Développement d'ingrédients alimentaires à partir d'érables. Valorisation des extraits des écorces d'érables et du sirop de qualité inférieure

Thèse

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Résumé

La tendance croissante des consommateurs à rechercher des sources alternatives d'aliments sains et naturels a incité l'industrie à rechercher des sources uniques, notamment à base de plantes. Le sirop d'érable, obtenu à partir de la sève d'érable à sucre et d'érable rouge, est largement consommé comme aliment ingrédient. De plus, les Premières nations utilisaient les écorces de ces espèces comme médicaments traditionnels et les consommaient sous forme d'infusion ou de thé. Avec l'industrialisation et l'utilisation intensive de produits forestiers, de grandes quantités de résidus d'écorce provenant de l'industrie sont devenues disponibles. Environ 17 millions de m^3 d'écorce sont produits chaque année au Canada, dont une fraction seulement sert à la production d'énergie par combustion et le reste est mis en décharge comme déchet. D'autre part, les industries acéricoles subissent un sérieux fardeau économique en raison de l'accumulation annuelle d'un sirop de qualité inférieure en tant qu'excédent. Ce dernier représente environ 21 à 38% de la production totale de sirop par an. Dans ce contexte, ce projet doctoral s'est focalisé sur la valorisation de l'écorce de l'érable et du sirop excédentaire afin de développer un produit innovant à base d'érable en tant qu'ingrédient naturel.

Premièrement, une étude des propriétés des extraits à l'eau chaude issus des écorces de l'érable à sucre et de l'érable rouge a été effectuée et a révélé que ceux-ci étaient riches en polyphénols antioxydants, ainsi qu'en nutriments organiques et inorganiques (oligo/polysaccharides, protéines et minéraux). En outre, l'étude sur l'effet des extraits bruts d'écorce de l'érable sur la viabilité des cellules de type neutrophiles a révélé leur non-cytotoxicité jusqu'à la concentration de 100µg/ml, suggérant ainsi leur utilisation potentielle comme agents alimentaires naturels. Deuxièmement, le sirop de qualité inférieure a été transformé en poudres d'érable à sucre (MSP) au moyen de la technique de lyophilisation (FD). Une approche systématique a été développée pour mettre en place le protocole FD afin de déshydrater avec succès le sirop d'érable de qualité inférieure. Le temps total par protocole FD a été réduit de manière significative de 25 à 38% (de 40 à 25 ou 30heures) après l'optimisation du protocole. Le MSP ainsi produit avait une propriété instantanée, ce qui lui conférait un potentiel énorme pouvant être utilisé comme boissons pour sportifs, dans un mélange de céréales instantanés, etc. Enfin, la dernière partie de ce projet était consacrée au développement de poudres d'érable à sucre enrichies en polyphénols par ajout des extraits

d'écorce dans le sirop de qualité inférieure avant le séchage. Cela a permis de valoriser à la fois l'extrait à l'eau chaude et le sirop d'écorce d'érable. Les poudres de l'érable à sucre enrichies en polyphénols ont été produites par FD (en utilisant le protocole FD développé et optimisé précédemment sur le sirop seul) et par séchage sous vide à l'aide du séchoir à double tambour (VDD). L'ajout d'extraits d'écorce de l'érable à seulement 0,01% w/v a permis d'enrichir le sirop en polyphénols de 13 et 20%, respectivement, pour les extraits d'écorce d'érable à sucre (SBX) et d'érable rouge (RBX). Les deux procédés de séchage ont provoqué la diminution significative des polyphénols totaux dans le produit final. Néanmoins, les composés phénoliques totaux étaient encore plus élevés (jusqu'à 10%) dans la poudre d'érable à sucre contenant des extraits d'écorce (en particulier avec le RBX) par rapport au témoin. La poudre d'érable à sucre produite par FD et par VDD présentait des propriétés physico-chimiques différentes (humidité, couleur, dissolution, coulabilité, microstructure, morphologie et taille des particules).

Quatre produits d'érable tels que les extraits d'écorce de l'érable en tant qu'aliments fonctionnels, les poudres instantanées et lyophilisées d'érable à sucre, et les poudres d'érable à sucre enrichies en polyphénols ont été conçus et produits dans le cadre du projet de recherche de cette thèse. Les résultats de ce projet pourraient s'avérer utiles aux industries acéricoles pour réduire les problèmes d'accumulation de résidus d'écorce et de surplus de sirop d'érable de faible qualité. Cette approche permettra également aux industries de s'aligner sur le concept d'économie circulaire à l'avenir.

Abstract

Rising consumers' inclinations toward alternative sources of foods that are healthy and natural have catalyzed the industry on finding unique sources, notably plant-based ones. Maple syrup, obtained from the sap of sugar and red maple trees, is widely consumed as food. In addition, barks of these species were used as traditional medicines by the First Nations and consumed in tea infusions. With the industrialization and extensive use of forest products such as pulp and papers, lumbers, etc., high volumes of bark residues from forest-based industries have become available. About 17 million m^3 of bark are produced annually in Canada, of which, only a fraction is used for direct energy production by combustion and the rest is landfilled as waste. On the other hand, maple industries are experiencing economical burden due to the year-over-year accumulation of low quality syrup as surplus, representing about 21-38% of total syrup production annually. In this context, this project has focused on valorizing the maple bark and surplus syrup to develop innovative maple product as natural food ingredients.

At first, an investigation on the properties of hot water extract of sugar and red maple barks has revealed that these are rich in antioxidant polyphenols, along with organic and inorganic nutrients (oligo/polysaccharides, proteins and minerals). Furthermore, the study on the effect of crude maple bark extracts on the viability of neutrophil-like cells has revealed their non-cytotoxicity up to the concentration of 100µg/ml, therefore suggesting their use as safe natural food agents. Secondly, a transformation of low quality syrup into maple sugar powders (MSP) was achieved by freeze-drying (FD). A systematic approach was adopted to formulate the FD protocol in order to successfully dehydrate a low quality maple syrup. The total FD time was significantly reduced by 25-38% (from 40h to 25 or 30h) after optimizing the protocol. The formulated FD protocol consisted of primary drying (T=-36 °C, t=15h), and secondary drying (T=30 °C, t=10h) conditions. Thus produced MSP had instant-like property (dissolution time<15s), signifying the huge potential for use in sport-drinks, instant cereal mix, etc. The last part of this project was dedicated to the development of polyphenols-enriched maple sugar powders by adding bark extracts into low quality syrup prior to drying. This allowed for the combined valorization of maple bark hot-water extract and syrup. Polyphenols-enriched maple sugar powders were produced by FD (using the optimized FD protocol developed previously on syrup alone) and vacuum double-drum drying (VDD).

Addition of maple bark extracts at only 0.01% w/v has allowed for the syrup enrichment in polyphenols by 13 and 20%, for sugar (SBX) and red maple bark (RBX) extracts, respectively. Both drying processes have caused the significant decrease in total phenolics in the final product. Nevertheless, the total phenolics were still higher (up to 10%) in maple sugar powders with bark extracts (particularly with RBX) than control. FD and VDD produced maple sugar powder with different physicochemical properties (moisture, color, dissolution, flowability, microstructure, morphology, and particle size). MSP produced by FD was amorphous, therefore demonstrated good dissolution property (dissolution time, <15s), whereas it exhibited poor to very poor flowability. Conversely, MSP produced by VDD was crystalline with free-flowing flow characteristics and satisfactory dissolution (dissolution time, within 30s).

Four maple products such as maple bark extracts as functional food agents, freeze-dried instant-like maple sugar powders, and polyphenols-enriched maple sugar powders have been designed and produced through the research project of this thesis. The results of this project may prove to be useful for the maple industries to mitigate the problems of the bark residues accumulation and of the low quality maple syrup surplus. This approach will also permit the industries to align with the concept of 'circular economy' in the future.

Table of contents

List of tables

List of figures

List of abbreviations

ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

ANOVA: analysis of variance

AV-FITC/PI: annexin-V with propidium iodide

a_w: water activity

BHA: Butylated hydroxyl anisole

BHT: butylated hydroxytoluene

CI: Carr's index

DAD: diode array detector

db-cAMP : N^6,2′-O-Dibutyryladenosine 3′,5′-cyclic monophosphate

DD: drum drying

DE: dry extract

DPPH: 2, 2-Diphenyl-1-picrylhydrazyl

DSC: differential scanning calorimetry

EMC: equilibrium moisture content

EtOAc: ethyl acetate

EtOH: ethanol

FD: freeze-drying

FDA: food and drug administration

FD:MS: freeze-dried maple sugar

FD:MS+SBX: freeze-dried maple sugar with sugar maple bark extract

FD:MS+RBX: freeze-dried maple sugar with red maple bark extract

FD^{PD}: primary drying phase of freeze drying

FD^{SD}: secondary drying phase of freeze drying

GAB: Guggenheim-Anderson-de Boer

GAE: gallic acid equivalent

GRAS: generally recognized as safe

HAT: hydrogen atom transfer

HPLC-RI: high performance liquid chromatography with refractive index detector

HPLC-MS: high performance liquid chromatography coupled with mass spectroscopy

HR: Hausner ratio

HWE: hot-water extraction

IC_{50}: concentration required to inhibit 50% of subject of interest (radical/cell population)

ICP-OES: inductively coupled plasma with optical emission spectrophotometer

MeOH: methanol

MS: maple syrup

MSP: maple sugar powders

ORAC: oxygen radical absorbance capacity

QE: quercetin equivalent

RDI: recommended dietary intake

RI: refractive index

RMB: red maple bark

RM-BX: red maple bark extract

RNS: reactive nitrogen species

ROS: reactive oxygen species

SET: single electron transfer

SMB: sugar maple bark

SM-BX: sugar maple bark extract

SEM: scanning electron microscopy

TC: thermocouples

T_c: collapse temperature

T_g: glass transition temperature

T_p: product temperature

t^{PD} : primary drying time

t^{SD}: secondary drying time

T_{shelf}: shelf temperature

TE: trolox equivalent

TFC: total flavonoid content

TPC: Total phenolic content

TUI: tolerable upper intake levels

VDD: vacuum double-drum drying

VDD:MS: vacuum double-drum dried maple sugar

VDD:MS+SBX: vacuum double-drum dried maple sugar with sugar maple bark extract

VDD:MS+RBX: vacuum double-drum dried maple sugar with red maple bark extract

XRD: x-ray diffraction

To my Grandparents (Late. Nirmala Devi Bhatta, and Late. Kam Dev Bhatta)

&

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"If I have the belief that I can do it, I shall surely acquire the capacity to do it, even if I may not have it at the beginning."
(Mahatma Gandhi)

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Foreword

The present thesis is submitted to the Faculty of Graduate and Postdoctoral Studies of Université Laval (Faculté des études supérieures et postdoctorales de l'Université Laval) to meet the requirements for obtaining the *Philosophiae Doctor es Sciences* (Ph.D) degree in Wood Science at the Faculty of Forestry, geography and geomatics (Faculté de foresterie, de géographie et de géomatique).

The research work presented in this thesis was financed by the Natural Sciences and Engineering Research Council (NSERC) of Canada and the companies, Levaco Inc., and DK-Spec, Quebec (Canada). This thesis was supervised by Professor Tatjana Stevanovic, Ph,D (Department of Wood Science and Forestry) and co-supervised by Professor Cristina Ratti, Ph.D (Department of Soil Science and Food Engineering), both from Université Laval.

Most of the research work was carried out at the laboratory of Renewable Material Research Centre (CRMR), and at different laboratories of Faculty of Agriculture and Food Science. Some of the analyses were performed at Research Center of Rheumatology and Immunology (CRRI) of Department of Medicine, and at Institute of Nutrition and Functional Foods (INAF).

Chapter 1 presents the literature review, and objectives of the thesis. The **chapters 2, 3,** and **4** include the experimental works and results, which are written in the form of scientific manuscripts. These research works have been either published or submitted for publication in relevant scientific journals, summarized below:

Chapter 2, **"**Nutrients, Antioxidant Capacity and Safety of Hot Water Extract from Sugar Maple (*Acer saccharum* M.) and Red Maple (*Acer rubrum* L.) Bark", published in *Plant Foods for Human Nutrition*, Vol.73 (2018), Issue 1, Pages 25-33. Authors: Sagar Bhatta, Cristina Ratti, Patrice E. Poubelle, and Tatjana Stevanovic.

Chapter 3, "Freeze-drying of maple syrup: efficient protocol formulation and evaluation of powder physicochemical properties", published in *Drying Technology* (2019), Pages 1-13. Authors: Sagar Bhatta, Tatjana Stevanovic, and Cristina Ratti.

Chapter 4, "Impact of drying processes on properties of polyphenol-enriched maple sugar powders", submitted to *Journal of Food Process Engineering*. Authors: Sagar Bhatta, Cristina Ratti, and Tatjana Stevanovic.

At last, the general conclusions and major findings of the present work and future perspectives are discussed.

In all the published and submitted articles, Sagar Bhatta is the first author who was responsible for conception, experimental design and execution of experimental works, result analysis and article writing. Author, Patrice E. Poubelle was involved in the part of experimental design of *in vitro* test performed in the first article (Chapter 2), and its correction and manuscript revision. Authors, Tatjana Stevanovic (Thesis supervisor) and Cristina Ratti (Thesis co-supervisor) were involved in conception, experimental design, scientific discussion, correction and revision of all manuscripts.

The results obtained in the context of the thesis were also presented in different occasions including national and international conferences:

- **Oral presentation** (as principal speaker), Bio2actives: From biomass & biorefinery to actives and ingredients, 2-4 July 2017, Quimper, France.
  Sagar Bhatta, Cristina Ratti, Tatjana Stevanovic, "Valorization of Canadian Maple Bark Extracts as Food Additives".
- **Seminar**, Course SBO 8001, 29 November 2017, Quebec, Canada.
  Sagar Bhatta, Cristina Ratti, Tatjana Stevanovic, "Potentiel des extraits d'écorces d'érables (*Acer saccharum* et *Acer rubrum*) pour produire un sucre d'érable enrichi en polyphénols antioxydants".
- **Oral presentation**, INAF student symposium, 24-25 April 2018, Quebec, Canada.
  Sagar Bhatta, Cristina Ratti, Tatjana Stevanovic, "Maple Bark Extracts: Cocktail of Nutrients, Minerals and Polyphenols".
  "*Best oral presentation*", and "*Coup de Coeur du public*" awards.
- **Poster presentation**, 5th International ISEKI_Food conference, 3-5 July 2018, Stuttgart, Germany.
  Sagar Bhatta, Tatjana Stevanovic, Cristina Ratti, "Development of Maple Sugar Powders by Freeze-drying".

- **Poster presentation**, 21st International Drying Symposium (IDS2018), 11-14 September 2018, Valencia, Spain.

  Sagar Bhatta, Tatjana Stevanovic, Cristina Ratti, "Development of polyphenols-enriched maple sugars by freeze- and vacuum drum drying technologies".
- **Oral and Poster presentation**, Green Food Tech 2018, 2-3 October 2018, Quebec, Canada.

  Sagar Bhatta, Tatjana Stevanovic, Cristina Ratti, "Valorization of Low-grade Maple Syrup to Polyphenol-enriched Maple Sugar".

  "*1st prize for oral presentation*"

Introduction

According to Natural Resources Canada (2015), a global market potential of 66 billion U.S. dollars is expected for the fine and platform chemicals (chemicals to be applied for the production of other chemicals) derived from biomass (Natural Resources Canada, 2015). Bark is one of the major biomass resources available as residues in large volume. Around 17 million m^3 of bark is produced annually in Canada by forest-based industries (Xing, Deng, Zhang, Riedl, & Cloutier, 2006). Despite of its valuable chemical compositions (lignin, polysaccharides, and extractives), it is largely used for calorific energy by combustion in industries or landfilled as waste (Feng, Cheng, Yuan, Leitch, & Charles, 2013). In recent years, extractives from inexpensive sources such as bark, seed and leaves have incited growing interest both from the food industry and scientific community, mainly due to the abundance of polyphenols with antioxidant activity among these extractives (Balasundram, Sundram, & Samman, 2006; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005).

Sugar maple (*Acer saccharum* M.) and red maple (*Acer rubrum* L.) species are socially and economically appreciated trees in Canada for the production of both maple syrup and lumber. Additionally, bark of these species has been used as traditional medicine by infusion to treat various ailments such as back pain, cataracts, sore eyes, diarrhea and as a diuretic by the Native Americans (Arnason, Hebda, & Johns, 1981). Extractives from maple bark have been successfully obtained by organic solvents or water extraction. Of various solvents used for the extraction of polyphenols, water is largely preferred, because it is green, low-cost, and readily available (Geoffroy, Fortin, & Stevanovic, 2018, 2017). In addition, it has advantages in terms of certifications and safety when extracts are to be used in food and health applications (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009). Recent phytochemical analyses of maple bark extracts have reported the presence of hydrolysable tannins, lignans, benzoic acid derivatives and simple phenolic compounds (Bi et al., 2016; Yuan et al., 2011; Yuan, Wan, Liu, & Seeram, 2012). Due to the presence of such polyphenols, maple bark extracts were reported to demonstrate antioxidant, antimicrobial, anticancer and anti-hyperglycemic activities (Geoffroy, Fortin, et al., 2017; González-Sarrías, Yuan, & Seeram, 2012; Royer, Diouf, & Stevanovic, 2011). Natural antioxidants from plant extracts are finding a broad application in foods, pharmaceuticals, and cosmetics.

This is due to the consumers' inclination to prefer natural products and the strict regulations on the use of synthetic antioxidants, particularly in foods. Considering the current trend to find alternative sources of antioxidants, maple bark extracts could become an interesting source of antioxidant as potential natural food agents.

Sugar and red maple trees are also known for obtaining maple syrup, a natural sweetener, which contains wide ranges of minerals, vitamins and polyphenols (Ball, 2007). Canada is the leading global producer of maple syrup, contributing to 71% of total world's production (Agriculture and Agri-food Canada, 2018). Around 21-38% of maple syrup produced has been stored as inventory surplus annually, and this quantity is growing year-over-year. Surplus syrup of low grade maple syrup, mainly 'dark' and 'very dark' color is represented a major handling problem for syrup producers in Canada (Aider, de Halleux, Belkacemi, & Brunet, 2007). This problem can be addressed by producing maple sugar; a dried product that can be sold as an alternative to conventional sugar. Unlike maple syrup, maple sugar is easy to store for longer period due to the absence of moisture, and more convenient for handling and transportation. However, the traditional technique such as evaporation method is still largely employed to produce maple sugar crystals, which is an exhausting method leading to inconsistency in the quality of final product. The implementation of new technologies and innovations could offer the maple producers an opportunity to make a greater contribution to the economy, especially in the North American food sector. The drying techniques that are commonly used in the food industries can be applied to transform maple syrup into maple sugar powders. However, drying of foods causes the modifications of its physicochemical properties. Freeze-drying (FD) is a well-known technique for drying foods containing heat-sensitive compounds prone to oxidation, such as polyphenols, because of the use of very low temperatures and high vacuum. Drying time and operation costs are major drawbacks of freeze drying (Ratti, 2001). Vacuum drum drying (VDD) is another technique that is widely used to dry viscous liquids or pastes (Daud, 2006). It operates at high temperature under vacuum, making it possible to dry faster in comparison to freeze-drying. No literature data have been found so far on the dehydration of syrup using FD and VDD techniques. Therefore, a brief overview of FD and VDD methods will be presented in order to highlight their possible use in syrup transformation. In addition, the most important quality parameters of food powders which define their potential applications

will be presented. These quality parameters of powders include moisture, color, microstructure, dissolution, particle size, flowability, morphology, etc.

Chapter 1 Literature review

1.1 Maple bark

1.1.1 Bark

Bark is defined as all the tissues outside of the vascular cambium of woody plants. It represents about 13-21% of the wood log on a dry weight basis (Harkin & Rowe, 1971). Bark is one of the major biomass produced as residues in large volume from forest-based industries. About 100-300 kg of bark waste is generated from each ton of pulp produced (Bajpai, 2015). In Canada, around 17 million m^3 of bark is produced annually by forest-based industries (Xing et al., 2006).

Bark comprises of valuable chemicals such as lignin, polysaccharides (celluloses and hemicelluloses), and extractives (**Table 1.1**) (Harkin & Rowe, 1971). It is known as the protective layer of plants, which defends plants from harmful UV radiation, herbivores, insects and pathogens. Such defensive attribute of bark is associated to the presence of secondary metabolites (Crozier, Jaganath, & Clifford, 2006). Secondary metabolites are structurally diverse and represent a vast range of organic compounds synthesized by plants. The most abundant secondary metabolites in plants are polyphenols and can be easily extracted from bark as extractives. In recent years, there has been an increasing interest on plant extracts containing polyphenols as protective dietary constituents in human nutrition research. Despite its valuable chemical compositions, bark is primarily used to generate calorific energy by incineration in industry or landfilled as waste (Feng et al., 2013). Therefore, utilisation of bark for high-grade applications, such as source of valuable chemical obtained by extraction for food, nutraceutical, and pharmaceutical applications, could add more value to bark transformation rather than using only for calorific energy.

Table 1.1 Chemical compositions of hardwood and softwood bark.

Compositions	Hardwood bark	Softwood bark
Lignin (%)	40-50	40-55
Polysaccharides (%)	32-45	30-48
Extractives (%)	5-10	2-25

1.1.2 Choice of maple bark

Maple (genus *Acer*) is widely distributed in the temperate regions of Eastern North America, East Asia and Europe (Van Gelderen, Jong, Oterdoom, & Dudley, 1994). Of 13 species of maple found in Canada, sugar maple (*Acer saccharum* M.) and red maple (*Acer rubrum* L.) species are economically and socially appreciated species mainly for the production of lumber from their wood, for producing maple syrup (a natural sweetener obtained by evaporating the maple sap) and for ornamental purpose.

Moreover, barks of these species have been documented for their use as traditional medicine to treat various ailments such as back pain, cataracts, sore eyes, and diarrhea by the Native Americans (Arnason et al., 1981; Bi et al., 2016). **Table 1.2** highlights some of the uses of maple barks as traditional medicine. Maple barks were also infused and consumed as tea regularly by the Native Americans.

Table 1.2 Sugar and red maple bark as traditional medicine.

Maple species	Uses
Sugar maple bark	Treating diarrhea, Treating sore eyes, back or limb pains, and hemorrhoids
Red maple bark	Treating sores, cough

Levaco Inc. and DK-Spec, companies based in Quebec, Canada, are interested in transforming the low-cost bark residues to valuable products that have potential to be used as natural ingredients in food and health applications. Therefore, the following section will discuss about major polyphenols in sugar maple bark (SMB) and red maple bark (RMB), beneficial effect of polyphenols derived from maple barks, and their potential application in foods.

1.1.3 Polyphenols from maple bark

Maple species contain diverse class of polyphenols such as tannins, lignans, flavonoids, benzoic acid derivatives, phytosterols, and simple phenolic compounds (Bi et al., 2016). Among these, the major polyphenols in sugar and red maple barks are tannins, lignans, benzoic acid derivatives, and simple phenolic compounds (**Table 1.3**).

1.1.3.1 Tannins

Tannins are abundantly available in numerous foods and beverages such as tea and red wines that are consumed regularly in our daily life. They are known for their astringent taste, which is noticeable while consuming these foods. Astringency occurs due to the binding of tannins with salivary proteins. Tannins are naturally occurring polyphenolic compounds abundant in bark extractives of several plant species such as barks of maple, cinnamon, willow, wild cherry, acacia mimosa, oak, seeds of cocoa and leaves of green tea (Bele, Jadhav, & Kadam, 2010; Crozier et al., 2006; Yuan et al., 2012).

Tannins are generally categorized into hydrolysable and condensed tannin. Hydrolysable tannins are derivatives of gallic acid that are simple mixtures of gallic acid, ellagic acid, pyrogallols, and esters of glucose with gallic or digallic acids. Gallotannins and ellagitannins are important hydrolysable tannin since they can be hydrolysed by acid into gallic acid and ellagic acid, respectively. On the other hand, condensed tannins are oligomeric/polymeric proanthocyanidins consisting of coupled flavan-3-ol (catechin) units (Feng et al., 2013).

About 41 tannins are identified in maple species, wherein twenty-five are found in plant parts of sugar and red maple species. They are mostly abundant in the red maple bark. Sixteen compounds were identified as tannins in RMB, where 14 of them are gallotannins, and the remaining are condensed tannins (Bi et al., 2016; Yuan et al., 2011, 2012) **(Table 1.3**).

Table 1.3 Major polyphenols identified in sugar and red maple bark.

	Polyphenols class	**Examples of identified polyphenols**
Red maple bark	Tannins	Gallic acid; Ginnalin A; Ginnalin B; Ginnalin C; Rubrumosides B; Maplexin A; Maplexin B; Maplexin C; Maplexin D; Maplexin E; Maplexin F; Maplexin G; Maplexin H; Maplexin I; Procyanidin A2; Procyanidin A6
	others	Rubrumosides A; Nymphaeoside A; methyl vanillate
Sugar maple bark	Lignans	Syringaresinol-*β*-D-glucopyranoside; Saccharumosides A; Icariside E4; Scopoletin
	Benzoic acid derivatives & simple phenolics	Saccharumosides B-D; Koaburside; Vanilloloside; 3,4,5 Trimethoxyphenyl-1-*O*-*β*-D-apiofuranosyl-(1,6)-*O*-*β*-D-glucopyranoside; 3,5-Dimethoxy-4-hydroxylbenzyl alcohol-4-*O*-*β*-D-glucopyranoside; 4-hydroxymethyl-2-methoxy-phenyl-1-*O*-*β*-D-apiofuranosyl-(1,6)-*O*-*β*-D-glucopyranoside

Gallotannins of RMB consist of gallic acid residues linked through ester bonds to hydroxyls from a 1,5 anhydro-glucitol core (**Figure 1.1**). First gallotannin, ginnalin A (or acertannin), was reported by Perkin in 1922 from the leaves of *Acer tataricum subsp. ginnala* (Maxim.). Later on, other gallotannins and their derivatives, mainly maplexins and ginnalins, were reported from red maple bark. Maplexins and ginnalins are gallotannins with 1,5-anhydroglucitol moieties. Although gallotannins are common in plant, those containing 1,5-anhydroglucitol moieties have only been isolated from the maple species so far. The differences among varieties of maplexins are the number and position of galloyl group to glucitol moiety (maplexin A-F), and also the presence of methyl functional group to galloyl group (maplexin G-I). Rubrumoside A, Nymphaeoside A are two lignans found in red maple bark. Methyl vanillate, a benzoic acid derivative, is also mentioned in RMB (**Table 1.3**).

Gallotannins from RMB are reported to demonstrate potential medicinal benefits related to bioactivities such as anticancer, antioxidant and α-glucosidase inhibition activities (González-Sarrías, Yuan, et al., 2012; Royer et al., 2011; Yuan et al., 2012).

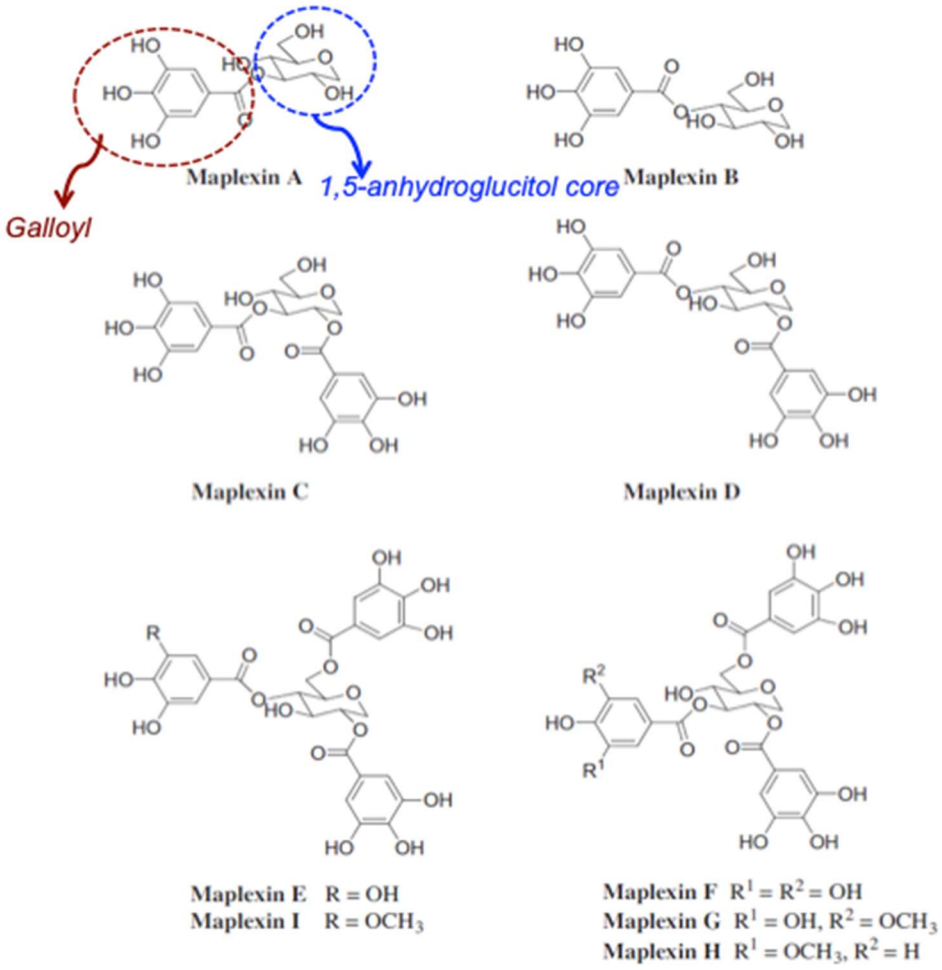

Figure 1.1 Maplexin A-I from red maple bark. Maplexin is a gallotannin with 1,5 anhydroglucitol moieties. Modified (González-Sarrías, Yuan, et al., 2012).

1.1.3.2 Lignans, and simple phenolics

In contrast to RMB extractives, lignans, benzoic acid derivatives, and other simple phenolic compounds were determined as major polyphenols in sugar maple bark (Yuan et al., 2011). Out of 15 lignans identified in sugar and red maple plant parts, six are found in the bark of these species (**Table 1.3**). Lignans are categorized into lignanes, neolignanes, oxyneolignanes, cyclolignanes, coumarins and simple phenylpropanoids (Bi et al., 2016). SMB contains four lignans; saccharumoside A, Icariside E4, syringaresinol-*β*-D-glucopyranoside, and scopoletin (listed in **Table 1.3**). The former two belong to neolignanes, third to lignanes, and the last one to coumarins. Lignan precursors are also found in a range of plant-based foods including legumes, grains such as flaxseeds, sesame seeds, fruits and

vegetables, tea, wines, etc, which is comprehensively reviewed in the literature (Landete, 2012). Lignans are reported to exhibit potential health beneficial effects such as antioxidant, anticancer, and prevention of cardiovascular diseases (Landete, 2012).

Benzoic acid derivatives and simple phenolic compounds are also main polyphenols found in SMB. Out of 13 benzoic acid derivatives and simple phenolic compound in different plant parts of RMB and SMB, eight are reported in the SMB (**Table 1.3**). Three benzoic acid derivatives are saccharumosides B, saccharumosides C, and saccharumosides D, and remaining five compounds are phenolic glycosides (see in Table 1.3). **Figure 1.2** highlights the structure of saccharumosides A-D, which are glycosides of benzoic acid derivatives and have been isolated from sugar maple bark (Yuan et al., 2011).

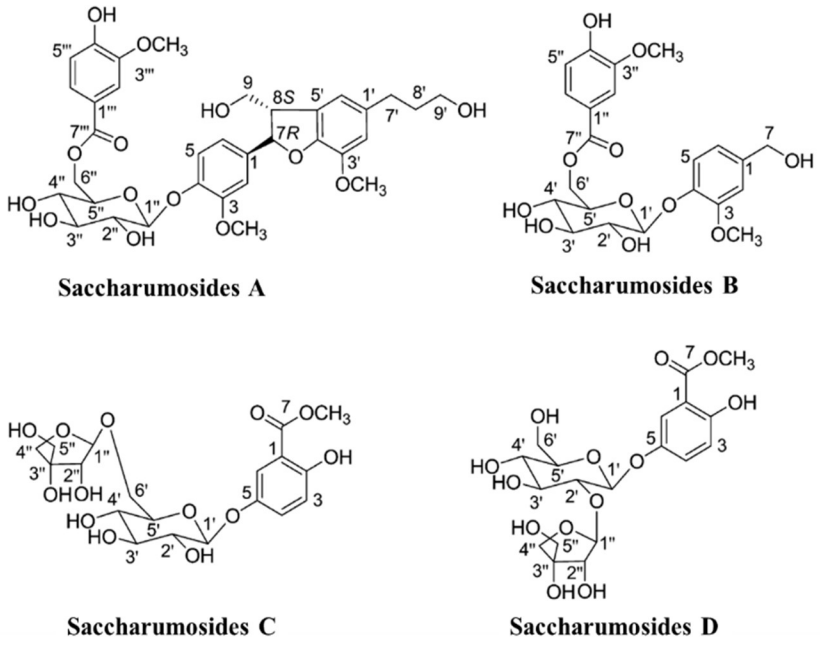

Figure 1.2 Structure of saccharumosides A-D elucidated from sugar maple bark. (Modified from Yuan et al., 2011).

1.1.4 Extraction of polyphenols

Polyphenols are originally present within the complex matrix of plants. Extraction plays a crucial role to release the polyphenols from the complex matrix. The extractions

commonly consist of three steps; (a) the diffusion of the solvent into the plant matrix, (b) solubilisation of the polyphenols within the plant matrix, and (c), diffusion of polyphenol-rich solvent out of the matrix. Extraction of polyphenols from plant source depends on several factors such as: solvent, temperature, extraction time, plant matrix (e.g. particle size), and extraction method used (Harbourne, Marete, Christophe, & Riordan, 2013; Naczk & Shahidi, 2004).

Prior to extraction, the sample preparation steps are performed which consist of drying and grinding. Generally, bark is air-dried at low temperatures of less than 40 °C to avoid the probable self-condensation of the extractives and undesirable bonding between the extractives and fiber or proteins that can negatively impact the extraction yield (Feng et al., 2013). Subsequently, dried bark is ground to obtain proper particle size for extraction. The smaller particle size increases the yield of extraction due to improved mass transfer resulting from the increase in specific surface area of particles. Geoffroy, Fortin, & Stevanovic, (2017) achieved higher extraction yield with smaller particle size, when the maple bark of two particle sizes (<250µm and 250-500µm) was studied.

The choice of solvent is one of the most important factors for extraction. As mentioned earlier, the extraction of polyphenols from plant matrix depends on the solubilisation of polyphenols into the solvent (Harbourne et al., 2013). Therefore, based on the polarity of targeted polyphenols, the best solvent can be selected. **Table 1.4** shows some of the common solvent used to extract polyphenols or bioactive compounds (Azmir et al., 2013).

Table 1.4 Example of some solvents used to extract polyphenols or bioactive compounds.

Water	Ethanol	Methanol
Tannins	Tannins	Anthocyanin
Saponins	Phenolic compounds	Terpenoids
Anthocyanins	Flavonol	Saponins
Terpenoids	Terpenoids	Tannins
	Alkaloids	Flavones
		Phenolic compounds

Different solvents such as methanol, ethanol, water and the combination of these solvents have been studied to extract polyphenols from sugar and red maple bark (Geoffroy, Fortin, et al., 2017; González-Sarrías, Li, & Seeram, 2012; Royer et al., 2011; F. St-Pierre, Achim, & Stevanovic, 2013). **Table 1.5** summarizes the solvent type used, extraction conditions, yield and total phenolic content (TPC) of maple barks reported by different authors. Regardless of types of solvent used, higher extractive yield were obtained from red than from sugar maple bark. The extractive yield of RMB is in the range of 8-21.2%, compared to 1.8-8.5% for SMB. Similarly, the TPC is typically higher in the extractives of RMB (322-540mg GAE/g dry extract) than SMB (170-328mg GAE/g extract), as shown in **Table 1.5**. Among studied solvents, hot water has mostly provided the higher extraction yield from maple barks than other solvents (methanol and ethanol). These results are due to the presence of hydrophilic polyphenols, such as tannins and phenolic acids, along with sugars and their glycosides, present in abundance in maple bark. It is important to note that high extraction yield may not necessarily indicate high TPC of extractives. For instance, Royer et al. (2011) reported that the use of hot water extraction (HWE) resulted in higher extraction yield from red maple stem bark but lower TPC when compared to maceration in 95% aqueous ethanol. However, HWE led to a higher total hydrocinnamic acid (2.7 times) and total flavonoids content (7.4 times) than 95% aqueous ethanol. This is due to the differences in the molecular affinity of solvent and solute. Besides the molecular affinity of solvents and solute, it is also important to consider environmental safety, human toxicity, and financial feasibility while selecting a solvent (Azmir et al., 2013; Harbourne et al., 2013).

Table 1.5 Extraction of sugar and red maple bark in different solvent, extraction yield and total phenolic content.

Species	Solvent used	Extraction conditions	Yield	Total phenolic content	Ref.
Sugar maple bark	EtOH	-5:1 (w/v) (bark:solvent) -Soaked for 48h	n/a	n/a	(Omar et al., 2000)
	MeOH	-4.2:10 (w/v) (powder:solvent) -Maceration at room temperature for 7days	6.1%	n/a	(Yuan et al., 2011)
	95% aq. EtOH	-1:10 (w/v) -Maceration at room temperature for 24h	1.8%	170mg GAE/g	(F. St-Pierre et al., 2013)
	MeOH	-1:10 (w/v) three times -Maceration at room temperature	8.5%	328.5mg GAE/g	(González-Sarrías, Li, & Seeram, 2012)
	Hot water	-Medium and fine particle sizes -1:5, 1:10,1:20 (bark:solvent) -At 60, 80, 100°C -Under reflux for 1h and 2h	6.9-7.3%, dry bark	298.6mg GAE/g	(Geoffroy, Fortin, et al., 2017)
Red maple bark	EtOH	-5:1 (w/w) bark powder:solvent -Soaked for 48h	-	n/a	(Omar et al., 2000)
	95% aq. EtOH	-1:10 (bark:solvent) -Maceration at room temperature for 24h	12.5%, dry bark	494.3mg TA/g	(Royer et al., 2011)
	Hot water	-1:10 (bark:solvent) -Under reflux for 1h	21.2%, dry bark	323.6mg TA/g	(Royer et al., 2011)
	MeOH	-1:10 (w/v) three times -Maceration at room temperature	8.0%	322.4mg GAE/g	(González-Sarrías, Li, & Seeram, 2012)
	Hot water	-Medium and fine particle sizes -1:5, 1:10,1:20 (bark:solvent) -at 60, 80, 100°C -Under reflux for 1h and 2h	18.2-20.2%, dry bark	528-540mg GAE/g	(Geoffroy, Fortin, et al., 2017)

EtOH, ethanol; MeOH, methanol; GAE, gallic acid equivalent; TA, tannic acid; n/a, not available.

It is reported that tannins are traditionally extracted with water. For instance, water has been widely used to extract polyphenols from bark of other trees including *Acacia mangium*, *Acacia auriculiformis*, *Rhizophora apiculate*, and *Larix leptolepis* (Makino, Ohara, & Hashida, 2009; Raju, Jonathan, & Rao, 2008; Yusoff, Chew, Ali, & Nasir, 1989). In recent years, hot-water extraction (HWE) method has been extensively used to extract polyphenols from maple barks (Geoffroy et al., 2018; Geoffroy, Fortin, et al., 2017; Royer et al., 2011). **Figure 1.3** depicts the schematic representation of the hot-water extraction (HWE) method. In HWE method, the bark powder, dispersed in water, is heated at controlled temperature for desired duration under reflux. A thermocouple (TC) probe is dipped in solution to set the appropriate extraction temperature. A mechanical stirrer can be used to homogenize the solution during extraction and facilitate the mass transfer.

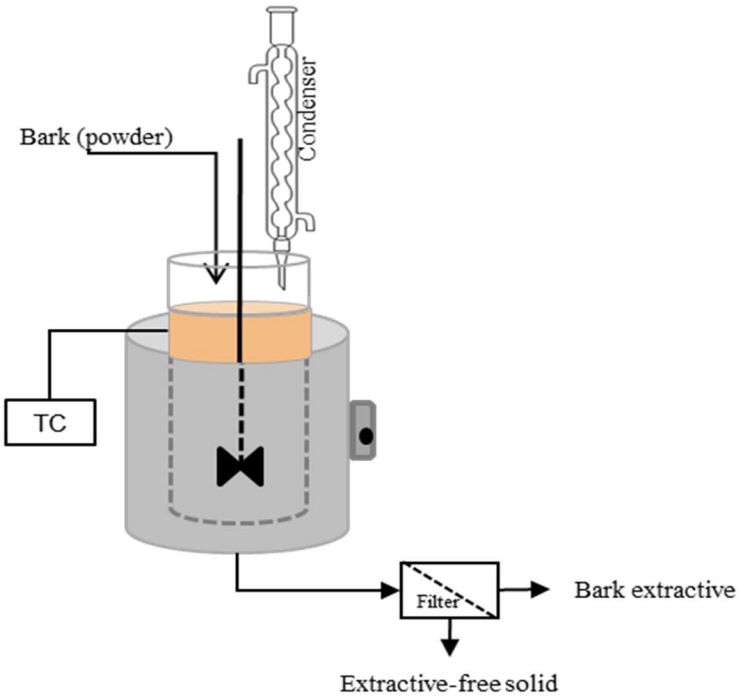

Figure 1.3 A schematic representation of hot-water extraction (HWE) method (TC, thermocouple).

HWE method has several advantages over extractions with organic solvents: (a) it uses water as the solvent which is an effective, low-cost and 'green' solvent; (b) it is the best suited method if the potential use of extract is in food applications; (c) it is relatively faster (normally takes about 1-2h) than maceration (more than 24h); (d) it does not use expensive

organic solvent and hence causes less harm to environment; and (e) it is easy to scale-up. More recently, HWE method was optimized and scaled-up to extract polyphenols from maple bark (Geoffroy, Fortin, et al., 2017). **Table 1.6** shows the optimized conditions determined: particle size of <250µm, 1:10 (bark: solvent) ratio, 80-100°C temperature for 1h (Geoffroy et al., 2018; Geoffroy, Fortin, et al., 2017).

Table 1.6 Optimized conditions for hot water extraction of sugar and red maple bark.

Variables	Studied conditions	Optimized condition
Particle size (µm)	<250	<250
	250-500	
T (°C)	60	80-100
	80	
	100	
Bark:water ratio	1:5	1:10
(w/v)	1:10	
	1:20	
time (h)	1	1
	2	

1.1.5 Bioactivities of maple barks' extracts

Sugar and red maple barks have a long history of use in traditional medicine (Arnason et al., 1981). This has led to numerous studies of the beneficial activities of the bark extracts in recent years. The crude extract and isolated polyphenols from the bark of these species have demonstrated many-fold beneficial effects such as antioxidant, anticancer, antimicrobial and α-glucosidase inhibitory activities. **Table 1.7** summarizes some of the bioactivities of sugar and red maple bark extracts.

1.1.5.1 Antioxidant activity

Polyphenols have gained immense attention mainly due to their antioxidant or free radical scavenging activity. In the living organisms, oxidative stress by free radicals is a significant event occurring in cells. These radicals are normally balanced by the endogenous enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase).

Antioxidants collectively act against free radicals to prevent their damaging effects to vital biomolecules and body tissues. However, exposure to several harmful factors, such as pollution, smoking, alcohol and chemicals, causes the overproduction of free radicals. Consequently, overproduction of free radicals leads to a condition called oxidative stress. Oxidative stress is considered as a precursor to a wide range of human degenerative diseases, such as cancer, cardiovascular diseases, Alzheimer's disease, and autoimmune disease (Auten & Davis, 2009; Laguerre, Lecomte, & Villeneuve, 2007). Therefore, antioxidant agents are of significant interest to control oxidative stress-mediated diseases.

Depending on the extraction method, and measurement method of the antioxidant activity, sugar and red maple bark extracts have shown wide range of antioxidant activities (Geoffroy et al., 2018; Geoffroy, Fortin, et al., 2017; Royer et al., 2011). In literature, the antioxidant activity of maple bark extracts is mostly compared with the commercial antioxidant agent, Oligopin®, an extract from the French maritime pine bark (**Table 1.7**). Hot water extract of RMB was reported to be a stronger radical scavenger than an ethanolic extract for scavenging different radicals; reactive oxygen species, ROS (O_2^-, $HO^\cdot$, $ROO^\cdot$, ClO^-, H_2O_2) and reactive nitrogen species, RNS (NO) (Royer et al., 2011). In addition, antioxidant efficiency of RMB extracts was superior to Oligopin®, mainly for scavenging O_2^-, $HO^\cdot$, ClO^-, and NO. Further, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity of hot-water extract of RMB was from 3.7 to 8.2 times, and 3 times higher than SMB extracts, and Oligopin®, respectively (Geoffroy et al., 2018; Geoffroy, Fortin, et al., 2017). Moreover, among isolated compounds (Ginnalin A and C) from RMB extract, Ginallin A exhibited stronger DPPH radical scavenging capacity than gallic acid, and ascorbic acid (Geoffroy, Meda, & Stevanovic, 2017).

However, there is no literature on antioxidant activity of maple bark extracts determined by other well-known assays such as 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and oxygen-radical absorbance capacity (ORAC) assays. The determination of antioxidant activity by ABTS assay is based on electron donor, a similar method to that of DPPH assay. On the other hand, ORAC assay is based on hydrogen atom donor method. Hence, it is important to investigate antioxidant activity of plant extracts using a method based on both mechanisms. Overall, the studies in the literature indicated that

phenolic compounds, mainly gallotannins present in maple bark extracts, are responsible for their antioxidant activity.

1.1.5.2 Anticancer and α-glucosidase inhibitory activities

Studies on animals or cultured human cell lines indicated that polyphenol plays an important role in reducing the risk of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, etc. (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Thomasset et al., 2007). Several authors have also investigated the effect of isolated compounds from SMB and RMB on anticancer and α-glucosidase inhibitory activities (**Table 1.7**).

Nine structurally related gallotannins (maplexin A-I) isolated from RMB were evaluated for anticancer effects against human tumorigenic (colon and breast) and non-tumorogenic (colon) cell lines. The maplexins C-D (containing two galloyl groups) showed greater antiproliferative effects than maplexins E-I (containing three galloyl groups), while no effect of maplexin A-B (one galloyl group) on cancer cell growth was noticed (González-Sarrías, Yuan, et al., 2012). For example, the IC_{50} (concentration that inhibit cell growth by 50%) value of maplexin C-D and maplexin E-I to inhibit the growth of colon cancer cell line was 59-67 and 73-165µM, respectively. The lower the IC_{50} value, the higher the antiproliferative effects. Similarly, antiproliferative effects of ginnalins A-C isolated from RMB were investigated on colon and breast cancer cell lines (González-Sarrías, Ma, Edmonds, & Seeram, 2013). Among studied compounds, ginnalin A inhibited 80% colon and 49% breast cancer cell lines, compared to ginnalin B and C, which inhibited only 50% colon and 30% breast cancer cell lines. In the case of antioxidant activity, (Geoffroy, Meda, et al., 2017) found that ginnalin A had better DPPH scavenging capacity than ginnalin C. These results suggest that a number of the galloyl groups attached to the 1,5-anhydro glucitol moiety of gallotannins have significant effect on antiproliferative as well as antioxidant activity.

In the case of SMB extract, twelve isolated compounds including saccharumosides A-D and other phenolic glycosides reported non-cytotoxicity on human tumorigenic and non-tumorigenic colon cell lines (Yuan et al., 2011). However, no detailed explanation on cytotoxicity or effectiveness of these isolated compounds from SMB on antiproliferative

effects was mentioned. It is also important to note that most of the antiproliferative activities of maple bark were carried out with isolated compounds; however, there could be synergistic effect on antiproliferative activity if crude bark extracts are tested on cell lines.

The incidence of diabetes is growing rapidly worldwide and there is increasing interest on exploring the existing natural therapeutic agents for controlling the prevalence of diabetes. One of the possible approach for type-2 diabetes management could be the inhibition of α-glucosidase, a carbohydrate hydrolyzing enzyme (Kawamori et al., 2009). Recent study on gallotannins isolated from RMB, maplexin F-I (IC_{50}= 7-16µM) were found to be 10-20 times more potent to inhibit α-glucosidase than the clinical drug (Acarbose, IC_{50}= 161µM). There is no literature on α-glucosidase inhibitory activity of crude or isolated compound from the bark of sugar maple tree. However, acertannin (2,6-di-O-galloyl-1,5-anhydro-D-glucitol) isolated from the methanolic extract of sugar maple leaves was reported to exhibit α-glucosidase inhibitory activity in both *in vitro* and *in vivo* experiments (Honma, Koyama, & Yazawa, 2010). Acertannins are galloyl derivatives, which showed similar inhibitory activity as tannins from different sources such as epigallocatechingallate from tea and ellagitannins from clove (Gamberucci et al., 2006; Toda, Kawabata, & Kasai, 2001).

1.1.5.3 Antimicrobial activity

Some studies have also demonstrated the potential of sugar maple and red maple bark extracts as antibacterial and antifungal agents (**Table 1.7**) (Jones et al., 2000; Omar et al., 2000). Antimicrobial activities with eight strains of bacteria (gram positive strains; *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycobacterium phlei*, *Bacillus subtilus*, and the gram negative strains; *Escherichia coli* wild strain, *Pseudomonas aeruginosa* 187 (wild), *Salmonella typhimurium*, and *Klebsiella pneumonia*) and six strains of fungi (*Saccharomyces cerevisiae*, *Crytococcus neoformans, Candida albicans, Aspergillus fumigatus, Microsporum gypseum* and *Trichophyton mentagrophytes*) were tested (Omar et al., 2000). Most of these microbial species are responsible for food spoilage, various infections and are toxic to human. The crude ethanolic extracts of both SMB and RMB were found to be active against tested bacteria, particularly against gram-positive bacteria (Omar et al., 2000). RMB extract was more effective against fungi than the SMB extract. Antimicrobial activities of maple bark extract could be attributed to the presence of phenolic compounds.

Table 1.7 Bioactivities of crude extracts or isolated polyphenols from sugar maple (SMB) and red maple bark (RMB).

Species	Bioactivities	Crude/isolated extract	Key results	Ref.
SMB	Antioxidant	Hot water crude extract	-Scavenge DPPH radical (1303-1673µmol TE/g), comparable to Oligopin ® (1930µmol TE/g)	(Geoffroy, Fortin, et al., 2017)
		Hot water crude extract	-DPPH (525-561µmol TE/g)	(Geoffroy et al., 2018)
	Anticancer	Twelve isolated compounds	-Cytotoxicity effect studied against human colon cancer cell lines -None of the studied compounds were cytotoxic	(Yuan et al., 2011)
	Antimicrobial /antifungal	Crude ethanolic extract	Active against strains of bacteria and fungi	(Omar et al., 2000); (Jones et al., 2000)
RMB	Antioxidant	Crude ethanolic/hot water extract	-Scavenge five ROS ($O_2^{\cdot-}$, $HO^{\cdot}$, $ROO^{\cdot}$, ClO^{-}, H_2O_2) and RNS (NO) -Mostly efficient than commercial antioxidant (Oligopin®) -Hot water extract was better than ethanolic extract - IC_{50} values of hot water extract: 0.057, 0.430, 0.588, 0.236, 0.683 and 0.919mg/ml for $O_2^{\cdot-}$, $HO^{\cdot}$, $ROO^{\cdot}$, ClO^{-}, H_2O_2, and NO, respectively.	(Royer et al., 2011)
		Hot water crude extract	-Scavenge DPPH radical (5510-6277µmol TE/g) compared to Oligopin ® (1930µmol TE/g)	(Geoffroy, Fortin, et al., 2017)
		Ginnalin A, C	-Ginnalin A was better in DPPH radical scavenging than gallic acid and ascorbic acid	(Geoffroy, Meda, et al., 2017)
		Hot water crude extract	-DPPH (4347-5275µmol TE/g)	(Geoffroy et al., 2018)

Anticancer	Maplexin A-I	-Tested against pool of colon and breast cancer cell lines -Maplexin C-D (IC_{50}=59-108µM) showed greater antiproliferative effects than Maplexin E-I (73-182µM), while no effect of Maplexin A-B was observed	(González-Sarrías, Yuan, et al., 2012)
	Ginnalin A-C	-Ginnalin A was more effective on antiproliferative effect than Ginnalin B and C against colon and breast tumorogenic cells	(González-Sarrías et al., 2013)
Antimicrobial	Crude ethanolic extract	Inhibit bacterial and fungal growth	(Omar et al., 2000)
α-glucosidase inhibitory activity	Maplexin F-I & Rubrumosides A-B	-Maplexins were effective in inhibiting α-glucosidase compared to rubrumosides -Maplexins found to be more potent than clinical drug Acarbose (IC_{50}=7-15 vs 161µM)	(Yuan et al., 2012)

1.1.6 Potential utilization of maple bark extracts as natural food ingredients

The results of the studies discussed previously indicate that sugar maple and red maple bark extracts have high antioxidant capacity when tested by DPPH radical and other radicals scavenging capacity assays. Hot water extract from RMB demonstrated strong antioxidant capacity, even 3-folds better than commercially available French maritime pine bark extracts (Geoffroy, Fortin, et al., 2017; Royer et al., 2011). The presence of phenolic compounds is found to be responsible for the strong antioxidant activity in maple bark extracts. In addition, crude extracts of maple bark demonstrated antimicrobial activity against a pool of bacterial and fungal strains (Jones et al., 2000; Omar et al., 2000). Due to their antioxidant and antimicrobial activities, maple bark extracts have potential to be used as natural food ingredients in order to preserve and improve the shelf life, and the overall quality of foods. Despite their promising potential as food ingredients, there is no record in the literature of using maple bark extracts in any food and food products.

In general, plant extracts rich in polyphenols can be utilized in food as ingredient for three main reasons, (a) to preserve, (b) to fortify, and (c) to enrich. When the ingredients are added in food for the above-mentioned reasons, they are collectively known as food

additives. There are numerous examples available in literature, wherein plant extracts such as kiam wood, chamomile, fennel, and olive leaves extracts were successfully incorporated in foods as ingredient to either preserve, fortify or enrich the food (Caleja et al., 2016; Caleja, Barros, Antonio, Oliveira, & Ferreira, 2017; Lalas et al., 2011; Maqsood, Benjakul, & Balange, 2012; Paiva-Martins, Correia, Félix, Ferreira, & Gordon, 2007). For instance, ethanolic extract of kiam wood (at 0.08% w/w) that is rich in tannic acid (545.5mg/g dry powder) was able to significantly retard lipid oxidation in fish emulsion sausages, hence preserving the quality during storage (Maqsood et al., 2012).

In another study, the water extracts of chamomile (rich in phenolic acids) and fennel (rich in quercetin-3-O-glucoside) were used as natural additives to fortify yogurts (Caleja et al., 2016). The effect of natural additives was also compared with synthetic additive (potassium sorbate, E202, used as preservatives in food to inhibit microbial growth). The fortified yogurts with chamomile extracts (40mg extract in 100g yogurt) showed significantly higher antioxidant activity than the fennel extract and potassium sorbate (fortified with same quantity in yogurt). In addition, chamomile extracts did not alter the nutritional properties of yogurt during a normal storage period. In the same way, fennel and chamomile aqueous extracts incorporated as natural antioxidants in biscuits showed similar performance when compared with butylated hydroxyl anisole (BHA, synthetic antioxidant) (Caleja et al., 2017). Butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) are commonly used synthetic antioxidants in food. However, consumers' preference to natural products as well as strict regulation on the use of synthetic antioxidants have directed research on finding natural antioxidants. The enrichment of foods with the polyphenols extracted from the same plant species are reported in literature. For instance, table olives and olive oil were found to be enriched with the addition of phenolic compounds extracted from olive leaves (Lalas et al., 2011; Paiva-Martins et al., 2007).

Overall, these studies suggest that maple bark extracts have huge potential to be used as natural food ingredients: (a) extracts can be used as a preservative agent in multiple foods such as fruits, meats, etc. Due to their high antioxidant capacity, extracts can limit the deteriorating events such as browning that is common in fruits, and lipid peroxidation in meat products; (b) extracts can be used to fortify cookies and dairy products that inherently lack polyphenols. In addition to their fortifying potential, maple bark extracts can act as

antimicrobial agents (instead of using synthetic antimicrobial) to the food products that are highly susceptible to the growth of harmful microorganism; and (c) maple bark extracts can be used to enrich a variety of maple-based products such as maple syrup, maple sugar, candy, cream, etc. to become rich in polyphenols.

Therefore, the following section of the literature review will discuss maple syrup and its derived product, maple sugar. The transformation process of syrup into maple sugar or maple sugar powder will be discussed. At last, different quality parameters of the produced powder will be reviewed.

1.2 Maple syrup

1.2.1 Chemical composition

Maple syrup is a non-timber forest product, produced by concentrating sap tapped from maple trees, mainly from sugar and red maple. Maple sap is a sweet water-like solution containing around 2-3% of sucrose, organic compounds, and minerals such as calcium, magnesium and potassium (Ball, 2007). The main organic compounds found in sap are amino acids, protein, and phenolic compounds (Legault, Girard-Lalancette, Grenon, Dussault, & Pichette, 2010).

Maple syrup is obtained by evaporation process that involves heating the sap until the sucrose concentration reaches to ca. 66%. The major sugar in syrup is sucrose followed by glucose and fructose. Besides sugars, a wide variety of compounds such as minerals, organic acids, amino acids and polyphenols are present at less than 1% in syrup (Ball, 2007; Sadiki & Martin, 2013). However, the composition of syrup may vary depending on the geographical location, sap and syrup processing and handling. The average composition of maple syrup is given in **Table 1.8**.

Table 1.8 Average composition of maple syrup containing sugars, minerals, and organic acids.

	Compositions	**Quantity/concentration**
Major constituent (%)	Sucrose	68.0±4.0
	Glucose	0.43±1.1
	Fructose	0.30±0.54
	Water	31.7±2.9
Organic acids (%)	Malic acid	0.47±0.11
	Fumaric acid	0.004±0.002
Minerals (in ppm)	Potassium	1300-3900
	Calcium	400-2800
	Magnesium	12-360
	Maganese	2-220
	Phosphorus	79-183
	Iron	0-36
	Zinc	0-90

Recently, several studies have focused on identifying the polyphenols in maple syrup due to their antioxidant activities. Maple syrup was reported to present numerous classes of polyphenols including lignans (lignanes, oxyneolignanes, coumarins, simple phenylpropanoids, etc.), simple phenolic compounds, and benzoic acid derivatives (Li & Seeram, 2010, 2011a, 2011b; Sadiki & Martin, 2013; Y. Zhang et al., 2014). **Table 1.9** highlights the fifty-eight polyphenols identified thus far in maple syrup. Majority of polyphenols in syrup come from the lignans family followed by simple phenolic and benzoic acid derivatives, and others such as stilbenes, furfural, etc.

Table 1.9 Major polyphenols identified in maple syrup (Li & Seeram, 2010, 2011a, 2011b; Sadiki & Martin, 2013; Y. Zhang et al., 2014).

Polyphenol *(No. of compounds)*	**Sub-class of polyphenols**	**Identified compounds** *(No. of compounds)*
Lignans *(32)*	Lignanes	Secoisolariciresinol; Syringaresinol; Buddlenol E; 7,9'-epoxylignanes derivatives *(3)*
	Neolignanes	Sakuraresinol; Dehydroconiferyl alcohol; Acernikol; 4',7-epoxy-8,3'-neolignanes derivatives *(2)*
	Oxyneolignanes	8,4'-oxyneolignanes derivatives *(9)*
	Cyclolignanes & coumarins	Lyoniresinol; Isolariciresinol; Fraxetin; Isofraxidin
	Simple phenylpropanoids	Syringenin; (*E*)-Coniferyl alcohol; Dihydroconiferyl alcohol; 2,3-Dihydroxy-1-(3,4- dihydroxyphenyl)-1-propanone; 2,3-Dihydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone; C-yeratroylglycol; Quebecol; 3-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-propan-1-one;
Simple phenolics & Benzoic acid derivatives *(14)*		Catechol (1,2-dihydroxybenzene); 4-Hydroxycatechol; Catechaldehyde; Vanillin; Syringaldehyde; 4-Acetylcatechol; 3',4',5'-Trihydroxyacetophenone; 3,4-Dihydroxy-2-methylbenzaldehyde; 2-Hydroxy-3',4'-dihydroxyacetophenone; 1-(2,3,4-Trihydroxy-5-methylphenyl)ethanone; 2,4,5-Trihydroxyacetophenone; 4-(Dimethoxymethyl); pyrocatechol; Syringic acid; Trimethyl gallic acid methyl ester
Other compounds *(12)*		(E)-3,3'-Dimethoxy-4,4'-dihydroxystilbene; 4,4'-Dihydroxy-3,3',5,5'-tetramethoxystilbene; 4,4'-Dihydroxy-3,3',5'-trimethoxystilbene; 1,2-Diguaiacyl-1,3-propanediol; (6R)-6-Hydroxy-3-(hydroxymethyl)-2- cyclohexanone; 3,4-Dihydro-5-(hydroxymethyl)pyran-2-one; 4-Hydroxy-2-(hydroxymethyl)-5-methyl-3(*2H*)- furanone; 5-(Hydroxymethyl)furfural; Benzenemethanol; Phaseic acid; 4-Methyl-1,2-venzenediol; 4-(Hydroxymethyl)-1,2-benzenediol

Number in parenthesis indicates the number of identified polyphenols.

1.2.2 Beyond sweetness: beneficial effect of maple syrup extract

Maple syrup is more than a natural sweetener as it contains a wide array of minerals, micronutrients, and important polyphenols. *In vitro* and *in vivo* biological studies of polyphenols rich-extract derived from maple syrup were reported to show potential antioxidant, anticancer, α-glucosidase enzyme inhibitory, and anti-inflammatory effects (Apostolidis, Li, Lee, & Seeram, 2011; González-Sarrías, Li, Seeram, & Gonza, 2012; Legault et al., 2010; Thériault, Caillet, Kermasha, & Lacroix, 2006; Y. Zhang et al., 2014). Some bioactivities of maple syrup extracts and polyphenolic compounds derived from syrup are listed in **Table 1.10**.

Maple syrup extracts have shown interesting antioxidant activities. Antioxidant activity of ethyl acetate extract of maple syrup was comparable to that of strawberry and orange juices when determined by oxygen radical absorbance capacity (ORAC) (Legault et al., 2010). Maple syrup extract has demonstrated strong diphenylpicrylhydrazyl (DPPH) radical scavenging activity (IC_{50}=77.5µg/ml), even superior to a synthetic antioxidant (butylated hydroxytoluene, BHT; IC_{50}=660µg/ml) (Li & Seeram, 2011a). BHT is widely used as a synthetic antioxidant in food industries. In addition, some of the isolated polyphenols, mainly the phenolic compounds such as catechaldehyde from maple syrup have shown stronger antioxidant (IC_{50}=31-51µM) than vitamin C (IC_{50}=58µM) as well as BHT (IC_{50}=2651µM) to scavenge DPPH radicals (Li & Seeram, 2010).

Maple syrup extracts have demonstrated antiproliferative activities (*in vitro*) on different conditions of colon cells (González-Sarrías, Li, Seeram, et al., 2012). The extracts were found to be more active against tumourigenic than non-tumourigenic colon cells. It was reported that syrup extract inhibited the nitric oxide (NO) overproduction in RAW264.7 murine macrophages, indicating its potential anti-inflammatory activity (Legault et al., 2010). About 75% of NO inhibition was achieved with a dose of 25µg/ml of syrup extract.

In another *in vitro* study, phenolic-enriched extracts of maple syrup exhibited potential agents for type-2 diabetes management (Apostolidis et al., 2011). The extract inhibited porcine α-amylase and rat α-glucosidase enzymes, enzymes that are responsible for hydrolyzing dietary carbohydrates to be absorbed by the small intestine, thus affecting the glucose blood level. Phenolic-rich maple syrup extract, obtained from the sap of sugar and

red maple species, exhibited strong antimicrobial activity and they found that catechol presented strong synergy with antibiotics against bacterial growth of Gram-negative strains (Maisuria, Hosseinidoust, & Tufenkji, 2015).

Moreover, an *in vivo* study highlighted that maple syrup showed lower response to glucose, insulin, amylin and gastric inhibitor polypeptide (GIP) than other studied natural sweeteners (corn syrup, brown rice syrup, and dextrose solution), thus, indicating that maple syrup could be an appropriate alternative to conventional sugar as a sweetener (P. St-Pierre et al., 2014).

Table 1.10 Bioactivities of maple syrup and its polyphenolic extracts.

Activities	**Author(s)**	**Syrup extract (solvent used)**	**Method used (Chemical/*in vitro/in vivo)***	**Medium (if present)**	**Results**
Antioxidant	(Thériault et al., 2006)	EtOAc	-TBARS assay -DPD discoloration	- -	-16.8-164.5% lipid peroxidation inhibition; -39.8-98.5% free radical scavenging capacity
	(Legault et al., 2010)	EtOAc	-ORAC -DCFH-DA *(in-vitro)*	- -WS1 normal cells	-15±5µmol TE/mg; -IC_{50}=6 µg/ml
	(Li & Seeram, 2010)	BuOH	DPPH assay	-	-some isolated compounds (phenolic derivatives) were stronger antioxidant than vitamin C and BHT
	(Li & Seeram, 2011a)	EtOAc	DPPH assay	-	-Syrup extract (IC_{50}=77.5µg/ml), superior to BHT (IC_{50}=660µg/ml)
Antiproliferative/ Anticancer	(Legault et al., 2010)	EtOAc	-*In vitro* cell	-lung carcinoma; colorectal adenocarcinoma; Normal fibroblasts	Moderately active against lung cancer cells (IC_{50}=42±6µg/ml)
	(Legault et al., 2010)	Diluted syrup	*In –vitro* cell	-WS1 normal cell -lung, colorectal, breast, prostate, brain cancer	-scarcely inhibit cell growth -28 to 74% inhibition

	(González-Sarrías, Li, Seeram, et al., 2012)	EtOAc; BuOH; MeOH	*In-vitro* cell	Human tumourigenic and non-tumourigenic colon cells	-all studied extracts were more active against tumourigenic than non-tumourigenic cells -antiproliferative activity greater for grade D than C syrup.
Anti-inflammatory	(Y. Zhang et al., 2014)	EtOH followed by EtOAc extract	*In-vitro* cell	RAW 264.7 macrophages	-extracts inhibited NO and PGE_2 levels in macrophages
	(Legault et al., 2010)	EtOAc	*in –vitro* cell	RAW 264.7 macrophages	75% NO inhibition/25µg/ml extract
α-glucosidase inhibition	(Apostolidis et al., 2011)	EtOAc; BuOH	*In-vitro*	-	-rat α-glucosidase and porcine α-amylase inhibitory activities -potential for type 2 diabetes management
	(P. St-Pierre et al., 2014)	Syrup	*In-vivo*	Male rats	-lower responses of glycaemia and insulinaemia compared to brown rice syrup, corn syrup and dextrose.

EtAc: ethyl acetate; EtOH: ethanol; BuOH: butanol; NO: nitrite; TBARS: thiobarbituric reactive substances; DPD: *N,N*-diethyl-*p*-phenylenediamine; DPPH: diphenylpicrylhydrazyl; ORAC: oxygen radical absorbance capacity; DCFH-DA: 2',7'-dichlorofluorescin diacetate; TE: trolox equivalent; IC_{50}: concentration of inhibition 50% of subject of interest; BHT: butylated hydroxytoluene; PGE_2: prostaglandinE2.

1.2.3 Production, and economical interest

Canada is the leading global producer of maple syrup accounting approximately for 71% of the world's production (Statistics Canada 2017). The province of Quebec is the major contributor with 90% of the nation's production share. In 2017, nearly 12.5million gallons (75.1million kilograms) of syrup was produced in Canada. Maple syrup industries make a significant contribution to the country's economy with a value of 494 million Canadian dollars. In addition, Canada is the largest exporter of maple products to different countries around the world including United States (62% market share), Germany (11%), Japan (6%), United Kingdom (5%), Australia (4%), France (3%) and others (9%). It has generated 382 million Canadian dollar from exports in 2017, and the numbers are increasing every year (35% increase over the last five years) (Agriculture and Agri-food Canada, 2018).

1.2.4 Grading, and surplus problem

In Canada, maple syrup generally must meet strict standards. The minimum solid content required in syrup is 66-67°Brix. However, maple syrups are available in different grades due to the differences in their color. The color of the syrup becomes darker as the season of tapping (sap) progresses, from early to late spring. In 2016, the grading system of syrup was updated by reducing the number of grades from five to four (**Table 1.11**). Accordingly, the new four grades of maple syrup are golden (light transmission not less than 75%), amber (50-74.9%), dark (25-49.9%), and very dark (less than 25%) are listed in **Table 1.11**. The color is determined by photometric light transmittance at the wavelength of 560 nm, considering glycerine as a reference (100% light transmittance).

Table 1.11 New and old grading system of maple syrup in Canada.

New grading system, 2016 onwards			Old grading system		
Light transmission (%)	**Color class**	**Taste**	**Light transmission (%)**	**Grade**	**Color**
>75.0	Golden	Delicate	>75.0	Canada No.1	Extra light (AA)
50.0-74.9	Amber	Rich	60.5-74.9	Canada No. 1	Light (A)
25.0-49.9	Dark	Robust	44.0-60.4	Canada No. 1	Medium (B)
<25.0	Very Dark	Strong	27.0-43.9	Canada No. 2	Amber (C)
			<27.0	Canada No. 3	Dark (D)

Very dark color maple syrup has strong taste and is considered as substandard and not consumed widely as table syrup. Hence, the surplus of very dark syrup accumulating each year is a major problem for syrup-producers in Canada. **Figure 1.4** clearly shows the difference in the production volume and domestic sales and exports, which has created a discrepancy between supply and demand of maple syrup (Agriculture and Agri-food Canada, 2018). In 2017, the surplus of syrup was more than 38% of maple syrup produced (**Figure 1.4**). Therefore, the valorization of surplus syrup to value-added maple products, such as maple sugar and novel maple products, is very important to alleviate the economic pressure.

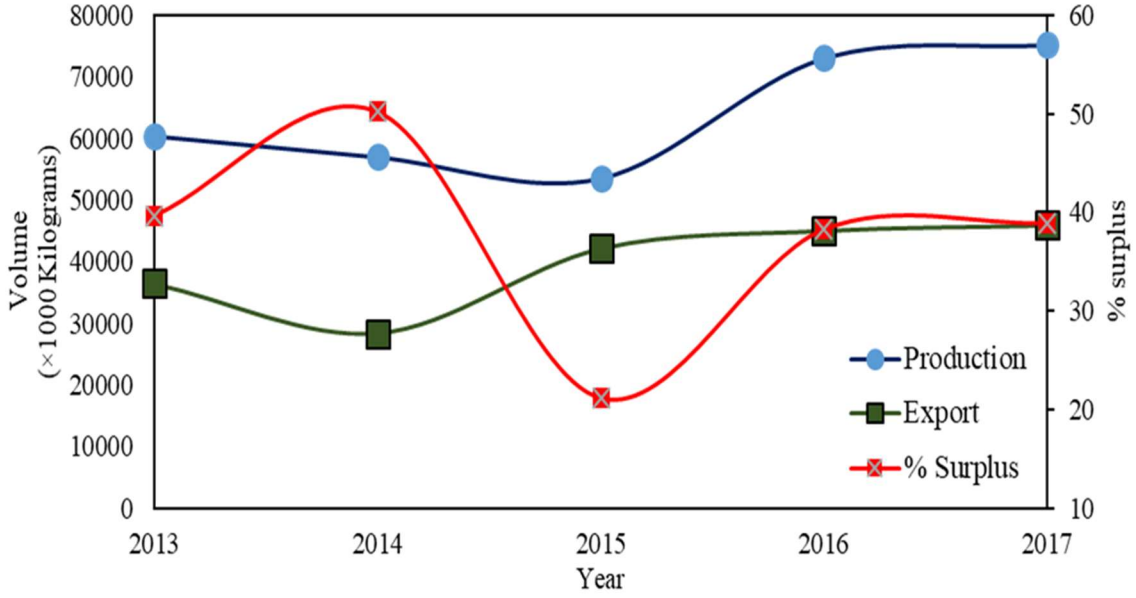

Figure 1.4 Changes in production, exports and estimated surplus of maple syrup in Canada, during 2013-2017. The percentage of surplus was estimated based on the differences of syrup production and exports data, collected from Agriculture and Agri-food Canada.

1.2.5 Transformation of maple syrup to powder

Depending on the form of raw materials (liquid or solid), various methods can be used to produce food powders. Grinding, crushing, milling, pulverization, and granulation are used for the conversion of solid materials into powder, whereas crystallization and drying are two methods used for the production of powders from liquid foods. Crystallisation has been traditionally used for the transformation of maple syrup to sugar crystals. However, there are no literature data about using drying techniques such as freeze-drying (FD), drum drying

(DD), spray drying, and belt drying for the conversion of syrup to powder. Therefore, the following discussion will briefly present the traditional method of maple sugar crystallisation followed by well-known drying techniques, mainly FD and DD, commonly applied in industries for the production of food powders, which can be used to transform syrup to powder. Decacer Inc., a maple syrup company in Quebec, uses a drum-drying technique to dehydrate the maple syrup to produce maple sugar powder and flakes. However, the scientific characterization of the final product properties and overall effect of drum drying on the polyphenols during the process has not been previously reported.

From a practical point of view, it is favourable to produce a dehydrated product, since the dry products are easier to store and transport than liquid ones. Many solid foods (fruits and vegetables) and liquid foods (juices, honey, and syrup) contain fibers, vitamins, minerals and bioactive compounds such as phenolic compounds (e.g. flavonoids, anthocyanins, etc), carotenoids, vitamins etc. Most of the time, fresh solid foods (fruits and vegetables) are processed into juice for longer-term consumption and distribution. However, their bioactive compounds are perishable and unstable due to their high sensitivity to moisture, temperature, light, air, pH conditions and other degradation-prone conditions (Fang & Bhandari, 2011). Therefore, drying of such food is necessary to preserve their intrinsic bioactive properties for long-term consumption.

1.2.5.1 Production of maple sugar crystals

In early days, maple sugar (also called stirred or Indian sugar) was the primary source of sweetener used by the Native Americans before the refined sugar was introduced to North America. During Colonial times, almost 100 percent of syrup produced was used to make maple sugar blocks. Due to the lack of appropriate jars for storing syrup, converting syrup to sugar blocks was the convenient way to store it for the long term. Sugar blocks have also been used as a form of a trade and exchange items for the coin and currency.

The recipe of preparing maple sugar is still being handed down from generation to generation among maple syrup farmers. **Figure 1.5** depicts the traditional approach of producing maple sugar crystals. It is prepared by direct heating of the syrup (66-67°Brix) in a metal vat to about 25 to 28°C above the boiling point of water and then cooled to

approximately 93°C. After cooling, cooked syrup is stirred either manually or mechanically until all the moisture is essentially removed and only crumbly and granulated sugar remains (Heiligmann, Koelling, & Perkins, 2006). During stirring, sugar crystals are sowed to initiate the maple sugar crystallisation. The obtained final products are further crushed and sieved through mesh to obtain a uniform size product. A liter of syrup can give around one kilogram of maple sugar. However, this approach is hard to control to produce a quality product, therefore the quality varies from one batch to another. In addition, it is a labour and energy-intensive method. Moreover, it requires additional steps such as drying, grinding, sieving, etc. after the formation of sugar, hence time-consuming. The production of sugar crystals from the very dark color syrup is challenging due to the presence of high percentage of invert sugar (>3%) (Aider, de Halleux, Belkacemi, et al., 2007).

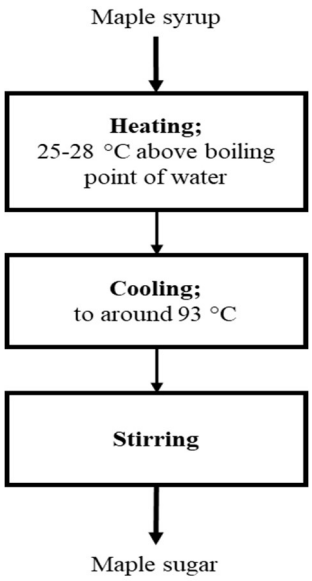

Figure 1.5 Traditional method of producing maple sugar crystals.

More than a decade ago, (Aider, de Halleux, Belkacemi, et al., 2007) used a laboratory-scale vacuum evaporator (3-liter Stephan mixer-cutter, UMC, Germany) to produce maple sugar crystals from grade D syrup (or very dark color syrup) **(Table 1.11)** in controlled conditions. Implication of vacuum helped to perform the evaporation at a lower temperature, of 90 °C, compared to the traditional method (125-128°C). High vacuum in evaporator also favored the crystallisation yield. Syrup with low invert sugar (less than 4%) resulted in higher yield, mixing speed having no significant impact on yield, while it

influenced the crystal size and shape. In another study performed by the same authors, syrup of grade D with 6% inverted sugar was used to produce granulated maple sugar in the lab-scale evaporator similar to above-mentioned (Aider, de Halleux, & Belkacemi, 2007). Only 50% crystallization was achieved with intense vacuum of 0.14 $\times 10^5$Pa (other studied vacuum pressures were 0.18 $\times 10^5$ and 0.22 $\times 10^5$Pa allowing for the crystallization of up to 40 and 17%, respectively), where the crystal growing time was 45 min. These studies highlighted the impact of technological parameters on transforming the low-grade syrup into sugar crystals. However, this technique is limited to batch process and its scaling-up could be another issue in future. Additional processing steps (removing remaining mother liquor, drying of crystals, etc.) after crystallization were still required, similar to traditional method. These studies focused only on the color parameters, and size and shape of maple sugar, whereas, effect of evaporation on nutritional properties as well as other quality parameters of final product were not investigated.

1.2.5.2 Production of maple sugar powder

Drying is the most commonly used industrial process for the production of powder from liquid food materials. Food powders have numerous practical and technological advantages such as improved shelf life, appropriate for long-term consumption and storage, facilitate its wider distribution and handling, and incorporation in solid food formulation, and ready-to-serve foods. The commercial powdered products such as instant coffee, milk powders, soups, infant formulas, energy drinks, etc. are few examples of powdered foods that are an integral part of our daily life.

1.2.5.2.1 Freeze-drying

Freeze drying (FD), also known as lyophilisation, is a well-known method for the production of high quality food powders (Karam, Petit, Zimmer, Baudelaire Djantou, & Scher, 2016; Ratti, 2001). It is a preferred method for drying the food containing thermally sensitive and prone to oxidation compounds since it operates at very low temperature and under high vacuum. FD is carried out sequentially in three-steps; the sample is frozen (freezing), sublimation of ice at sub-atmospheric pressure (primary drying, FD^{PD}), and desorption of the remaining unfrozen/bound water (secondary drying, FD^{SD}).

Freezing is the first separation step in the FD process that solidifies the food materials. During freezing, liquid food can show either eutectic (crystalline) or glass forming (amorphous) behaviour. The freezing temperature of eutectic and glass-forming samples can be determined from eutectic and glass transition temperature (T_g), respectively. It is known that the aqueous solution of low molecular weight carbohydrates (such as sucrose, glucose, and fructose) are glass forming (Levine & Slade, 1988). T_g is defined as the temperature above which an amorphous system goes from the glassy to the rubbery state. For instance, the glass transition temperatures of maximally-freeze concentrated 20% sucrose, glucose and fructose aqueous solutions are at -32, -43, and -42°C (Levine & Slade, 1988). This indicates that the freezing should be done below -32°C for 20% sucrose solution. The rate of freezing is also important for the formation and size of ice crystals, slow rate of freezing forming bigger ice crystals and vice-versa. Accordingly, the size of ice crystals formed affects the rate of drying, wherein large ice crystals are easier to sublimate and hence increase the rate of primary drying (Ratti, 2013).

In primary drying, vacuum is created and the shelf temperature is increased to commence the sublimation, however it should be noted that the shelf temperature must be 2-3°C below the product collapse temperature (T_c). Collapse temperature is the temperature above which the product loses macroscopic structure and collapses during FD process (Tang & Pikal, 2004). T_c is practically difficult to determine therefore, it is associated with the glass transition temperature (T_g). When a substance is in frozen state T_c can be generally at a temperature of 2°C higher than T_g (Patel, Doen, & Pikal, 2010; Pikal, 2004). **Figure 1.6** depicts the typical temperature profile of a product during each step of freeze-drying process.

Secondary drying is performed at elevated temperatures to remove the remaining unfrozen or bound water by desorption. However, it is challenging to identify the endpoint of primary drying or the beginning of the secondary drying phase. If temperature is increased before all of the ice is sublimated (endpoint of primary drying phase), it could collapse the product and hence, affect the final quality. Patel et al. (2010) have suggested some techniques to determine the endpoint of primary drying such as using a Pirani pressure gauge, dew point monitor, tunable diode laser absorption spectroscopy (TDLAS), gas plasma spectroscopy, thermocouple (TC) and condenser pressure. Among these techniques, Pirani, dew point,

TDLAS and TC were found to be effective for determining the endpoint of primary drying phase.

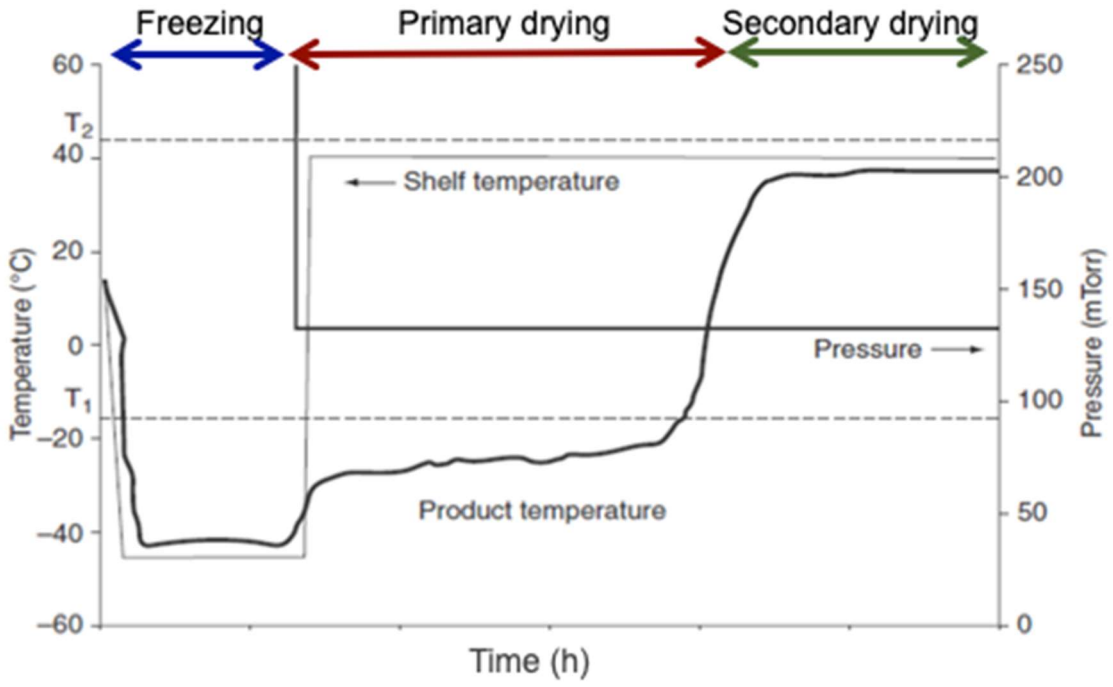

Figure 1.6 Temperature profile of product during freeze-drying process. Modified from Ratti (2013).

The quality (color, shape, aroma and nutritional value) of dried food obtained from this method is superior compared to other drying processes (Ratti, 2001). This is due to the application of a low temperature (-50 to -20°C) and vacuum during the process. Therefore, FD is widely used for the drying of high-value products including nutraceuticals, pharmaceuticals, various plant extracts, etc. However, FD is associated to a high operation cost due to the high vacuum required and it is a time-consuming process. If under-optimized, it can take from few days to a week to completely dry the product. Among the three steps of FD, FD^{PD} is the longest step, consuming nearly $2/3^{rds}$ of the total process time. Several studies reported by Pikal and other researchers have highlighted the optimization of the process and effective determination of each FD cycle (Franks, 1998; Patel et al., 2010; Pikal, 2004; Schneid, Gieseler, Kessler, Luthra, & Pikal, 2011). These literatures data can be used as a guideline to freeze-dry maple syrup to produce high quality powder.

1.2.5.2.2 Drum-drying

Drum drying (DD), is a commonly used process to dry viscous, concentrated solutions, and slurries/pastes. Powder from variety of foodstuffs such as fruit purées, mashed potatoes, dry soup mixtures, and pre-gelatinized starches are produced using drum dryers (Daud, 2006; Galaz et al., 2017; Henríquez, Córdova, Almonacid, & Saavedra, 2014; Pua, Hamid, Rusul, & Rahman, 2007; Pua et al., 2010).

The main components of drum dryer are a horizontally placed drum, steam, and motor to control the rotation speed of the drum. The size of drums may vary and are usually from 0.45 to 1.5m in diameter, from 1 to 3m in length, with 2 to 4cm of drum wall thickness (Daud, 2006). DD can be classified based on the configuration and number of rotating drums and the use of pressure (atmospheric or vacuum). Accordingly, there are five types of DD, atmospheric single drum dryer, atmospheric double drum dryer, atmospheric twin drum dryer, enclosed drum dryer, and vacuum double drum dyer. A schematic diagram of a double-drum dryer is shown in **Figure 1.7**. The choice of DD types depends on the quality requirement for the final product. For instance, vacuum double-drum drying (VDD) is appropriate for drying raw materials that involve oxygen-sensitive compound like polyphenols because of the use of vacuum. In addition, VDD has advantage of higher throughput due to increase in drying surface (two drums) over single drum dryer. Hence, our focus will be mainly on vacuum double-drum dryer.

Drum dryers conduct an indirect heat transfer through a solid surface. DD are usually fed at the nip between the drums by a pendulum nozzle in a header (**Figure 1.7**). The sample starts boiling and most of the moisture is removed at water boiling temperature at the applied pressure (vacuum) (Daud, 2006). The counter-rotating drums draw the liquid food from the nip and spread it into a thin sheet. The residence time of the sample on the drum varies from 10 to 30 seconds (Mujumdar, 2006), depending on the rotation speed of the drum. After about three-quarters of a rotation of the drums from the point of feeding, the sample is dried and scrapped off from the drum with static doctor's blade, as shown in **Figure 1.7**.

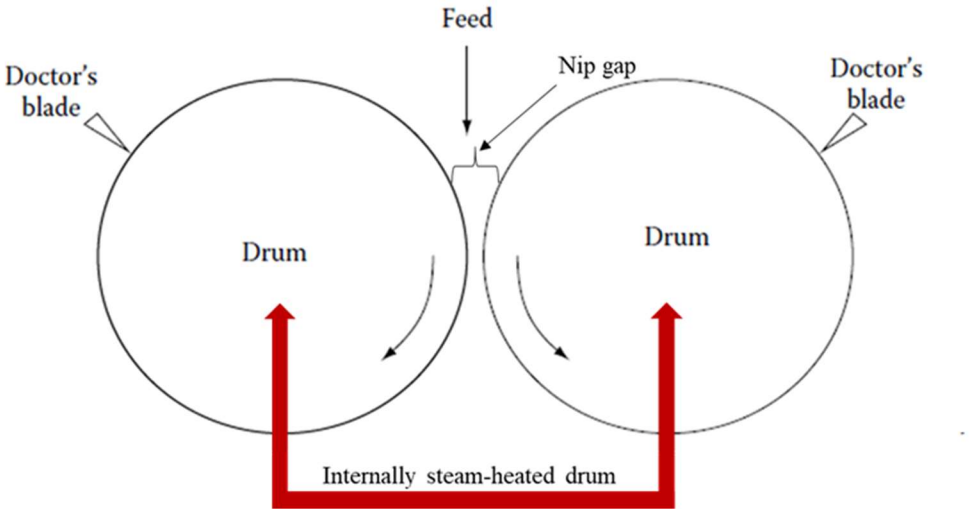

Figure 1.7 A double-drum dryer with nip feed. Modified from (Daud, 2006).

There are multiple advantages of drum drying method. The most important one is that DD can dry viscous foods that cannot be easily dried with other methods. The drum dried products have shown good rehydration property (J. Tang, Feng, & Shen, 2010), while the equipment itself is easy to operate and maintain. DD normally have high energy efficiency compared to spray dryer, tunnel or rotary dryers (Mujumdar, 2006). However, there are some pitfalls of this method, such as high sugar content foods are not easy to scrape-off from the drum. A possible scorching of the product may occur due to direct contact with the high temperature at the drum surface. The latter problem can be reduced using vacuum, which can facilitate moisture removal and hence minimize the residence time of the product on the drum surface. However, the use of vacuum increases the capital and operational costs. No literature data is available on the vacuum-double drum drying of maple syrup.

1.2.6 Quality of powders

In general, a powder is composed of particulate solid-state materials of sizes ranging from nanometers to millimeters. Different terminologies are used to designate such particulate materials, fine powder (50-200µm), granules (200-4000µm), flour (100-5000µm), and dust (5-100µm) (B. Bhandari, 2013).

There are vast numbers of properties for measuring the quality of a powder. In general, powder properties can be classified under fundamental and functional properties. The former include moisture, microstructure, bulk density, shape, size, surface composition and morphology. The latter categories include flowability, reconstitution and instantaneous properties (B. Bhandari, 2013). The functional property of a powder is greatly influenced by its fundamental property. Some key fundamental and functional properties of powder that will be discussed in the following section are shown in **Figure 1.8**.

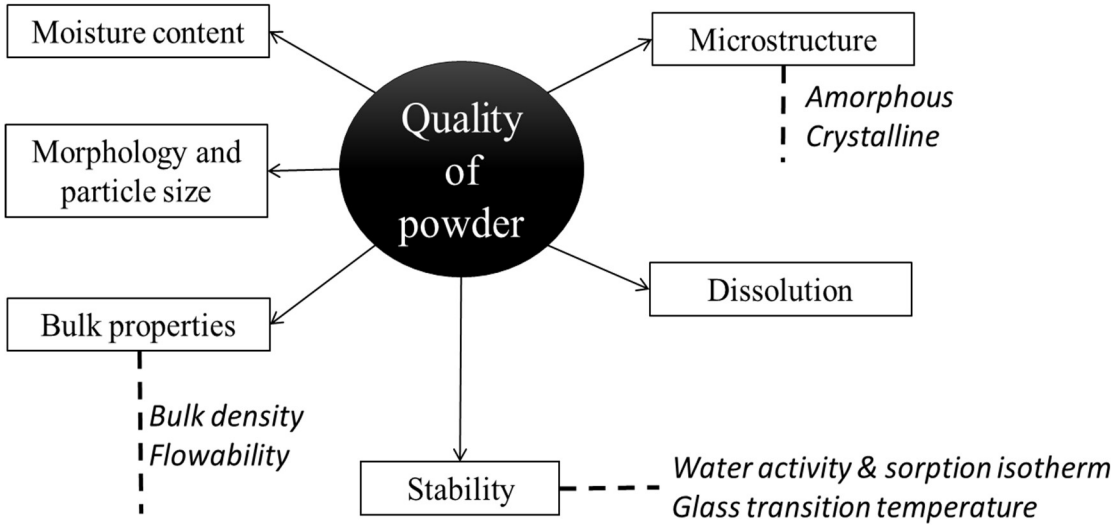

Figure 1.8 Some important fundamental and functional properties of powders.

1.2.6.1 Moisture content

Moisture content (MC) is an important parameter of a powder that affects many aspects of powder handling and processing. It influences powder cohesiveness that may result in undesirable caking and stickiness (Fitzpatrick, 2013). However, it can be useful in agglomeration and granulation processes of powder. For the dried product, moisture content is also a key factor to understand the drying performance. It gives an idea on effectiveness of drying methods to remove the moisture from the food.

Moisture content is defined in two ways, either on a dry basis or on a wet basis. Dry basis is calculated as the ratio of the mass of water in powder to mass of dry powder, whereas wet basis is the ratio of mass of water in powder to total mass of powder (including water). MC is widely measured by gravimetric method, where the sample is weighed before and after

the moisture removal by evaporation. Other methods for the determination of MC in food include Karl-Fischer titration, gas chromatography, spectroscopy, nuclear magnetic resonance, near infrared, and microwave, can be found elsewhere in the literature (Mohamed Mathlouthi, 2001).

1.2.6.2 Microstructure

Depending on the drying method used, the powders can be produced in amorphous, crystalline or in mixed forms. The type of microstructure state of powders is distinguished based on their molecular arrangement. Powders in crystalline state have well-defined molecular alignment in the long-range order, whereas for the amorphous state, the alignment is disordered with a short-range order molecular alignment that consists of high and low density regions. Thermodynamically, amorphous structures are meta-stable while crystalline structures are in equilibrium state (B. Bhandari, 2013).

Amorphous and crystalline powder exhibits different properties. For example, the degree of hygroscopicity is different even though the powder has similar chemical composition. Amorphous powders have more open and porous structures and molecules acquire more sites for external interactions that can incorporate large quantities of moisture. Unlike amorphous powders, crystalline powders are dense and their molecular matrix, except for some at the air-crystals interface, absorb no significant quantities of moisture (Palzer, Dubois, & Gianfrancesco, 2012). Crystalline powders are important for food that requires excellent physical stability. It does not exhibit caking but enters to deliquescence point (relative humidity at which powder dissolves completely) when the critical relative humidity is reached (Palzer et al., 2012). For instance, crystalline sucrose has deliquescence point at relative humidity of 86%, which means that sucrose can remain stable below 86% relative humidity (Yao, Yu, Lee, Yuan, & Schmidt, 2011). On the other hand, amorphous polymers can be used for encapsulation during drying due to their excellent film forming properties (Y. H. Roos, 2010). In contrast to crystalline, amorphous can entrap compounds in the void of loosely packed molecules.

Dissolution property of powder is also significantly affected by its microstructure. An amorphous powder dissolves faster than a crystalline one, as less energy and time are needed

to dissociate the weakly bound molecules in an amorphous structure (Marabi et al., 2008). Additionally, an amorphous powder produced by freeze-drying has a porous structure, with higher specific surface area, which helps to dissolve the powder quicker than for a crystalline one. Therefore, the microstructure property of a powder is an important aspect that affects its stability and functional properties.

X-ray diffraction method is a powerful tool used in the food industry for determining the microstructure property of a powder (Lian Yu, 2001). It helps to qualitatively distinguish amorphous and crystalline structures, and determine the degree of crystallinity. Other techniques to measure the degree of crystallinity are differential scanning calorimetry, near-infrared spectroscopy, and polarized light microscopy. To observe the structure, particularly the surface morphology of powder, scanning electron microscopy is also useful.

1.2.6.3 Bulk properties

1.2.6.3.1 Density

Bulk density is defined as the mass of powder divided by its bulk volume. The measurement of density of powder provides the necessary information for determining the size of packaging and storage. There are two types of bulk density; loose and tapped bulk densities or simply called bulk density and tapped bulk density, respectively. A volume occupied by a specified mass of powder in a graduated cylinder indicates its bulk density. Tapped density is the volume occupied by the powder after a specified number of taps (for settling of the particles). The bulk (ρ_b) and tapped densities (ρ_t) are defined in *Eq. (1)* and *(2)*. It varies as a function of the compactness of the powder and it is also useful for determining the flow property of powder.

$$\rho_b = \frac{m_s}{V_b} \qquad Eq.(1.1)$$

$$\rho_t = \frac{m_s}{V_t} \qquad Eq.(1.2)$$

where, m_s, V_b, V_t refer to mass of powder sample, bulk volume, and tapped volume, respectively.

1.2.6.3.2 Flowability

Powder flowability is a desirable property of a powder that signifies the ease of flow during transporting, storing, dispensing, and mixing (B. Bhandari, 2013). It mainly depends on the extent of surface interaction between particles due to cohesion and internal friction forces. Cohesion force is the force of attraction between the particles and consists of van der Waals, electrostatic, and magnetic forces. On the other hand, internal friction is the resistance of particles with each other under normal pressure. If powder overcomes the resistance created by surface interaction between particles in contact, then it is a free-flowing powder. If the powder has poor flowability, additional assisting energy such as gravitational, compression, aerodynamic, vibration and mechanical energy is required, which increases the operating cost. Other factors that affect powder flow are moisture content, size, shape and surface roughness.

Increasing moisture content in powder significantly affects powder flowability due to the formation of liquid bridges and capillary forces between the particles. Most of the powders are hygroscopic, hence susceptible to absorbing moisture from surrounding atmosphere at ambient condition. This leads to the formation of powder caking (formation of hard crust or lumps on the exposed surface of the powder) therefore causes severe problem to flowability and the quality of the powder. Decrease in particle size (less than 200μm) may worsen the powder flowability (Fitzpatrick, 2005). This is due to increase in surface area and hence availability of more surface area for cohesive forces as well as internal frictional forces to resist the flow (Shamlou, 2013).

There are several methods of measuring the flow characteristics of powder such as the standard shear testing technique, powder flow function and wall friction based on Jenike's shear testing by mass and funnel flow mode, and empirical tests (Fitzpatrick, 2013; Johanson, 2005). The former two are more reliable for designing the powder flowability when the hopper and silos are used for powder storing and processing. Conversely, empirical tests such as Hausner ratio (HR), Carr's (CI) index, and angle of repose (θ) are simple and fast methods that may be useful in quality control (Carr, 1965; Teunou, Vasseur, & Krawczyk, 1995). HR (*Eq. 1.3*) and CI (*Eq. 1.4*) are calculated using the loose and tapped bulk densities of powders (explained in section 1.2.6.3.1). HR is indicative of the cohesiveness of a powder that can be

applied to provide an index of powder flow property. Angle of repose (θ), determines the flow properties of a powder, and is defined as the angle formed by the free surface of a pile of powder with the horizontal plane, shown in *Eq. (1.5)*. The flow character of a powder according to HR, CI and angle of repose is presented in **Table 1.12.**

$$HR = \frac{\rho_t}{\rho_b} \qquad Eq.(1.3)$$

$$CI = 1 - \left(\frac{\rho_b}{\rho_t}\right) \times 100\% \qquad Eq.(1.4)$$

$$\theta = tan^{-1}\left(\frac{h}{r}\right) \qquad Eq.(1.5)$$

where h is for height of the conical pile, and r is the radius of horizontal plane of pile of powder.

Table 1.12 Flow characteristic of powder calculated based on Hausner ratio, Carr's index, and angle of repose.

Flow character	Hausner ratio	Carr's index (%)	Angle of repose (°)
Excellent	1.00-1.11	<15	<20
Good	1.12-1.18	15-20	20-30
Fair	1.19-1.25	21-35	30-34
Passable	1.26-1.34	-	-
Poor	1.35-1.45	36-45	35-40
Very poor	>1.45	>45	>40

1.2.6.4 Powder dissolution

One of the quality indicators of food powder is complete and rapid reconstitution in an aqueous system. Consumers highly appreciate powders with good dissolution property especially for liquid applications. On the contrary, powder with poor dissolution property would trouble manufacturers resulting in long processing time, increased production costs and eventually poor quality or appearance of foods (Selomulya & Fang, 2013). Powders that dissolve in water (hot or cold) with a minimum of stirring and without formation of lumps are denoted as instant powders (Forny, Marabi, & Palzer, 2011). Generally, a powder's

reconstitution in water occurs in four steps: (a) wetting of agglomerates followed by the penetration of the water into the pores, (b) immersion of the agglomerates into the water, (c) dispersion of particles, and (d) complete dissolution of powder particles in the solution (Schubert, 1987). These steps occur simultaneously rather than sequentially and are difficult to distinguish.

The instant property of a powder depends on the microstructure, size, powder compositions, and condition of dissolution solvent (temperature, pH, etc.). Different methods used to analyse the dissolution property of powders can be found in the literature (Fang, Selomulya, & Chen, 2008; Forny et al., 2011).

1.2.6.5 Stability of powder

1.2.6.5.1 Water activity and sorption isotherm

Water activity (a_w) is defined as the ratio of the equilibrium partial vapor pressure of water in a food system to the equilibrium partial vapor pressure of water at the same temperature. The value of a_w of a food is always less than one (a_w=1 for pure water). It can be presented as the percentage of relative humidity (%RH) divided by 100. Water activity is widely used as a tool for understanding the stability of food products. It measures the free water available for any chemical and biochemical reaction in the food. The values of water activity below 0.40 generally ensure the stability of food against the hydrolytic reactions, biological and enzymatic activities (Marques, Ferreira, & Freire, 2007). Of several methods used for determining a_w of food, activity meter based on dew-point technique is widely used since it is quick and easy to operate. Other techniques that can be used to measure water activity are electric hygrometry, wet bulb-dry thermometric, and hair hygrometer (Mathlouthi, 2001).

The measurement of the water activity of foods may not be sufficient in order to understand the behaviour of food products in different relative humidity conditions during storage. Most of food powders are in amorphous state or thermodynamically unstable, therefore, the powders would tend to attain stable state by reaching equilibrium with the surrounding atmosphere. Sorption isotherms give the relationship between water content and water activity when the food powder is exposed to a range of relative humidity at constant

temperature. Water sorption isotherms are useful for multiple purposes such as to predict shelf life, prevent caking and sticking problems (particularly for amorphous powder), in order to choose an appropriate packaging and storing condition (Mohamed Mathlouthi, 2001). Powders with different microstructure properties demonstrate dissimilar sorption isotherm curves. For instance, **Figure 1.9** shows the general adsorption isotherm curves of amorphous and crystalline sugars. The hygroscopicity of amorphous sucrose is observed by noticing the increase in moisture content at low a_w, whereas crystalline sucrose remains unchanged for a large range of water activities (until 0.85-0.86 a_w). For amorphous sucrose, an increase in moisture content allows the increase in molecular mobility therefore molecules arrange themselves to form a more stable crystalline structure. At or above 0.86 a_w, an abrupt increase in water content results a deliquescence (dissolve into solution) of crystalline sucrose.

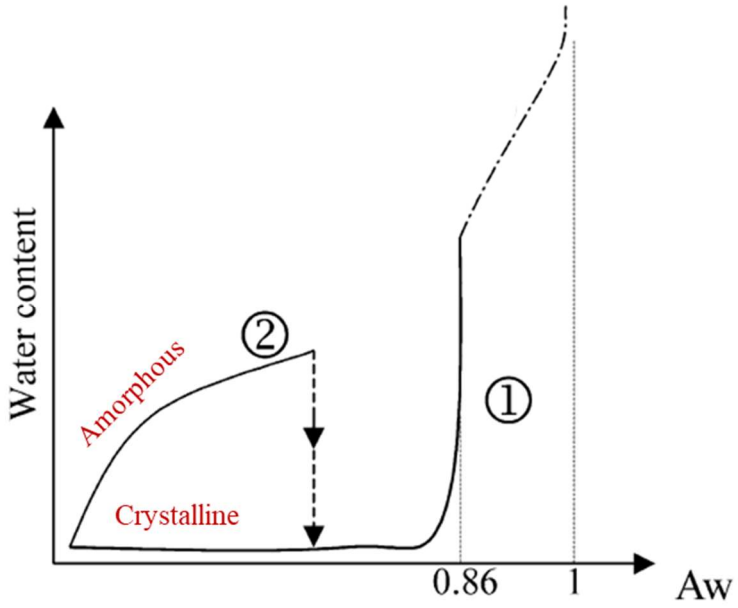

Figure 1.9 General shape of adsorption isotherm curves of crystalline sucrose (1), and amorphous sucrose (2). Modified from M. Mathlouthi & Rogé (2003).

There are three methods for the determination of water sorption isotherms, (a) gravimetric, (b) manometric, and (c) hygrometric. The most common technique is the gravimetric method, which involves the measurement of mass changes. This method uses thermostatted jars filled with supersaturated salt solutions at the bottom to maintain the required relative humidity as defined in the European project COST90 (Baucour & Daudin, 2000). Many mathematical models are used to describe the sorption isotherms of food

powders. Such models are the Langmuir, Brunauer-Emmett-Teller (BET), Guggenheim-Anderson-de Boer (GAB), Oswin, Halsey, and Henderson equations. Comprehensive review on these models can be found in the literature (Al-Muhtaseb, McMinn, & Magee, 2002; Andrade & Lemus, 2011; Basu, Shivhare, & Mujumdar, 2006).

1.2.6.5.2 Glass transition temperature

Glass transition temperature (T_g) is defined as the temperature at which an amorphous system changes from the glassy to the rubbery state (Ratti, 2001; Y. H. Roos, 2010). It is considered as an indicator of the onset of deteriorating mechanisms. Below T_g, molecular mobility of food matrix is extremely slow due to high viscosity. Conversely, at temperature above T_g, there is an increase in molecular mobility due to a drastic decrease of viscosity. These changes may result in extensive effects on physical state of food powders. The determination of T_g of dried powder can provide an idea of best storage temperature. Food powders stored at a temperature below their T_g can be considered stable. However, there are other factors that can affect the T_g of powders during storage. Such factors are moisture content and food compositions. Water acts as a plasticizing agent; therefore, increase in moisture content depresses the glass transition temperature. The T_g of sucrose with 3.5% moisture content is about 32 °C, whereas for anhydrous sucrose, it is in the range of 57-74 °C (Yeting Liu, Bhandari, & Zhou, 2006). Therefore, T_g is useful in understanding and predicting the behaviour of food powders during storage.

Differential scanning calorimetry is a powerful technique for identifying the glass transition temperature and has been used to determine the T_g of various foods (Khalloufi, El-Maslouhi, & Ratti, 2000; Yeting Liu et al., 2006; Y. H. Roos, 2010).

1.3 Problematic, hypothesis and objectives of the research

1.3.1 Problematic

The aim of this project is to address two major problems faced by forest-based industries related to maple trees. First, huge quantities of maple bark are produced as residues from the industry and second, low quality maple syrup is accumulated as surplus.

Several studies have demonstrated that maple bark extracts contain important classes of polyphenols. Most of the studies were focused on their primary use as pharmaceutical agents. However, the potential of maple bark extracts as natural food ingredients has yet to be investigated. Along with polyphenols, hot-water extract contain polysaccharides, proteins, and minerals. Identifying their nutrients and polyphenols contents shall enhance the prospect of using maple bark extracts as food ingredients. From the literature, we observed that bark extracts were tested *in vitro* on ranges of cancer cells, however, no records on the cytotoxicity of bark extracts are available. Knowing the cytotoxicity limit can provide a primary indication for using these extracts as food ingredients.

Regarding maple syrup, studies have identified its content with a number of various polyphenols. Isolated polyphenols from syrup were studied to explore their potential as therapeutic agents. However, scientific work on developing novel maple products by using surplus syrup is still fragmentary. In the last 12years, only two literature references are available that have focused on valorizing low quality syrup to maple sugar crystals. However, the nutritional and functional properties of final product were not reported in those studies. The crystallization of syrup still represents a challenge, in particular with the traditional processing, as it is a batch process, producing inconsistency in product quality, and additional processing steps are required after crystal formation. Therefore, one of the possibilities of valorizing low quality syrup could be the production of maple sugar powder using drying techniques that are common to food industries. If these two problems are well addressed, it will help to boost the economy of maple industries in Canada.

1.3.2 Hypothesis

The present work hypothesizes that drying techniques will produce stable polyphenol-enriched maple sugar powders from maple syrup with enhanced antioxidant activity.

1.3.3 Objectives

The general objective of this work is to develop functional food ingredients from low-quality maple syrup by adding maple bark extracts and applying an appropriate drying

technology. To achieve this objective, the project is divided into two parts that include three sub-objectives in total.

Part-I: Valorization of maple barks (production of hot water extract from maple bark)

Objective 1: Study the composition, antioxidant capacity and safety of hot water extracts of sugar and red maple bark.

- Identify the major nutrients in maple bark extracts
- Analyse the antioxidant capacity using ORAC and ABTS assays
- Investigate the safety of these extracts by *in vitro* cytotoxicity on neutrophil-like cells
- Determine the maximal safe dose that can be added for development of food ingredients

Part-II: Valorization of substandard maple syrup (production of maple sugar powders)

Objective 2: Develop the freeze-drying protocol to produce maple sugar powders from pure maple syrup.

- Formulate the freeze-drying cycles to obtain dried syrup in minimum drying time
- Assess the effect of primary drying time on the physicochemical properties of maple sugar powders

Objective 3: Develop polyphenol-enriched maple sugar powders by adding maple bark extracts into the low-quality syrup

- Investigate the effect of addition of sugar and red maple bark extracts into substandard maple syrup on the total polyphenolic content
- Identify the major polyphenols in syrup and bark extracts mixture
- Study the effect of FD and VDD on the physicochemical and functional properties of maple sugar powders.

Chapter 2 Nutrients, antioxidant capacity and safety of hot water extract from sugar maple (*Acer saccharum* M. and red maple (*Acer rubrum* L.) bark

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2.1 Résumé

L'extraction à l'eau chaude des écorces d'érable à sucre (*Acer saccharum* M) et d'érable rouge (*Acer rubrum* L.) a permis d'investiguer pour la première fois le potentiel de leurs extractibles en tant qu'antioxydants alimentaires. Les nutriments organiques et inorganiques extraits ont été identifiés et leur non-toxicité sur neutrophiles a été établie. L'analyse immédiate (proximate analysis) a montré que les deux extraits d'écorce ont une faible teneur en eau et en gras. La teneur en protéines et en matières inorganiques est plus élevée pour l'extrait d'écorce d'érable à sucre (SM-BX) que pour l'extrait d'écorce d'érable rouge (RM-BX). De plus, SM-BX montre une teneur en sucres totaux plus élevée que RM-BX, tandis que leur teneur en sucres complexes (oligo- et/ou polysaccharides) est comparable. D'autre part, SM-BX contient une gamme étendue de minéraux essentiels (K, Ca, Mg, P, Na, Fe and Cu) en quantités supérieures à celles retrouvées chez RM-BX, à l'exception toutefois de Zn et Mn. L'analyse des composés phytochimiques a montré de plus fortes teneurs en composés phénoliques totaux et en flavonoïdes pour RM-BX. Suivant cette tendance, RM-BX a montré une activité antioxydante bien supérieure à celle de SM-BX: 2.85 plus importante par test de piégeage du radical ABTS et 1.9 fois supérieure par test ORAC. Enfin, la non-toxicité à forte dose a été établie pour les deux extraits. Jusqu'à 100µg/mL, ceux-ci ne modifient pas la viabilité des neutrophiles comme déterminé par cytométrie en flux avec l'annexine V-FITC et le propidium d'iode comme marqueurs. En conclusion, cette étude *in vitro* confirme le potentiel des extraits d'écorces d'érable à sucre et d'érable rouge comme additifs alimentaires.

Mots clés : Extraits d'écorce d'érable, Nutriments, Antioxydants, Additifs alimentaires, Neutrophiles, Viabilité

2.2 Abstract

Sugar maple (*Acer saccharum* M.) and red maple (*Acer rubrum* L.) barks were treated with hot water to extract nutrients in order to explore, for the first time, their potential as safe dietary antioxidants. The organic and inorganic nutrients of these extracts, as well as their safety on human PLB-985 cells differentiated into neutrophils-like cells, were determined. Proximate analysis showed that both bark extracts were low in moisture and fat. Sugar maple bark extract (SM-BX) showed crude protein and ash content higher than those found in red maple bark extract (RM-BX). In addition, SM-BX had total sugars higher than those evaluated in RM-BX, while complex sugars (oligo- and/or poly-saccharides) were similarly abundant in both bark extracts. Furthermore, SM-BX demonstrated a wide array of vital minerals (K, Ca, Mg, P, Na, Fe and Cu) in quantity larger than that evaluated in RM-BX, whereas RM-BX had Zn and Mn levels higher than those found in SM-BX. Phytochemical analyses showed that RM-BX exhibited total phenolic and flavonoid contents higher than those measured in SM-BX. Consequently, RM-BX presented an antioxidant activity higher than that of SM-BX: 2.85-fold ABTS radical cation scavenging capacity and 1.9-fold oxygen radical absorbance capacity. Finally, RM-BX and SM-BX were deemed safe since, at concentration up to 100µg/ml, they did not modify the viability of neutrophils as determined by flow-cytometry assay using Annexin V-FITC/Propidium Iodide as markers. In conclusion, our *in vitro* studies indicate that both red and sugar maple bark extracts have a real potential as food additives.

Keywords: Maple bark extracts, Nutrients, Antioxidants, Food additives, Neutrophils, Viability

2.3 Introduction

Acer saccharum M. (sugar maple) and *Acer rubrum* L. (red maple) are widely distributed in the temperate region of eastern north America (Van Gelderen et al., 1994). These maple species are of great importance for their use as food (maple sap and syrup) and traditional medicines. Maple sap is consumed as a tonic and is reported to have health beneficial properties (Yuan, Li, Zhang, & Seeram, 2013). Maple syrup, a natural sweetener obtained by concentrating the sap, has high nutritional value containing sugar, polyphenols, minerals, amino acids and vitamins (Ball, 2007). As traditional medicines, plant parts (particularly the bark) of these species were used by Native Americans in the treatment of various ailments such as sore eyes, diarrhea, back pains and as a diuretic (Arnason et al., 1981; Bi et al., 2016). Additionally, maple bark was infused and consumed as a tea regularly.

Previous studies dealing with sugar maple and red maple bark extracts have highlighted the presence of many classes of polyphenols, such as gallic acid derivatives, ellagic acids, lignans and flavonoids. Phytochemical analysis pointed out the major polyphenols presented in maple bark are maplexins, which are gallotannins with a 1,5-anhydro-glucitol moiety (Yuan et al., 2012). Gallotannins belong to the hydrolysable tannins, which are also listed as GRAS (generally recognized as safe) by the Food and Drug Administration (US Code of Federal Regulations, 2016). Maple bark extracts, mainly those from red maple, reportedly showed *in vitro* radical scavenging ability, hence a potent antioxidant (Royer et al., 2011). In addition, phenolic-rich bark extracts from sugar maple and red maple were demonstrated to have glucidase inhibitory and anticancer activities (Yuan et al., 2011, 2012). These abovementioned health beneficial activities of maple bark extracts were mainly credited to their phenolic compounds.

Phenolic compounds from plants have gained significant attention as protective dietary constituents, attributable to their antioxidant property (La Vecchia, Altieri, & Tavani, 2001). Antioxidants are also used to prevent lipid oxidation in foods, thus increasing their shelf life, while preserving their nutritional value. Furthermore, consumers' inclination to natural product as well as the strict regulations on the use of synthetic antioxidants has led to research in finding natural antioxidants sources, such as plant extracts (Amensour et al., 2010). Maple bark extracts represent, therefore, a potential source of antioxidant compounds.

Hence, a well-documented study on nutrients and other active compounds in maple bark extracts would enhance their prospect as functional food ingredients.

Therefore, the goal of this study was to evaluate the potential of sugar and red maple bark water extracts as sources of safe and natural dietary antioxidants. Characterization of hot water bark extracts, in terms of organic and inorganic nutrients, phytochemical contents, and antioxidant activities was performed to highlight their potential as functional food ingredients. The effects of crude bark extracts on human leukocytes such as neutrophils were examined to get an indication of the safety of these extracts.

2.4 Materials and methods

2.4.1 Chemicals and plant samples

All the chemicals and standards, unless otherwise stated, were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals and solvents used were of analytical grade. Sugar maple (SM) and red maple (RM) bark from pure maple stands were provided by Decacer Inc (Dégelis, QC, Canada). Bark samples were subsequently air-dried and ground to 250-500 microns particle size as mentioned by Geoffrey et al. (Geoffroy, Fortin, et al., 2017).

2.4.2 Preparation of the hot water bark extracts

250 g of ground sugar maple bark (moisture content in wet basis of 5.6%) and red maple bark (9.5%) was individually extracted with 2.5L of water under reflux for 1h at 90°C (Geoffroy, Fortin, et al., 2017). The solids were separated by vacuum filtration through a Whatman No. 1 filter paper on a Büchner funnel and washed with 0.6L of hot water. The aqueous filtrate was freeze-dried and then stored at -80°C before analysis. The freeze-dried extract obtained from sugar and red maple bark are labelled hereafter as “SM-BX” and “RM-BX”, respectively.

2.4.3 Analyses of crude bark extracts

2.4.3.1 Nutrients

2.4.3.1.1 Proximate analysis

The crude bark extracts were analyzed for macronutrients (moisture, fat, carbohydrate, protein and ash) following AOAC methods (AOAC, 1995), except for protein content in the samples, which was estimated by Nitrogen-analyzer (2410 Series II, Perkin Elmer). The determined percentage of nitrogen was multiplied by a factor of 6.25 to evaluate protein content.

2.4.3.1.2 Water-soluble sugars

Water-soluble sugars of the extract were analyzed by HPLC using Sugar-Pak-I column 6.5×300 mm (Waters, MA, USA), packed with a micro-particulate cation-exchange gel in calcium form. Refractive index (RI) detector (Hitachi, L-7490) was used for sugar identification as performed by P. St-Pierre et al. (2014). Sugar components were identified and quantified using retention times of standards (sucrose, glucose and fructose) and peak area, respectively. As for the complex sugar, it was quantified using sucrose as internal standard.

2.4.3.1.3 Minerals content

Minerals were analyzed by using inductively coupled plasma with optical emission spectrophotometer (ICP-OES; PerkinElmer, Waltham, USA). Ten elements (K, Ca, P, Mg, Zi, Fe, Cu, Na, Mn, and Pb) were quantified according to the intensity measurement and calibration standards. The data are expressed as ppm, which is then converted to mg per 100g of dry extract, based on dry extract mass used for preparing the solution for the analysis.

2.4.3.2 Determination of total phytochemicals and antioxidant activities

2.4.3.2.1 Total phytochemicals

The total phenolic content (TPC) and flavonoid content (TFC) of the crude extracts were determined using spectrophotometry as performed by Royer et al. (Royer et al., 2011).

TPC and TFC are expressed as grams of gallic acid equivalents and grams of quercetin equivalents per 100 gram of dry extract samples, respectively.

2.4.3.2.2 ABTS assay

The free radical scavenging capacity of SM-BX and RM-BX was determined by ABTS (2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) free radical colorimetric assay (Re et al., 1999). 10µL of aqueous extract or Trolox was added to 300µL of ABTS+* solution and the absorbance reading was taken after incubating for 6 min at 37°C in the microplate reader (Fluostar Omega, BGM labtech, NC, USA). Trolox was used for the calibration curve. The values were expressed as mmol Trolox equivalent/100g of dry extract (mmol TE/100g DE).

2.4.3.2.3 Oxygen radical absorbance capacity (ORAC) assay

Antioxidant capacity of SM-BX and RM-BX was also determined by ORAC assay. Prior to ORAC assay, samples were extracted with acetone/water/acetic acid (AWA, 70:29.5:0.5 v/v/v) to perform hydrophilic-ORAC test following the procedure described by Prior et al. (Prior et al., 2003) with slight modification as followed by Dudonne (Dudonne et al., 2009). The ORAC values were expressed as mmol Trolox equivalent/100g of dry extract (mmol TE/100g DE).

2.4.3.3 Viability of neutrophil-like cells

2.4.3.3.1 Cell culture and differentiation

PLB-985 cell line (DSMZ; German collection of microorganisms and cell culture) were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin at 37°C, in a humidified atmosphere of 5% CO_2. The PLB-985 cells were then differentiated into neutrophil-like cells by culturing them in medium supplemented with 0.3mM db-cAMP ((N^6,2′-O-Dibutyryladenosine 3′,5′-cyclic monophosphate) sodium salt; Sigma-Aldrich, ON, Canada) for 72hours (Pivot-Pajot, Chouinard, Amine El Azreq, Harbour, & Bourgoin, 2010).

2.4.3.3.2 Incubation of cells with crude bark extracts

Neutrophil-like cells (1×10^6 cells per well) were incubated in a 96-well plate, in which SM-BX and RM-BX with varying concentrations [0 (control), 0.1, 1, 10, 100, 500µg/ml] were added and kept in the incubator (37°C and 5% CO_2) for 24h.

2.4.3.3.3 Annexin V/PI apoptosis assay

Analysis of cell viability was carried out using Annexin V-FITC/PI detection kit (BD Pharmingen™, BD Biosciences, ON, Canada). Cells (1×10^6) were stained with annexin V-FITC (AV) to identify the process of apoptosis and co-stained with propidium iodide (PI) for determining cells under necrosis. The protocol was followed as mentioned by Meda et al. (Meda, Poubelle, & Stevanovic, 2017). Flow cytometry instrument (BD FACSCalibur, BD Biosciences, ON, Canada) was used for the acquisition of cell viability, apoptosis and necrosis. Ten thousand events were collected and analyzed by CellQuest Pro (BD Biosciences) to acquire the percentages of viable, apoptotic and necrotic cells.

2.4.4 Statistical analysis

The results are presented as the mean±standard deviation (S.D.) of triplicated data. The results were analyzed using one-way analysis of variance (ANOVA) by SigmaPlot version 12.5 program. If the differences among the results were found significant ($P<0.05$), ANOVA was followed by Holm-Sidak method with $\alpha=0.05$.

2.5 Results and Discussion

2.5.1 Nutrients in crude bark extracts

2.5.1.1 Macronutrients

Proximate analysis provided immediate nutritional evaluation of crude bark extracts, summarized in **Table 2.1**. The moisture content of bark extracts was less than 6%, which is

consistent with moisture contents of freeze-dried fruits co-products (Selani et al., 2016). Low moisture content of food materials is desirable as it provides stability during storage and increase of shelf life. Ash and protein contents were determined to be in SM-BX higher by 2.6-folds and 1.7-folds than in RM-BX, respectively. The ash content in SM-BX was similar to that reported for *Ampelozizyphus amazonicus* aqueous bark extract (8.22%), while higher than some of fruit co-products (2.43 to 5.24%) (Selani et al., 2016; Simen et al., 2016). The presence of high ash content also indicates that SM-BX could be a good source of inorganic nutrients (minerals). In comparison to above-discussed fruit co-products, the protein content was low in the maple bark extracts but higher than that in *Ampelozizyphus amazonicus* bark extract (0.50%). In both samples, crude fat was the lowest (less than 0.50%) among the macronutrients. The low fat content in both water extracts can be explained by low affinity of water for fats. Studies on hot water extraction of bark and leaves of other plants have also reported low fat content, below 1% (Berté, Beux, Spada, Salvador, & Hoffmann-Ribani, 2011; Simen et al., 2016).

Carbohydrates are an essential part of a balanced human diet. It was the most abundant macronutrients of both bark extracts. Comparing two samples, it represented significantly higher in RM-BX (89.43%) than in SM-BX (82.33%) ($P<0.05$). *Ampelozizyphus amazonicus* aqueous bark extract had lower carbohydrates content (84.96 %) than that present in RM-BX but higher than that present in SM-BX (Simen et al., 2016). Consequently, the energetic values calculated for RM-BX were also significantly higher than those for SM-BX (366.97 *vs* 343.80kcal/100g DE). Thus, according to proximate composition, maple bark extracts could be used as food ingredients, both bark extracts having low in moisture and fat contents. At the same time, SM-BX was determined to have high ash content (inorganic nutrients) and protein, while RM-BX showed higher energetic values.

Table 2.1 Macronutrients and water-soluble sugar composition in crude SM-BX and RM-BX.

Traits	Macronutrients		Compositions	Water-soluble sugars	
	SM-BX (%)	RM-BX (%)		SM-BX (g/100g DE)	RM-BX (g/100g DE)
Moisture	5.75±0.09[a]	5.30±0.15[b]	Complex sugars	25.05±1.38[a]	18.85±0.77[b]
Ash	8.84±0.05[a]	3.40±0.07[b]	Sucrose	10.94±0.11[a]	5.58±0.08[b]
Protein	2.65±0.13[a]	1.50±0.12[b]	Glucose	5.28±0.04[a]	3.51±0.06[b]
Fat	0.43±0.12[a]	0.36±0.18[a]	Fructose	5.56±0.03[a]	4.50±0.02[b]
Carbohydrates	82.33[a]	89.43[b]	Total sugar content	46.83±1.51[a]	32.44±0.92[b]
Energy[A]	343.80[a]	366.97[b]			

Values represent means (n=3) ± S.D; [a,b] different superscript letters in the same row are significantly different (P<0.05) according to Holm-Sidak method; [A]energy value expressed in kcal/100 g DE; DE, dry extract; SM-BX, sugar maple bark extract; RM-BX, red maple bark extract.

2.5.1.2 Water-soluble sugar composition

The types and contents of water-soluble sugars in crude bark extracts are also presented in **Table 2.1**. The concentration of all studied individual sugars were higher in the SM-BX. As a result, SM-BX showed a significantly higher total sugar content (46.82 ±1.51g/100g DE) compared to RM-BX (32.44 ±0.92g/100g DE), approximately by 44% (P<0.05). Interestingly, among water-soluble sugars, the amount of complex sugars (oligo/poly-saccharides) was the highest followed by sucrose and monosaccharide in both maple bark extracts. These complex sugars may consist of polysaccharides such as glucan and derivatives, oligosaccharides, pectin and water-soluble fibers that are commonly present in woody plants (J. Liu, Willfo, & Xu, 2015). The presence of complex sugars has also been mentioned in maple syrup, a natural sweetener obtained from sugar and red maple, reporting 2% of polysaccharides of total sugars (P. St-Pierre et al., 2014). In the case of maple bark extracts, the complex sugars are presented in larger proportion, 53.49 and 58.10% of total sugars in SM-BX and RM-BX, respectively. The use of natural polysaccharides for development of functional foods and nutraceuticals is becoming very important. Polysaccharides from the aqueous extract of inner bark of Norway spruce have been reported to possess immune-stimulating activity in macrophages, which play important role in host defense and inflammation (Le Normand et al., 2014). In addition, pectin and dietary fibers

are beneficial to digestive system by slowing down the movement of foods in digestive tract as well as by reducing the rate of sugar absorption from food in blood (J. Liu et al., 2015). Therefore, the presence of complex sugars in concentration superior to those of simple sugars in maple bark extracts could have important impact in nutrition and pharmaceutics. However, further elucidation of structure of polysaccharides from maple bark extracts and their biological activities would be required.

2.5.1.3 Mineral contents

Plants assimilate minerals from their growing environment. Therefore, it is essential to have knowledge on mineral composition in plant extract. The composition of macroelements (K, Ca, P, Mg, and Na) and trace elements (Zn, Fe, Mn and Cu) determined for the maple bark extracts are reported in **Table 2.2**. All macroelements as well as some trace elements (Fe and Cu) are found in higher concentrations ($P<0.05$) in SM-BX, while Zn and Mn were higher ($P<0.05$) in RM-BX. Among the studied minerals, Fe was found in low quantity, while toxic element such as Pb was below the detection limit of ICP-OES (0.04mg/kg). The concentration of minerals higher in SM-BX than in RM-BX is also associated with higher ash content (presented in **Table 2.1**) that explains this difference. Reports on minerals present in maple sap and syrup are available; conversely, data on analyses of minerals present in maple bark are scarce. It has been reported that maple sap and syrup mainly contains K, Ca, Mg and trace elements but in lower concentration than in studied maple bark extracts (Ball, 2007). Macroelements (Ca, P and Mg) and trace elements are essential for physiological processes such as development of bone, tissue growth and as cofactor of various enzyme systems (Institute of Medicine, 1997).

Table 2.2 Minerals present in crude bark extracts and values of estimated daily dietary intake (RDI) and tolerable upper intake (TUI) levels for the reference.

	Mineral content (mg/100g DE)									
Sample	**K**	**Ca**	**P**	**Mg**	**Na**	**Zn**	**Fe**	**Mn**	**Cu**	**Pb**
SM-BX	3025.3 ±80.2[a]	746.9 ±9.4[a]	240.2 ±1.6[a]	196.4 ±2.3[a]	35.1 ±1.3[a]	17.0 ±0.5[a]	3.1 ±0.3[a]	17.2 ±0.2[a]	6.5 ±0.2[a]	BDL
RM-BX	546.4 ±5.0[b]	577.7 ±4.2[b]	111.9 ±3.8[b]	137.1 ±1.3[b]	13.3 ±1.6[b]	21.4 ±1.4[b]	2.1 ±0.2[b]	53.9 ±0.4[b]	2.8 ±0.1[b]	BDL
RDI[A,B]	4500-4700	1000-1300	700-1250	240-420	1200-1500	8-11	8-18	2-5	0.7-0.9	-
TUI[A,B]	-	2000-3000	3000-4000	-	2200-2300	23-40	40-45	6-11	5-10	-

Values represent means (n=3) ± S.D; [a,b] different superscript letters in the same column are significantly different ($P<0.05$) according to Holm-Sidak method; SM-BX, sugar maple bark extract; RM-BX, red maple bark extract; BDL, below detection limit; RDI, estimated daily dietary intakes based on Recommended dietary intake; TUI, tolerable upper intake levels; DE, dry extract [A]From Dietary reference intakes (1997, 2001); [B]value expressed in mg/day.

The obtained results were also compared to average daily dietary intake (RDI) and tolerable upper intake (TUI) levels of studied elements (Institute of Medicine, 1997, 2001) (also presented in **Table 2.2**). K, Ca and Mg levels in SM-BX were very close to RDI level, while trace elements (mainly Zn, Mn and Cu) were found in abundance in both bark extracts, at level higher than RDI values. All determined values were below TUI limit, except for Mn that was higher in both bark extracts: 17.2 and 53.9 mg/100g dry extract for SM-BX and RM-BX, respectively compared to TUI limit of Mn (6-11mg/d). However, it is important to note that only a small percentage (1.3 to 5%) of dietary Mn is retained in the body (Institute of Medicine, 2001). It should also be noted that these values apply to pure dry extract, which is likely to be used in mixtures, upon dilution. In summary, the presence of wide range of essential minerals in maple bark extracts, mainly in SM-BX, highlighted their potential as an alternative to mineral supplements as well as to fortify mineral-deficient foods.

2.5.2 Total phytochemicals and antioxidant activities of crude bark extracts

2.5.2.1 Total phytochemicals

Maple bark is reported to contain of wide range of extractable phenolic compounds including gallic acid derivatives and flavonoids such as quercetin glycosides, rutin and kaempferol (W. Liu, Ouyang, & Wan, 2013; Yuan et al., 2011, 2012). These aforementioned compounds are of increasing interest due to their ability to scavenge free radicals. The total phytochemicals in crude bark extracts estimated as total phenolic (TPC) and flavonoid (TFC) content are presented in **Table 2.3**. RM-BX showed significantly higher TPC (40.12 ± 0.86g GAE/100g dry extract) than SM-BX. Similar trend was found in a previous work, in which hot-water extract from red maple bark was determined to have a higher total phenolic than sugar maple bark (Geoffroy, Fortin, et al., 2017). The total phenolic contents determined for two maple barks in our study are higher than those reported for some tropical fruits co-products (0.37-0.46g of GAE/100g dry matter) and açai fruits (3.00-12.30g of GAE/100g dry matter), while green tea extract (29.80g of GAE/100g dry matter) was determined to show higher total phenolic content than that of SM-BX but lower than RM-BX (Gordon et al., 2012; Selani et al., 2016; Yin, Becker, Andersen, & Skibsted, 2012). Total phenolic content in RM-BX was found to be higher than that from commercial *Pinus maritima* extract (36.00g of GAE/100g sample) (Dudonne et al., 2009). In the case of flavonoids, RM-BX was found to have higher flavonoid content ($P<0.05$) than SM-BX, 1.58 *vs* 1.46g QE/100g DE. Flavonoid content in RM-BX is consistent with previous studies (Royer et al., 2011), but it must be noted that the literature on flavonoid content for water-extracted sugar maple bark is limited.

Table 2.3 Total phenolics, flavonoids and antioxidant activities by ABTS and ORAC assays of the crude bark extracts.

Sample	TPC (g GAE/100g DE)	TFC (g QE/100g DE)	ABTS assay (mmol TE/100g DE)	ORAC assay (mmol TE/100g DE)
SM-BX	19.04±0.58[a]	1.46±0.01[a]	45.20±1.49[a]	372.17±19.51[a]
RM-BX	40.12±0.86[b]	1.58±0.01[b]	128.71±0.39[b]	714.13±39.61[b]

Results are expressed as means (n=3)±S.D; [a,b] different superscript letters in the same column are significantly different (P<0.05) according to Holm-Sidak method; SM-BX, sugar maple bark extract; RM-BX, red maple bark extract; TPC, total phenolics content; TFC, total flavonoids content; GAE, gallic acid equivalent; QE, quercetin equivalent; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acidradical cation scavenging assay; ORAC, Oxygen radical absorbance capacity; TE, trolox equivalent; DE, dry extract.

2.5.2.2 Antioxidant activities

Antioxidant activity of the putative antioxidants are mainly determined by their ability to scavenge free radicals either by single electron transfer (SET) or by hydrogen atom transfer (HAT) mechanism. Hence, the maple bark extracts were analyzed by a method based on each of these mechanisms: ABTS radical cation scavenging capacity (SET-based method) and Oxygen radical absorbance capacity (ORAC; HAT-based method). It is important to note that ABTS is not found in mammalian biology tests and thus considered as a "non-physiological" radical. On the other hand, ORAC method uses the peroxyl radical, a radical that has a biological significance because of its involvement in lipid oxidation and autoxidation. Therefore, it can provide a better analogy with *in vivo* systems (Tan & Lim, 2015).

The results of ABTS radical cation (ABTS+*) scavenging capacity of both bark extracts are presented in mmol trolox equivalent (TE) per 100g of dry extract **(Table 2.3)**. Both bark extracts showed scavenging activity but at different level. The ABTS radical scavenging capacity of RM-BX was significantly higher (P<0.05), around 2.85 times more powerful, than that of SM-BX. The higher scavenging capacity of RM-BX than SM-BX could be related to its superior phenolic content. The ABTS+* scavenging capacity of both maple bark extracts determined in our study are higher than those reported for açai fruits (0.27-1.7mmol TE/100g dry matter) and extracts from various anatomical parts of amaranth (3.01-40.6mmol TE/100g extract) (Gordon et al., 2012; Kraujalis, Venskutonis, Kraujalienė,

& Pukalskas, 2013). No comparative study was found so far on the measurement of antioxidant activity of maple bark extracts by ABTS assay. Previous study on hot water extract of maple bark also indicated that red maple bark extract had a better scavenging capacity for 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical than sugar maple bark (Geoffroy, Fortin, et al., 2017). DPPH is another popular method based on SET-mechanism. One of the limitation of ABTS assay is that ABTS+* must be generated either by chemical (potassium peroxide and manganese dioxide) or enzymatic (peroxidase and myoglobin) reactions and this reaction time can take up to 16hours. However, ABTS assay has the advantage over DPPH assay to eliminate color interference, since the absorption is read at 734nm (Dudonne et al., 2009). To compare the results from different studies, it is suggested to use the same methods and the same extraction solvent. As the crude maple bark extracts had demonstrated ABTS+* scavenging activity, it will be interesting to determine the activity of individual antioxidant compounds present in these extracts.

The results of antioxidant capacity of the crude bark extracts determined by ORAC assay are presented in **Table 2.3**. In ORAC assay, RM-BX showed higher antioxidant capacity than SM-BX (714.13±39.61 *vs* 372.17±19.51mmol Trolox equivalent/100g DE), which was expected from the higher phenolic content in RM-BX (discussed earlier). Reported ORAC value of commercial extract of *Pinus maritima*, was slightly higher than current studied extracts (Dudonne et al., 2009). Wang et al. (2017) reported the ORAC values of varieties of blueberries that were within the value of 2.62-6.74mmol TE/100g fresh weight, indicating lower value than both maple bark extracts (Wang et al., 2017). Thus, superior antioxidant capacities of maple bark extracts suggest their potential use in food application as natural antioxidants.

2.5.3 Effects of crude bark extracts on viability of neutrophil-like cells

To evaluate the safety of hot water maple bark extracts in humans, we investigated the effects of these extracts on the viability of neutrophil-like cells. PLB-985 cell line was chosen to obtain neutrophil-like cells, because this cell line is reported to provide a very suitable neutrophilic cellular model, especially when differentiated with dibutyryl cyclic-AMP (dbc-AMP) (Pivot-Pajot et al., 2010). Neutrophils, the most abundant circulating

leukocytes in human blood, are strongly implicated in host defense against invading pathogens as well as in the inflammatory process and, thus, are an essential part of the immune system. Additionally, neutrophils are terminally differentiated cells that spontaneously undergo apoptosis (programmed cell death) to maintain immune homeostasis. Any imbalance of apoptosis is associated with a wide range of diseases including immunological and development pathologies, cancer and neurodegenerative disorders (Fuchs & Steller, 2011). Therefore, the safety of our bark extracts on vital cells like neutrophils is a major issue to study through their effects on the pattern of neutrophil apoptosis. A few studies have demonstrated the anti-proliferative effects of phenolic-enriched maple bark extracts against a panel of cancer cells (González-Sarrías, Li, & Seeram, 2012). However, reports on the effect of maple bark extracts on neutrophils are very fragmentary.

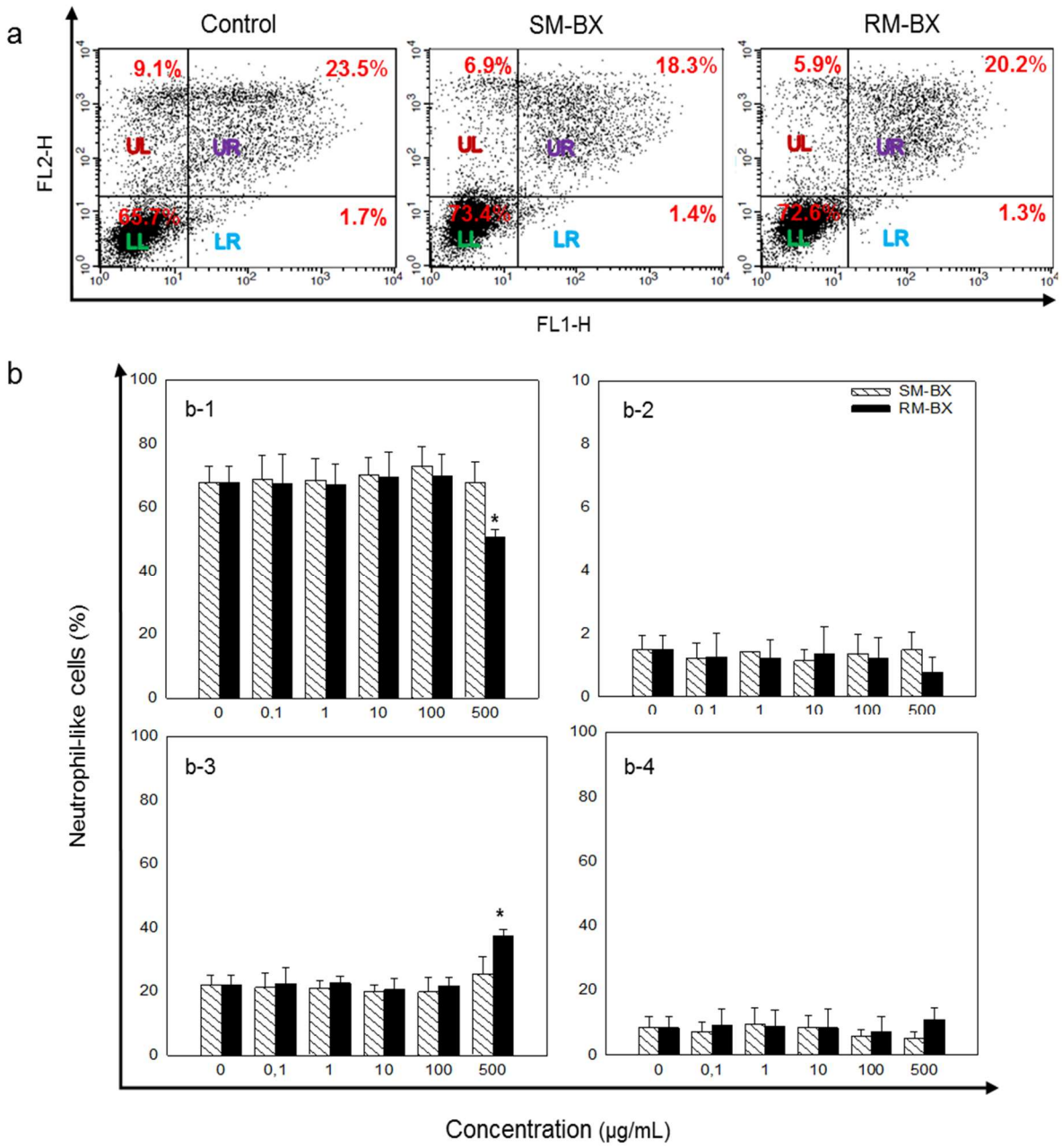

Figure 2.1 Effects of SM-BX and RM-BX on the viability of neutrophil-like cells. PLB-985 cells were differentiated into neutrophil-like cells with dc-AMP and then incubated with graded concentrations of the extracts for 24h. (a) Example of a flow cytometry analysis showing the Control (cells incubated with vehicle) and samples (cells incubated with 100 µg/ml of SM-BX or RM-BX) using Annexin V (FL1-H) and PI (FL2-H) as tracers. Four quadrants are individualized as: LL (lower left, viable cells: AV-/PI-); LR (lower right, early apoptotic cells: AV+/PI-); UR (upper right, late apoptotic cells: AV+/PI+); UL (upper left, necrotic cells: AV-/PI+). Ten thousand cells were counted and percentages of cells in each quadrant are indicated. (b) Neutrophil-like cells were incubated with vehicle (Control) or graded concentrations of SM-BX and RM-BX.

Viable cells (LL, b-1); early apoptotic cells (LR, b-2); late apoptotic cells (UR, b-3); and necrotic cells (UL, b-4). Results are expressed as means±SD of 3 independent experiments. One-way ANOVA followed by Holm-Sidak method was performed to compare extract-treated cells *vs* vehicle-treated cells (Control) and (*) indicates a significant difference; significance was set at $P<0.05$.

The effect of SM-BX and RM-BX on cell viability of neutrophil-like cells were evaluated by flow cytometry after a 24h incubation of cells with the extracts followed by labeling the cells using annexin V-FITC (AV) and propidium iodide (PI) (**Figure 2.1**a). AV/PI distinguishes apoptotic cells from necrotic cells. The population of viable, apoptotic and necrotic cells are presented in **Figure 2.1**b: b-1) viable cells [AV negative, PI negative (AV-/PI-]; b-2) early apoptotic cells [AV positive, PI negative (AV+/PI-)]; b-3) late apoptotic cells (AV+/PI+); and b-4) necrotic cells (AV-/PI+).

The effects of SM-BX and RM-BX on the viability of neutrophil-like cells were compared to cells incubated with vehicle (control). In **Figure 2.1**b-1, the percentage of viable cells were not significantly different for all of the concentrations of SM-BX tested, while the percentage dropped significantly ($P<0.05$) for RM-BX at 500µg/ml, the highest concentration tested. In addition, there is a slight increase (not significant) of the percentage of viable cells at 100µg/ml of both extracts, mainly for SM-BX. Recently, Meda et al.(Meda et al., 2017) showed a comparable tendency of neutrophil viability when cells were treated with hot water extract of red maple bud. This increase of viability is suggestive of a stimulation/activation of neutrophils at this concentration of extracts, as described by McCracken et al (McCracken & Allen, 2014). This could be possible as the fingerprint of phenolic compound in maple bark and bud are somewhat similar (Meda et al., 2017; Yuan et al., 2011, 2012). In the case of early apoptosis (**Figure 2.1**b-2), no significant difference was found between the control and cells treated with bark extracts ($P=0.228$). Flow cytometry showed that a significant percentage of cells treated with 500µg/ml RM-BX were in late apoptosis (**Figure 2.1**b-3), which explains the decrease of the percentage of viable cells. In addition, the percentage of necrotic cells in control conditions were similar to those in cells treated with each of maple bark extracts at all tested concentrations (**Figure 2.1**b-4). This indicates no modification of the pattern of apoptosis at concentrations of both extracts up to 100µg/ml, and no modification of the pattern of cell necrosis at concentration of both extracts up to 500µg/ml. In summary, SM-BX and RM-BX at concentrations up to 100µg/ml did

induce neither apoptosis nor necrosis on the studied neutrophil-like cells. Hence, SM-BX and RM-BX are non-cytotoxic and considered safe for this cell type.

2.6 Conclusions

Acer saccharum M. (sugar maple) and Acer rubrum L. (red maple) bark extracts were reported to present important phenolic contents. Therefore, several studies have been primarily focused on their use as potential pharmaceutical agents. Nevertheless, the possibility of these maple bark extracts as food ingredients had yet to be explored. Therefore, the detailed study performed in the present report was aimed at exploring the potential of sugar and red maple bark extracts as dietary antioxidant. SM-BX was abundant in proteins, total sugars and minerals. Total phytochemicals (total phenols and flavonoids) and antioxidant activities were determined to be more important for RM-BX than for SM-BX, indicating RM-BX as a powerful antioxidant. In addition, *in vitro* cell viability of neutrophil-like cells revealed that RM-BX and SM-BX did not induce apoptosis at concentrations up to 100µg/ml and without any effect on cell necrosis at concentrations up to 500µg/ml, therefore being safe and non-cytotoxic. Considering the overall results, the hot water extract from maple bark not only showed antioxidant potential but also indicated to be a rich source of organic and inorganic nutrients. All these findings indicate that the studied sugar and red maple bark extracts have a very good potential to be used as food indregients.

2.7 Acknowledgements

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Chapter 3 Freeze-drying of maple syrup: efficient protocol formulation and evaluation of powder physicochemical properties

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3.1 Résumé

A partir de la détermination de la température de transition vitreuse, de l'historique thermique en direct et de la cinétique de séchage, un procédé de séchage efficient constitué de deux étapes a été mis au point pour la lyophilisation du sirop d'érable foncé. Des propriétés physicochimiques ont été mesurées sur les poudres de sucre d'érable (MSP) issus de différents temps de séchage (25 et 30h). Les teneurs en humidité des MSP oscillaient entre 3 à 4% alors que les valeurs de l'activité de l'eau (a_w) étaient comprises entre 0,19 à 0,21a_w. Ces poudres présentaient des caractéristiques d'écoulement passable à médiocre en raison de leur cohésion, mais ont montré des propriétés instantanées de dissolution (temps inférieurs à 14secondes). Enfin, les MSP étaient très hygroscopiques, nécessitant ainsi d'être conservés en dessous de 0,36a_w à température ambiante.

Mots clés : lyophilisation, température de transition vitreuse, sucre d'érable en poudre, propriétés physicochimiques, isotherme de sorption

3.2 Abstract

An efficient two-step protocol for freeze-drying of dark grade maple syrup was developed from glass transition temperature determinations, online-thermal history and drying kinetics. Maple sugar powders (MSP), produced under two drying times (25 and 30h), were analyzed for their physicochemical properties. Moisture content and water activity (a_w) of MSP were determined to be in the range of 3-4% dry basis and 0.19-0.21a_w, respectively. The powders exhibited fair to poor flow characteristic owing to their cohesiveness. However, they have instant-like properties with dissolution times shorter than 14s. MSP were highly hygroscopic, requiring to be stored below 0.36a_w at ambient temperature.

Keywords: freeze-drying, glass transition temperature, maple sugar powder, physicochemical properties, sorption isotherm

3.3 Introduction

Maple syrup, a natural sweetener with high nutritional value, is primarily composed of a mixture of sugars (66% sucrose, 0.4% glucose, and 0.5% fructose), minerals and water, and traces of organic acids, proteins and polyphenols (Ball, 2007; Perkins & van den Berg, 2009). Canada accounts for more than 70% of the world maple syrup production (Statistics Canada, 2017). Maple syrup is produced by thermal evaporation of watery sap (1-5% sugars, and traces of minerals and polyphenols) collected from maple trees during early to late spring season. While transforming sap into syrup, the syrup developed the characteristics flavors, colors, and some new polyphenols are also derived (due to the chemical reactions) during the heating process (Perkins & van den Berg, 2009). With the progress of sap tapping season, the color of syrup becomes darker. According to the Canadian Food Inspection Agency, maple syrup is categorized in four grades based on its color, measured as the percentage of light transmission at 560nm; golden color (>75% of light transmission, delicate taste), amber (50-75%, rich taste), dark (25-49%, robust taste) and very dark (<25%, strong taste). Generally, maple syrup of less than 30% light transmission is considered as substandard quality for table syrup and hence, not consumed widely. Consequently, the surplus of dark maple syrup accumulated each year represents a major problem for the syrup producers in Canada due to the requirement of additional inventory space, storage jars, controlled storage temperature, etc. If the accumulated syrup is not properly stored, undesirable chemical changes may also occur.

Drying of syrup in order to produce a maple sugar could be an appropriate solution to this problem, since the product shelf life is thus increased, while easing handling and transportation. In addition, dried sugar powders can be directly used in the formulation of foods, as ready-to-serve products and instant powders for drink preparations. However, viscous liquid foods like honey, having a high sugar content, are difficult to dehydrate by most of common drying techniques (B. R. Bhandari, Datta, & Howes, 1997; Nurhadi & Roos, 2016). Therefore, dilution of viscous syrups to the maximum of 10 to 25% total solid content has been reported to successfully produce honey powders by drying (Nurhadi & Roos, 2016; Shi, Fang, & Bhandari, 2013).

For the present study, freeze-drying (FD) was chosen as a dehydration method to produce maple sugar powders with superior free-flowing and instant-like properties. FD of sucrose solutions is known to produce amorphous sugars (Roos & Karel, 1991; Roos & Karel, 1990). Despite of caking and handling problem of amorphous powder, it has advantages of fast dissolution and could be used to encapsulate bioactive compound due to porous microstructure (Palzer et al., 2012). However, FD is a time-consuming and expensive drying method, especially if under-optimized. FD typically consists of three steps: freezing, primary drying, and secondary drying phase. Among them, primary drying phase is the longest, time- and energy-consuming crucial step. The best way to reduce drying time is to operate the FD at the maximum possible temperature. However, to produce an acceptable freeze-dried product, it is necessary to freeze dry a sample at a temperature lower than the collapse temperature (T_c), at which the material loses the microscopic structure and collapses. T_c is normally 2°C higher than the glass transition temperature (T_g) (X. Tang & Pikal, 2004). T_g is defined as a temperature above which amorphous food material enters from solid glassy state to rubbery state, which means that a significant change in molecular mobility and physical properties occur at this critical temperature (Ratti, 2001; Roos & Karel, 1991). Therefore, the FD is generally carried out at shelf temperatures 2-3°C below the T_g (X. Tang & Pikal, 2004). Also, identification of optimal primary drying time can adequately reduce the overall drying time. Various techniques such as comparative pressure measurement, dew point monitor, Lyotrack (gas plasma spectroscopy), and thermocouples to follow temperature profiles are used to identify the endpoint of primary drying (Patel et al., 2010). Among these techniques, the method with thermocouples is frequently used in determining the endpoint because it is simple to operate and need no complex interpretation of results since the time at which the product temperature reaches the FD shelf temperature, indicates the end of primary drying (Patel et al., 2010).

Moisture content and water activity (a_w) are key stability parameters to characterize food powders. Water activity is defined as the ratio of the equilibrium vapor pressure of water in a system, to the vapor pressure of pure water at the same temperature. In general, moisture content represents both free and bound water in a food system, whereas a_w measures the availability of free water that is liable for any biochemical reaction in the food (Seerangurayar, Manickavasagan, Al-Ismaili, & Al-Mulla, 2017). As well, color of final

product is equally important for the first appearance of food and must be appealing to the consumers. Reconstitution capacity of a powder determines its instant property. Hence, powders that disperse in water in less than 20s with a minimum of stirring and without formation of lumps are termed as 'instant' powder (Forny et al., 2011). For example, skimmed milk powder is referred as instant, when it dissolves within 15 s (Sharma, Jana, & Chavan, 2012).

Flowability is another vital physical property of a food powder that effects its processing, handling and storage (Amrutha, Hebbar, Prapulla, & Raghavarao, 2014; Tze et al., 2012). It is generally determined by Hausner ratio (cohesiveness of powder), Carr's index (compressibility index) and angle of repose. Hausner ratio and Carr's index are calculated from the bulk and tapped density of the powder (Seerangurayar et al., 2017). Sorption isotherm describes the relation between equilibrium moisture content of a food and relative humidity at constant temperature, providing important information to assure product stability during storage. When food materials are amorphous and hygroscopic, an efficient packaging and optimal storing conditions are required for quality preservation.

Hence, the main objectives of this study were to formulate a suitable freeze-drying protocol to successfully dry maple syrup in minimum drying time, and to study the physical and flow properties of final powders. This is, to the best of our knowledge, the first report on successful freeze-drying of maple syrup.

3.4 Materials and methods

3.4.1 Maple syrup

Maple syrup was provided by Levaco Inc. (Quebec, Canada). Syrup samples used for this study presented 65.75±0.06 °Brix (Pocket refractometer, Atago, Japan) and light transmission of 28.30±0.13% (Spectrophotometer at 560 nm, glycerol as reference), hence classified as 'Dark color and Robust taste' according to Canadian Food Inspection Agency 2016. Syrup was stored at room temperature as recommended by the manufacturer (Decacer Inc., Dégelis, Canada), but when opened, it was stored at 4°C.

3.4.2 Drying equipment

Freeze-drying (FD) was done in a freeze dryer (Freeze Mobile 25EL, Virtis Company Inc., NY, USA) equipped with a Unitop 400L (Virtis Company Inc., NY. USA) drying chamber. The temperature of product (T_p) and shelf (T_{shelf}) was measured online during FD process with a precision very fine wire T-type insulated thermocouples of 0.076 mm probe diameter (model 5SRTC-TT-T-40-36, OMEGA, CT, USA). Thermocouple reading was recorded with 8-channel Thermocouple Temperature recorder (OM-CP-OCTTEMP, OMEGA, USA) and retrieved using the Omega 2.0 software (OMEGA, USA). The schematic representation of the freeze-dryer and the equipment is shown in **Figure 3.1**.

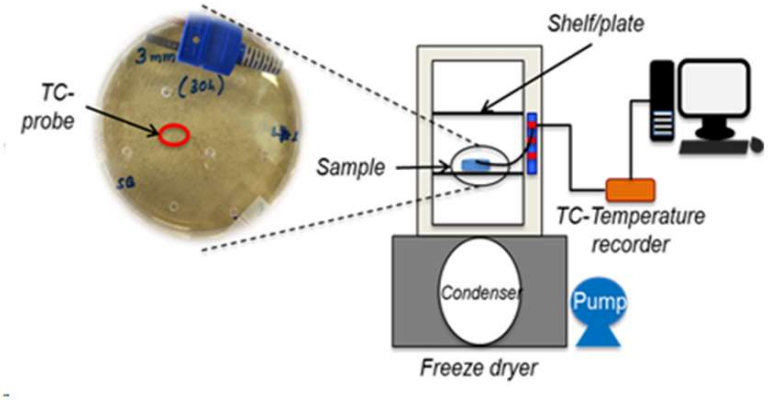

Figure 3.1 Schematic representations of freeze dryer and set-up for on-line temperature recording. *TC*, thermocouple.

3.4.3 Freeze drying experiments

For the freeze-drying (FD) experiments, pure maple syrup was diluted with water to 20 °Brix (from preliminary study, concentrations of 5, 10, 20 and 30 Brix were assessed). Maple syrup solution (MS, 20°Brix) was poured in polystyrene sterile Petri dishes (90mm outer diameter with perforated cover) up to different product thickness. Then, samples were frozen at -40°C for about 24h in a Sanyo medical freezer (MDF 235, Gunma, Japan). Prior to freezing, a thermocouple-probe was carefully placed in the center of the sample to read on-line product temperature (T_p) during the experiment (as shown in **Figure 3.1**). T_{shelf} was determined by attaching another thermocouple-probe on the shelf surface with insulation

tape. For all the studies, freeze-dryer condenser was operated at -90°C and vacuum was maintained below 30mtorr.

3.4.4 FD protocol development

To achieve freeze-dried maple sugar powders (MSP) with optimal characteristics, three different cases were studied (drying conditions summarized in **Table 3.1**). Case I was used as the base to formulate the final two-step protocol for MS freeze-drying. In Case I, a single-cycle FD for 40hours at a shelf temperature of -36°C was performed for two sample thicknesses, 3 and 5mm. Sample of 3 and 5mm thickness freeze-dried in Case I will be abbreviated as 3mm-40h and 5mm-40h, respectively, in the remaining text. Primary shelf drying temperature (T^{PD}) was determined from knowledge of glass transition temperature (T_g) of the initial humid product. Then, the minimal end-point of primary drying (t^{PDmin}) was identified from the sample thermal history (using thermocouples), and drying kinetics of Case I. Finally, the secondary shelf drying temperature (T^{SD}), was determined from the measurements of glass transition temperature of the dry product.

Table 3.1 Freeze drying of maple syrup at various drying conditions.

Case	Sample thickness	T^{PD} (°C)	t^{PD} (h)	T^{SD} (°C)	t^{SD} (h)	Total drying time (h)	Abbreviation used in the text
I	3mm	-36	40	-	-	40	3mm-40h
	5mm						5mm-40h
II	3mm	-36	15	+30	10	25	PD-15h
III	3mm	-36	20	+30	10	30	PD-20h

Three independent experiments were carried out for each Case; T^{PD}, primary drying temperature; t^{PD}, primary drying time; T^{SD}, secondary drying temperature; t^{SD}, secondary drying time; PD, primary drying; SD, secondary drying.

Cases II and III consisted of using the formulated protocol for the 3mm-thickness sample (shorter drying time than 5mm), where T^{PD} and T^{SD} were set at -36 and +30°C, respectively, but during different primary drying times. For case II, t^{PD} was 15 h and 20h for Case III, while secondary drying time (t^{SD}) was set to be constant (10h) for both Cases II and

III. Hereafter, the maple powder obtained from Cases II and III will be abbreviated as PD-15h and PD-20h, respectively.

3.4.5 Measurement of drying kinetics

In order to determine the freeze-drying kinetics, the vacuum was interrupted after certain time (t) and samples were taken out of the freeze-dryer and weighed with an electronic balance (Mettler AT250, Mettler Instrument, Switzerland). Moisture content in the product during experiment was calculated based on initial moisture content of the sample and mass loss. For the following time, the experiment was started again from the beginning. Regarding the protocol formulation (Case I of Table 1), times explored for establishing the drying kinetics were 0, 3, 7, 12, 24 and 40h. For Case II, times were 0, 3, 7, 12, 15, 20 and 25h, whereas for Case III, 0, 3, 7, 12, 15, 20, 25 and 30h. The experiment was repeated twice.

3.4.6 Glass transition temperature

The glass transition temperature (T_g) of samples was measured with differential scanning calorimetry (DSC 823^e, Mettler Toledo, USA). A calibration of instrument was done with indium (melt temperature, T_m=156.59°C; heat of fusion, ΔH_m = 28.57J/g). A 10-20mg sample was prepared in a 40-µL aluminum pan, and hermetically sealed. An empty pan was used as a reference. For obtaining the T_g of 20°Brix maple syrup solution (humid product), sample was scanned from -65 to -25°C at a scan rate of 10°C/min. For freeze-dried maple sugar powders, scanning was done from -10 to 110°C at a scan rate of 20°C/min with heating-cooling-heating cycle. Liquid nitrogen was used for cooling the calorimeter. The T_g was calculated using the STAT^e SW software version 9.30 (Mettler Toledo, USA).

3.4.7 Analysis of freeze-dried maple sugar powders

Freeze-dried maple syrup was ground manually with flat stainless steel spatula over a flat surface until no lump of powder was noticed. All the samples were ground under inflatable polyethylene AtmosBag (Aldrich® AtmosBag, SIGMA-ALDRICH, Canada) to avoid moisture gain from the environment. The freeze-dried MSP obtained from cases II

(PD-15h) and III (PD-20h) were analyzed for their physical properties, flowability and sorption isotherm.

3.4.7.1 Water content and water activity

Water content in the MSP was determined by vacuum-oven drying (Fisher Scientific, OH, USA) at 70°C and vacuum pressure of 558mm Hg for 5h (Park & Bell, 2004). Water activity of the powder was measured using a water activity meter (AquaLab Series 3, ON, Canada). During the water activity measurement, the recorded temperature was at 24±0.5°C. All the measurements were done in triplicate.

3.4.7.2 Dissolution test

The dissolution test was modified from (Quek, Chok, & Swedlund, 2007). About 0.5g of MSP was dissolved in 10mL of water in mini-beaker using magnetic stirrer (THERMIX 210 T, FISHER, Canada), stir set at number 4. The dimension of magnet was 12.8×3.4mm (L×diameter). The complete dissolution of sample was measured by visual observation. The time required by powder to completely reconstitute in water by vortexing at ambient temperature is termed here as dissolution time (s).

3.4.7.3 Color analysis

Color of the sample was measured with a CR-300 Chroma colorimeter (Konica Minolta, Japan), referring to color space CIE $L^*a^*b^*$, against a white ceramic reference plate (Y=92.6, x=0.3136, y=0.3195). The freeze-dried MSP was determined by measuring the specific color parameters, where, L^*, a^* and b^* signifies color brightness, red parameter and yellow parameter, respectively. Chroma value indicates the color intensity or saturation of the sample and it was calculated using the formula, $(a^{*2}+b^{*2})^{1/2}$. Data were mean of three measurements.

3.4.7.4 Flow characteristics measurement

The measurement of flow characteristics of MSP was performed under inflatable polyethylene AtmosBag (Aldrich® AtmosBag, SIGMA-ALDRICH, Canada) to work in

isolated environment and avoid any change in moisture content. AtmosBag was of 38in.×51.5in. (W×L) dimensions with built-in gloves. The AtmosBag was inflated and desiccant was placed on a tray to control the humidity at 12 ± 3% during the measurements. Bulk density of MSP was determined by pouring the sample in a graduated cylindrical glass tube of 10mL and recording the mass (m_s) and volume of the powder (V_b). For determining the tapped density, the cylindrical glass tube with constant mass of powder (m_s) was repeatedly dropped from the vertical distance of 14±0.5mm high manually until negligible difference in volume between succeeding measurements were recorded (V_t). The powder bulk (ρ_b) and tapped densities (ρ_t) were calculated using *Eq. (3.1)* and *(3.2)*, respectively.

$$\rho_b = \frac{m_s}{V_b} \qquad Eq.(3.1)$$

$$\rho_t = \frac{m_s}{V_t} \qquad Eq.(3.2)$$

The Carr's index (CI) and Hausner ratio (HR) labels the flowability and cohesiveness of a powder, respectively. Both CI (*Eq. (3.3)*)and HR (*Eq. (3.4))* were calculated using the above measured value of bulk and tapped densities of a powder as follows:

$$CI = 1 - \left(\frac{\rho_b}{\rho_t}\right) \times 100\% \qquad Eq.(3.3)$$

$$HR = \frac{\rho_t}{\rho_b} \qquad Eq.(3.4)$$

Angle of repose (θ), determines the flow properties of a powder, and is defined as the angle formed by the free surface of a pile of powder with the horizontal plane. θ was measured by following the described method with slight modification (Seerangurayar et al., 2017). Briefly, the glass funnel of 6cm in diameter with a stem of 3.5cm long (1cm of stem diameter at the opening) was attached to a stand. The distance between the end of the funnel stem and flat surface was maintained at 4cm. About 3g of sample was poured in the funnel and the diameter of the base of the powder cone (measured at three different positions and averaged) and height of cone were recorded using a digital caliper (STM, S B Simpson Group Inc., ON, CA). Then, θ was calculated by using *Eq. (3.5)*.

$$\theta = tan^{-1}\left(\frac{h}{r}\right) \qquad Eq.(3.5)$$

where h for height of conical pile, and r, radius of horizontal base of powder.

Scanning electron microscopy (SEM, JSM-6360 Version 1.0, JEOL Inc., USA) was operated at an accelerating voltage of 20kV. The powder samples were mounted on stubs and coated with thin layer of gold before photographed by SEM at a magnification of 100× (at scale bar of 100μm).

Particle size of powder was estimated using ImageJ software version 1.52i (National Institutes of Health, USA) of scanning electron micrograph of samples. Subsequently, SEM image was accessed with ImageJ software and calibrated to set the scale of image from pixels to μm. The particle size was measured using the particle analyze tool. As FD powder particles were not round but close to a rectangular shape, particle sizes in SEM images were characterized by their longer and shorter lengths. Then, an equivalent circle diameter was calculated from the average area of the rectangle and reported as an equivalent particle size. The sizes of at least fifty particles was measured and averaged. The results are expressed as average±S.D.

3.4.7.5 Sorption isotherm

The sorption isotherms of freeze-dried MSP were obtained by placing 0.5g of sample in triplicate in desiccators at room temperature over various saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, NaBr, NaCl and KCl of water activities, 0.11, 0.22, 0.33, 0.58, 0.75 and 0.86, respectively). Water activity of solution was checked with water activity meter (Aqualab Series 3, ON, Canada). Weight of sample was measured initially after 3 days, and then at every day during one week until constant weight was noticed, as mentioned by (Farahnaky, Mansoori, Majzoobi, & Badii, 2016). The equilibrium moisture content was then determined by dehydration in vacuum oven at 70°C for 24h.

The Guggenheim-Anderson-de Boer (GAB) model (*Eq. (3.6)*) was applied to fit the experimental data of sorption isotherm of maple sugar powders.

$$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \qquad Eq.(3.6)$$

where X is the equilibrium moisture content (kg H_2O/kg dry solid), X_m is the monolayer moisture content (kg H_2O/kg dry solid), C and K are constants related to the heat of sorption of the monolayer and multilayer region, respectively (Basu et al., 2006). The model was fitted to experimental data using regression equation wizard in SigmaPlot 12.5 (Systat Software Inc., USA).

3.4.8 Statistical analysis

Analysis of physical and flow properties of the freeze dried maple powders were done in triplicate of three independent experiments and the values are presented as Mean±S.D. Significant differences between the sample obtained from freeze drying conditions were determined by one-way ANOVA (SigmaPlot 12.5) followed by Tukey's test at α=0.05.

3.5 Results and discussion

3.5.1 Formulation of freeze-drying protocol

The two-step protocol for the formulation of freeze-drying of maple syrup, as described in materials and methods (section 3.4.4), was followed.

3.5.1.1 Glass transition temperature (T_g)

Figure 3.2 shows the results of T_g for (a) initial maple syrup solution, and (b) final freeze-dried sugar powders. From **Figure 3.2a**, the T_g of maple syrup solution (MS, 20°Brix) was found to be -33.20±1.63°C (onset glass transition temperature, $T_{g,onset}$), comparable to that of the 20% sucrose solution (T_g'= -32°C) (Levine & Slade, 1988). The $T_{g,onset}$ of maple sugar was determined at 52.8°C (shown in **Figure 3.2b**). Roos and Karel reported the T_g of anhydrous amorphous sucrose at 57°C (Roos & Karel, 1991), higher than the current obtained result. T_g significantly depends on moisture content of the sample (Roos & Karel, 1991), specially at lower water contents. As the maple sugar was not completely dried, hence the presence of some residual moisture (3.7%, dry basis) may have resulted in lower T_g in this study compared to that obtained by Roos and Karel. Additionally, the lower T_g values of

maple sugar than the ones for pure sucrose could be due to the presence of simple sugars (glucose and fructose) in maple sugar, since the T_g of glucose (31°C) and fructose (5°C) are lower than for sucrose (Yrjö H. Roos, 1993).

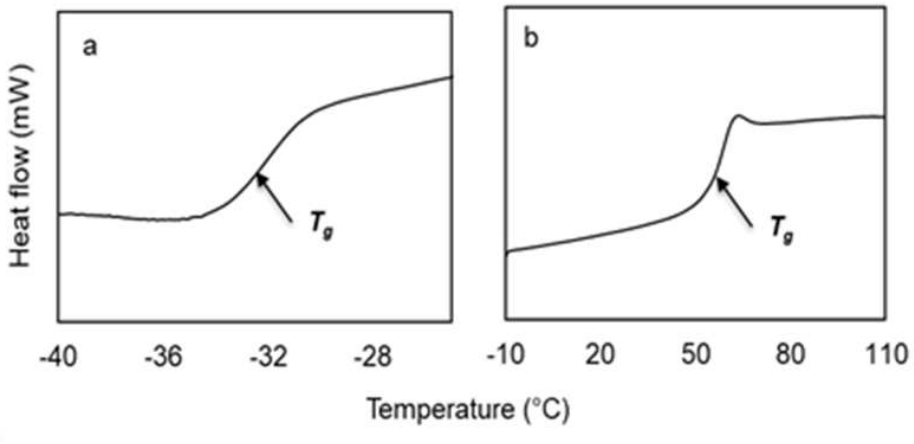

Figure 3.2 DSC thermograms of 20°Brix maple syrup solution (a) and maple sugar (b) showing the glass transition temperature (T_g).

3.5.1.2 Identification of primary and secondary freeze-drying temperature using T_g

As the T_g indicates a critical maximum temperature to avoid quality defects during processing, therefore FD needs to be done below this temperature. Hence, the T^{PD} was selected below T_g of sample (**Figure 3.2a**), at -36°C. Similarly, the secondary drying temperature (T^{SD}) was estimated using the T_g of freeze-dried maple sugar (**Figure 3.2b**). The T^{SD} for secondary drying was selected to be 30°C, which was sufficiently lower than the obtained T_g of maple sugar powder.

3.5.1.3 Single-cycle freeze-drying kinetics and determination of minimum primary drying duration

After determining the primary and secondary drying temperatures, the next step was to identify the respective drying time. Primary drying (PD) is considered the longest step in freeze-drying. Therefore, the identification of its minimum endpoint can help to reduce the overall drying time and operating cost. This was done from temperature and moisture content

evolution during single-cycle FD experiment at constant shelf temperature of -36°C for 40hours (Case I, Table 1). Results are shown in **Figure 3.3**.

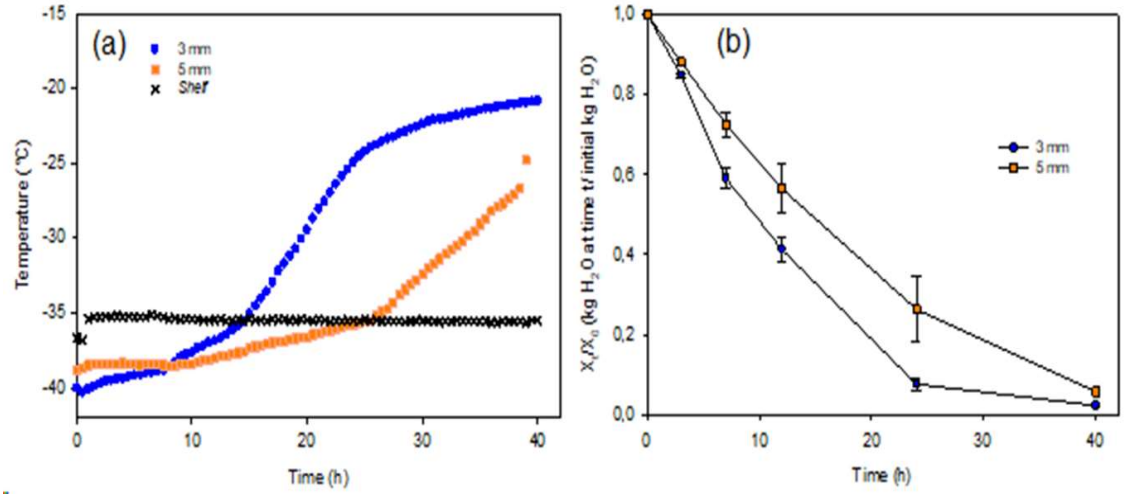

Figure 3.3 Change in product-/shelf-temperature (a) and drying kinetics (b) for two sample thicknesses (3 and 5mm), during single-cycle freeze-drying (T^{PD}= -36°C) of 40h.

The evolution of product (T_p; 3 and 5mm thickness) and shelf temperature (T_{shelf}) during freeze-drying was recorded, shown in **Figure 3.3a**. When drying started, the product temperature (T_p) increased with time due to solids concentration in the matrix, following the ice removal. After some time, the increasing product's temperature equals the shelf temperature. In the present case, the temperature of 3 and 5mm-thickness samples reached T_{shelf} at 15 and 25h, respectively. When temperature of shelf equals that of the product, it indicates the end of primary drying (Patel et al., 2010). The difference in endpoint for the two samples is strongly related to the differences in their thickness. An endpoint of primary drying for 5mm sample thickness was nearly 1.6-times longer than for 3mm thick sample. In previous studies, freeze-drying time was reported to depend linearly or to the square of sample thickness (Shishehgarha, Makhlouf, & Ratti, 2002).

In **figure 3.3a**, it can also be observed that the T_p continued to increase after it equals to T_{shelf}, but with a steeper slope indicating the end of sublimation. At the end of primary drying, increase in dry cake resistance also causes some increase in T_p (Ratti, 2013). Additionally, the formation of dry layer and its thickness has an impact on mass transfer rate, which will be discussed in the following paragraph on drying kinetics.

The drying kinetics was studied to perceive the moisture removal rate from the samples of 3 and 5mm thickness (shown in **Figure 3.3b**) during the single-cycle FD at -36°C of 40h. As expected, the decrease of X_t/X_0 was exponential for both sample thicknesses. The tendency of moisture removal for both sample thicknesses can be divided into two phases: first phase, where the drying rate was fast (primary drying), and second phase, where the rate slowed down (secondary drying). From **Figure 3.3b**, the first phase for 3 and 5mm sample thickness was around 15 and 25h, respectively. This can also be related to the results obtained in the case of shelf and product temperature evolution (**Figure 3.3a**), which indicates the end of primary drying at 15 and 25h for 3 and 5mm sample, respectively. Obviously, the rate of moisture removal was faster for 3mm sample than for 5mm. It was reported that the increasing sample thickness considerably slowed down the moisture removal rate during freeze-drying of strawberry slices (Shishehgarha et al., 2002). In the secondary phase, there was a decrease in moisture removal rate that can be expected from the increase in dry layer thickness and thus, resistance to mass transfer during the drying process (Ratti, 2013). Therefore, in order to facilitate the removal of moisture by desorption during secondary drying phase and thus reduce overall freeze-drying time, it is recommended to increase the drying temperature after T_p equals T_{shelf}.

3.5.1.4 Formulation of two-step protocol

Considering the results reported in the previous paragraphs, only the 3mm sample was used for the succeeding studies. As discussed above, in the first step of protocol formulation, primary and secondary drying temperatures were determined to be -36 and +30°C, respectively. In the second-step, the minimum endpoint of PD for 3mm sample was identified at 15h using thermocouples. However, it is important to note that the product temperature read by thermocouple may not represent the whole sample during the freeze-drying experiment, therefore, a soak period of 10-30% is generally added to primary drying time to compensate for the bias (Patel et al., 2010). Thus, the experiment was also conducted by considering a soak period to be added to the primary drying time, where t^{PD} was increased from 15 to 20h (a soak period of around 30% of the primary drying time). On the other hand, secondary drying time (t^{SD}) was selected to be constant at 10h, using the general 1/3rd rule of drying time. As a result, the FD of maple syrup was done for two primary drying times

(mentioned in Table 3.1), Case II without considering a soak period (PD-15h) and Case III, with a soak period (PD-20h), in both cases followed by 10hours of secondary drying time.

Thus formulated two-step cycle FD conditions were used to study drying kinetics (discussed in section 3.5.2), and to study the physicochemical properties of thus obtained powders (discussed in section 3.5.3 to 3.5.5).

3.5.2 Drying kinetics with improved FD conditions (two-step cycle)

Figure 3.4 depicts the drying kinetics of maple syrup solution (3mm-thickness) dried with the formulated two-step cycle FD conditions for different primary times (PD-15h and PD-20h). For comparison, the sample dried under single cycle freeze-drying (40h) was considered as a control. It can be observed from **Figure 3.4**, that moisture removal was faster with both formulated FD conditions than the control. At 20h, the moisture in PD-15h sample significantly dropped (to less than 5% moisture content), compared to PD-20h and control. Rapid decrease in moisture for PD-15h was due to the increase of shelf temperature (T^{SD}= 30°C) at 15h, when the secondary drying started. Similar drop in moisture for PD-20h sample was achieved at 25h, only when the temperature (T^{SD}= 30°C) was increased at 20h. Therefore, increasing the shelf temperature (secondary drying phase) is important to enhance the removal of moisture from the sample to achieve an optimally freeze-dried maple syrup in minimal drying time (less than 25h) than performing freeze-drying in a single-cycle.

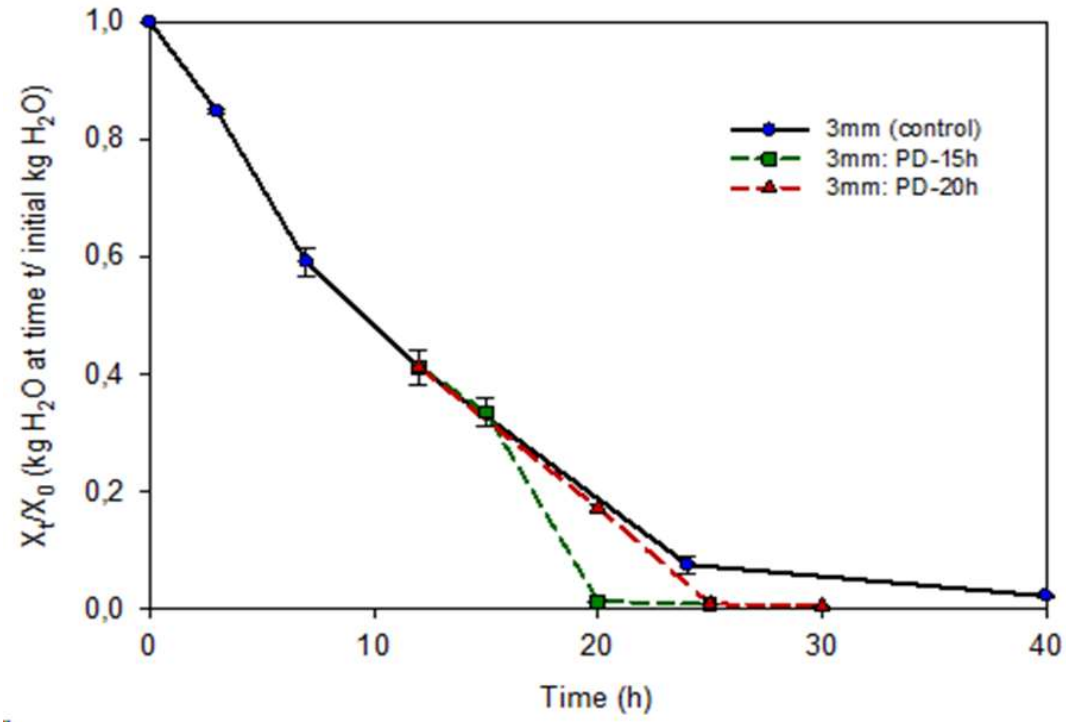

Figure 3.4 Change on drying kinetics curve (3mm-thickness) under new formulated FD conditions (PD-15h and PD-20h). Line plot indicates single-cycle FD of 40h (as control, from protocol formulation) and dashed plot indicates extended drying kinetics, PD-15h (square) and PD-20h (triangle).

3.5.3 Physicochemical properties of maple sugar powders

3.5.3.1 Moisture content

Moisture content of freeze-dried maple sugar powders (MSP) obtained from two sets of drying conditions was below 5% (dry basis), as shown in **Table 3.2**. Moisture content of MSP was significantly lower ($P<0.05$) for PD-20h sample (3.18%, dry basis) than that for PD-15h (4.18%, dry basis), which was obvious due to longer primary-drying time, consequently total drying time.

Depending on the drying conditions and food materials, freeze-dried foods usually have moisture content less than 10%. A study of freeze-and spray-drying of blackberry juice with carrier agents reported that the moisture content in final product was 6-6.11% and 3.4-3.7%, respectively (Franceschinis, Salvatori, Sosa, & Schebor, 2014). The present results were more comparable to spray-dried blackberry powder. Another study of foam-mat freeze-

dried date powder reported the final moisture content of 7.1-12.5% based on the types of carrier agent used (Seerangurayar et al., 2017). Discrepancies in moisture content could be due to the differences in sample material and drying conditions. For instance, FD of date pulp was done at -40°C (sample thickness, 1.5cm; drying time, 72h). FD was done in a single-cycle (one temperature throughout the drying period), despite of possibility of drying the sample at higher temperature (in secondary drying phase). In addition, sample thickness is positively related to the time required for drying. Hence, increased sample thickness and the absence of secondary drying phase could be the reason of the presence of higher moisture content found for FD of date pulp as compared to the current study.

Table 3.2 Moisture content, water activity and dissolution of the freeze-dried MSP.

Samples	Moisture content (%, db)	Water activity	Dissolution time (s)
PD-15h	4.18±0.76[a]	0.24±0.05[a]	13.66±1.49[a]
PD-20h	3.18±0.10[b]	0.19±0.05[b]	13.93±1.30[a]

Values are presented as mean (n=3) ± SD. [a,b] different letters in column means significant difference by Tukey's test. db, dry basis; MSP, maple sugar powder.

3.5.3.2 Water activity

Water activity (a_w) of MSP obtained from both drying conditions was less than 0.25a_w (**Table 3.2**). PD-20h maple sugar powder showed significantly ($P<0.05$) lower water activity (0.19a_w) compared to that of PD-15h (0.24 $_w$). It can be concluded that longer primary drying time (15 *vs* 20h) significantly decreased the water activity, similarly to what was observed for the moisture content (discussed in 3.5.3.1). The obtained a_w result for PD-15h is comparable to that of the foam-mate freeze-dried date powders (0.25-0.27a_w) (Seerangurayar et al., 2017), whereas FD blackberry juice powder produced with carrier agents (0.19a_w) (Franceschinis et al., 2014) was similar to that for PD-20h sample. The higher a_w found in date powder could be related to the presence of hydrophilic fibers in dates, which contributes to increasing the water binding capacity. Moreover, water activities of maple powder, particularly obtained from PD-20h, were comparable to the mango powder obtained from freeze-drying (0.174a_w) as well as from other drying processes including spray and vacuum

drying (0.169 to 0.17a_w) (Caparino et al., 2012). Water activity of food samples depends on various factors such as food matrix, composition and its structure (sample water-binding capacity). Generally, the values of water activity within the range of 0.20 to 0.40a_w ensure the food stability against the hydrolytic reactions, biological and enzymatic activities (Marques et al., 2007). In this study, a_w of maple sugar powders was in the range of 0.19 to 0.24a_w and therefore, can be considered as biochemically stable.

3.5.3.3 Dissolution of maple sugar powders

Dissolution of food powder is a key quality indicator for consumers when the target application is to use in drinks. Dissolution times of studied MSPs are presented in **Table 3.2**. MSP showed quick dissolution in water, within 14s. No significant difference (P=0.730) in dissolution time was observed between the MSP obtained from PD-15h and PD-20h. However, it was slightly higher in the case of PD-20h than that of PD-15h. Faster dissolution of PD-15h maple powder can be related to the presence of higher moisture content in it than PD-20h powder. Similar tendency was also observed for the spray-dried watermelon powders, where positive relation between moisture content and dissolution was reported (Quek et al., 2007). The explanation proposed was that food powders with high moisture content have greater agglomeration affinity, which can facilitate the reconstitution of powder. It is reported that FD of food resulted in a porous-like structure due to sublimation of ice during freeze-drying process (Sablani & Rahman, 2002). As a result, it increases the specific surface area of the food. This can be reason why FD maple sugar powders have a quick dissolution.

3.5.3.4 Color analysis

Color of food powder is the first sensory aspect noticed by consumers. Additionally, powder's color greatly affects the final color when it is reconstituted. **Table 3.3** shows the color parameters (L^* a^* b^*) determined for FD maple sugar powders under different drying conditions. With the increase in primary drying time from 15 to 20h, no significant difference (P=0.131) in L^* parameter was observed. The powders obtained from both drying conditions showed high L^*, within the range of 84 to 85. High L^* values of MSP may be due to the absence of browning, since the maple syrup was dried at low temperature and under vacuum.

Other studies have also reported that freeze-dried pumpkin flour and soursop fruit pulp powder demonstrated high *L** value (Ceballos, Giraldo, & Orrego, 2012; Que, Mao, Fang, & Wu, 2008). However, chroma value was found significantly different ($P<0.05$) between the studied maple sugar powders. Chroma value represents the true color intensity or saturation of the sample. The higher chroma value for PD-15h can be associated to its higher redness and yellowness parameters (**Table 3.3**), compared to that of PD-20h. Also from the digital photographs (**Figure 3.5**), it can be observed that MSP produced from both drying conditions are light in color; however, PD-15h powder is more yellowish than PD-20h sample (similar to the result obtained from colorimeter).

Table 3.3 Color analyses of freeze-dried maple sugar powders.

Samples	Color parameters			
	L* ***(lightness)***	***a**** ***(redness)***	***b**** ***(yellowness)***	**Chroma value**
PD-15h	84.93±0.33[a]	0.14±0.05[a]	15.70±0.28[a]	15.70±0.28[a]
PD-20h	85.72±0.68[a]	-0.11±0.04[b]	14.09±0.34[b]	14.09±0.34[b]

Values are presented as mean (n=3) ± SD. [a,b] different letter in column means significant difference by Tukey's test.

Figure 3.5 Digital photographs of maple sugar powders obtained from PD-15h and PD-20h conditions.

3.5.4 Flow properties of the freeze-dried maple sugar powders

Flowability is an important property of powder to analyze, because of its relation to bulk material handling, processing and formulations (Amrutha et al., 2014; Tze et al., 2012). It is even vital to investigate flow property for sugar-rich food products such as maple sugar

(>95% sucrose, dry weight) since amorphous sucrose is known to be very hygroscopic in nature and therefore, can greatly affect the flowability of powder (Szulc & Lenart, 2012). In order to evaluate the flow properties of MSP, the bulk and tapped densities were measured (**Table 3.4**). Bulk density of the PD-15h maple sugar powder sample was significantly ($P<0.05$) higher than that of PD-20h. However, no significant difference ($P=0.226$) in tapped density was found for MSP produced under increased primary drying time. In both drying cases, the tapped density was higher than bulk density as tapping allowed the smaller particles to occupy the voids between larger particles and thus, achieve denser packing.

According to Hausner ratio (HR) (Hausner, 1967), the cohesiveness of powder is considered high for HR value greater than 1.4, intermediate for the values between 1.2 and 1.4, and low for values less than 1.2. From the **Table 3.4**, the freeze-dried MSP were highly cohesive in nature, since the HR of powders were greater than 1.4. No significant difference ($P=0.084$) in HR was observed for MSP obtained from both drying conditions. High cohesiveness of MSP could be due to the predominant presence of sugar, mainly sucrose.

Furthermore, Carr's index (CI) of MSP was calculated to measure the flowability of powder (**Table 3.4**), but no significant difference ($P=0.099$) in CI was found for powders produced from two drying conditions. CI categorized the powder's flowability as very good for the value less than 15%, good flow is denoted by values between 15 and 20%, fair flow by values between 21 and 35%, bad flow by values between 36 and 45%, and very bad flow when values of CI is greater than 45% (Carr, 1965). The freeze-dried MSP showed CI between 31 to 36%, indicative of fair to bad powder flow characteristics.

Finally, the angle of repose *(θ)* determined for MSP is also listed in **Table 3.4**. Powder with repose angle less than 20° indicates free flowing, θ value between 20 and 30° represents good flow, value between 30 and 34° represents fair flow and greater than 35 or 40° indicates poor flow (Amrutha et al., 2014). From **Table 3.4**, MSP showed fair flow, and no significant difference ($P=0.792$) for θ were observed for the powders obtained at PD-15h and PD-20h. Angle of repose can provide a basic idea of powder flowability. However, results might not be consistent since they depend on various factors such as cone shape, funnel opening, fall height of the powder, personal error, etc. during the measurement. In the current measurement of angle of repose, the coefficient of variation falls in between 12.5 to 20.2%.

However, if measured under controlled conditions or with automated equipment, magnitude of repose angle can provide an important information for the designing of conveyors to remove bin discharge and powder unloading device (Amrutha et al., 2014).

Table 3.4 Flow characteristics of freeze-dried maple sugar powder.

Flow measurements	**PD-15h**	**PD-20h**	**Flow characteristics**
Bulk density (g/ml)	0.29±0.01[a]	0.26±0.02[b]	-
Tapped density (g/ml)	0.43±0.05[a]	0.41±0.02[a]	-
Hausner ratio	1.46±0.10[a]	1.60±0.21[a]	Highly cohesive
Carr's index	31.13±4.55[a]	36.60±8.78[a]	Fair to bad flow
Angle of repose *(ϑ)*	30.45±6.16[a]	31.17±3.91[a]	Fair flow

Values are presented as mean (n=3)±SD. [a,b] different letter in row means significant difference by Tukey's test.

Considering the results obtained from flow characteristics measurement, MSP produced under PD-15h condition showed lower (not significant) CI and angle of repose value, which means better flowability, than PD-20h. Despite of higher moisture content of PD-15h powder than in PD-20h, better flowability for PD-15h sample could be explained by decrease in friction and interlocking which caused by surface roughness since moisture acted like a lubricant. Similar result was also found for spray dried pitaya fruit powders, in which powder with higher moisture content showed better flowability than that of lower one (Tze et al., 2012).

From SEM images, freeze-dried MSP presented irregular particle size, similar to published data on freeze-dried date syrup and blackberry juice powder (Franceschinis et al., 2014; Seerangurayar et al., 2017). In this study, MSP presented a wide distribution of particle sizes with an equivalent diameter of 77.73±31.76µm, higher than reported particle sizes of FD date syrup powder, which presented free-flow to fairly cohesive flowability (Seerangurayar et al., 2017). Overall, freeze-dried MSP obtained under both conditions showed fair to bad flow characteristics, which could be due to FD powder particle irregularity that may cause mechanical interlocking between the particles, thus reducing powder flowability.

3.5.5 Sorption isotherm

The sorption isotherm experimental data and the fitted Guggenheim-Anderson-de Boer (GAB, *Eq. 3.6*) for MSP obtained for PD-15h and PD-20h conditions are presented in **Figures 3.6a and b**, respectively. For the studied water activity range (0.11 to 0.86a_w), MSP from both drying conditions showed a typical sigmoidal shape curve of type II according to Brunauer's classification, at constant room temperature. Type II is the most common isotherm curve for many food products, mainly for amorphous and sugar-rich foods. The amount of moisture uptake for powders from both drying conditions was similar for studied water activities. Equilibrium moisture content increased linearly for the MSP placed under 0.11 to 0.36a_w. From 0.36 to 0.56a_w, there was a decrease in moisture content that could be ascribed to increase in molecular mobility at higher water activities, probably allowing a change from amorphous to crystalline state. This is a general adsorption isotherm shape of amorphous sucrose (M. Mathlouthi & Rogé, 2003). Above 0.76a_w, there was an abrupt increase in moisture content and MSP reached the deliquescence point at 0.86a_w.

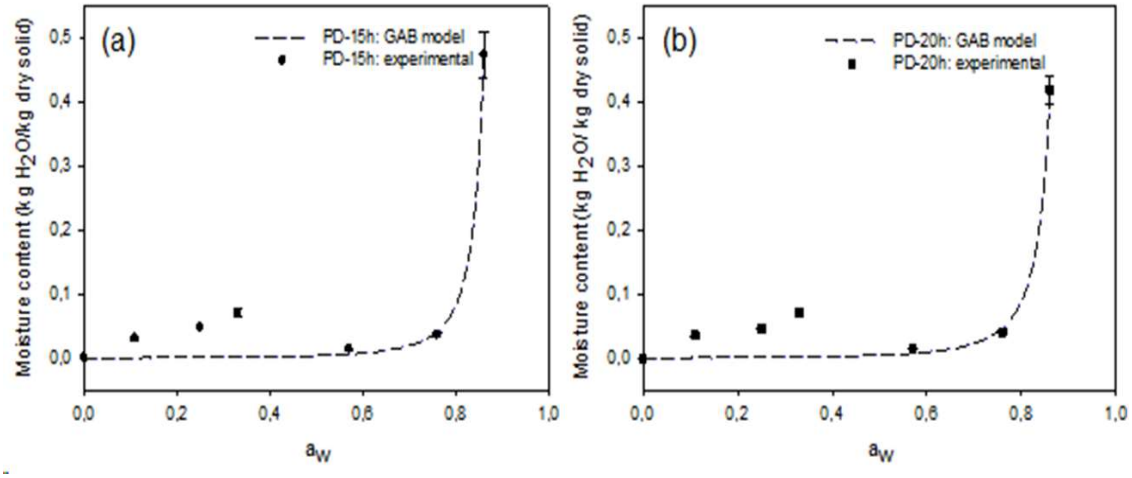

Figure 3.6 Sorption isotherm of freeze-dried maple sugar powders obtained from PD-15h (a) and PD-20h (b) conditions, fitted with GAB model (dashed line). PD, primary drying.

Table 3.5 shows the GAB model parameters for MSP obtained from different drying conditions. The fitness of GAB model was slightly higher for PD-15h (R^2=0.9521) than for PD-20h (0.94). Monolayer moisture content (X_m) is the amount of water strongly adsorbed to specific sites at the surface of food materials and an important parameter for assuring food stability. The value of X_m was determined to be 0.079 and 0.052 (kg water/kg dry solids) for

PD-15h and PD-20h sample, respectively. The moisture contents of MSP (**Table 3.2**) obtained from both drying conditions were below their respective X_m values, indicative of good product stability over time. Other studies have also reported the similar X_m value for sugar-rich foods such as freeze-dried mango pulp powder (0.087, g water/g solid) and vacuum-dried honey powder using different maltodextrin ratios (0.049 to 0.062) at room temperature (Fongin, Kawai, Harnkarnsujarit, & Hagura, 2017; Nurhadi & Roos, 2016). Likewise, Farahnaky et al. reported X_m value (0.064 to 0.185) for vacuum-dried date syrup powder studied at different sorption temperature of 5 to 60°C, when fitted on GAB model (Farahnaky et al., 2016). The *C* values obtained from GAB model for this study were lower (0.016-0.032) than those obtained for the above reported food materials (0.60-1.87, 2.12-8.84, and 3.0-5.8 for date syrup powder, mango pulp powder, and honey powder, respectively). This variation could be due to the mathematical compensation between *C* and *K* parameters. On the other hand, *K* values usually vary between 0.7 to 1.0, for many food products (Rahman, 1995). In our case, the value of *K* was slightly higher than one (1.10 and 1.11) for the studied freeze-dried MSPs (**Table 3.5**), which is common for sugar-rich foods. Similar to our results, *K* values were reported to be greater than one for freeze-dried mango pulp powder (1.009) and vacuum-dried honey powder (1.10-1.20) (Fongin et al., 2017; Nurhadi & Roos, 2016).

Table 3.5 The GAB model parameters of maple sugar powders from PD-15h and PD-20h.

GAB parameters	**PD-15h**	**PD-20h**
X_m	0.079	0.052
K	1.112	1.107
C	0.0162	0.032
R^2	0.95	0.94

X_m, monolayer water content (kg water/ kg dry solids); PD, primary drying.

3.6 Conclusions

A simple freeze-drying protocol for dark grade maple syrup was formulated in two-steps:

a. Using the glass transition temperatures for humid and dry products, the primary and secondary drying temperatures were determined,
b. End of primary drying was determined from online shelf and product temperatures reading, using thermocouples, and subsequently confirmed with the drying kinetics.

The proposed systematic protocol for FD of maple syrup can also be used to freeze-dry other high sugar-rich liquid foods. Maple sugar powders (MSP) produced from PD-15h and PD-20h conditions were determined low moisture (less than 5%, dry basis) and water activity (less than 0.21a_w), hence biochemically stable. Prolonged primary drying time, from 15 to 20h, had no impact on powder lightness, however chroma value for PD-15h was higher than for PD-20h. Powder showed satisfactory flow characteristics when determined by Hausner ratio, Carr's index and angle of repose. MSP were highly hygroscopic and the sorption isotherm study demonstrated typical Type II isotherm curve. When fitted with GAB model, the values of monolayer moisture content and K were in the range of 0.052-0.079 (kg water/kg dry solid) and 1.10-1.11, respectively. From the obtained physical and flow properties, FD can be done without considering the soaking period to primary drying phase, unless very low moisture and water activity are targeted. Freeze-dried MSP could be used in instant-drinks owing to its quick dissolution in water as well as in other food applications, particularly for natural food products. However, the effect of FD on nutritional properties including polyphenols and long-term storage stability should be further studied to anticipate other food applications.

3.7 Acknowledgements

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Chapter 4 Impact of drying processes on properties of polyphenol-enriched maple sugar powders

Short title: Drying of substandard quality maple syrup

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4.1 Résumé

Le but de cette recherche était de développer un ingrédient alimentaire naturel à base de poudres de sucre d'érable (MSP) enrichies en polyphénols. En incorporant 0,01% (p /v) d'extrait d'eau chaude (extraction à l'eau chaude: 90 ° C et 1h; écorce / eau, 1/10 p/v) à partir de sucre et d'écorce d'érable rouge dans du sirop d'érable de qualité inférieure, ce dernier a été enrichi de 13 à 20% en contenu phénolique total (TPC). Les mélanges (sirop et extraits) ont été séché par lyophilisation (FD: à -36°C pendant 15h et ensuite à 30°C pendant 10h) ou par double-cylindre sous vide (VDD: à 80°C et 660Torr) pour produire des poudres. L'influence des processus de séchage sur le TPC, la capacité antioxydante et les propriétés physiques des poudres a été étudiée. Les deux processus de séchage ont entraîné une diminution du TPC et de l'activité antioxydante du MSP. Néanmoins, les extraits d'écorce ont contribué à un TPC plus élevé (8 à 10%) et à une capacité antioxydante (>40%) par rapport au MSP témoin. La teneur en humidité des poudres de VDD (0,63 à 0,71%, sur base sèche) était significativement inférieure à celle des poudres à base de FD (4,10 à 4,40% sur une base sèche). Les MSP produits par FD étaient amorphes et ceux produits par VDD sont cristallins. Les poudres de FD présentaient des propriétés instantanées (temps de dissolution de 12-13s), alors que celles produites par le VDD étaient moins cohésives (ratio de Hausner, 1,13 à 1,21) avec un excellent écoulement.

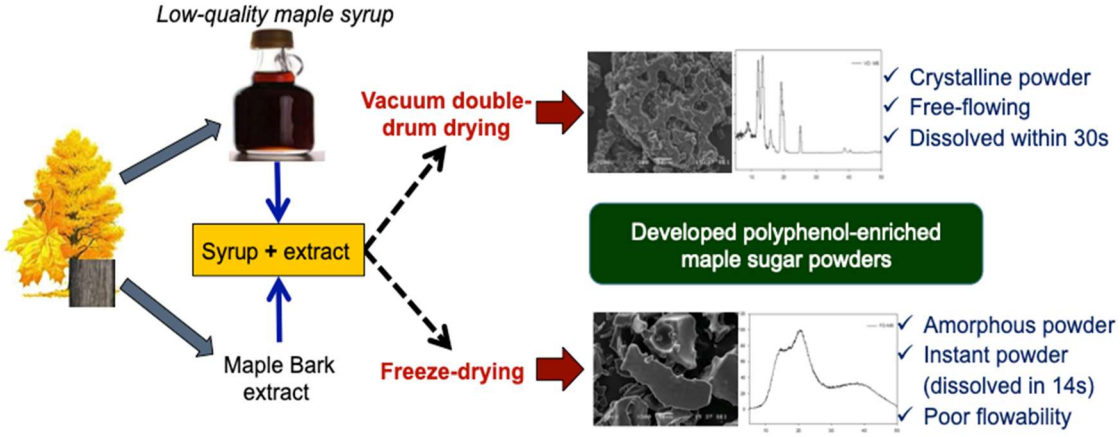
Mots clés: lyophilisation, séchage sous double tambour, propriétés de la poudre, sirop d'érable, ingrédients alimentaires naturels, polyphénols

4.2 Abstract

The aim of this research was to develop a natural food ingredient based on maple sugar powders (MSP) enriched in polyphenols. By incorporating 0.01% (w/v) of hot water extract (hot water extraction: 90°C and 1h; bark/water, 1/10 w/v) from sugar and red maple bark into substandard quality maple syrup, the latter was enriched by 13-20% in total phenolic content (TPC). The mixtures (syrup and extracts) were dehydrated using freeze-drying (FD: at -36°C for 15h and subsequently at 30°C for 10h) or vacuum double-drum drying (VDD: at 80°C and 660Torr) to obtain the powders. Influence of drying processes on TPC, antioxidant capacity, and physical properties of powders was studied. Both drying processes caused a decrease in TPC and antioxidant capacity of MSP. Nevertheless, bark extracts contributed to higher TPC (8-10%) and antioxidant capacity (>40%) compared to control MSP. The moisture content of VDD powders (0.63-0.71%, dry basis) was significantly lower than that of FD powders (4.10-4.40 %, dry basis). MSP produced by FD were amorphous and those produced by VDD crystalline. FD powders had instant-like properties (dissolution time of 12-13s), whereas those produced by VDD were less cohesive (Hausner ratio, 1.13-1.21), with excellent flowability.

Keywords: freeze-drying, vacuum double-drum drying, powder properties, maple syrup, natural food ingredients, polyphenols

Graphical abstract



Practical applications

Consumers are increasingly attracted by natural food products. Canada is the world major producer of maple syrup, a nutritious natural sweetener exclusively obtained from maple trees sap. Unfortunately, a 'very dark' color syrup is accumulated as surplus in large quantity in Canada as it is considered of substandard quality. In this research, freeze-drying (FD) and vacuum-double drum drying (VDD) techniques were studied to produce maple sugar powders from this substandard surplus syrup. This syrup was additionally enriched in polyphenols by adding hot water extracts from maple barks. The obtained polyphenol-enriched maple sugar powders (MSP) have shown interesting qualities for application as natural sweeteners, such as free flowing or instant-like powder. Our results indicate that FD and VDD are suitable techniques for substandard syrup conversion into value-added maple product. MSPs have a huge potential of application as natural food ingredients of instant drinks, cereal mix, cookies and energy bars.

4.3 Introduction

Growing consumers' proclivity towards natural foods has led research on identifying diverse sources of natural foods, particularly plant-based ones. Maple trees, notably the sugar maple and red maple, are socially and economically appreciated North American forest species because of their use as traditional medicine (bark) and food (maple syrup). As traditional medicine, maple bark has been used by Native Americans to treat several ailments by bark infusion (Arnason et al., 1981). Bark extracts of these species have been reported to contain dietary polyphenols such as gallic-acid derivatives, lignans and flavonoids. The major polyphenols in maple bark were found to be maplexins and ginnalins (about 15.6%, w/w), which belong to the family of gallotannin with 1,5-anhydroglucitol as carbohydrate moieties (Geoffroy, Meda, et al., 2017). Gallotannins are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) at the concentration ranging from 10-400ppm as food ingredients (Maqsood et al., 2012). Beside polyphenols, the crude maple bark extracts were reported to present a wide array of organic and inorganic nutrients beneficial to human health (Bhatta, Ratti, Poubelle, & Stevanovic, 2018). Therefore, maple bark extracts have been determined as promising therapeutic and potent antioxidant activities (Bhatta et al., 2018; González-Sarrías, Li, & Seeram, 2012; Royer et al., 2011).

Maple syrup is widely consumed, obtained by evaporating the sap of sugar and red maple trees. It is mainly composed of sugars (primarily sucrose at 65-67%, fructose and glucose), and other nutrients (<1%) such as minerals, organic acids, and polyphenols (Ball, 2007). The presence of polyphenols is well-appreciated in syrup, even though they are found at low concentration (0.0015% w/v, St-Pierre et al., 2014). The polyphenols found in syrup have also shown potential antioxidant, anticancer, α-glucosidase enzyme inhibitory, and anti-inflammatory effects (Apostolidis et al., 2011; González-Sarrías, Li, Seeram, et al., 2012; Thériault et al., 2006).

Maple syrup is predominately produced in Canada (71% of world production), which contributes about 494 million dollars to country's economy (Agriculture and Agri-food Canada, 2018). Despite of important successes of maple industry, it faces serious challenges due to the important volume of the surplus maple syrup, estimated to be more than 38% of annual syrup production in 2017 (Agriculture and Agri-food Canada, 2018). Syrup is

classified into four grades based on its color; golden, amber, dark and very dark (Canadian Food Inspection Agency, 2016). A 'very dark' colored syrup is considered to be substandard and not consumed widely as table syrup. As a result, huge quantities of very dark syrup are accumulated as surplus each year. Therefore, valorization of surplus syrup is a current need of the maple industry. One possible avenue is by enriching this substandard grade with polyphenols-rich maple bark extracts. The application of polyphenol-enriched maple syrup can be then enhanced by dehydrating syrup to produce a free-flowing maple sugar powder.

Drying of sugar-rich foods such as maple syrup is a process involving several problems. The high hygroscopicity of simple sugars, the increase in solubility with temperature, a low glass transition temperature of sugars (fructose, glucose, and sucrose; T_g= 5, 31, and 62°C, respectively (Roos, 1993), and the stickiness problem in the drying equipment (Bhandari, Datta, & Howes, 1997) are some of the obstacles to overcome. Recently, we have applied freeze drying (FD) to produce dried maple syrup powder with instant-like properties (Bhatta, Stevanovic, & Ratti, 2019). However, the dilution of maple syrup at 20 °Brix was required to successfully produce a powder. Additionally, the obtained powder displayed satisfactory to poor flowability. FD is a well-known technique for drying foods containing thermo-sensitive molecules that are prone to oxidation. However, long drying time and operation costs are still the major drawbacks of FD process (Ratti, 2001). Vacuum double-drum drying (VDD) is another technique that is mostly used to dry pastes or viscous liquids such as maple syrup (Daud, 2006). It operates under vacuum but high operating temperatures make it possible to dry foods faster than by FD. It may also preserve the molecules sensitive to oxidation due to the use of vacuum and shorter processing times. Many foods, such as mango puree, jackfruit juice, molasses and honey, mashed potatoes and other starchy foods have been dehydrated using drum-drying technique (Caparino et al., 2012; Daud, 2006; Pua et al., 2010). However, there is no scientific report available in literature about dehydration of maple syrup by vacuum double-drum dryer. The choice of drying methods can greatly affect the final quality of powder. Moisture content, surface morphology, size, density and microstructure (crystalline or amorphous) of powder are important quality parameters influencing their functional properties including dissolution, flowability, etc. (Bhandari, 2013).

Therefore, the objective of the present study was to incorporate polyphenol-rich maple bark extracts to substandard syrup and perform drying of the mixture using FD and VDD techniques, so as to produce polyphenol-enriched maple sugar powders with improved functional properties.

4.4 Materials and methods

4.4.1 Chemical and reagents

HPLC grade methanol, acetonitrile, ethyl acetate, formic acid and sodium carbonate were purchased from Fischer Scientific (Fair Lawn, NJ, USA). Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), and gallic acid were purchased from Sigma-Aldrich (St-Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

4.4.2 Sample preparation

4.4.2.1 Hot water extraction of maple barks

Plant samples (sugar maple and red maple bark) were provided by Levaco Inc. (Quebec, Canada). Samples were air-dried and ground to 250-500µm particle size as previously mentioned (Geoffroy, Fortin, et al., 2017). Hot water extraction of maple barks was done following the method previously used (Bhatta et al., 2018). The concentration of extractives in the filtrate was calculated by oven-dry method at 105±2°C and expressed as mg extractives/ml filtrate. The filtrate was kept at -20°C for further use. The extractives from sugar maple and red maple bark are named as SBX and RBX, respectively, throughout this paper.

4.4.2.2 Addition of maple bark extracts to maple syrup

Maple syrup (MS) was provided by Levaco Inc., Canada. Syrup samples used for this study were determined 66.63±0.21°Brix (Pocket refractometer, PAL-2, Atago, Japan) and

560nm wavelength light transmission of 10.02± 0.32% (Cary® 50 UV-vis spectrophotometer, Varian Inc., USA), glycerol as reference, hence classified as 'Very dark color and Strong taste'(Canadian Food Inspection Agency, 2016). Hot water maple bark extracts were added to maple syrup as to make the final concentration of extract in syrup of 0.01% w/v (based on toxicology results obtained from a previous study (Bhatta et al., 2018)). Hereafter, pure maple syrup without extracts will be denoted as MS (control), while maple syrup with sugar and red maple bark extract as MS+SBX and MS+RBX, respectively.

4.4.2.3 Polyphenol identification by HPLC-MS

To determine polyphenol in syrup by HPLC-MS, samples were treated following the methods described in the literature with slight modifications (Kermasha, 1995; P. St-Pierre et al., 2014). Ten milliliters of maple syrup samples were dissolved in 50mL of water. Then, 250µL of gallic acid (5mg/mL) was added to the sample as external standard. Subsequently, the resulting solution was extracted with ethyl acetate in three successive extractions using the volumes of 50, 25 and 25mL. The organic fractions obtained from three successive extractions were evaporated in a rotary evaporator (Rotavapor R-215, Buchi Labortechnik AG, Switzerland) at a temperature of 45°C. The dried extract was dissolved in 1.5mL of 80% aqueous methanol and filtered using a 0.45µM syringe filter before performing the HPLC analysis. HPLC analyses were performed on Agilent 1100 series HPLC (Agilent Technologies Inc., USA) equipped with a quaternary pump system, an autosampler, a column compartment, and a diode-array detector (DAD). A Zorbax® SB-C18 column (250mm × 4.6mm, 5µm) was used for the separations. The solvent system consists of mobile phase A, water/acetonitrile/formic acid (94/5/1, v/v/v), and mobile phase B, water/acetonitrile/formic acid (69/30/1, v/v/v) (Thériault et al., 2006). The elution gradient was: 100-87% A from 0 to 5min, 87-35% A from 5 to 4 min, isocratic (35% A) from 45 to 55min, 35-0% A from 55 to 56 min, 0-100% A from 56 to 64min, and then kept constant for 10min for equilibration before another injection. 10µL of sample was injected, the flow rate was 0.7mL/min, and column temperature was set at 30°C. The DAD detection was set at 280nm. HPLC analysis was coupled to Agilent 6210 series high-resolution mass spectrometer with Time-of-Flight (MS-TOF), equipped with an electrospray ionization interface. The analysis was performed in negative mode (ESI-). The parameters applied were gas temperature, 325°C; drying gas,

5L/min; nebulizer pressure, 30psig; capillary volatage, 4000V; cone voltage, 65V; skimmer, 60V; and fragmentor, 70V. Data acquisition was achieved using the MassHunter Workstation software (Version B.02.00, Agilent Technologies Inc., USA).

4.4.3 Drying experiments

Freeze drying (FD) of syrup samples were done by following the previously formulated protocol for syrup only (Bhatta et al., 2019). Briefly, a freeze dryer (Freeze Mobile 24, Virtis Company Inc., NY, USA) equipped with a Unitop 400L (Virtis Company Inc., NY, USA) drying chamber was used. Maple syrup was diluted to 20°Brix and poured in Petri dishes to a sample-thickness of 3mm. Then, samples were frozen at -40°C overnight before freeze-drying. The FD experiment was done by setting the shelf temperature at -36°C for 15h and successively at +30°C for 10h. The freeze-dryer condenser was operated at -90°C and vacuum was maintained below 30mTorr. Freeze-dried maple sugar powder without extract (control), with SBX, and with RBX will be indicated as FD:MSP, FD:MSP+SBX, and FD:MSP+RBX, respectively. The FD samples were ground with spatula on flat surface until no lump of powder was noticed.

A laboratory-scale vacuum double drum dryer (Buflovak Group, Buffalo, USA) such as the one depicted in **Figure 4.1** was used to produce powder of polyphenol-enriched maple sugar. Vacuum double-drum dryer (VDD) was comprised of two metal drums that were internally heated by steam at 12psig and operated under fixed vacuum of 660Torr. Nip gap (clearance between two drums) was adjusted at 0.03mm for allowing maple syrup to flow when drum rotates. From preliminary experiments, the rotational speed of drum was selected to obtain the homogenous dried maple sugar (less than 5% moisture content, dry basis). Maple syrup was fed with the aid of suction (created by vacuum) over the nip area. A constant feed level was maintained by adjusting the feed flow rate with the valve. A thin layer of maple syrup initiated to dry in approximately three fourth of the revolution of the drums. The dried maple sugar was scraped off from the drum surface using the in-built scrapers. The initial and final drum surface temperature measured by infrared thermometer (RAYMT6U, Raytek, QC, Canada) was 80±5 and 103±2°C, respectively. The obtained maple sugars were the mixture of maple big to small flakes, rolls and powder. For further analysis, only flakes

and powders were considered. Most of the big flakes were crumbled to small flakes or coarse granules when they were vacuum-packaged. As a result, VDD samples were mixture of coarse granules and powders. Vacuum double-drum dried maple sugar powder without extract (control), with SBX, and with RBX will be labelled as VDD:MSP, VDD:MSP+SBX, and VDD:MSP+RBX, respectively.

Each drying experiment was conducted three times. Maple sugar powder (MSP) was vacuum packed and then stored at 4°C for future analysis.

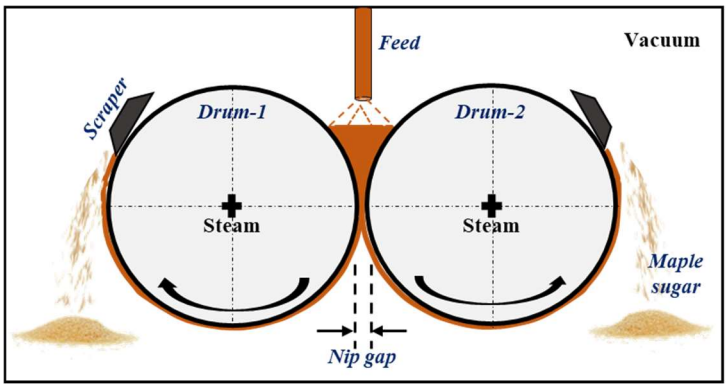


Figure 4.1 Schematic diagram of vacuum double-drum dryer (VDD) used for the development of maple sugar powder. Drum-1 and Drum-2 were internally heated with steam; vacuum was applied in the chamber.

4.4.4 Analyses of sample

4.4.4.1 Sugar composition

Sugar compositions of samples were determined by HPLC-RI (high performance liquid chromatography with refractive index) following the method previously used (Bhatta et al., 2018). Column and detector used for sugar analysis was Sugar-Pak-I (6.5×300mm; Waters, MA, USA) and refractive index detector (Hitachi L-7490), respectively. Sugar compositions were quantified using sucrose, glucose and fructose as standard as expressed in milligram per gram sample on dry basis (mg/g dry sample).

4.4.4.2 Total phenolic content and antioxidant activity

The total phenolic content (TPC) was determined by the Folin-Ciocalteu method in 96-well plates using the method described in literature (Q. Zhang et al., 2006). Briefly, 20µL of standards or samples were mixed in 100µL of Folin-Ciocalteu reagent (diluted, 1:10 v/v in water). After 5 minutes, sodium carbonate (80µl, 75g/L) was added to each well and kept in the dark for 30 minutes before the absorbance was read at 760nm using microplate spectrophotometer (X Mark^{TM}, Bio-Rad Laboratories, USA). The analysis was performed in triplicate. Gallic acid was used for the standard calibration curve and the results are expressed in milligram gallic acid equivalent per gram of sample on dry basis (mg GAE/g dry sample).

Antioxidant activity of samples was determined by the radical scavenging capacity of the samples against 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH analysis was performed in 96-well microplate as described by (Geoffroy, Fortin, et al., 2017) with slight modification. Briefly, a volume of 100µL of Trolox (standard) or samples (20mg/mL) was mixed with 150µL DPPH (40mg/mL in methanol). The mixture was subsequently incubated at room temperature in the dark for 30 minutes before the absorbance was read at 517nm with microplate spectrophotometer (X Mark^{TM}, Bio-Rad Laboratories, USA). Trolox (0 to 250µmol/L) was used for the standard curve and the results were reported in µmol trolox equivalents per gram sugar on dry basis (µM TE/g dry sample).

4.4.4.3 Moisture content

Moisture content in maple sugar powder was determined by vacuum-oven dry method (Park & Bell, 2004).

4.4.4.4 Density, Hausner ratio and Carr's index

Bulk density was determined by weighing about 1g (m_s) of sample in 10mL graduated cylinder and recording the volume occupied by the powder (V_b). For determining the tapped density, the cylindrical glass tube with constant mass of powder (m_s) was repeatedly dropped from the vertical distance of 14±0.5mm high manually until negligible difference in volume between succeeding measurements were recorded (V_t). The powder bulk (ρ_b) and tapped densities (ρ_t) were then calculated using *Eq. (4.1)* and *(4.2)*, respectively.

$$\rho_b = \frac{m_s}{V_b} \qquad Eq.(4.1)$$

$$\rho_t = \frac{m_s}{V_t} \qquad Eq.(4.2)$$

Hausner ratio (HR) and Carr's index (CI) indicates the cohesiveness and flowability of powder, respectively. HR (*Eq. (4.3)*) and CI (*Eq. (4.4)*) were calculated using the measured value of bulk and tapped densities of a powder as follows:

$$HR = \frac{\rho_t}{\rho_b} \qquad Eq.(4.3)$$

$$CI = 1 - \left(\frac{\rho_b}{\rho_t}\right) \times 100\ \% \qquad Eq.(4.4)$$

4.4.4.5 Dissolution time

The dissolution test was modified from (Quek et al., 2007). In brief, about 0.5g of sample was dissolved in 10mL of water in mini-beaker using magnetic stirrer (THERMIX 210 T, FISHER, Canada), stir set at number 4. The dimension of magnet was 12.8×3.4mm (L×diameter). The dissolution time (in seconds) was recorded when the dried sample was fully reconstituted by visual observation.

4.4.4.6 Color determination

Color of the sugar samples was measured with a CR-300 Chroma colorimeter (Konica Minolta, Japan), referring to color space CIE $L^*a^*b^*$, where, L^*, a^* and b^* signifies color brightness, red parameter and yellow parameter, respectively. Chroma value indicates the color intensity or saturation of the sample and it was calculated using the formula, $(a^{*2}+b^{*2})^{1/2}$. Results are presented as averages of three measurements.

4.4.4.7 X-ray diffraction analyses

The crystallinity of maple sugars was analyzed with a powder X-ray diffractometer (SIEMENS/Bruker, Germany). The powder samples were prepared in 0.5mm diameter capillary glass tube (Charlessupper Company, Natick, MA, USA). Samples were measured

under operational conditions of 40kV and 40mA using Co ($K\alpha_{1+2}$) radiation and scanned at a diffraction angle (2θ) between 0 to 50° with a stepped increase of 0.02° 2θ/0.5s.

4.4.4.8 Powder morphology by SEM

The morphology of powder samples was examined by scanning electron microscopy (SEM, JSM-6360 Version 1.0, JEOL Inc., USA) operated at an accelerating voltage of 20kV. The samples were mounted on stubs and coated with thin layer of gold before analysis. All micrographs were photographed at a magnification of 100×, 300× and 1000× at scale bar of 50 and 10µm, respectively. Micrographs of 100× were used to estimate the powder particle size using Image J software version 1.52i (National Institutes of Health, USA). Micrographs were accessed with ImageJ software and calibrated to set the scale of image from pixels to µm, following the method previously used (Bhatta et al., 2019). The particle size was measured using the particle analyze tool. The sizes of at least fifty particles was measured and averaged. The results are expressed as average±S.D.

4.4.4.9 Sorption isotherm

FD and VDD maple sugars (without bark extracts) was studied for their sorption characteristics. About 0.5g of sample in duplicate at room temperature over various saturated salt solutions (LiCl, CH_3COOK, $MgCl_2$, NaBr, NaCl and KCl of water activities, 0.11, 0.22, 0.33, 0.58, 0.75 and 0.86, respectively) was kept in desiccators. Sample weight was measured initially and after 3 days, and then at every day during one week until constant weight, as mentioned by (Farahnaky et al., 2016). The equilibrium moisture content was then determined by vacuum-oven drying method. The Guggenheim-Anderson-de Boer (GAB) model (*Eq. (4.5)*) was applied to fit the experimental data of sorption isotherm of maple sugar powders.

$$\frac{X}{X_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \qquad Eq.(4.5)$$

where X is the equilibrium moisture content (kg H_2O/kg dry solid), X_m is the monolayer moisture content (kg H_2O/kg dry solid), C and K are constants related to the heat of sorption

of the monolayer and multilayer region, respectively. The model was fitted to experimental data using regression equation wizard in SigmaPlot 12.5 (Systat Software Inc., USA).

4.4.5 Statistical analysis

Analyses of the physicochemical properties of maple sugar powders obtained by FD and VDD were performed in triplicate, unless otherwise stated. The values are presented as the Mean±S.D. Significant differences among the sample with and without bark extracts as well between the two drying methods were determined by one-way ANOVA (SigmaPlot 12.5, Systat Software Inc. USA) followed by Tukey's test at α=0.05.

4.5 Results and discussion

4.5.1 Characterization of polyphenol-enriched maple syrup

The total phenolic content (TPC) of MS, MS+SBX and MS+RBX were found to be 8.04±0.31, 9.09±0.21, and 9.62±0.44mg GAE/g syrup (on dry basis), respectively. This corresponds to an average TPC increase in syrup by 13.05% and 19.65% for SBX and RBX, respectively. Accordingly, antioxidant capacity of MS (17.43±4.72µM TE/g syrup, in dry basis) increased by an average of 21 and 43% with SBX (21.10±0.39), and RBX (25.00±1.32), respectively. Overall, addition of maple bark extracts, notably red maple bark extract, enriched syrup significantly in polyphenolic content and antioxidant activities.

Figure 4.2 highlights the ESI (-) chromatogram of ethyl acetate extracted maple syrup (MS, control; **Figure 4.2a**), syrup with sugar maple bark extract (MS+SBX; **Figure 4.2b**) and with red maple bark extract (MS+RBX, **Figure 4.2c**). Polyphenolic compounds were identified based on their mass spectra and HPLC (UV-vis at 270 nm) results, and compared with the available mass spectra data reported in literature on maple products (Geoffroy, Meda, et al., 2017; Yongqiang Liu, Rose, DaSilva, Johnson, & Seeram, 2017; P. St-Pierre et al., 2014). The identified compounds are summarized in **Table 4.1**. From results presented in **Table 4.1** and **Figure 4.2a**, indicate that phenolic acids and lignans were the major polyphenols present in maple syrup, which were also previously reported in the literature

(Yongqiang Liu et al., 2017; P. St-Pierre et al., 2014). All three syrup samples were determined to contain 17 compounds in common; however, four additional phenolic compounds (peaks 1, 4, 5, and 7) were identified in MS+RBX (**Figure 4.2c** and **Table 4.1**). These compounds were identified to be maplexin A (retention time, t_R=7.768 min; peak 1), Ginallin 3,6 (t_R=10.118 min, peak 4), Maplexin D (t_R=10.426 min, peak 5) and Ginnalin A (t_R=11.887, min peak 7). Previous studies have also reported the presence of these four phenolic compounds in the hot-water extract of red maple bark and buds (Geoffroy, Meda, et al., 2017; Meda et al., 2017).

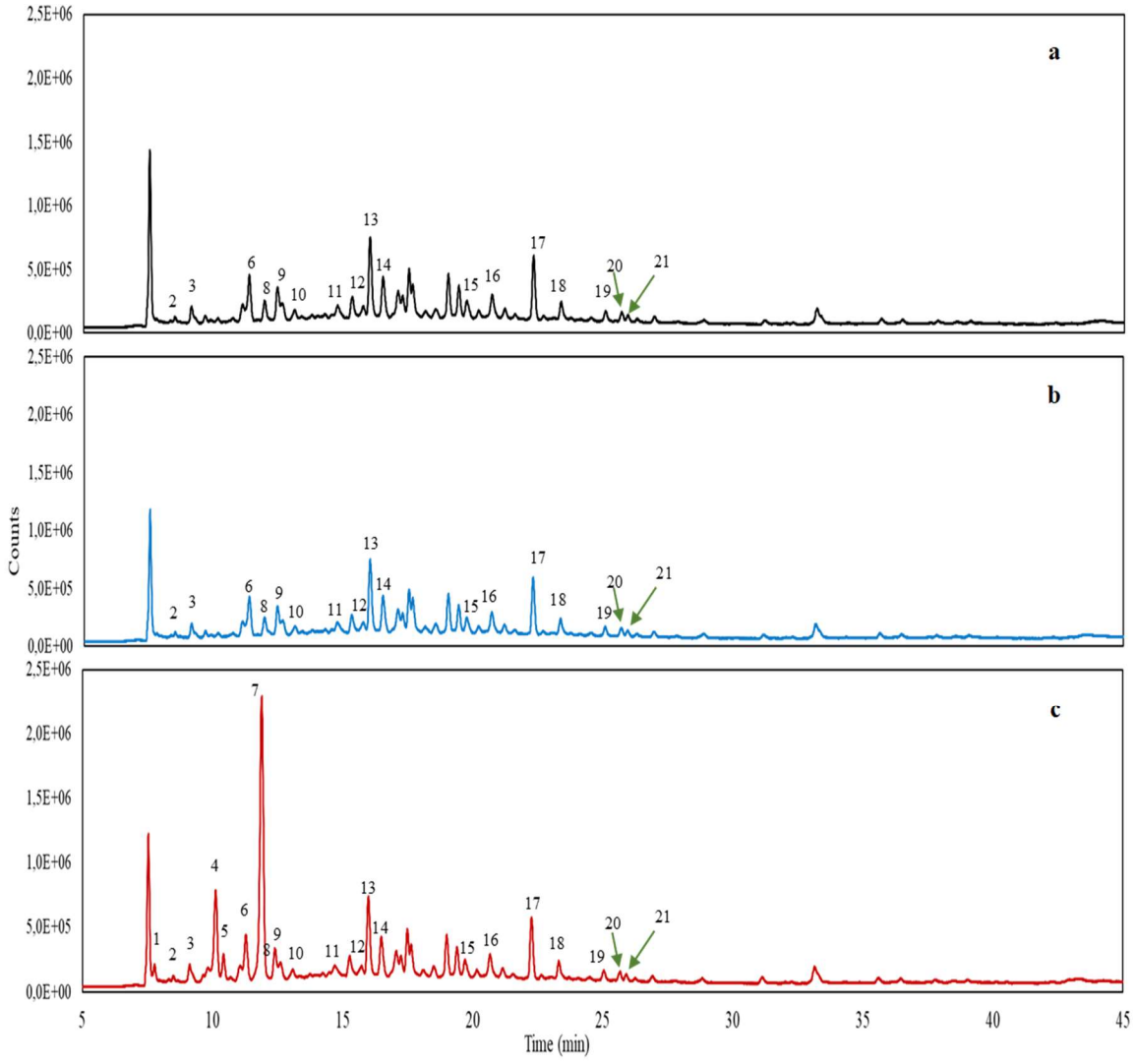


Figure 4.2 ESI(-) chromatogram of ethyl-acetate extracts of (a) maple syrup (MS, control); (b) maple syrup with sugar maple bark extract (MS+SBX); (c) maple syrup with red maple bark extract (MS+RBX). Compound names are listed in Table 4.1.

Table 4.1 Major polyphenols identified in ethyl acetate extracts of maple syrup samples.

Peak	t_R (min)	HRMS (-)	Mass (g/mol)	Calculated mass (g/mol)	Molecular Formula	Compounds identification
1	7.768	315.0722	316.0809	316.0794	$C_{13}H_{16}O_9$	Maplexin A
2	8.500	167.035	168.0441	168.0423	$C_8H_8O_4$	Vanillic acid
3	9.139	109.0295	110.0385	110.0368	$C_6H_6O_2$	Catechol
4	10.118	467.0831	468.0921	468.0904	$C_{20}H_{20}O_{13}$	Ginnalin 3,6
5	10.426	467.0831	468.0926	468.0904	$C_{20}H_{20}O_{13}$	Maplexin D
6	11.365	137.0244	138.0332	138.0317	$C_7H_6O_3$	Catechaldehyde
7	11.887	467.0831	468.0925	468.0904	$C_{20}H_{20}O_{13}$	Ginnalin A
8	11.955	109.0295	110.0384	110.0368	$C_6H_6O_2$	Catechol
9	12.445	151.0401	152.0490	152.0473	$C_8H_8O_3$	Vanillin
10	13.101	515.1923	516.2023	516.1995	$C_{27}H_{32}O_{10}$	Leptolepisol
11	14.754	179.035	180.0440	180.0423	$C_9H_8O_4$	Caffeic acid
12	15.743	419.1711	420.1815	420.1784	$C_{22}H_{28}O_8$	Lyoniresinol
13	16.009	377.1606	378.1702	378.1679	$C_{20}H_{26}O_7$	*threo*-guaiacylglycerol-*β*-O-4'-dihydroconiferylalcohol
14	16.499	377.1606	378.1704	378.1679	$C_{20}H_{26}O_7$	*erythro*-guaiacylglycerol-*β*-O-4'-dihydroconiferylalcohol
15	19.721	361.1657	362.1759	362.1729	$C_{20}H_{26}O_6$	Secoisolariciresinol
16	20.702	403.1398	404.1493	404.1471	$C_{21}H_{24}O_8$	Lignanes derivatives
17	22.296	585.2341	586.2439	586.2414	$C_{31}H_{38}O_{11}$	Acernikol
18	23.351	585.2341	586.2439	586.2414	$C_{31}H_{38}O_{11}$	Neolignanes derivatives
19	25.062	613.2291	614.2395	614.2363	$C_{32}H_{38}O_{12}$	Lignanes derivatives
20	25.677	583.2185	584.2287	584.2258	$C_{31}H_{36}O_{11}$	Buddlenol E
21	25.918	809.3026	810.3131	810.3099	$C_{42}H_{50}O_{16}$	Lignan derivatives

It is interesting to note that there is no peak 7 (Ginnalin A) in the chromatograms related to the pure maple syrup, nor in the chromatogram obtained for the syrup enriched in SBX. It is, on the other hand, the major peak in the chromatogram obtained for maple syrup enriched with RBX.

4.5.2 Properties of maple sugar powders

4.5.2.1 Sugar composition

The sugar compositions of maple syrup samples were MS (sucrose, 975.26±1.21; glucose, 37.26±0.25; fructose, 22.09±1.59mg/g dry sample), MS+SBX (sucrose, 985.04±1.21; glucose, 38.25±0.14; fructose, 22.85±1.40mg/g dry sample), and MS+RBX (sucrose, 976.57±2.30; glucose, 38.05±0.15; fructose, 21.05±0.58mg/g dry sample). From the above result, it was found that the sucrose content increased significantly in MS with the addition of SBX. Similarly, glucose content in syrup significantly increased with the addition of SBX as well as RBX. It can be due to the presence of sugars in SBX (sucrose, 10.94±0.11; glucose, 5.28±0.04; and fructose, 5.56±0.03g/100g dry extract) and in RBX (sucrose, 5.58±0.08; glucose, 3.51±0.06; and fructose, 4.50±0.92g/100g dry extract) (Bhatta et al., 2018). However, no significant change ($P>0.05$) in fructose content was noticed for all syrup samples. The sugar composition of FD and VDD maple sugar powders is presented in **Table 4.2**. No significant difference in sucrose content was observed among FD sugar powder samples. On the contrary, MSP+SBX produced from VDD demonstrated the highest sucrose content among all the samples. This could be due to the combined effect of high temperature in VDD and high sucrose content in MS+SBX syrup sample.

4.5.2.2 Total phenolic content and antioxidant activity

Table 4.2 summarizes the total phenolic content (TPC) and antioxidant activity of maple sugar powders obtained from FD and VDD process. Drying resulted in a significant decrease in TPC value of powders, by an average of 13-20% and 8-16.7% for FD and VDD, respectively, when compared with initial TPC of syrup samples obtained upon addition of bark extracts. Despite at the higher temperature applied in VDD than in FD, the powders produced by VDD were determined to have somewhat higher TPC (7.40-8.01mg GAE/g dry sugar) than those obtained by FD (6.97-7.64mg GAE/g dry sugar). High temperatures in VDD could have caused the cleavage of the glycoside bonds in phenol-glycosides, releasing phenols. As a result, more phenolics become available to form a complex with the Folin's reagent for TPC determination, compared to the FD sample. Similar results were reported in the literature for heat-treated *Citrus unshiu* peels and Shiitake extracts, where TPC was found

to increase with higher temperatures during the treatment (Choi, Lee, Chun, Lee, & Lee, 2006; Jeong et al., 2004). Jeong et al., (2004) identified that low molecular weight phenolic compounds such as vanillic acid, ferulic acid, etc. were newly formed after the heat treatment of citrus peel extract.

Regardless of the type of drying process used, the powders containing bark extracts had higher TPC than the control (MSP). In particular, MSP+RBX presented significantly higher ($P<0.05$) TPC than control. This is in agreement with previous results from LC-MS (section 4.5.1). Interestingly, among the dried syrup TPC values, VDD:MSP+RBX represented similar TPC ($P>0.05$) determined for pure maple syrup (8.04±0.31mg GAE/g syrup, in dry basis). Thus, an addition of RBX to syrup (0.01% w/v) seems to compensate for the negative effect of the drying process itself on phenolic content causing its reduction.

The results of antioxidant activities of maple sugar powders determined by DPPH assay are shown in **Table 4.2**. Similarly to TPC results, decrease in antioxidant activity was noticed when syrup samples were dried by FD and VDD. MSP containing bark extracts were determined to have higher antioxidant activity than of the control. MSP with RBX had the highest antioxidant activity among studied sugar powders. However, the results were neither statistically significant ($P>0.05$) among syrup with bark extracts added, nor between the studied drying processes.

Table 4.2 Total phenolic content, antioxidant activity and sugar compositions of maple sugar powders.

Process	Sample	TPC (mg GAE/g dry sample)	DPPH (µM TE/g dry sample)	Sugar compositions (mg/g dry sample)		
				Sucrose	Glucose	Fructose
FD	MSP	6.97±0.07^{bcf}	8.20±3.04^{a}	863.95±2.55^{a}	32.93±0.22^{a}	18.73±0.15^{a}
	MSP+SBX	7.26±0.23^{ce}	10.63±1.13^{a}	872.06±1.81^{a}	33.01±0.76^{a}	18.54±1.32^{a}
	MSP+RBX	7.64±0.17^{ae}	11.99±1.50^{a}	865.06±3.55^{a}	30.69±0.11^{cd}	18.34±1.06^{a}
VD	MSP	7.40±0.03^{def}	7.90±2.93^{a}	826.13±0.66^{b}	31.05±0.60^{bd}	18.40±0.47^{a}
	MSP+SBX	7.59±0.33^{ae}	10.28±1.09^{a}	894.92±1.03^{a}	33.78±0.18^{a}	18.86±1.27^{a}
	MSP+RBX	8.01±0.32^{a}	11.57±1.45^{a}	834.60±0.12^{a}	32.35±0.09^{ad}	17.43±0.53^{a}

Values represent mean±S.D. Means with difference superscript letters in columns are significantly different by Tukey's test ($P<0.05$). GAE, gallic acid equivalent; TE, trolox equivalent.

4.5.2.3 Microstructure of powder

Figure 4.3 depicts the X-ray diffractograms of powders produced by FD and VDD. For amorphous material, the diffractograms patterns are large and disperse due to disorderly arranged molecules, whereas XRD patterns of crystalline material shows sharp and defined patterns indicating that the molecules are present in a highly ordered state. As seen from **Figure 4.3**, powders obtained from FD (**Figure 4.3 a-c**) were amorphous in nature and there was no observable effect of the type of bark extract added (**Figure 4.3 b-c**). This could be explained by low mobility of solutes in frozen state, lacking enough energy to rearrange in a more ordered, crystalline form (Palzer et al., 2012). It is reported that FD of sucrose solutions and other sugar-rich foods such as mango powder, produced usually an amorphous powders (Caparino et al., 2012; Harnkarnsujarit & Charoenrein, 2011; Roos & Karel, 1991).

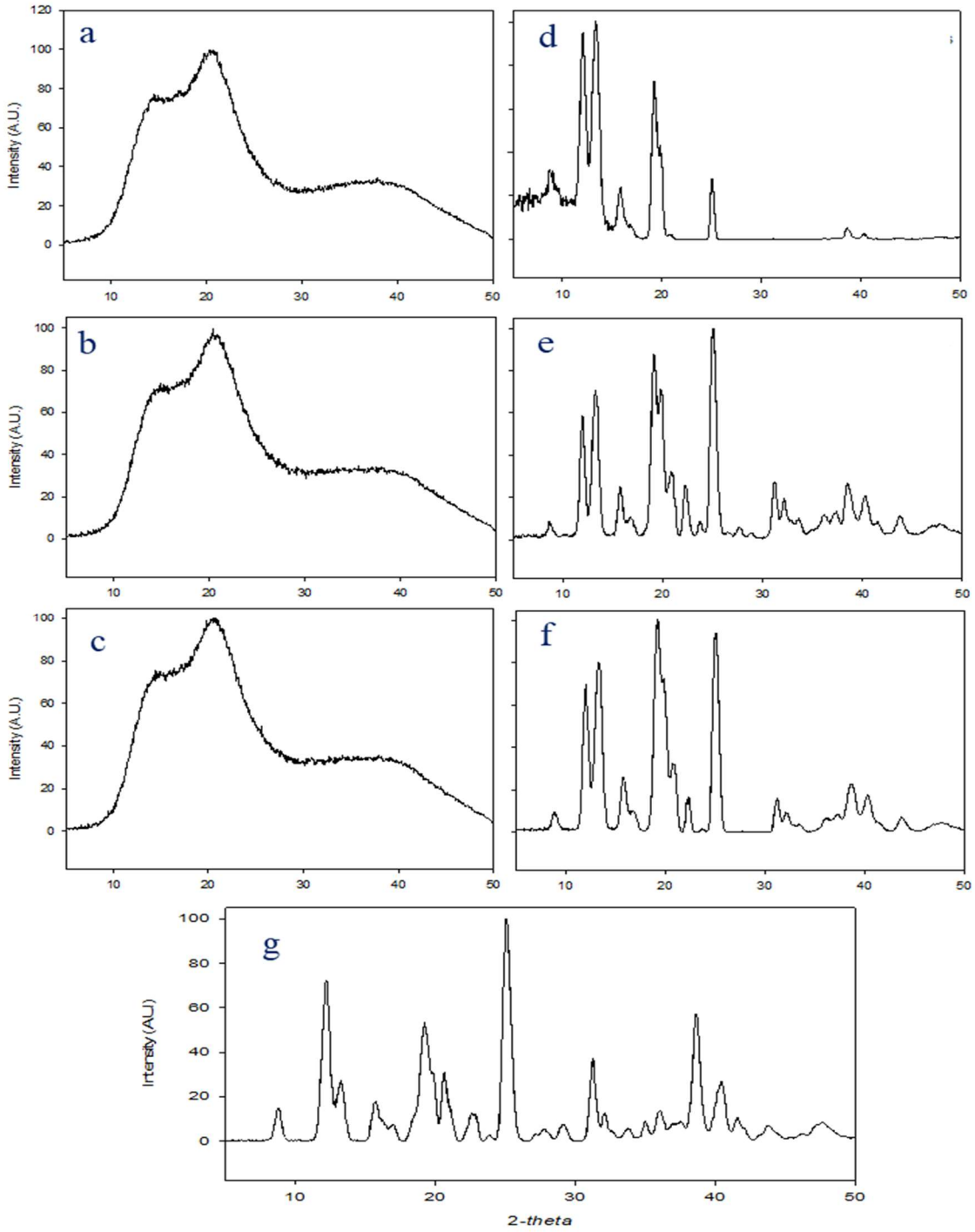


Figure 4.3 X-ray diffractograms of maple sugar powders achieved from FD (a-c) and VDD (d-f) methods. (a) FD:MSP; (b) FD:MSP+SBX; (c) FD:MSP+RBX; (d) VD:MSP; (e) VD:MSP+SBX; (f) VD:MSP+RBX; and (g) powdered sucrose crystal for the reference.

The X-ray diffractograms of powders obtained by VDD has the characteristic crystalline pattern (**Figure 4.3d-f**). A significant effect of addition of bark extract on

crystallinity has been noticed in terms of XRD patterns. VDD:MSP (control, in **Figure 4.3d**) presented lesser sharp peaks (indicating the presence of amorphous and crystalline states) as presented for VDD:MSP+SBX (**Figure 4.3e**) and VDD:MSP+RBX (**Figure 4.3f**). The sharper peaks observed in diffractograms of VDD:MSP+SBX compared to VDD:MSP+RBX can be associated to their difference in sugar composition, discussed in section 4.5.2.1. The sucrose added through extracts may have contributed to the initiation of sugar crystallization during the VDD process. The crystallinity of powders obtained from VDD could also be related to the high drying temperature. During experiment, the drum surface temperature increased from 75°C (before vacuum is applied) to 105°C (by the end of experiment), therefore increasing the product temperature. An increase in the difference of product temperature and glass transition temperature (T_g) of sucrose (62°C, (Roos, 1993)) may have lowered viscosity, finally resulting in the formation of crystals during VDD process. Similarly to our results, Islam & Langrish reported that increase in T-T_g initiated the crystallization of lactose during spray drying, which was explained by the William-Landel-Ferry theory (Islam & Langrish, 2010; Islam, Sherrell, & Langrish, 2010). Above T_g, the food materials change from glassy to rubbery state which results in decrease in system viscosity and thus allowing for the rearrangement of molecules to form crystals. The pattern of the VDD diffractograms, particularly for powder containing bark extracts, were comparable to that of pure sucrose powder (included for comparison, **Figure 4.3g**).

4.5.2.4 Morphology and particle size

The morphology of sugar powder particles was studied by SEM. **Figure 4.4** illustrates the morphology of powders obtained by FD (**Figure 4.4a-f**) and VDD (**Figure 4.4g-l**). As seen in **Figure 4.4a-c**, particles of FD powders presented smooth flat surface with elongated shape and no homogeneity. Similar results were reported for freeze-dried blackberries and date powders (Franceschinis et al., 2014; Seerangurayar et al., 2017). Porous-like structure can also be observed on the magnified images (1000×) (**Figure 4.4d-f**), characteristic of freeze-dried food powders (Palzer et al., 2012). The particle size ranges of FD powders were determined to be between 113.0±82.7 and 131.0±73.5µm, similar to the results reported for FD date powders (Seerangurayar et al., 2017). The particles of FD:MSP powder (113.0±82.7µm) was relatively smaller than those of powder with bark extracts, 131±73.5

and 114±82.2µm for FD:MSP+SBX, and FD:MSP+RBX, respectively. The presence of sugars in bark extracts in different proportion could have resulted in discrepancies in the particle size.

On the other hand, VDD powders also displayed irregular particles with rough and grainy surfaces (**Figure 4.4**, g-i and j-l for the magnification of 300 and 1000×, respectively). VDD:MSP powder showed islands of grains (**Figure 4.4 g** and **j**), these grains (sugar crystals) are found to be surrounded by amorphous sugars (liquid/glass like). This is similar to the XRD pattern observed of VDD:MSP (section 4.5.2.3), indicating the presence of both amorphous and crystalline states. VDD powders with maple bark extracts (MS+SBX, **Figure 4.4h** and **k**, and MS+RBX, **Figure 4.4i** and **l**) have considerably different particle morphology and size distribution from the control (MSP, **Figure 4.4g** and **j**). For instance, the surface of VDD:MSP+SBX particles has remarkably textured appearance due to the presence of sucrose crystals in larger proportion than in VDD:MSP+RBX and VDD:MSP. It is also observed that the surfaces of some particles of VDD powders were smooth, while rough and corrugated on other side (clearly visible in **Figure 4.4i**). The smooth surface could be due to the direct contact of sample with drum surface, which is a common characteristic of VDD. Similar observation was reported when mango powder was produced by double-drum drying (Caparino et al., 2012).

In comparison to FD, powders obtained by VDD presented smaller particle size, ranging between 58.0±23.3 and 94.0±60.4µm. Among VDD powder, the particle size of VDD:MSP+SBX was smaller (58.0±23.3µm) than VDD:MSP+RBX (94.0±60.4µm) and VDD:MSP (83.1±66.7µm). Besides sucrose, SBX was reported to be rich in complex sugars (oligo/poly-saccharides) (Bhatta et al., 2018). The presence of sucrose may have assisted sucrose crystallization (as discussed in section 4.5.2.3), but further growth of crystals may have been disturbed by the presence of complex sugars in SBX. It has been described in the literature (Hartel & Shastry, 1991) that the presence of macromolecules such as proteins, oligosaccharides, and dextrans can inhibit the growth of sucrose crystal and influence the mode of attachment of sugar molecules to the crystal lattice when such macromolecules are absorbed to crystal surface. Overall, FD and VDD produced MSP with different surface morphology and particle size that may affect their functional properties such as dissolution and flow characteristics.

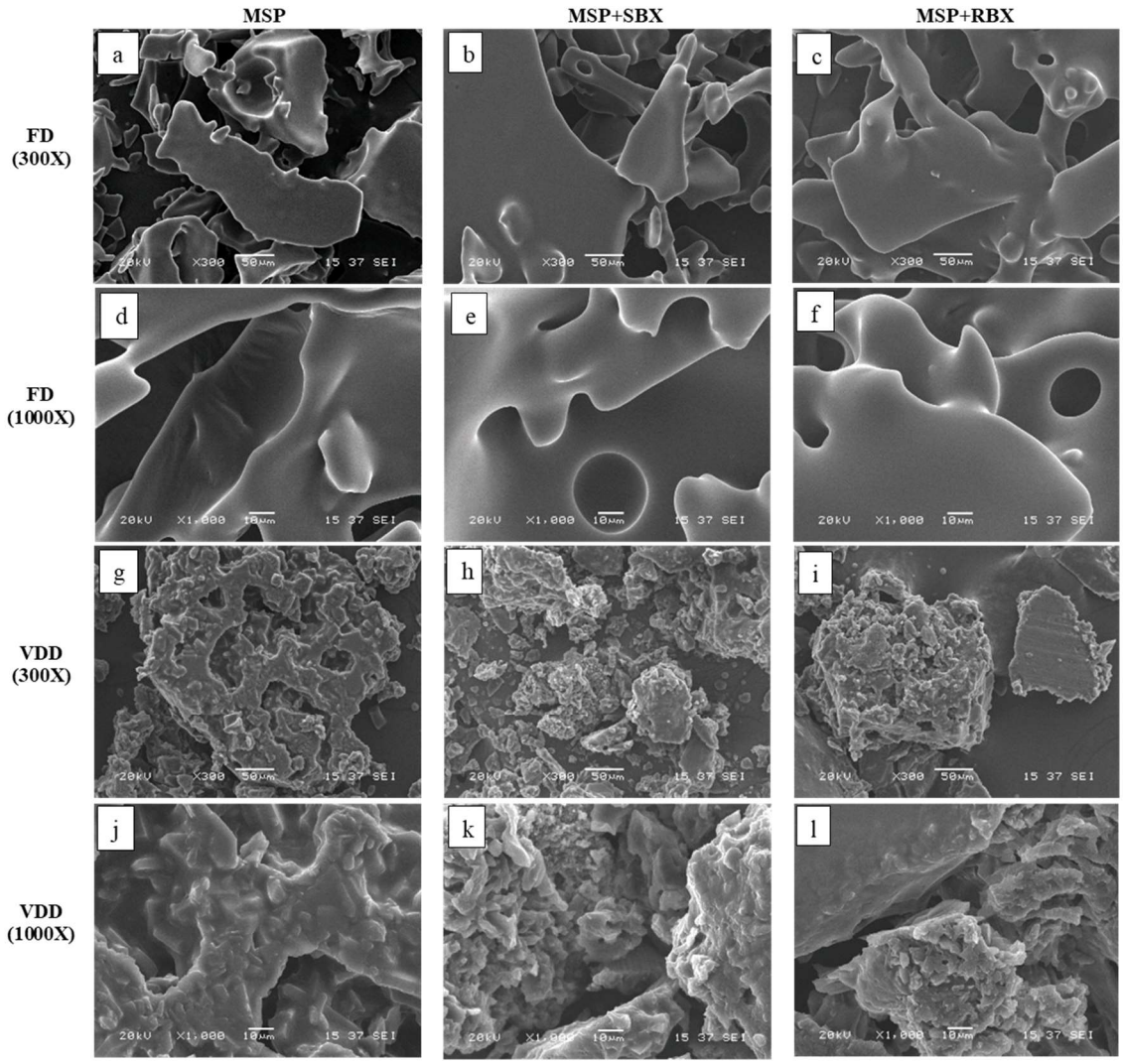


Figure 4.4 Morphologies of maple sugar powder achieved from FD (a-f) and VDD (g-l) using scanning electron microscopy. 300× (a-c for FD:MSP, FD:MSP+SBX, and FD:MSP+RBX, respectively; g-i for VDD:MSP, VDD:MSP+SBX, and VDD:MSP+RBX, respectively); and 1000× (d-f for FD:MSP, FD:MSP+SBX, and FD:MSP+RBX, respectively; j-l for VDD:MSP, VDD:MSP+SBX, and VDD:MSP+RBX, respectively).

4.5.2.5 Moisture content, color and dissolution time of maple sugar powders

Moisture contents of FD and VDD maple sugar powders are presented in **Table 4.3**. No significant ($P>0.05$) difference in moisture content was found between different powder samples if produced by the same drying process. However, a significant effect ($P<0.05$) of drying processes on moisture content is found. The moisture content of FD maple sugar

powders was in the range of 4.10 to 4.40% on dry basis. It is in agreement with the reported value (less than 5% in dry basis) of FD maple sugar (Bhatta et al., 2019). The moisture content of VDD powders was in the range of 0.63 to 0.71%, dry basis. Lower moisture content in VDD powders can be attributed to higher drying temperature in VDD (>80°C), providing greater driving force for moisture removal, than in FD. Similarly, the moisture content of mango powder obtained by double-drum drying (1.3%, dry basis) was reported to be lower than obtained by FD (Caparino et al., 2012). Generally, low moisture content is preferred for food powders to avoid caking and stickiness during handling and processing (Fitzpatrick, 2013).

Color of food powder is a very important attribute from the consumer's point of view. The color paramaters ($L^*a^*b^*$) and chroma value of powders produced by FD and VDD are presented in **Table 4.3**. L^*-value was higher for FD powders (78.68-80.64) in comparison to VDD (72.16-73.20). This means VDD produced darker maple sugars than FD, probably due to the higher drying temperature during the process. Such effect has been confirmed on mango and jackfruit puree powder, in which high drying temperature process produced dark colored powder (Caparino et al., 2012; Chun Kiat Pua et al., 2010). Maple syrup contains sucrose, glucose and fructose (Ball, 2007). Therefore, brown color can be induced by Maillard reaction or caramelization due to the chemical reactions between sugars and proteins which are present both in maple syrup and in the extracts (Caparino et al., 2012; Chun Kiat Pua et al., 2010). L^*-value of FD powders in this study is consistent with the values previously reported from our studies (Bhatta et al., 2019). Powder produced by VDD have shown significantly higher a^*- and b^*- values, indicating more redness and yellowness in color, respectively, than powders produced by FD (**Table 4.3**). Increase in redness of VDD powders can be associated to increase in browning or decrease in L^* value. At last, higher Chroma value or vividness was determined for VDD powders than for FD powders. Different surface morphologies could explain the differences in color parameters of MSP obtained by FD and VDD. As the color is measured by the amount of light reflected from the dried surface, therefore changes in surface morphology or porosity of FD and VDD powders have considerably affected the amount of light reflected (Ozkan, Cemeroglu, & Kirca, 2003; Palzer et al., 2012).

Table 4.3 Moisture content, color and dissolution time of maple sugar powders.

Process	Sample	Moisture (% db)	Dissolution (s)	Color analysis			
				*L**	*a**	*b**	Chroma value
FD	MSP	4.40±0.38[a]	12.90±1.0[b]	78.68±1.48[a]	0.67±0.02[b]	17.07±0.01[b]	17.08±0.01[b]
	MSP+SBX	4.16±0.74[a]	12.80±0.57[b]	78.97±0.03[a]	0.99±0.01[b]	18.40±0.01[b]	18.42±0.01[b]
	MSP+RBX	4.10±0.64[a]	12.20±1.75[b]	80.64±0.25[a]	0.59±0.07[b]	16.83±0.01[b]	16.84±0.01[b]
VDD	MSP	0.63±0.30[b]	28.80±3.51[a]	73.20±0.05[a]	2.10±0.02[a]	22.75±0.05[a]	22.85±0.04[a]
	MSP+SBX	0.69±0.05[b]	28.80±2.25[a]	72.62±1.08[a]	1.86±0.17[a]	21.06±0.42[a]	21.14±0.43[a]
	MSP+RBX	0.71±0.29[b]	27.1±2.20[a]	72.16±2.34[b]	1.94±0.45[a]	21.97±1.05[a]	22.06±1.08[a]

Values represent mean±S.D. Means with difference superscript letters in columns are significantly different by Tukey's test ($P<0.05$). db, dry basis.

Table 4.3 shows the dissolution time of powders produced by FD and VDD. The dissolution characteristics of powder can provide an important information for its use as instant-powder. FD maple sugars dissolved significantly ($P<0.05$) faster than VDD maple sugars. From **Table 4.3**, the dissolution time for FD maple sugars was less than 13s, whereas it was around 29s for VDD maple sugars. Dissolution time of MSP obtained by FD was comparable to the literature (Bhatta et al., 2019). Foods and food powders produced by FD are generally highly porous (discussed in section 4.5.2.4) resulting in an increase of surface area for dissolution (Palzer et al., 2012). Furthermore, FD powder is amorphous; therefore, it was easier to dissociate molecules compared to highly ordered crystalline VDD powder. Dissolution times of FD and VDD maple sugar powder with bark extracts were not significantly different ($P>0.05$) from those for control. Skimmed milk powders that dissolve in less than 15s is termed 'instant' (Sharma et al., 2012). Therefore, FD powder showed instant-like property in this study. Dried syrup powders can be used as natural food ingredients, for example, in instant-drinks, dairy powder mix, sweet snacks, beverage, ice cream, candy, etc.

4.5.2.6 Flowability of powder

The results on densities and calculated flowability of powders produced by FD and VDD are presented in **Table 4.4**. Addition of bark extracts resulted in no significant difference on powder densities and flowability when produced by the same drying process.

Bulk densities of FD powders (0.26 to 0.27g/ml) were significantly lower ($P<0.05$) than those of VDD powders (0.33 to 0.35g/ml). Similarly, the bulk density of FD mango powder was reported to be lower than drum-dried powder (Caparino et al., 2012). High bulk density of VDD maple sugar powder can be associated to its small particle size (presented in section 4.5.2.4), which allows for the particles to rearrange in a lesser space. Similar tendency of increase in bulk density with decrease in particle size was observed for date syrup powders (Seerangurayar et al., 2017). Tapped density of maple sugar powders was higher than bulk density because tapping allowed the smaller particles to occupy the void spaces and thus achieve the dense packing conditions (Seerangurayar et al., 2017). The tapped densities of MSP obtained from the two drying processes were not significantly different ($P=0.074$).

Hausner ratio (HR) was calculated using the bulk and tapped density of powder, and the obtained values are presented in **Table 4.4**. According to the literature (Hausner, 1967), the cohesiveness of powder is considered high for HR values higher than 1.4, intermediate for the values between 1.2 and 1.4, and low for values lower than 1.2. The HR values of MSP produced by FD and VDD were 1.45-1.47 and 1.14-1.22, respectively. These values indicate that FD powders are highly cohesive, whereas VDD powders are low to intermediate in cohesiveness (**Table 4.4**). The HR value of FD maple sugar powders was comparable to a previous study (Bhatta et al., 2019). HR value is inversely related to powder flowability (Fitzpatrick, 2013).

The flowability of MSP produced by FD and VDD was characterized by Carr's index (CI), shown in **Table 4.4**. Based on CI, the powder's flowability is considered as very good for the value less than 15%, good flow by values between 15 and 20%, fair flow by values between 21 and 35%, bad flow by values between 36 and 45%, and very bad flow when values of CI is greater than 45% (Carr, 1965). The CI values of MSP produced by FD and VDD in this study are 30.82-32.13 and 12.34-17.69%, respectively. These values designate the fair flowability for FD powder, whereas good to very good flowability for VDD powders. Differences in flowability of FD and VDD powders can be explained by differences in previously discussed values of cohesiveness (HR values). Moreover, better flowability of VDD powders than FD can be related to smaller particle size. A similar finding of better flowability with the decrease in particle size was reported for ginger and date syrup powder

(Seerangurayar et al., 2017; Zhao et al., 2009). Overall, powder produced by VDD demonstrated low cohesiveness and better flowability than FD.

Table 4.4 Density and flow characteristics of maple sugar powders

Pro-cess	Sample	Bulk density (g/ml)	Tapped density (g/ml)	Hausner ratio	Cohes-iveness	Carr's Index (%)	Flow property
FD	MSP	0.27±0.01[a]	0.39±0.00[a]	1.45±0.03[a]	High	30.82±1.43[a]	Fair
	MSP+SBX	0.27±0.01[a]	0.39±0.01[a]	1.48±0.07[a]	High	32.13±3.04[a]	Fair
	MSP+RBX	0.26±0.01[a]	0.38±0.01[a]	1.47±0.06[a]	High	31.75±2.62[a]	Fair
VDD	MSP	0.35±0.01[b]	0.40±0.01[a]	1.14±0.02[b]	Low	12.34±1.73[b]	Very good
	MSP+SBX	0.33±0.02[b]	0.40±0.01[a]	1.20±0.04[b]	Low	16.78±3.10[b]	Good
	MSP+RBX	0.33±0.01[b]	0.40±0.00[a]	1.22±0.02[b]	Inter-mediate	17.69±1.38[b]	Good

Values represent mean ± S.D. Means with difference superscript letters in columns are significantly different by Tukey's test ($P<0.05$).

4.5.2.7 Sorption isotherm

The sorption isotherm curves of FD and VDD maple sugar powders at ambient temperature is presented in **Figure 4.5**. Only dried samples without bark extract were studied to understand the sorption characteristics of amorphous (produced by FD) and crystalline (VDD) maple sugar powder matrices. As expected from XRD results, MSP obtained from FD and VDD showed similarities in terms of characteristic isotherm curves for amorphous and crystalline sucrose, respectively (Mathlouthi & Rogé, 2003). The distinct differences in the two curves are observed in the encircled section of the **Figure 4.5a**. For FD:MSP, the equilibrium moisture content (EMC) increased abruptly from 0.11 to 0.36a_w, which was followed by decrease in EMC from 0.36 to 0.56a_w. The decrease in EMC can be associated with amorphous FD sucrose crystallization, since the molecular mobility of sucrose at higher water activity may have increased leading to the formation of sucrose crystals (Mathlouthi & Rogé, 2003). For VDD:MSP, one can note that the powder absorbs very little moisture over the range of 0.11 to 0.56a_w. However, both samples showed isotherm curves with similar characteristics with an exponential increase in EMC above 0.76a_w. Such exponential increase in EMC at high water activity (> 0.76a_w) was also reported for crystalline sucrose and lactose

powder (Bronlund & Paterson, 2004; Mathlouthi & Rogé, 2003). At high a_w, there is a phase transition from solid to solution, induced by water uptake from surrounding environment. The relative humidity at which the crystalline sucrose adsorbs water in large quantities from the atmosphere is often called deliquescence point (Palzer et al., 2012).

The fitted Guggenheim-Anderson-de Boer (GAB) model and its parameters for MSP obtained from different drying methods are presented in **Figure 4.5b**, and **Table 4.5**, respectively. The fitness of GAB model to describe the sorption isotherm was better for VDD:MSP (R^2=0.9999) than for FD:MSP (0.9797). Monolayer moisture content (X_m) refers to the amount of water strongly adsorbed to specific sites at the surface of food materials, and it is an important parameter for assuring food stability. The value of X_m was determined to be 0.021 (g water/g solid) and 0.008 for FD:MSP and VDD:MSP samples, respectively. The moisture contents of MSP (Table 4.2) obtained from both drying methods were below or similar to their respective X_m values, indicative of good product stability over time. In the present study, the value of K was found to be 1.13 and 1.14 for FD:MSP and VDD:MSP samples, respectively. The obtained K values are comparable to those determined for other sugar-rich dehydrated food products, ranging between 1.009 for freeze-dried mango pulp powder and 1.10-1.20 for vacuum-dried honey powder (Fongin et al., 2017; Nurhadi & Roos, 2016). Considering the studied sorption isotherms (Figure 5a), the obtained powders should be stored below 0.36a_w for FD:MSP, whereas below 0.56a_w for VDD:MSP, at ambient temperature, to avoid the physicochemical changes in powder.

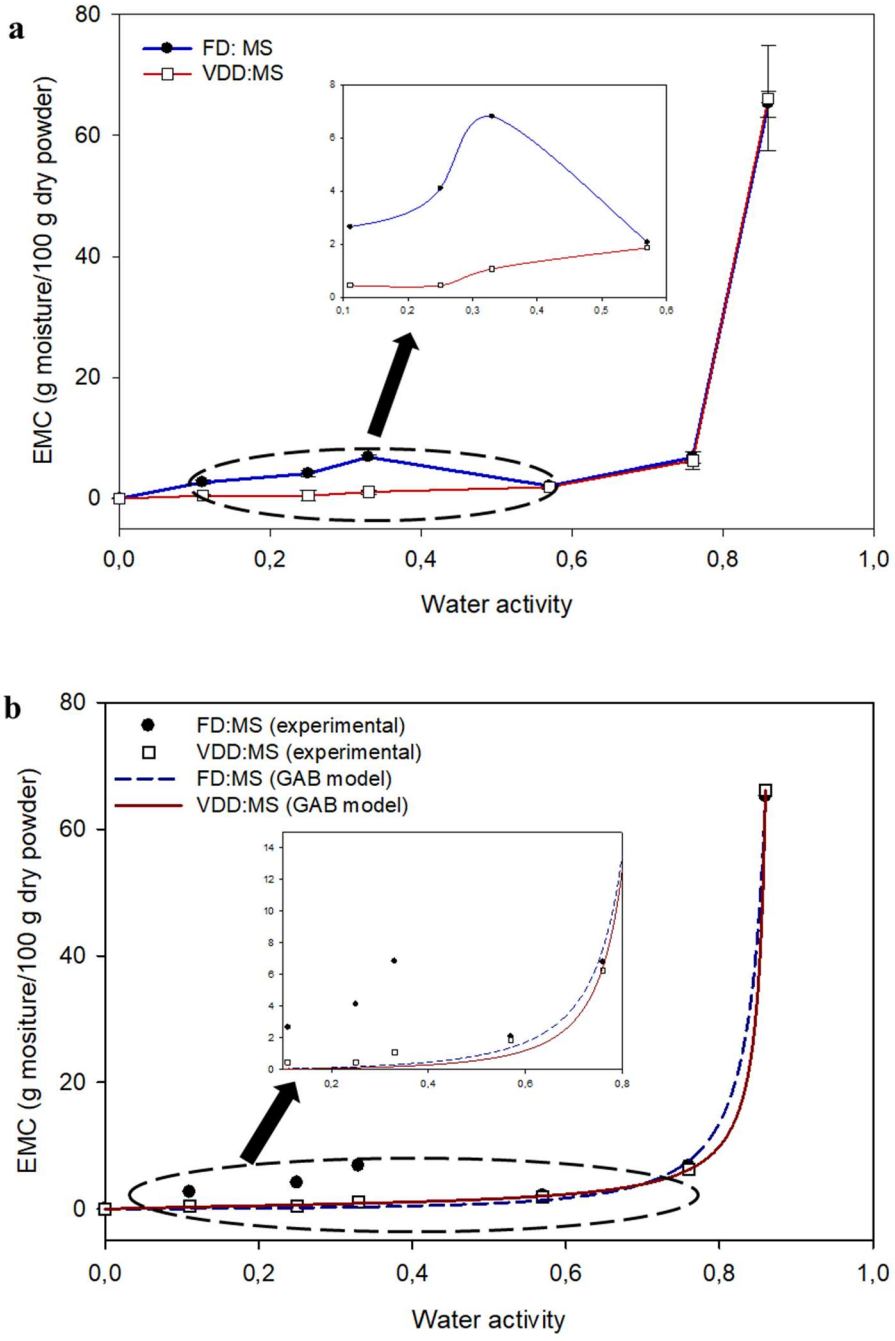


Figure 4.5 Sorption isotherm curve of FD and VDD maple sugar powder. (a) Experimental data; (b) curve fitted with GAB model. FD:MSP (filled circle); VDD:MSP (open square); EMC, equilibrium moisture content.

Table 4.5 The GAB model parameters of maple sugar powders from FD and VDD.

GAB parameters	FD:MSP	VDD:MSP
X_m	0.0211	0.0082
K	1.130	1.148
C	0.168	3.200
R^2	0.9797	0.9999

X_m, monolayer water content (g water/g dry solids).

4.6 Conclusion

The addition of maple bark extracts to low quality maple syrup is an interesting way of valorizing the surplus of this substandard grade of syrup. The addition of sugar and red maple bark extracts at low level of 0.01% w/v resulted in syrup enrichment in polyphenols by 13 and 20% respectively. The LC-MS results confirmed the presence of 17 phenolic compounds in syrup and extract enriched syrup samples; however, four additional phenolic compounds were identified in syrup with RBX. Furthermore, in order to facilitate the distribution and to widen its application as natural food ingredients, syrup was successfully dehydrated using FD and VDD to produce free-flowing maple sugar powder. After drying of maple syrup with bark extracts, the obtained maple sugar powders were determined to have 8-10% higher total phenolic content with RBX than the control. The differences in physicochemical properties of powders are mainly due to the differences in the drying method. FD produced amorphous and instant-like powder, whereas VDD produced crystalline powder with excellent flow characteristics. Apart from the effect of bark extracts on crystallinity of VDD powder, no significant differences were observed in terms of properties of powder such as moisture content, dissolution, densities and flowability. Overall, MSPs have huge potential of use in instant foods, and as functional food ingredients, particularly for the development of natural food products. Future studies shall focus on the storage stability of polyphenols within different microstructural MSP matrices in order to enhance their use as natural ingredients.

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General conclusion

Three objectives, identified in this project, have facilitated to accomplish the main goal of valorizing the maple barks (sugar and red maple) and syrup as natural food ingredients.

The first objective of this project has allowed for the implementation of the concept of bio-refinery by integration of extractions of bark residues using a green solvent (hot water extraction). In this objective (**Chapter 2**), hot water extracts of maple barks were analyzed, for the first time, as potent functional food ingredients. Beside polyphenols, we identified the presence of organic and inorganic nutrients in maple bark extracts that are known to benefit human health. These nutrients were presented at different concentrations in both bark extracts. Sugar maple bark extract (SM-BX) was found to be rich in complex sugars (oligo/poly saccharides) and minerals (K, Ca, Mg, P, Zn, Fe, etc.). On the other hand, red maple bark extract (RM-BX) presented superior antioxidant capacity when determined by ABTS radical scavenging and ORAC assays. Antioxidant capacity of RM-BX was found to be better than green tea extract and oligopin®. At last, the safety of maple bark extracts on neutrophil-like cells indicated no cytotoxicity at concentrations up to 100µg/ml. Cytotoxicity test was crucial to determine the safe concentration of bark extracts that will be incorporated in maple syrup for future studies.

The next two objectives were valorizing the low quality maple syrup to novel maple products. Maple syrup was valorized to produce maple sugar powders (MSP). Despite the well-known difficulties involved in drying sugar-rich foods, we were able to formulate a successful protocol to dry maple syrup and thus allow for the multiple potential applications of dried syrup powder. For examples, MSP can be used in instant drinks, instant cereal mix, and as natural ingredients for various solid food formulations. Freeze-drying (FD) and vacuum double-drum drying (VDD) were the choice of drying methods. FD was chosen as reference drying method as a technique of choice to produce high quality foods. VDD was considered for this study, as it is a common technique to dry viscous liquids and pastes. In addition, the industrial partner of this project is interested in further exploring the effectiveness of VDD on transforming syrup to maple sugar powders.

FD is a well known drying method to obtain food with high quality, but at the same time, known for being an expensive and time consuming process (especially if not optimized). Due to the lack of literature data on FD of maple syrup, an effort was made to formulate the FD protocol for pure maple syrup. Realising the second objective, an efficient FD protocol was formulated to obtain an instant-like maple sugar powders. FD protocol was formulated with the aid of thermocouples, glass transition temperature (T_g), and drying kinetics techniques. T_g was useful to determine the FD temperatures, while thermocouples and drying kinetics were essential to allocate the duration of drying phases. The optimized protocols formulation allowed for the FD time reduction from 40h (non-optimized) to 25 or 30h, corresponding to 25 or 38% reductions. The properties of MSP produced under two optimized FD conditions (25 and 30h) were compared. No significant difference on powder properties (dissolution time, flowability, and sorption characteristics) were noticed for powder produced under the two FD conditions. Additionally, both conditions produced stable powder (water activity, below 0.30) with low moisture content (<5%, dry basis). Consequently, the FD protocol with minimum drying time (25h) was considered for objective 3. The formulated condition for FD was as follow; primary drying (-36°C, 15h) and secondary drying (30°C, 10h). Another contribution of this objective was that the systematic approach developed to formulate the FD protocol for maple syrup drying could also be applied to freeze-drying of other sugar-rich liquid foods.

Third objective of this project was of central importance as it collectively valorized maple bark extracts and low quality syrup. In this objective, possibility of using maple bark extracts as food ingredient (results from objective 1) was examined by incorporating it to maple syrup. As expected, the addition of both bark extracts allowed for the enrichment of maple syrup and maple sugar powders in polyphenols by 13-20% and 8-10%, respectively. Interestingly, VDD allowed for the retention of higher total phenolics in MSP than FD. MSP with red maple bark extracts produced by VDD demonstrated similar total phenolics as pure maple syrup. This indicates that the addition of bark extract to syrup has compensated the negative effect of drying on polyphenols. The major differences between MSP produced by FD and VDD were found in terms of their microstructural properties. Powders produced by FD were amorphous, whereas, VDD powders were crystalline, as determined by x-ray diffraction. A significant effect of bark extracts on crystallinity of powder was also detected

during VDD process. Powders obtained from FD and VDD were determined to be significantly different in terms of their physicochemical properties (morphology, particle size, moisture content, color, dissolution time, flowability, and sorption characteristic). For instance, dissolution time of FD powders was shorter (<14s) than VDD powders (28-30s). On the other hand, powder obtained by VDD had shown better flowability than that produced by FD. These differences in physicochemical properties of powders can be attributed to the differences in microstructural property. Sorption characteristic were used to identify the critical relative humidity for storing the powders. It was found that FD and VDD powders should be stored below the relative humidity of 36 and 56%, respectively to avoid physicochemical changes.

Overall, the results of this doctoral thesis indicate that it is possible to valorize maple barks (in form of hot water extracts) and substandard maple syrup to develop natural food ingredients. Maple bark extracts were determined to be not only rich in polyphenols but also in other organic and inorganic nutrients. In future, it would be interesting to elucidate the types and structure of complex sugars present in sugar maple bark extracts, which will enhance the prospect of using extracts as functional ingredient. Considering the last objective, both drying methods have produced polyphenol-enriched maple sugar powders with improved quality and therefore, confirming the hypothesis of this study. This work also offers valuable insights about dehydration of sugar-rich liquid food by FD and VDD. From practical standpoint, VDD process is found to be effective, fast, and has higher throughput for transforming the maple syrup than FD process. Moreover, VDD and syrup with red maple bark extract was found to be the best combination on producing polyphenol-enriched maple sugar powders from this study. All these findings indicate huge potential of maple products to be used as natural food ingredients. Yet, future studies such as to determine the stability of polyphenols in maple sugar matrices, and bioavailability on *in vivo* model will be helpful to increase their prospect as functional food ingredients. At last, other complimentary studies such as life cycle assessment could provide a better insight on the sustainability of the process.

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Annexe 1: Conference proceedings 21st IDS symposium 2018 (Valencia, Spain): Development of polyphenols-enriched maple sugars by Freeze-and vacuum drum drying technologies

DOI: 10.4995/IDS2018.2018.7804

Abstract

Hot water extract of sugar and red maple bark was added to maple syrup and dried by freeze (FD) and vacuum drum drying (VD) techniques. Addition of maple bark extracts to syrup helped to develop polyphenols-enriched maple sugar. X-ray diffraction revealed that sugar obtained from FD was amorphous in nature, while crystalline when dried by VD. Furthermore, the observation of maple sugar samples under scanning electron microscopy showed smooth and porous surface for FD sugar, while rough and grainy surface for VD sugar. Hausner ratio indicated that sugar produced by VD showed better flow characteristics than FD sugar.

Introduction

Drying of foods, solid (fruit and vegetables) or liquid (juices and syrups), increase their shelf life along with ease in handling and transportation. However, drying of foods cause the modifications of its physicochemical properties. In general, drying involving high temperatures effect thermo-labile compounds. Freeze drying (FD) is well-known technique for drying foods containing heat-sensitive and oxidative compounds such as polyphenols, because of the use of very low temperature and high vacuum. However, drying time and operation costs are major drawbacks of freeze drying (Ratti, 2001). Vacuum drum drying (VD) is another technique that operates at high temperature under vacuum, making it possible to dry food faster in comparison to freeze drying. Similar to FD, it may also preserve oxidative compounds due to the use of vacuum. Physical properties of powder such as moisture, flowability, morphology, etc depends on the type of drying method used [2, 3]. Therefore, drying techniques ought to be selected carefully, depending on the desired quality of the final product.

Canada accounts for approximately 71% of maple syrup production in the world (Statistics Canada, 2016). Maple syrup is a natural sweetener with high nutritional value based mainly on its sucrose, minerals, proteins and polyphenols (Ball, 2007). Depending on the percentage of light transmission (at 560nm), maple syrups are classified into four grades: golden, amber, dark and very dark (Canadian Food Inspection Agency). A class of "very dark" maple syrup, which has light transmission less than 25%, is considered as substandard. Therefore, drying of such substandard maple syrup to produce a maple sugar can add value to a natural sweetener containing minerals, polyphenols, etc. However, the polyphenols are present at a very low concentration in maple syrup. When dried, the concentration of polyphenols may further diminish. Therefore, the main objective of this work was to produce polyphenol-enriched maple sugar by adding, red and sugar maple bark extracts to maple syrup, and subsequently drying the mixture.

For this study, freeze-drying and vacuum drum drying technologies were explored. Thus obtained maple sugars were examined for total phenol content, moisture content, flow characteristics, crystallinity and morphology in order to study the effect of drying methods on developing the polyphenol-enriched sugars.

Materials and Methods

Hot water bark extraction and addition of extracts to maple syrup

Maple barks (sugar and red maple) were extracted using hot water following the method previously described by Geoffroy et al. (Geoffroy, Fortin, et al., 2017). Hot water extracts of maple barks were then added (at 0.01 % w/v) to maple syrup (very dark, 66 ºBrix) and subsequently dried. Maple syrup without any extract was considered as control. Hereafter, dried maple syrup (without extract, control) will be denoted as MS, while maple syrup with sugar and red maple bark extract are identified as MS-SX and MS-RX, respectively.

Drying experiments

Freeze drying (FD) was done by using a laboratory freeze dryer (Freeze Mobile 24, Virtis Company Inc., Gardiner, NY). Sample was frozen at -40 °C overnight and freeze-dried for 25h. The heating plate temperature was initially set at -36 °C, vacuum level was below 30

mtorr and the condenser at -90 °C. For this study, a laboratory-scale vacuum drum dryer (Buflovak, Buffalo, USA) was used. Sample was fed between the nips of two rotating drums, where drum temperature increased up to 110 °C. The dried maple sugar powder were scraped off using in-built scrapers. The obtained FD and VD samples were vaccum packed and stored in 4 ºC for further analyses.

Analyses of maple sugar powder

Total phenolic content

Total phenolic content (TPC) in maple sugar powder was determined using the Folin-Ciocalteu method using microplate spectrophotometer (X Mark^{TM}, BIO-RAD) following the method used by Zhang et. al (Q. Zhang et al., 2006). The results were expressed in milligram gallic acid equivalent per gram of oven dry sample (mg GAE/100g sample, db). TPC was measured in triplicates.

Moisture content

Moisture content of maple sugar powder was measured by vacuum-oven method at 70 °C and -22 in.Hg vacuum for 24h. Analyses were done in triplicates.

Flow properties

Hausner ratio (HR) was used to obtain the indication on flow characteristic of maple sugar powders. HR was calculated using the measured bulk and tapped density of the sample. Bulk density was measured by pouring the known mass of sample (m) to a cylindrical glass tube and volume occupied (V_b) was noted. For the tapped density, the glass tube was tapped from the vertical distance of 12±2 cm until no successive differences in volume (V_t) was recorded. Bulk and tapped densities were calculated using the following formula; m/V_b and m/V_t, respectively. Measurements were done in triplicates.

X-ray powder diffraction

The crystallinities of freeze and vacuum dried maple sugars were analyzed by powder X-ray diffractometer (SIEMENS/Bruker, Germany). Samples were measured at a diffraction angle (2θ) between 0 to 50° under operational conditions of 40 kV and 40 mA using Co ($K\alpha_{1+2}$) radiation.

Microstructural analysis by environmental scanning electron microscopy (ESEM)

Freeze and vacuum dried maple sugar samples were analyzed by environmental scanning electron microscopy (ESEM). The samples were scanned at 500X magnification at 20 kV.

Results and discussion

Effect of drying on total phenolics of maple sugar powder

Regardless of the type of drying techniques used, TPC of sugars containing maple bark extracts showed higher values than the control. Sugar containing red maple bark extract presented higher TPC value than other sugar samples. This can be explained by the higher phenolic content of the red maple bark extract (Bhatta et al., 2018). MS-RX obtained from VD had around 8% higher TPC than the control (MS). In general, FD method is preferred to dry a food containing a thermo-labile compound such as phenolics. On contrary, we observed that VD maple sugars had relatively higher TPC than those of FD sugars. This could be due to high temperature in VD that could have caused the breaking of the phenolic-glycoside bonds. Resulting in more phenolics becoming readily available to form complex with Folin's reagent while determining TPC by Folin-Ciocalteu method. However, it has yet to be validated by identifying the phenolic profile in maple sugar powders using techniques of chromatography and mass spectrophotometry.

Moisture content

Moisture content of freeze-and vacuum dried maple sugars are presented in Fig. 1. The moisture content in VD maple sugar (0.6-0.7%, on dry basis) was signigicantly lower than that of FD (4 to 5 %, dry basis). The drying temperature in VD was higher (110 °C) than in FD. Therefore, it was obvious that VD produced sugars had lower moisture content than that of FD. Similar tendency was also observed for the production of mango powder by freeze and drum drying (Caparino et al., 2012).

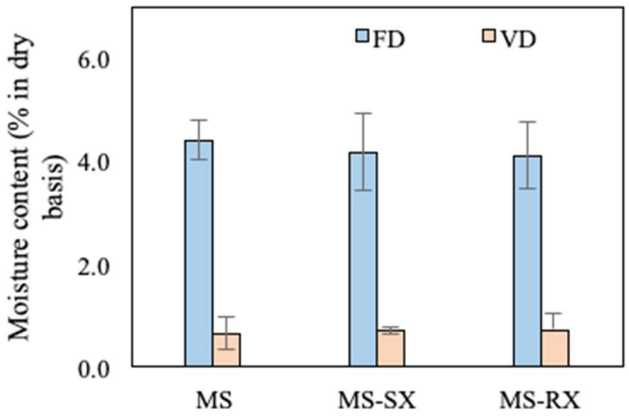


Fig. 1 Moisture content of freeze-and vacuum drum dried maple sugars. MS: maple sugar, MS-SX: maple sugar with sugar maple bark extract, MS-RX: maple sugar with red maple bark extract, FD: freeze-drying, VD: vacuum drum drying.

Flow characteristics of maple sugar

The calculated Hausner ratio of FD and VD maple sugars are presented in Table 1. Lower the Hausner ratio, the better is the flow properties (Seerangurayar et al., 2017). The Hausner ratio was signigicantly higher ($P<0.05$) for the FD maple sugars (1.45 to 1.48, poor to very poor) than that for VD sugars (1.14 to 1.20, good to fair flow). Therefore, VD maple sugars showed better flow characteristic than that of FD.

Table 1. Flow characteristics of freeze-and vacuum dried maple sugars.

Drying techniques	Sample	Hausner ratio
FD	MS	1.45 ± 0.03^{a}
	MS-SX	1.48 ± 0.07^{a}
	MS-RX	1.47 ± 0.06^{a}
VD	MS	1.14 ± 0.02^{b}
	MS-SX	1.20 ± 0.04^{b}
	MS-RX	1.20 ± 0.05^{b}

Values represent Mean±S.D. ^{a,b}different superscript letters in the same column are significantly different ($P<0.05$) by Tukey's test.

Effect of drying on crystallinity of maple sugar

Fig. 2 depicts the profile of X-ray diffractogram of maple sugars produced by FD and VD techniques. Amorphous material shows large and disperse peaks due to disorderly arraged molecules in amorphous state. On the other hand, crystalline material shows sharp and defined peaks since the molecules are present in a highly ordered state. From Fig. 2, it can be observed that freeze dried maple sugar (FD_MS) is in amorphous state, whereas vacuum dried maple sugar (VD_MS), in crystalline state. However, the degree of crystallinity has yet to be identified. Crystalline state is important for the stability of food powders. Considering this results, VD sugars seemed to have an advantage of stability.

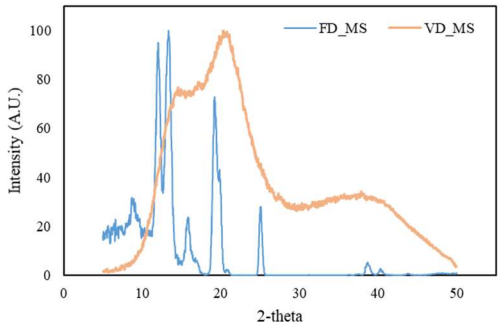
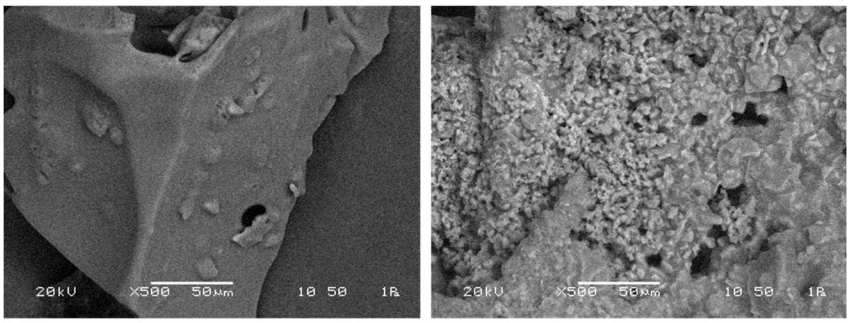


Fig. 2 X-ray powder diffractogram of maple sugars produced by different drying methods. FD_MS, freeze dried maple sugar, VD_MS: vacuum dried maple sugar.

Microstructural analysis

Microstructural analysis of maple sugar obtained from FD and VD techniques are shown in Fig. 3. FD maple sugar showed irregular and porous structure (Fig. 3a). Freeze-dried food normally shows porous structure due to the fact that ice removal by sublimation during drying prevents shrinkage and volume reduction (Ratti, 2001). Vacuum-dried maple sugar resulted in rough and grainy surface (Fig. 3b). The difference in microstructure of FD and VD maple sugars can also be related to the the difference in their crystallinity (shown in Fig. 2).



a **b**

Fig. 3 Enviromental scanning electron micrograph (ESEM) of freeze-dried (a) and vacuum dried (b) maple sugar.

Conclusion

Considering the overall results, polyphenol-enriched maple sugar obtained either by FD or VD has potential applications in foods, particularly as sweetener for natural food products. For instance, FD maple sugar could be used in instant-drinks owing to its amorphous powder nature and VD sugar as functional food ingredient due to its high phenolic content as well as its better flow characteristics.

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Annexe 2: Poster presentation at ISEKI_Food2018 (Stuttgart, Germany): Development of maple sugar powders by Freeze-drying

5th International ISEKI_Food Conference
ISEKI_Food 2018
3 - 5 July 2018, University of Hohenheim, Stuttgart, Germany
"The Food System Approach: New Challenges for Education, Research and Industry"

Development of Maple Sugar Powders by Freeze-drying

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Background

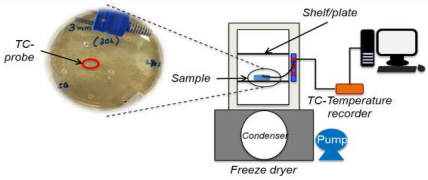
Canada accounts for more than 70% of world maple syrup production (Statistics Canada, 2017). Maple syrup is a natural sweetener with high nutritional value based on its composition (sucrose, minerals, proteins and polyphenols). Unfortunately, the surplus of maple syrup accumulated each year is a major problem among the syrup producers in Canada. Drying of syrup could be an appropriate solution to this problem, since it increases product shelf life and eases handling and transportation. In addition, dried powders can be straightforwardly used in the formulation of foods. Freeze-drying (FD) was chosen as a dehydration method to produce maple powders due to an outstanding final product quality. However, it is a time-consuming process, specially if under-optimized. Also, foods having high sugar or fat content are difficult to dehydrate by this method.

Objectives

The main objectives of this study were:

- to formulate a suitable freeze-drying protocol to successfully dry maple syrup in minimum drying time, and
- to study the physicochemical and flow properties of final powders.

Materials & Methods



Sample preparation
Maple syrup Grade: Strong (Robust)
%Light Transmittance: <30
Syrup concentration: 20 °Brix
Sample thickness: 3 mm

Part 1: Formulation of FD protocol

- **Differential scanning calorimetry**
  (Primary & secondary drying temperature)
- **Thin-wire thermocouple**
  (Duration of drying phases)
- Drying kinetics
- Control: single-cycle FD at -36 °C (40 h)

Part 2: Physicochemical and flow properties

- Moisture content & water activity
- Color analysis by colorimetry
- Dissolution time
- Flow properties
  Hausner ratio
  Angle of repose (θ)

Part 3: Sorption isotherm

Results

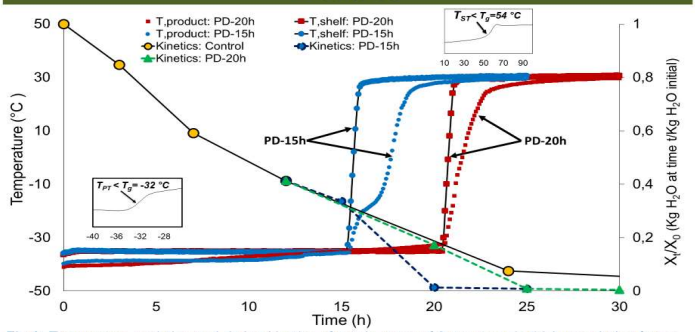


Fig.1: Temperature evolution and drying kinetics of maple syrup of 3 mm sample thickness during freeze-drying under different primary drying time **(PD, 15 & 20 h)** while constant secondary drying time **(SD,10 h)**
Primary drying temperature, T_{PT}= -36 °C; Secondary drying temperature, T_{ST}= +30 °C

Table 1: Physicochemical and flow properties of maple sugar powders obtained under optimized freeze-drying conditions (PD-15h and PD-20h).
**Mean±S.D. values followed by different superscript letters in the same row are significantly different (P<0.05) by Tukey test.*

		PD-15h	PD-20h
Moisture content (%, db)		4,18±0,76[a]	3,18±0,10[b]
Water activity, A_w		0,24±0,05[a]	0,19±0,05[b]
Dissolution time (s)		13,66±1,49[a]	13,93±1,30[a]
Color analysis	*L**	84,93±0,33[a]	85,72±0,68[a]
	Chroma	15,70±0,28[a]	14,09±0,34[b]
Bulk density (g/ml)		0,29±0,01[a]	0,26±0,02[b]
Tapped density (g/ml)		0,43±0,05[a]	0,41±0,02[a]
Hausner Ratio *(Cohesiveness)*		1.46±0,10[a] *(High)*	1,60±0,21[a] *(High)*
Angle of repose (θ) *(Flowability)*		30,45±6,16[a] *(Fair)*	31,17±3,91[a] *(Fair)*

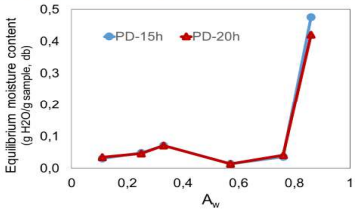


Fig. 2: Sorption isotherm curve of maple sugar powders obtained from PD-15h and PD-20h.

0,11 to 0,36 A_w: linear increase

0,36 to 0,56 A_w: decrease in moisture *(probably due to change from amorphous to cystalline state)*

At 0,86 A_w: deliquescence point

Conclusions

- This study proposes a **simple and efficient method** to develop a freeze-drying protocol for sugar-rich liquid foods
- Freeze-dried maple sugar powders was **stable** (<0,25 A_w), showed **instant-like powder** ($t_{dissolution}$<14s) with **fair flowability**
- Powders are **very hygroscopic** in nature, therefore, need to be carefully stored (< 0,36 A_w)
- Natural **sweetener powder** for various food applications, mainly for **natural food products**

Acknowledgement

The authors are grateful for a financial support from National Science and Engineering Research Council (NSERC), Levaco Inc. and Decacer Inc., Canada.

Annexe 3: Poster presentation at GFT2018 (Quebec, Canada): Valorization of low grade maple syrup to polyphenol-enriched maple sugar

Valorization of Low Grade Maple Syrup to Polyphenol-enriched Maple Sugar

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Introduction

Background The surplus of dark grade maple syrup is a major problem among the syrup producers in Canada. Drying of syrup could be an appropriate solution, since it increases product shelf life, eases handling and produces ready-to-serve food.

Challenges Drying is an energy-intensive process as well as sugar-rich foods are difficult to dehydrate owing to low glass transition temperature of simple sugars.(1) Thermal-sensitive compounds like polyphenols which are present in syrup may also degrade during the process. Therefore, the choice of an appropriate drying technique is essential to produce a desirable final product with better quality.

Study Freeze-drying (FD) and vacuum drum drying (VD) were chosen as dehydration methods to produce maple sugar powders. Considering that polyphenols are sensitive to thermal process, maple syrup was enriched with hot-water maple bark extracts, as they are rich in polyphenols (results from our previous study).(2)

Objectives

The main objectives of this study were:

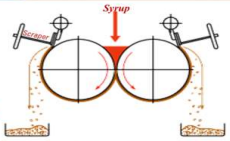
- to produce polyphenol-enriched maple sugar by adding sugar (SX) or red maple bark extracts (RX),
- to study the effect of FD and VD on nutritional and physicochemical properties of final powders, and
- to compare the FD and VD process based on their production yield and drying time.

Materials & Methods

Sample Preparation → Experiment → Analyses

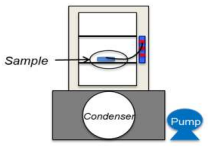
Dark Syrup

Addition of maple bark extracts (0,01%(w/v))



Vacuum Drum Dryer (VD) (T_{drum}=75°C, vacuum=660 torr)

Freeze-dryer (FD) (T=-36°C & +30°C; vacuum=30mtorr)



Nutritional properties
- Phenolic compounds by HPLC-MS
- Total phenolic content

Physicochemical & flow properties:
- Moisture content & water activity
- Dissolution time
- Flow characteristics
- X-ray diffraction
- Morphology analysis

Process comparison:
- Production yield
- Drying time

Results

Nutritional properties

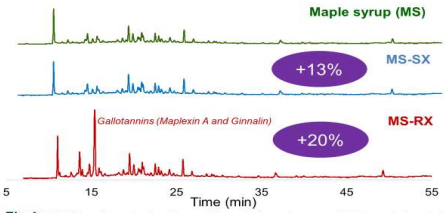


Fig.1 LC-MS profiles showing the enrichment of maple syrup (MS) in polyphenols by incorporating the maple bark extracts. MS-SX, maple syrup with sugar maple bark extract; MS-RX, maple syrup with red maple bark extract.

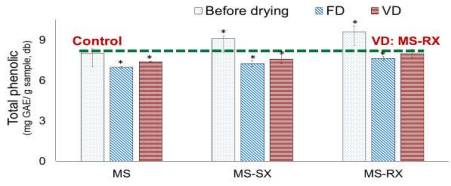


Fig.2 Effect of drying processes (FD and VD) on total phenolics of maple syrup (MS, control) and syrup treated with bark extracts (MS-SX and MS-RX). MS-SX, maple syrup with sugar maple bark extract; MS-RX, maple syrup with red maple bark extract.
*indicates significantly different values compared to MS (control) by Tukey's test.

Physicochemical & flow properties

Table 1: Physicochemical and flow properties of maple sugar powders obtained FD and VD conditions.

Processes	Sample	Moisture (% db)	A_w	Dissolution (s)	Hausner ratio
FD	MS	4,10-4,40	0,19-0,21 (Stable)	12,2-12,9 (Instant-like)	1,44-1,47 (Poor flow)
	MS-SX				
	MS-RX				
VD	MS	0,63-0,71*	0,29-0,32* (Stable)	27,1-28,8*	1,14-1,21* (Good to fair flow)
	MS-SX				
	MS-RX				

*No significant difference was observed among samples obtained from the similar drying method; Values were average of three independent experiments; * Significantly different values by Tukey's test.*

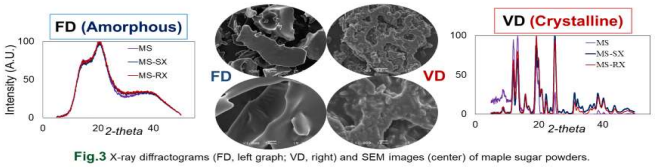


Fig.3 X-ray diffractograms (FD, left graph; VD, right) and SEM images (center) of maple sugar powders.

Process comparison

Table 2: Comparison of drying processes based on their yield, drying time and production capacity.

Drying processes	Feed concentration	Yield (%, db)	Drying time (min)	Production capacity (g sugar/min)
FD	20 °Brix	94,2±0,7	1500	0,04**
VD	66 °Brix	73,3±5,8	10 to 15*	16,22

*VD is continuous process, where drying time varies depending on the volume of syrup need to be dehydrated, 400 to 450 mL of syrup was dried in 10-15 min.
**FD production capacity was calculated based on dry maple sugar obtained from each batch when FD was operated on full capacity (in 2 shelves); db: dry basis.*

Conclusions & Potential Applications

- Maple syrup was **enriched by 13 and 20%** in total phenolics with SX and RX, respectively. However, both drying processes resulted loss in total phenolics.
- FD and VD produced **stable** (<0,40 water activity) maple sugar powders.
- **FD** maple sugar powders demonstrated **instant-powder** like property (amorphous), while **VD** produced powder with **better flowability** than FD.
- Unlike FD, VD was fast and no dilution of syrup was required.
- Natural **sweetener powder** for various food applications, mainly for **natural food products.**

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