 25 and 35 °C. At 35℃ the lowest value was obtained from OJ/MD 60:40 powders followed by 50:50, 40:60 and 30:70 powders and OJ/MD 30:70 powders at 10 ℃ exhibited the highest *D*

value during storage. The lowest D values of OJ/MD 60:40 powders indicated that the higher thermal damage of ascorbic acid occurred this type of powders when stored at a higher temperature. The similar results were obtained by (M. S Uddin et al., 2002) for storage of guava juice at different temperature and also mentioned that degradation rate of ascorbic acid was minimum at lower storage temperature .

Temperature dependent ascorbic acid degradation was investigated by Arrhenius plots as given in Figure 5-2 and values are presented with their standard deviations and coefficients of determination (R^2) in Table 5-3. The high R^2 value (0.97 to 0.99) justify the satisfactorily fitted our data to the models. The activation energy of VSD MWM orange powders was significantly varied from 18.30 to 30.05 kJ/m as shown in Table 5-3. The activation energy values were comparable to literature values reported by several authors. Alvarado and Palacios Viteri (1989) reported that activation energy values were in the range of 38-46.5 kJ/m, and also mentioned that the Arrhenius equation satisfactorily describes ascorbic acid degradation in the temperature ranges of 20 to 92℃. The higher activation energy of OJ/MD 60:40 powders indicated that degradation of ascorbic acid was more dependent on temperature than other samples. The lower values of OJ/MD 30:70 powders suggest that in the temperature range studied (10-35℃), ascorbic acid was more stable. This is in agreement with the results reported by (Vikram et al., 2005) for degradation kinetics of orange juice.
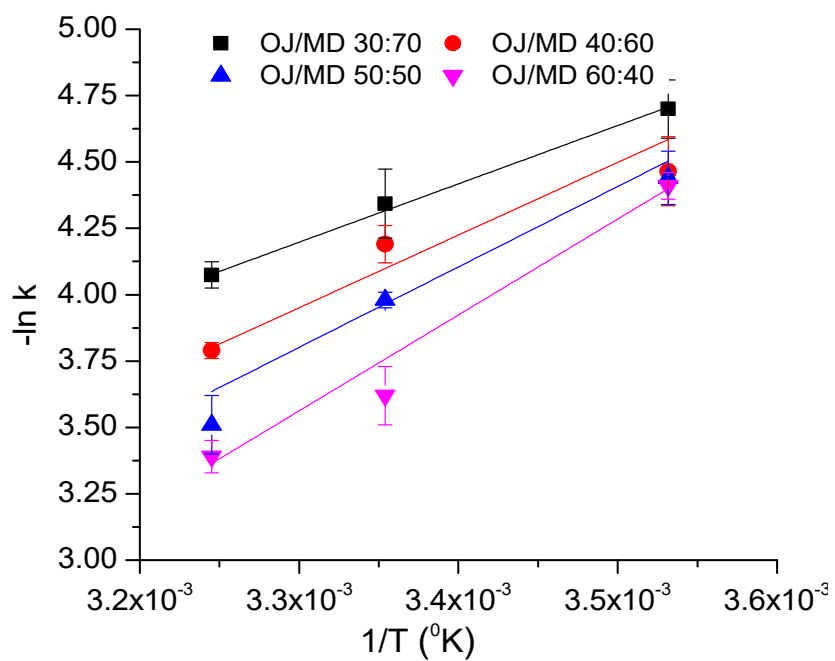
Figure 5-2. Arrhenius plots of ascorbic acid degradation of VSD MWM orange juice powders

5.3.4 Stability of antioxidant activity

The antioxidant activity of different orange powders was determined using DPPH scavenging method. Figure 5-3 represents the antioxidant activities of MWM orange juice powders during the 90 days of storage. Antioxidant activity of OJ/MD (60:40, 50:50, 40:60 and 30:70) powders at the beginning of storage were 83.23, 77.54, 72.12 and 68.23 mgTE/g of dry solid respectively as shown in Table 5-1. The powder produced with higher maltodextrin content showed lower antioxidant activity because maltodextrin itself does not show any antioxidant activity. This finding resembles that of (Mishra et al., 2014a) for spray drying Amla juice powders with maltodextrin. Storage study revealed that antioxidant activity of all orange powders increased with the increase of storage temperature as well as the time of storage, but slightly decreased after 60 days. This behavior was in similar with spray dried blackberry powders when stored for 150 days (Ferrari et al., 2013). This phenomenon may be because higher temperature conditions trigger Maillard reactions producing Maillard products, which can act as antioxidants and can enhance their activity at the beginning of storage (Nicoli et al., 1997). Powders produced with higher juice solids (OJ/MD 60:40) and stored at 35℃ showed significantly higher antioxidant activity compared to the other powders as well as other storage temperatures. This study was in accordance with the production and storage of acai juice powders Tonon et al. (2010) where the authors confirmed that higher antioxidant activity of acai powders was attained as storage temperature increased. The authors attributed this phenomenon to possible factors: antioxidant activity was increased due to the non-enzymatic browning reaction (Maillard reaction) and/or presence of some compounds other than polyphenols that contributed to its antioxidant activity. Storage at 35℃ could have increased the bioavailability of these unknown compounds, thus increasing the antioxidant potential. The authors also verified that increase on storage temperature could have resulted in the higher formation of Millard reaction and consequently higher antioxidant activity. This finding also resembled that of spray dried cherry juice powders during 9 months of storage at 15, 25, 35 and 45℃ (Righetto and Netto, 2006). In the present

study, the overall antioxidant activity of VSD orange powders increased at higher temperatures, although there was a significant loss of ascorbic acid which is a natural antioxidant. As shown in Table 5-3, VSD powders produced with the higher maltodextrin solid ratio (OJ/MD 30:70) showed lower degradation rate of ascorbic acid at a higher temperature compared to other powders, indicating that carrier agents offered protection against the degradation of ascorbic acid. (Taxi et al., 2003) reported that maltodextrin protects the heat sensitive compounds like ascorbic acid during spray drying. Maltodextrin protecting bioactive compounds due to its film-forming properties and protect ingredients that are sensitive to light, oxygen and free radical degradation and higher percentages of maltodextrin retained maximum bioactive compounds during spray drying of Jaboticaba peel extract reported by (Ibrahim Silva et al., 2013). According to Tonon et al. (2010) the higher degradation rate can be attributed to the materials are not encapsulated because those shows greater contact with light, oxygen, and higher water adsorption at the beginning of the storage are also responsible for the degradation of the bioactive compounds.

(a) OJ/MD 30:70
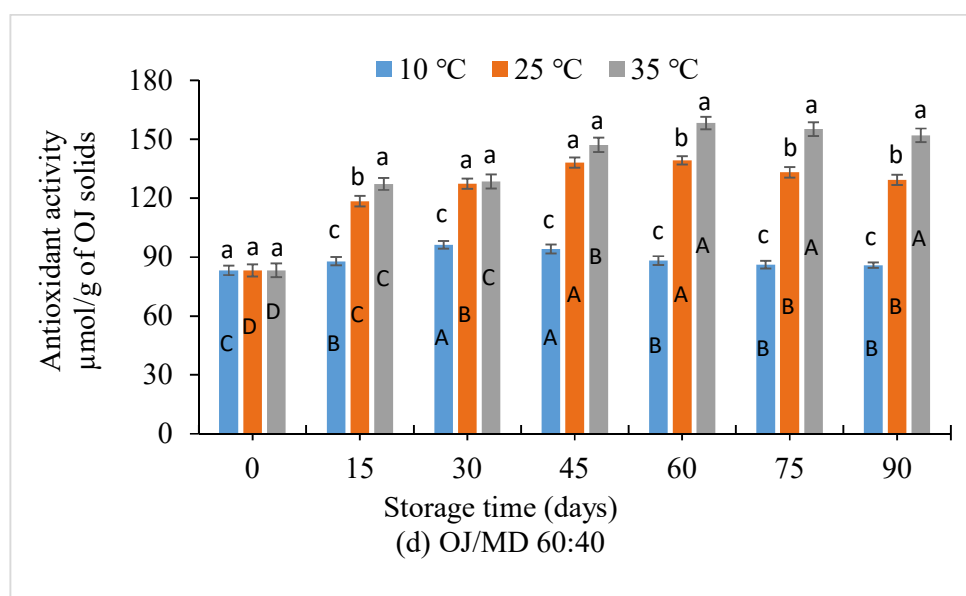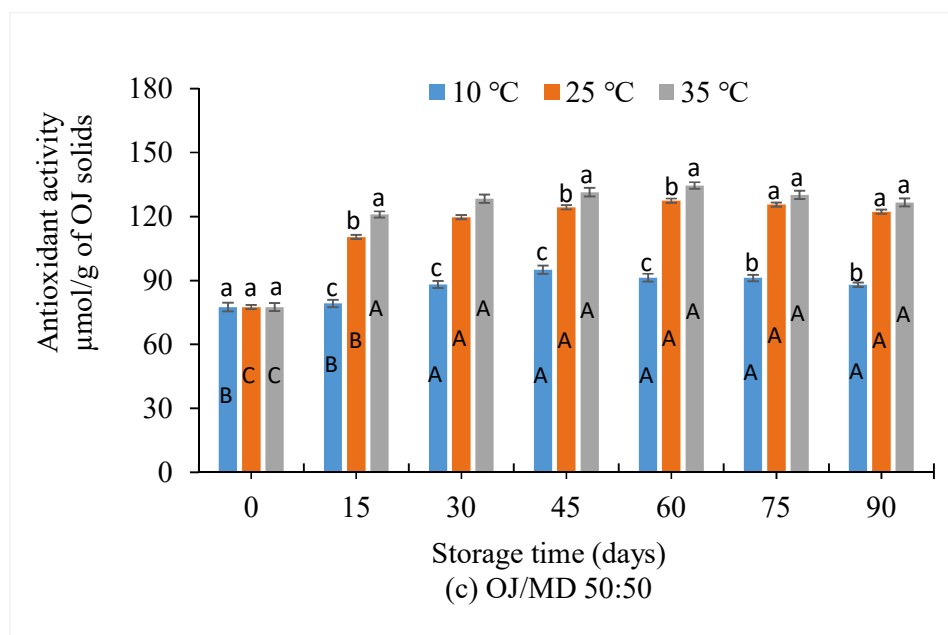

(b) OJ/MD 40:60
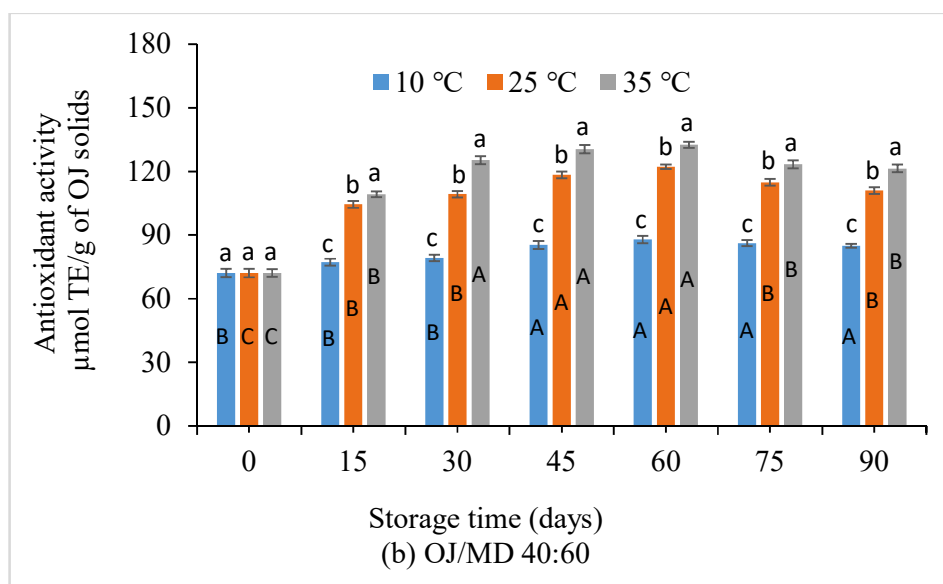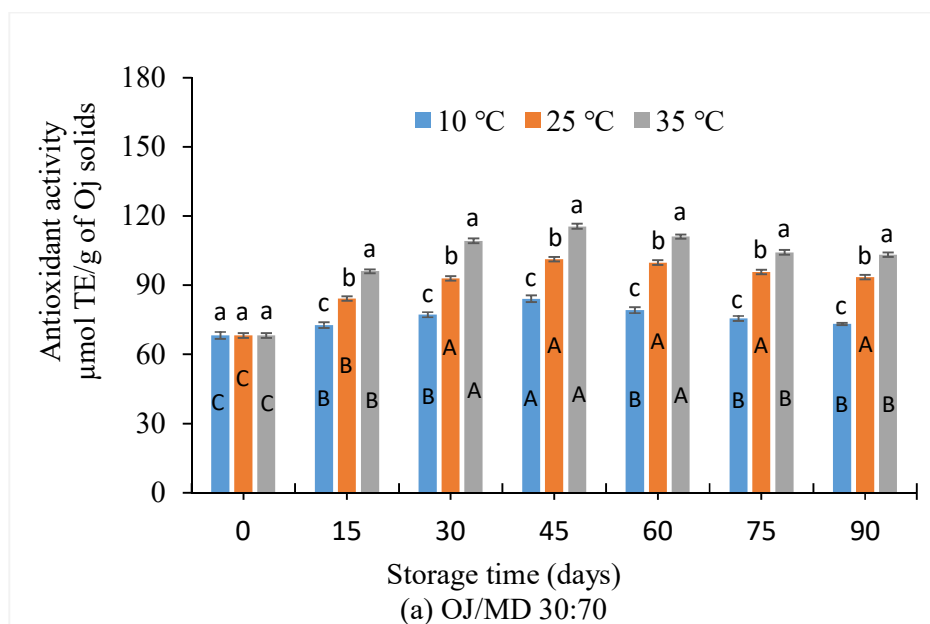
Figure 5-3. Antioxidant activity of VSD MWM orange juice powders (excluding carrier agent) during storage at 10, 25 and 35°C for 90 days.

Bars represent the mean with standard error. The means with different lower case letter above column differed significantly and uppercase letter inside of the column of each temperature during the storage differed significantly (p ≤ 0.05)

5.3.5 Product stability based on water activity and glass transition

Glass transition temperature and water activity have both been used extensively to predict the stability of dried food products during storage. Glass transition temperature and water content are indicators of the onset of deteriorating mechanism of food products (Peleg, 1996; Roos, 1995; Slade and Levine, 1991). Prediction of food stability based only on sorption data such as moisture content and water activity can be inaccurate because these data are not enough to explain some physicochemical and structural processes, namely stickiness, crispness, and amorphous-to-crystal transformation, which are highly correlated to glass transition temperature. (Peleg, 1996) stated that when the ambient temperature is higher than glass transition temperature, the quality of foodstuffs seriously altered. Collapse (Slade and Levine, 1991) and sugar crystallization (Roos, 1995) in amorphous food also been shown. The another cause of the quality loss of low moisture food is Non-enzymatic browning reactions. (Karmas, 1992) stated that although the browning rate in different foods is strongly related to the temperature and moisture content, but it is also affected by the glass transition temperature. The Millard reactions also favored at a lower temperature than the glass transition temperature and browning reactions occurred slowly even 30℃ below the Tg (Lievonen and Roos, 2002) and increased rapidly at temperature 10 – 20 ℃.

Several authors have coupled the data of sorption isotherms with those of glass transition temperature, in order to determine the critical conditions for food storage (Caparino et al., 2013; E M Kurowska et al., 2000; Roos, 1995; Tonon et al., 2009b). They have also stated that determination of glass transition of food products over a wide range of humidity would be particularly useful (Caparino et al., 2013; Khalloufi et al., 2000b; Ostrowska-Ligęza et al., 2014; Tonon et al., 2009a). The critical water content and/or water activity are the value at which the glass transition temperature is equal to the ambient temperature (Roos, 1995; Tonon et al., 2009b).

In order to find the critical storage conditions for VSD MWM orange juice powders, powders were stored at 25℃ with different water activity levels. After reaching the equilibrium conditions (near to 4 weeks), the equilibrium moisture content and glass transition temperature of each powders with their respective water activity were determined by differential scanning colorimetry (DSC 60, Shimadzu, Japan). The obtained data are presented on the Table 5-4.

Table 5-4. Equilibrium moisture content and glass transition temperature of the VSD OJ powders with their different water activity levels

Water activity	OJ/MD 30:70		OJ/MD 40:60		OJ/MD 50:50		OJ/MD 60:40	
	EMC*	Tg**	EMC	Tg	EMC	Tg	EMC	Tg
0.11	0.0198	83.67	0.0201	77.35	0.0213	65.45	0.0221	61.98
0.22	0.0347	75.34	0.0332	62.13	0.0375	56.23	0.0341	54.11
0.33	0.0455	62.12	0.0472	56.43	0.0516	50.11	0.0533	47.78
0.43	0.0544	51.11	0.0588	44.54	0.0628	35.23	0.0652	30.12
0.53	0.0657	40.65	0.0697	33.88	0.0755	21.42	0.0818	23.89
0.64	0.1085	23.98	0.1177	17.12	0.1354	13.23	0.1518	11.76
0.71	0.1457	6.92	0.1544	2.21	0.1825	-3.12	0.2014	-7.65
0.75	0.1769	-6.87	0.2052	-9.34	0.2189	-17.15	0.2217	-21.87
0.84	0.2617	-26.76	0.2928	-36.44	0.3219	-42.23	0.3416	-47.34

*EMC = Equilibrium moisture content, g water/g of dry solids

**Tg = Glass Transition Temperature, ℃

To find out the critical conditions during storage of VSD orange juice powders, sorption isotherm (GAB model Eq. 8) and Tg (Gordon Taylor model Eq. 09 and Khalloufi model Eq. 10) data were plotted as a function of water activity and moisture content and constructed combined diagram with Origin Pro 8.5 software.
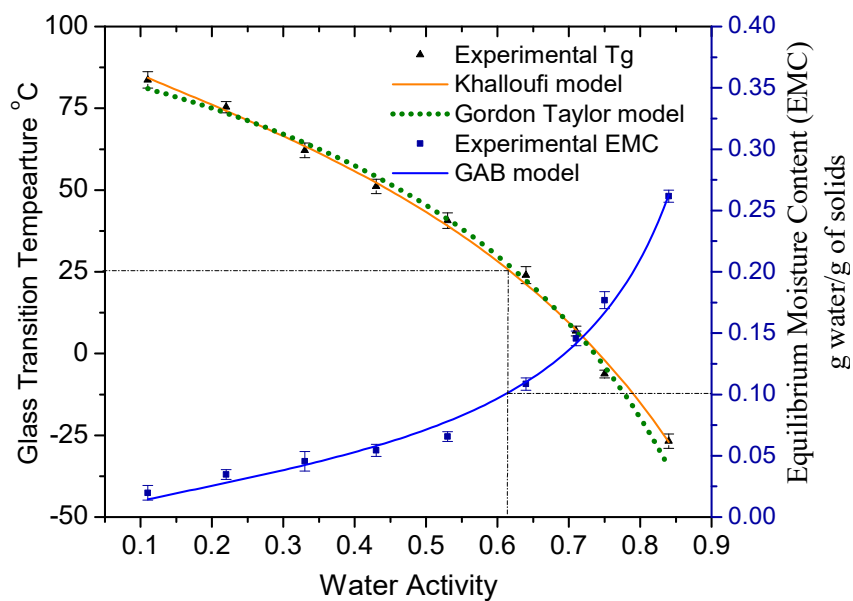
(a) OJ/MD 30:70 Powder
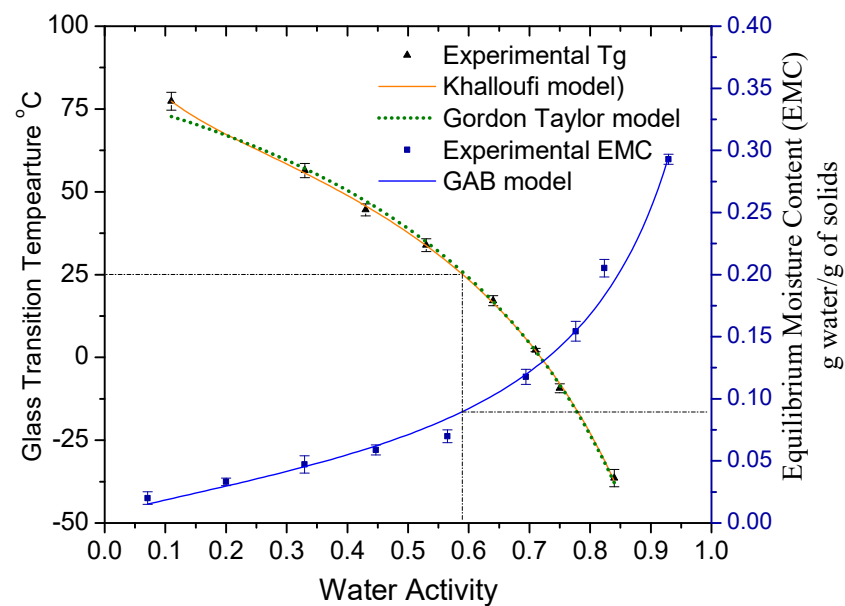
(b) OJ/MD 40:60 Powder
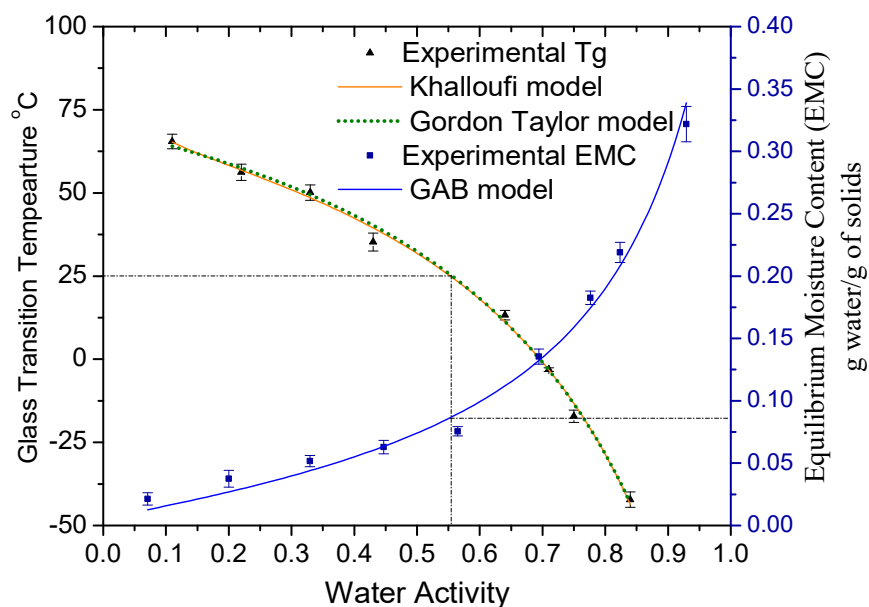
(c) OJ/MD 50:50 Powder
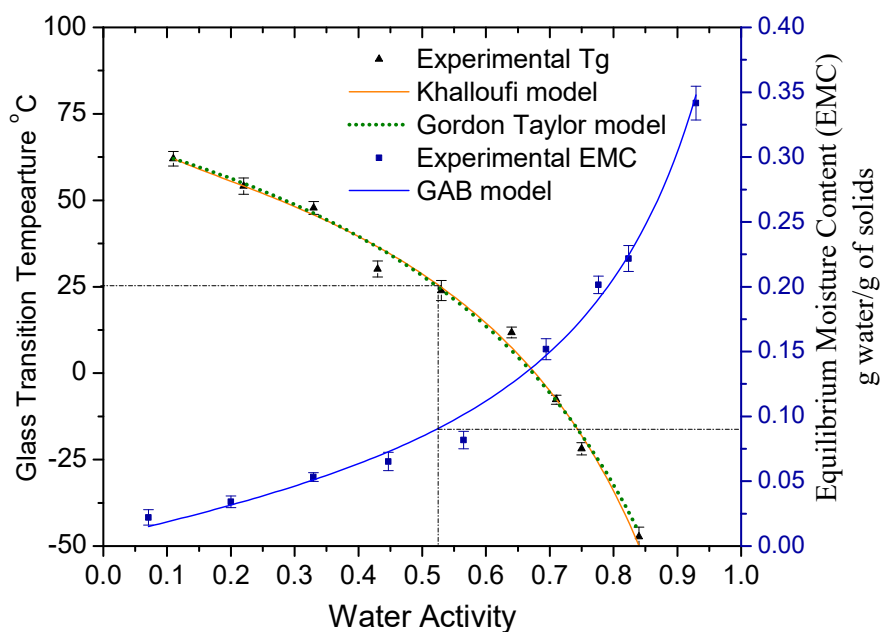
(d) OJ/MD 60:40 Powder

Figure 5-4. Variation of glass transition temperature and sorption isotherm as a function of water activity for VSD MWM orange juice powders

The critical water activity and critical moisture content were obtained by considering glass transitions temperature 25℃ equivalent to the ambient temperature of the OJ powders during storage and results are shown in Figure. 5-4 and predicted data are presented in Table 5-5

The GAB models were widely used to predict final moisture and safe moisture content of the dried foods over the wide range of water activity (Edrisi Sormoli and Langrish, 2015; Tonon et al., 2009b). Considering the values for R^2 and %E the GAB equation cab be considered satisfactory for fitting the adsorption of VSD orange powder. The strength of the binding for the water molecules to the primary binding sites on the product surface can be explained by the parameter c. The larger value of c represents the stronger bonds between the water molecules in the monolayer. The parameter k represents the values which determine the correction factors for multilayer molecules relative to the bulk liquid, such as when k =1, which describe the same characteristics of the molecules beyond monolayer and pure water (Quirijns et al., 2005). Sorption isotherm of VSD orange juice powders followed type III (J-shape) behavior, typical of sugar-rich products (Rahman and Labuza, 1999). The monolayer moisture content (X_m) is considered as the safest moisture content for long time storage with minimum quality loss at a given temperature. (Karel, 1975) stated that monolayer moisture content of dried foods varies between 0.04-0.11 kgH_2O/kg dry solid. This low water content does not support deteriorative reactions, and hence contributes to the stability of food product. VSD orange powders showed monolayer moisture content in the ranges between 4.9-7.6 % dry weight basis. The low percentage error mean deviation (3.2 – 4.6 %) and (2.2 -3.5%)values indicated that Gordon Taylor and Khalloufi model respectively were also satisfactorily fitted with the experimental data of VSD orange powders. As shown in Fig 5-4, glass transition temperature decreased with increasing water activity due to the plasticizing effects of water. The same trend was observed by the several author for spray drying berries, oranges, and acai juice powders (Goula and Adamopoulos, 2010; Khalloufi et al., 2000b; Tonon et al., 2009a). As shown in Table 5-4, the

predicted glass transition temperature by the Gordon Taylor model increased with the increase

of maltodextrin solids.

Table 5-5. Estimated model parameters for VSD orange juice powders

Model	Model Parameters	Types of orange powders (OJ/MD)			
		60:40	50:50	40:60	30:70
Gordon Taylor	T_{gs}	67.5 ± 1.0^d	70.3 ± 0.5^c	80.0 ± 1.3^b	87.0 ± 2.5^a
	k	0.24 ± 0.02^a	0.21 ± 0.01^a	0.22 ± 0.02^a	0.23 ± 0.01^a
	R^2	0.97	0.98	0.99	0.99
	%E	4.64 ± 1.2^a	4.26 ± 1.4^a	3.48 ± 1.7^a	3.19 ± 1.4^a
Khalloufi	A	-264.1 ± 6.4^d	-2245.5 ± 4.2^a	-1777.3 ± 3.6^c	-1909.5 ± 5.2^b
	B	72.6 ± 4.5^d	1427.8 ± 3.7^a	1127.0 ± 7.4^c	1279.7 ± 2.7^b
	C	71.1 ± 3.2^d	86.8 ± 2.4^c	106.7 ± 3.6^a	97.0 ± 3.7^b
	α	-2.5 ± 0.5^c	-19.1 ± 2.3^a	-14.0 ± 1.3^b	-10.89 ± 3.6^b
	β	2.3 ± 0.4^c	23.1 ± 2.1^a	17.0 ± 2.3^b	15.3 ± 3.2^b
	R^2	0.98	0.99	0.98	0.99
	%E	3.5 ± 1.2^a	3.2 ± 1.7^a	3.5 ± 1.0^a	2.2 ± 1.3^a
GAB	X_m	0.076 ± 0.01^c	0.065 ± 0.02^b	0.053 ± 0.02^a	0.049 ± 0.02^a
	C_{GAB}	1.5 ± 0.7^b	1.7 ± 1.1^{ab}	2.8 ± 1.3^a	2.9 ± 1.2^a
	K_{GAB}	0.96 ± 0.01^b	0.97 ± 0.01^{ab}	0.98 ± 0.01^a	0.98 ± 0.01^a
	R^2	0.99	0.98	0.98	0.99
	%E	4.7 ± 1.3^a	5.5 ± 1.1^a	4.5 ± 1.6^a	3.5 ± 1.7^a

The values are mean ± S.D of three independent determinations. The means with different lowercase superscripts in a row differs significantly ($p \leq 0.05$).

The critical water activities of all the powders varied from 0.61 to 0.53 and moisture content from 0.10 to 0.08 g/g of dry solid as indicated by the dotted line in Fig 5-4. Powders produced with higher maltodextrin solids (OJ/MD 30:70) can be considered as the most stable, and it showed the highest critical water activity values as 0.61. VSD powder produced with higher juice solids are susceptible to deterioration, due to its lower critical water activity as well as moisture content. When OJ/MD (60:40) powders stored at a relative humidity higher than 53% (at 25 ℃) or at a higher temperature (at a_w = 0.53), the powder will suffer physical transformations such as collapse, stickiness and caking. The results were in accordance with the findings reported by different authors for the stability study of acai juice powder, fridge dried mango and berry powders (Caparino et al., 2013; Khalloufi et al., 2000b; Tonon et al., 2009a). The critical storage parameters for OJ/MD (30:70) was higher than the results reported by (Tonon et al., 2009a) for acai juice powder, which may be due to the presence of higher maltodextrin solids in orange juice powders as well as the lower maltodextrin DE 13 values used in this study. Finally, according to (Roos, 1995), such information shows the combined effects of water activity and temperature on the physical state and provides an important tool for the prediction of behavior in processing, handling and storage of VSD orange juice powders.

5.4 Conclusion

Ascorbic acid in VSD orange powders decreased with an increase in storage temperature as well as the time of storage. Ascorbic acid degradation exhibited first-order kinetics throughout the storage. Orange powder with higher maltodextrin solids and lower storage temperature (at 10℃) showed the lowest reaction rate. The activation energy for the reaction involving ascorbic acid was predicted by using Arrhenius equation. OJ/MD (30:70) powder showed better color stability at a lower temperature. Antioxidant activity of VSD orange powders was increased although natural antioxidant (i.e. ascorbic acid) were lost during higher temperature and storage. The glass transition temperature of powders stored at different water activities was measured and it was

decreased with an increase in water activity. The critical storage (at 25℃) study based on water activity and glass transition demonstrated that powder produced with higher maltodextrin showed better stability in higher humid conditions. The results generated provide valuable information for predicting the stability and suitable storage conditions of VSD MWM orange juice powders.

Chapter 6. Conclusion and Recommendation

The effects of micro wet milling and two different spray drying methods on the processing of fresh and commercial orange juice powders have been investigated in this study. The micro wet milled and commercial orange juice were also studied. In a comparison of micro wet milled and commercial orange juice, micro wet milled resulted in superior products in terms of color, nutrients, and antioxidant potentials. The result demonstrated that micro wet milled orange juice had lower particle sizes of 55 μm (median) and higher glass transition temperature of -21°C whereas commercial orange juice particle sizes of 115 μm (median) and glass transition temperature of -42°C. In terms of antioxidants, micro wet milled orange juice contained higher ascorbic acid, total polyphenol, total flavonoid and also showed the higher antioxidant activity as well.

The present study investigated the effects of vacuum spray drying on the water sorption and glass transition temperature of commercial orange juice powders with maltodextrin (DE 12) as a carrier agent. Orange powders were produced in the weight ratios of orange juice solids to maltodextrin solids of 60:40, 50:50, 40:60 and 30:70. Vacuum spray drying was successfully produced commercial orange powders at 50 % or higher combinations of maltodextrin solid ratios. Due to the lower glass transition phenomenon of the commercial orange juice sticky powder produced and vacuum spray drying was not successful at 60:40 combinations. Overall, the sorption behavior of the orange juice powder exhibited a type III sigmoid curve, and the highest and lowest water adsorption occurred at a_w values above and below 0.53, respectively. The experimental water adsorption data were satisfactorily correlated by both the BET and GAB models. Based on the stability and product recovery the present study concluded that OJ/MD 30:70 and 40:60 by weight can be used in industrially to produce orange juice powder.

In a comparison between vacuum spray drying and spray drying of micro wet milled orange juice powders were also determined. Powders were produced with above mention combinations of maltodextrin to micro wet milled orange juice solids. Vacuum spray drying was able to produce powders at higher combinations of juice solids (60:40) whereas spray drying was not successful when juice to maltodextrin solid ratios of 50:50 or more. The powder quality in terms of physicochemical and antioxidant properties was investigated. Vacuum spray dried powders had the superior quality in terms of color, physical properties and also the antioxidant properties. Vacuum spray drying was able to retain maximum ascorbic acid (74.0%) whereas spray drying retains only 39.7%. The product recovery of the vacuum spray drying and spray drying process were not significantly varied for OJ/MD 30:70 and 40:60 powders.

Finally, vacuum spray dried micro wet milled orange juice powders were also investigated for storage stability in terms of degradation kinetics, color and antioxidant activity of powders throughout the storage of 90 days. Powders were stored at 10, 25, 35℃ and relative humidity of 33%. Temperature and storage time negatively influenced the stability of ascorbic acid and color, whereas antioxidant activity increased at the beginning of storage at a higher temperature then decreased slightly after 60 days. For stability study, powders were stored at different water activities (0.11 to 0.84) in order to determine the plasticizing effects of water on glass transition temperature. Vacuum spray dried orange juice solids/maltodextrin solids (30:70) ratio powder considered as the most stable, once it showed the higher critical water activity ($a_w = 0.61$) and moisture content of 0.10 g water/g of dry solid.

We concluded that the combination of maltodextrin additions and use of vacuum spray drying of micro wet milled orange juice was proven to be an effective way of producing heat resistant fruit juice powders. Further study is needed to evaluate the biochemical changes during processing and storage of powders. It is also required to analyze the cost and feasibility study for industrial scale production.

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Abstract

Orange juice is the most appreciated juice beverage due to its pleasant aroma and healthful properties. Moreover, it is a good source of vitamin C, polyphenol, carotenoid, flavonoid and other antioxidant constituents. To lengthen the shelf life and for convenient uses, oranges are usually preserved in the form of juice and powder. During industrial processing, orange juice is passed through the finisher for separating juice from pulp and seeds, and undergoes some thermal treatments that extend its shelf life but may deteriorate the color and flavor, and substantially decrease vitamins and functional compounds. In addition, color, flavor and functional compounds also deteriorate during powder processing with high temperature. To overcome these limitations and reduce the loss of functional components, we exploited two new techniques namely micro wet milling (MWM) and vacuum spray drying (VSD) process for producing orange juice with pulp powders.

The present study includes 5 chapters. Chapter 1 describes the mentioned background of this study. Furthermore, the limitations and advantages of different methods for processing of orange juice and powders are illustrated in detail.

In Chapter 2, detailed application of VSD for processing of orange juice powders from the concentrated commercial orange juice is depicted. According to the experimental results, the physical properties of orange juice powder with four different combinations of juice solids to maltodextrin solids at 60:40, 50:50, 40:60, and 30:70 were determined. VSD was unable to produce powder from higher juice solids combinations such as 60:40, due to the lower glass transition temperature of the rich sugar. The experimental water adsorption data were satisfactorily correlated by both the Brunauer–Emmett–Teller and Guggenheim–Anderson–de Boer models. Based on the physical properties and product recovery, this study concluded that OJ/MD 30:70 by weight can be used industrially to produce concentrated orange juice powder.

In Chapter 3 a new technique for producing concentrated orange juice with pulp by MWM system was introduced. The suitable milling conditions were achieved by varying the feeding rate and rotational speed of the mill. Feeding rate of 15 mL/min and rotational speed of 50 rpm can able to produce orange juice with smaller particle size and better color quality than the industrially extracted orange juice. In case of antioxidants, MWM orange juice contained higher ascorbic acid, total polyphenol, total flavonoid contents and antioxidant activity than the commercial orange juice. We concluded that MWM can be used to minimize the losses in fruit juice processing and can also provide fiber enriched fruits juice with higher nutrient values.

The aim of the study in Chapter 4 was to produce concentrated MWM orange juice powders by the application of VSD and spray drying (SD) process. A comparative study based on the effects of VSD and SD on physicochemical and antioxidant properties of MWM orange juice powders produced with four different weight ratios of juice solids to maltodextrin solids; 60:40, 50:50, 40:60 and 30:70 were investigated. The experimental results demonstrated that spray drying (SD) was not successful in producing MWM orange juice powders at 50:50 solid ratios or higher, whereas VSD can produce them successfully. The analyzed physical properties of OJ/MD 40:60, 30:70 of both SD and VSD powders were not significantly different, but the ascorbic acid and other bioactive compounds differed significantly. VSD was able to retain maximum 73.97% ascorbic acid whereas SD retained only 39.72%. VSD with a low temperature of 50-60 °C using superheated steam as a heating medium was able to produce powders with higher quality than the SD.

Chapter 5 depicted the ascorbic acid degradation, color and antioxidant activity of VSD and MWM orange juice powders throughout the storage of 90 days. Powders were stored at 10, 25, 35°C and relative humidity of 33%. Orange powder with higher maltodextrin solids and lower storage temperature (at 10 °C) showed the lower degradation of physical properties as well as the antioxidants. Both water activity and glass transition temperatures were used to predict the

critical conditions for storage. The critical water activities of all the powders varied from 0.61 to 0.53 and moisture content from 0.10 to 0.08 g/g of dry solid. The results provide valuable information for predicting the stability and suitable storage conditions of VSD MWM orange juice powders.

The present study demonstrated that vacuum spray drying and micro wet milling was able to produce orange juice powders with better quality in terms of color, stability and antioxidant properties than the conventional spray drying process.

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