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Antioxidative capacity of rosehip polyphenols and their potential role in type 2 diabetes mellitus prevention and management

ZHAO ZHAO

Master of Science Thesis

Supervisor: Cecilia Svelander and Marie Alminger

Examiner: Marie Alminger

Department of Chemical and Biological Engineering

Food Science

CHALMERS UNIVERSITY OF TECHNOLOGY

Gothenburg, Sweden, 2012

Abstract

The health benefits derived from a diet rich in phenolic compounds have been suggested to be partly through effects generated in the gastrointestinal (GI) tract. Elevated blood glucose level plays an important role in the development of type 2 diabetes mellitus. The inhibition of digestive enzymes in the GT tract involved in starch degradation may lead to beneficial effects on blood glucose control after the intake of starch containing foods. The aim of this master thesis was to investigate the ability of rosehip extracts to inhibit α-amylase and α-glucosidase activity during *in vitro* digestion.

Aqueous and methanol extracts of rosehip powder were analyzed for total phenolic content and antioxidant capacity. Folin-Ciocalteau method (FC) and oxygen-radical absorbance capacity (ORAC) assays were optimized for respective purposes. Porcine pancreas α-amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) were used in enzyme inhibition assays. Acarbose, a commercial pharmaceutical α-amylase inhibitor used as a therapeutic approach to control blood glucose levels, was used as a positive control.

Rosehip extracts were found to have a high content of phenolic compounds and high antioxidant capacity. The extracts were able to exert a weak inhibitory effect on α-amylase and a strong inhibitory effect on α-glucosidase. Acarbose displayed an opposite pattern to these inhibition assays, which was consistent with its reported side effects.

The results from this study show that rosehip extracts had high ability in inhibiting starch hydrolyzing enzymes and thereby decreasing potential blood glucose responses *in vivo*.

Acknowledgement

I am grateful to a number of persons who supported me to finish this thesis work, namely:

My supervisors, Professor Marie Alminger and Doctor Cecilia Svelander, for the patience in instructing my work and all the constructive opinions throughout this master thesis work;

All my colleges and friends in the department, for kindly offering expertise and suggestions to assist this project work moving on, and for all the good memories shared together;

My parents and family members, for supporting and encouraging me to pursuit knowledge and development.

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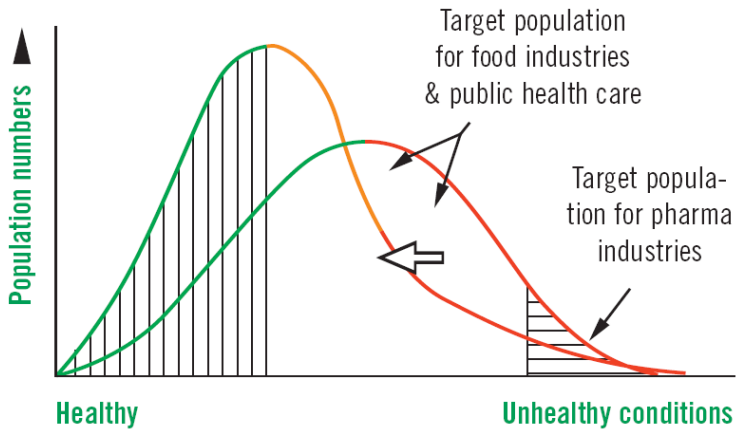
1. Introduction

Diabetes mellitus type 2 is a metabolic disorder described as hyperglycemia due to insulin resistance and insufficient insulin secretion. The raised high blood sugar level, a typical clinical syndrome of type 2 diabetes, always leads to other diseases if not intervened by special medication in time. Retinopathy, nephropathy, neuropathy, coronary heart disease, peripheral vascular disease and hypertension had been reported as complications linked with type 2 diabetes (Amos et al., 1997, Apostolidis et al., 2006), and the life span of the patient is reduced significantly by these long-term risks (Olshansky et al., 2005). This normally adult-onset disease has been observed as a rapidly-increased incidence in young population (Rosenbloom et al., 1999), which is a major current concern in disease control and public health. Frequent diets with excessive calorie and unhealthy fatty acids has been associated with negative effects on glucose metabolism (Hu et al., 2001), thus, disease prevention can be achieved either by introducing a healthy and balanced diet to ordinary people or by consuming food with certain anti-diabetic effects on a daily basis (**Figure 1**). Dietary intake of food ingredients with inhibitory effects on digestive enzymes which affect starch degradation and glucose metabolism is a potential approach to alleviate postprandial glucose increase and subsequent diabetes development.



Source: Green MR and van der Ouderaa F, Nature Pharmacogenomics, 2003

Figure 1. Schematic illustration of improving human health by pushing the most population into the area, where preventative actions can be taken by healthy food intake other than pharmaceutical preparations.

α-Amylase and α-glucosidase are involved in starch degradation in the human body. α-Amylase initiates starch hydrolysis into maltose and then α-glucosidase cleaves maltose into glucose which is later transported to the blood stream. Thus, inhibition of these two digestive enzymes can be used to reduce the postprandial response after consumption of starch-containing foods. Based on this rationale, acarbose, voglibose and miglitol (van de Laar et al., 2005), three specific inhibitors for the enzymes are used in diabetic therapy by limiting starch hydrolysis (α-amylase) and glucose release (α-glucosidase). However, long-term administration of these pharmacological preparations can bring about gastrointestinal discomfort as a result of undigested starch passing through to the colon. Flatulence,

abdominal cramps and diarrhea are reported as the most common side effects after medication with acarbose (Lam et al., 1998, Chiasson et al., 2002). The explanation to these unpleasant side effects are not only because of excessive inactivation of α-amylase, but also the increased bowel movement which gives less time for the enzyme to act on their substrates (Weaver et al., 1997). Therefore, natural food components exhibiting less α-amylase but more α-glucosidase inhibition will be ideal for diabetic management and prevention (Pinto Mda et al., 2010).

Polyphenols are secondary metabolic products from plant resources as a defense mechanism produced under particular stimuli and stresses (Beart et al., 1985), and they are considered as beneficial to human health. Researchers have reported plant-derived polyphenols exert anti-proliferative (McDougall et al., 2008b), anti-cancer (Seeram, 2008), anti-pathogen (Puupponen-Pimia et al., 2005), anti-microbial (Puupponen-Pimia et al., 2001, Oliveira et al., 2008), anti-inflammatory (Winther et al., 1999) properties *in vitro* tests. Several studies on animal models have shown that polyphenol-rich diet can lower serum cholesterol concentration (Martín-Carrón et al., 1999) and reduce risk of lipid peroxidation (Ramirez-Tortosa et al., 2001), and these properties are suggested to be associated with their strong antioxidant character. Recent studies have also shown that α-amylase and α-glucosidase can be inhibited by polyphenols from spices (McCue et al., 2004), herbs (Iwai et al., 2006) and soft fruits (McDougall et al., 2005). Thus, foods rich in phenolic compounds are promising components of a healthy diet that can help to stabilize fluctuating blood sugar levels after a meal by controlling the rate and extent of glucose regulation.

Rosehip is a red fleshy berry and although many kinds of roses can produce fruits, only rosehips from *Rosa canina* and *Rosa rugosa* are edible. Rosehip is a rich source of vitamin C, carotenoids and also contain smaller amount of essential long-chain fatty acids (Zlatanov, 1999). Its usage differs from culture to culture. In Turkey, rosehips are more often used for medical purposes (Ercisli, 2007), while in Sweden, rosehip are consumed as the main ingredient in dishes, such as rosehip soup, *nypon soppa* in Swedish. Medical research has shown rosehip powder has significant anti-inflammatory properties (Winther et al., 1999) and may effectively reduce the pain of patients suffering from osteoarthritis in knee and hip (Winther et al., 2005). However, these health benefits have mainly been suggested to be related to high content of vitamin C and carotenoids in rosehips. Thus, the health benefits derived from phenolic compounds in rosehips may be underestimated. In the present study, the total content of phenolic compounds and the antioxidant capacity of rosehip extracts as well as their ability to inhibit starch hydrolyzing enzymes were evaluated. The potential of launching rosehip products with anti-diabetic ability is also discussed in this thesis work.

2. Background

2.1 Diabetes mellitus

Diabetes mellitus is a chronic metabolic disorder caused by insufficient insulin secretion by pancreas β cells, less sensitivity towards insulin of target cells or structurally-flawed insulin production. Based on pathogenesis studies, diabetes mellitus is categorized into two different major types: type 1 and type 2. Although the causes of these two types are not similar, they both disturb the normal metabolism to a degree.

Within hours, carbohydrates from ingested foods can be converted to glucose, which is the main sugar in the blood stream. When the glucose level of blood is raised after a meal, β cells in pancreas synthesize and secret insulin into the blood. By blood circulation, insulin reaches to target cells in relevant tissues and organs, where they are recognized by specific receptors in order to elicit a cellular response that accelerate the utilization of glucose from the blood stream. Any abnormality in this whole process can lead to the development of diabetes mellitus.

Type 1 diabetes mellitus, also known as insulin-dependent diabetes, is regarded as a kind of autoimmune disease. Patient self-immune system destroys insulin-secreting pancreas β cells and in that way making the person unable to regulate glucose uptake by insulin production (Amos et al., 1997). Normally, to compensate for the impaired control over carbohydrates metabolism, exogenous insulin is needed. Because genetic factor plays an important role in its onset, no preventative measures are certified against type 1 diabetes mellitus successfully by now.

The number of subjects with non-insulin-dependent diabetes or type 2 diabetes mellitus has increased dramatically recently and the increasing prevalence is closely related to cultural and social changes, aging populations and urbanization, which seems not easy to change in the near future. Therefore, actions that can benefit a large population globally should be taken to slow down the increasing prevalence. Unlike type 1 diabetes mellitus which normally needs special medication, non-insulin dependent diabetes, or type 2 diabetes mellitus can be prevented and alleviated by improving lifestyles such as moderate exercises and a healthy diet. Aerobic exercise can help to improve glycemic control, keep fitness and reduce the incidence of cardiovascular diseases. Resistance exercise has also been recommended for the patients with type 2 diabetes mellitus. However, it might need qualified exercise assistance to guarantee the positive outcome. Dietary control is as important as proper exercise. Either high-fat diet or high-sugar diet is detrimental to human health as causing obesity and insulin resistance or deficiency (Hu et al., 2001), which are often associated with type 2 diabetes mellitus. In a dietary pattern study, a “western” dietary pattern (characterized as high consumption of red meat, processed meat, French fries, high-fat dairy products, refined grains, and sweets and desserts) showed a strong association with the risk for type 2 diabetes mellitus (van Dam et al., 2002) . Meanwhile, a

prudent dietary pattern (characterized as high consumption of vegetables, fruits, fish, poultry and whole grains) only showed a moderately-lowered diabetes risk.

As a result, keeping a healthy diet rich in vegetables and fruits as well as regular physical activities are recommended to prevent type 2 diabetes mellitus for a large population. In addition, identification of new approaches like the use of specific food components may provide potential prevention and management of type 2 diabetes.

2.2 Phenolic compounds

Phenolic compounds are the secondary metabolic products of plants or microorganisms, and plant skins, roots, leaves and fleshes of some vegetables and fruits have remarkably high content of them. Apart from vegetables and fruits, polyphenols are also detected in cereals, processed food products and beverages, for example, teas and wines.

The compositions of polyphenols from different kinds of vegetables and fruits differ considerably. The diversity was not found only in different species, but also in the same cultivar. Location of growth (Latti et al., 2008), temperature change between day and night (Zhou and Yu, 2004, Wang and Zheng, 2001), ultra-violet irradiation (Kalt et al., 1999), sun light exposure (Bąkowska et al., 2003) and post-harvest treatment (Zauberman et al., 1991) have been demonstrated to affect accumulation and stability of phenolic compounds in foods. In cereals, phenolic compounds can bind to polysaccharides covalently by ester bonds (Vitaglione et al., 2008). Quantification of food phenolics is difficult both due to the complex formation between their constituents and the high variability between different genotypes. Despite of those variations, based on a recent comparative study by Phenol-Explorer database, seasonings have the highest content in phenolic compounds if comparison is made by portion size, followed by fruits and seeds (Perez-Jimenez et al., 2010).

In general, phenolic compounds can be synthesized by two different ways. For instance, gallotannins and ellagitannins are formed from shikimic acid pathway directly, while benzoic acids, flavonoids and lignins require a more complicated process with shikimic acid pathway as a prerequisite. In the latter case, phenylalanine produced in shikimic acid pathway plays a crucial role in all subsequent chemical reactions, including the formation of cinnamic acid and tyrosine, two primary precursors of various phenolic compounds after various chemical modifications (Parr and Bolwell, 2000). These phenolic compounds biosynthesis pathways can be enhanced under external stresses, such as wounding, pathogen invasion and fertilizer utilization, because by producing more phenolic compounds, plants are able to resist diseases and insects invasions (Tomás-Barberán and Espín, 2001).

Structurally speaking, polyphenols have more than one phenol hydroxyl group attached to the benzene ring and they can be very different in size, from the simplest hydroxybenzoic acids to polymerized condensed tannins with high molecular weights. **Figure 2** shows a detailed classification of phenolic compounds. Phenolic compounds are usually acting as

effective hydrogen or electron donors in chemical reactions, and due to their chemical properties, they have wide applications in food industry via improving food quality during processing and storage.

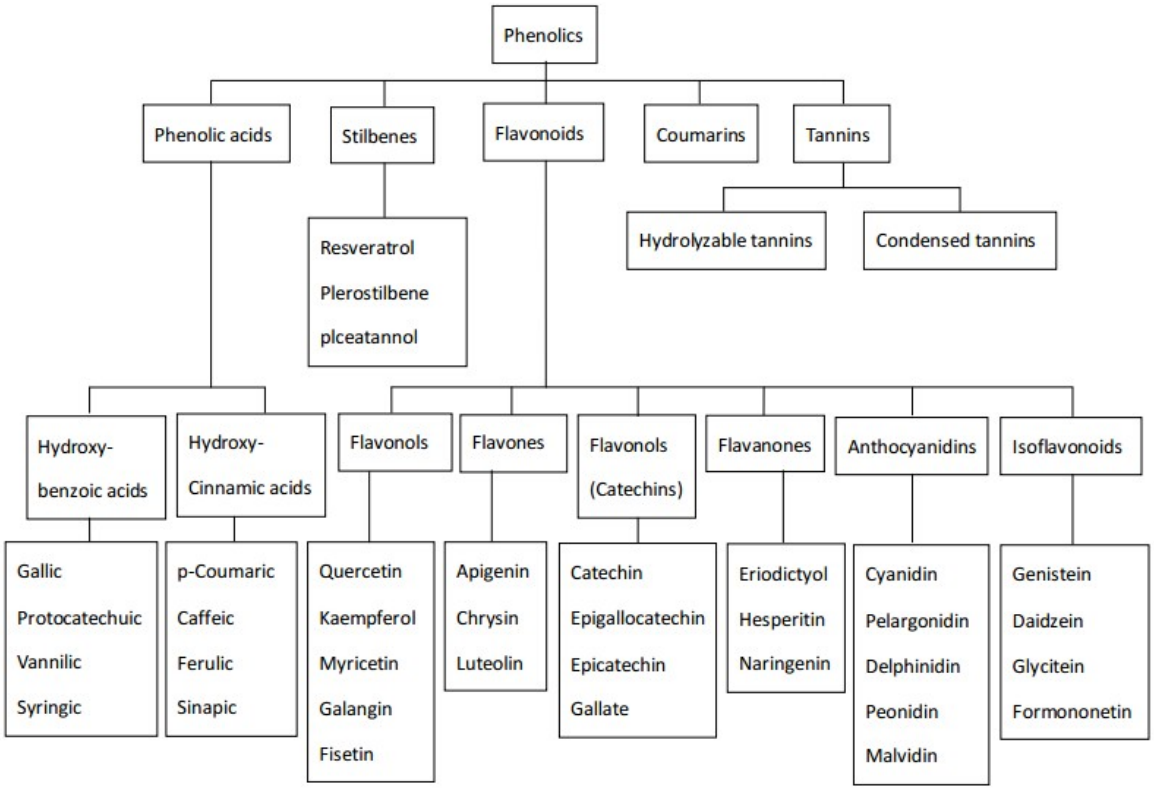


Figure 2. Classification of phenolic compounds. Source from Liu Ruihai's work with modifications (Liu, 2004).

To start with, phenolic compounds can be incorporated in food products as natural antioxidants to prevent rancidity caused by lipid peroxidation. Some phenolic compounds such as caffeic acid, quercetin and catechin, are more effective than the synthetic antioxidants BHA and BHT used in the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (von Gadow et al., 1997), a method used to evaluate antioxidant capacity. In addition, the application of these natural antioxidants can avoid the toxicity from traditional additives. Phenolic compounds can also effectively inhibit microbial and bacterial growth in stored foods. They have weak acidity, and can be added to neutral or acidic foods for preservation purposes with only little sensory changes. Further, phenolic compounds are used as clarifying agents for their ability to precipitate proteins and other suspended solids. Phenolic hydroxyl binds to protein or other compounds through hydrogen bonds to form large complexes, which is a critical step in wine-making.

In addition to their applications in food processing, phenolic compounds themselves are regarded as beneficial to human health. They have been suggested to have anti-inflammatory, anti-tumor, anti-pathogen, anti-carcinogenic and anti-proliferative properties.

2.3 Analytical methods and assays

Schematic outline of the methods used in the study is presented in **Figure 3.**

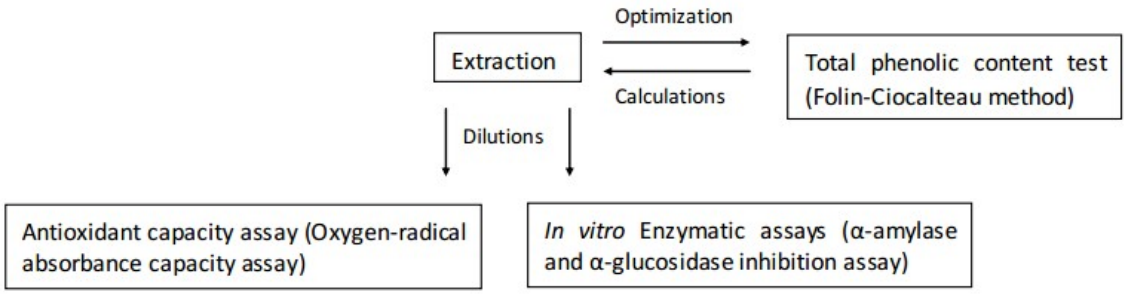


Figure 3. Outline of the methods used in the present study

Extraction is the very first step of a series of assays. To gain insight into the effect of the used of different extraction solutions, a number of different extraction solvents were evaluated and compared. Mechanical disruption treatments were also introduced in this step to improve the extraction.

Folin-Ciocalteau test, the redox colorimetric assay, was selected to quantify the amount of phenolic compounds in rosehip extracts. Oxygen-radical absorbance capacity (ORAC) assay was used for assessment of the antioxidant capacity. Measurements of enzyme activities were made after determination of total phenolic contents, and phenolic contents was estimated from a standard curve of gallic acid.

2.4 Extraction

An efficient extraction procedure is crucial for the assessment of total phenolic contents and antioxidant capacity. Numerous published methods have been reviewed by comparing different solvents, numbers of extractions, and extraction duration and so on.

During extraction, polyphenols undergo certain conversion or degradation depending on the nature of solvents used. In aqueous solution, new compounds can be produced from anthocyanins by proton transfer and hydration reactions (Cheynier, 2005). Methanol, a common extraction solvent, can activate polyphenol oxidase that catalyzes the oxidation of phenol groups and causes undesirable fruit browning by melanin formation (Janovitz-Klapp et al., 1990). Both of these may lead to misjudgments of characterization and quantification of which phenolic acid is originally present in the sample.

In addition, due to the different solubility of the various compounds, both lipophilic and hydrophilic solutions should be used accordingly. A more comprehensive image of the total phenolic contents can be obtained, but it is still oversimplified just to add these results together.

As described in the literature, many new techniques have been developed for more reliable measurement of polyphenol content and among which, the assist of ultrasonication in extraction seems practical and advantageous and had been applied in this study. Ultrasonication has been reported to increase the efficacy of ethanol extraction, which is a poor solvent in normal conditions (Albu et al., 2004), and it is likely that the use of ultrasonication can be efficient for aqueous and methanol extracts as well. Ultrasonication is normally used to disrupt cell membranes and organelles to release cellular compounds. By ultrasonication, more bioactive compounds will be released from broken cellular structure (Takahama, 2004, Bonoli et al., 2004) and the mechanical force accompanied can give a greater penetration of solvents into the solid phase of a test sample by enlargement of the contact area. Furthermore, no other interfering chemicals need to be used in ultrasonication, which may otherwise adversely change the stability of the compounds of interest (Wang and Weller, 2006). In the present study, since the rosehip aqueous extracts were rather thick and viscous, the use of ultrasonication became a useful tool in making a homogenous mixture and more effective than merely vortexing and shaking.

Except for ultrasonication, more advance technologies such as pressurized liquid extraction, supercritical fluid extraction, microwave-assisted extraction and ultrasound-assisted extraction have been reported (Alonso-Salces et al., 2001, Wang et al., 2008) and reviewed by Wang (Wang and Weller, 2006). However, they also pointed out that the efficacy of those techniques were closely associated with the nature of the tested samples. Thereby, the optimal extraction condition varies from species to species according to the degree of similarity in chemical composition and molecular configuration. Furthermore, the way of sampling and material preparation is decided largely by the experimental set-up and researchers' subjective preference and convenience that also contribute to the variances.

2.5 Determination of total phenolic content

Folin-Ciocalteau method is widely used to determine humic substances in soils, freshwaters and lake sediments in environmental studies, and have become a robust method to quantify phenolic compounds from various foodstuffs. The principle behind it is that the phosphomolybdic and phosphotungstic acids in Folin-Ciocalteau phenol reagent can be reduced to a blue complex in an alkaline environment. When a proper standard curve is established, the color depth of the reduced products can be correlated to the amount of phenolic compounds in a linear pattern. Various chemicals have been reported to create the standard curves as reference. In order to compare the results with previous studies, gallic acid was used as the standard reference in both Folin-Ciocalteau method and ORAC assay in the present study.

Several basic substances can help to create an alkaline environment, while Na_2CO_3 has been preferred by most scientists because it gives higher reproducibility than the others. For example, NaOH solution may cause haze formation because of complexing with minerals.

As summarized by Box (Box, 1983), after evaluation of alkaline solutions as supporting medium, a mild alkali like Na_2CO_3 was superior to a strong alkali since changes in total volume for introducing less variation.

However, the concentration of sodium bicarbonate solution used in Folin-Ciocalteau method varies considerably, ranging from 2% to 35% (**Table 1**) and no explanation for this variation has been found. Thus, in the present study, different Na_2CO_3 concentrations were compared to investigate if the varying concentration influences the results. Besides, the impact of light exposure and the presence of trifluoroacetic acid (TFA) on polyphenol retention were also evaluated.

Table 1 List of different concentration of sodium bicarbonate solution used for analysis of total phenolic contents using the Folin-Ciocalteau assay

Concentration of Na_2CO_3 solution in Folin-Ciocalteau method (w/v)	References
35%	(Turkmen et al., 2006)
20%	(Su et al., 2007, Yi et al., 2008)
7.5%	(Aaby et al., 2007, Lapornik et al., 2005)
7%	(Chu et al., 2002)
2%	(Oktay et al., 2003, Ercisli, 2007)

2.6 Antioxidant capacity assay (ORAC)

Phytochemicals, like allyl sulfide (Borek, 2001), carotenoids (Krinsky, 1989), and flavonoid (Heim et al., 2002), has been extensively studied for decades, and some of the proposed health benefits have been found associated with their antioxidant capacity (Nakayama et al., 1992). Natural pigments within the group of polyphenols have also been studied for their potential role as effective free radical scavengers (Scalbert et al., 2005).

The chemical diversity of antioxidants makes it difficult to quantify antioxidants in food and extracts. Different methods have been established and modified to measure the total antioxidant capacity and these assays can be broadly classified as HAT (hydrogen atom transfer) or ET (electron transfer) (**Figure 4**). HAT means that the possible antioxidant passes a hydrogen atom onto the radical and then becomes a new radical itself by doing so. The mechanism of ET is similar to that of HAT in general but an electron is transferred by the antioxidant which changes into a cation radical afterwards. Both of the new radicals derived from the original antioxidants in these two mechanisms have higher stability than their hydrogen/electron acceptors, thus they are not expected to react with the substrates. To assess the level of antioxidant capacity of a given substance, the oxygen-hydrogen bond dissociation enthalpy and ionization potential are key parameters for HAT and ET mechanisms respectively (Leopoldini et al., 2004).

assays involving hydrogen atom transfer reactions	ORAC (oxygen radical absorbance capacity)
$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$ $ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}$	TRAP (total radical trapping antioxidant parameter) Crocin bleaching assay IOU (inhibited oxygen uptake) inhibition of linoleic acid oxidation inhibition of LDL oxidation
assays by electron-transfer reaction	TEAC (Trolox equivalent antioxidant capacity)
$M(n) + e$ (from AH) $\rightarrow$ $AH^{\bullet+} + M(n-1)$	FRAP (ferric ion reducing antioxidant parameter)
	DPPH (diphenyl-1-picrylhydrazyl) copper(II) reduction capacity total phenols assay by Folin–Ciocalteu reagent
other assays	TOSC (total oxidant scavenging capacity) (*90*) inhibition of Briggs–Rauscher oscillation reaction (*91*) chemiluminescence (*92*) electrochemiluminescence (*93*)

Figure 4. The mechanisms behind different kinds of in vitro antioxidant capacity assays from Huang's work (Dejian Huang 2005).

Obviously, the antioxidant capacity measured by an individual assay reflects only the chemical reactivity under the specific conditions applied in the assay. It is therefore misleading to generalize the most suitable indicator of total antioxidant capacity activity. However, oxygen-radical absorbance capacity (ORAC) assay was chosen among these available assays with careful consideration.

Oxygen-radical absorbance capacity (ORAC) assay was reported by Cao and his colleagues in 1993 (Cao et al., 1993). The principle behind this assay is to compare the loss of fluorescence after the addition of Trolox (a water-soluble vitamin E analogue) and tested sample to the blank. During the reaction period, the reactive oxygen species derived from the thermal decomposition of the peroxyl radical will quench the fluorescence, and the efficacy of antioxidant capacity can be compared by the changes in fluorescence intensity between different treatments (**Figure 5**). Several modifications have been made for specific usages (Huang et al., 2002), which makes the application of this method more extensive. Thus, it has become a robust method with relatively good reproducibility to measure sample's antioxidant capacity.

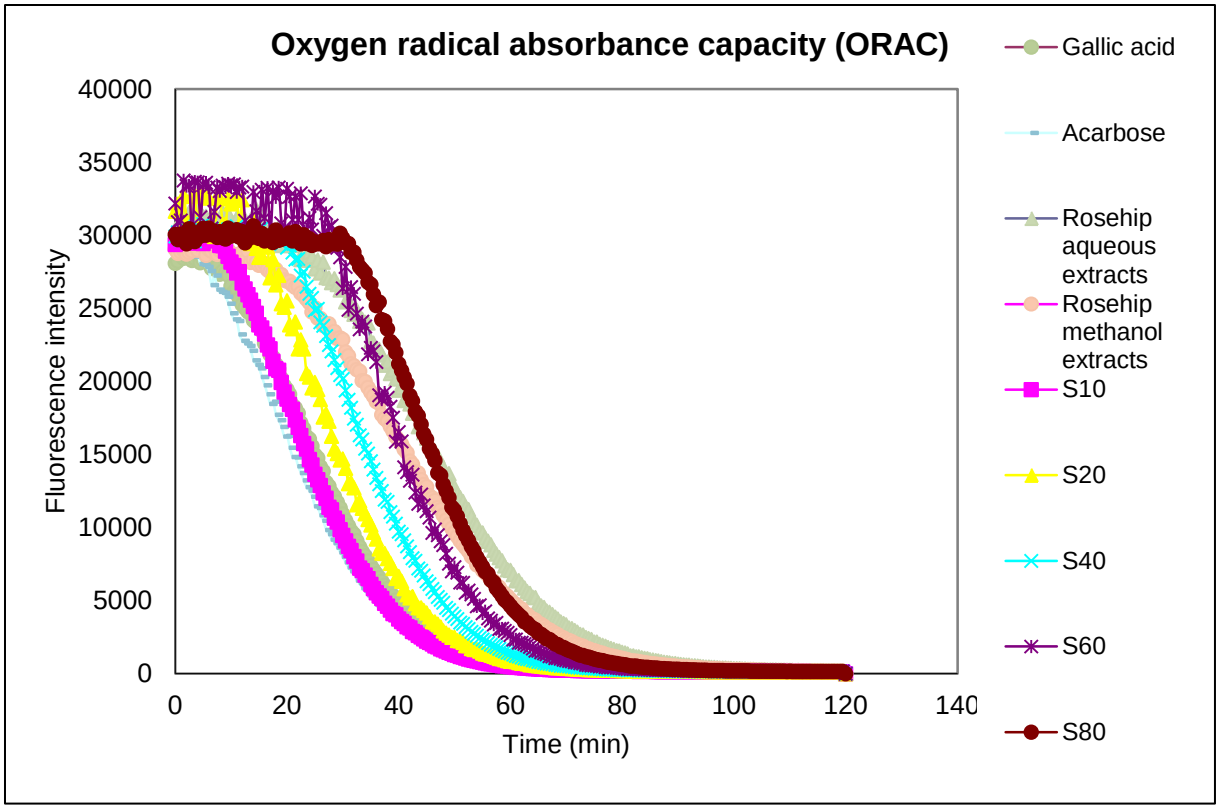


Figure 5. A schematic illustration of fluorescence decay curve from ORAC assay. Rosehip aqueous and methanol extracts were tested with gallic acid and acarbose as positive control. S10, S20, S40, S60 and S80 were used to establish a standard curve.

First of all, ORAC can mimic the real biological environment better than other methods. Although anti-oxidant phytochemicals can eliminate free radicals, it depends on the type of radicals. Superoxide radical, hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide, peroxynitrite and hypochlorous acid are all reactive oxygen species generated in human body, while only peroxyl radical from lipid peroxidation is the most common one in human biology (Wu et al., 2004b). Given that, a peroxyl free radical, called AAPH, is used in ORAC method, so it is sometimes considered superior to other antioxidant capacity assays using other radicals that are not likely to appear in biological systems.

Secondly, the ORAC method is more appropriate among those assays because of phenolic compound antioxidant reaction mechanism of chain-breaking. Antioxidants scavenge free radicals by different mechanisms, such as blocking oxidation chain reaction to suppress radical accumulation, chelating metals to inhibit catalyzed radical formation, competing for oxidative agents as reductants, and stimulating enzymatic oxidative defenses (Yu et al., 2002). Phenolic antioxidants can form stable phenoxyl compound with peroxyl radicals, and the reaction rate is much faster than that between peroxyl radicals and their substrates, thus they can protect the substrates from oxidation and prevent the initiation of chain reactions. Based on its role in the reaction, polyphenols are categorized into chain-breaking

antioxidants, to which the employment of ORAC is more chemically relevant and specific (Ou et al., 2002).

Thirdly, considered the other phytonutrients from rosehip, the advantage of ORAC assay over other methods is that it might avoid interference. In a recent article, ascorbic acid gave a higher value using the Trolox equivalent antioxidant capacity (TEAC) method compared with ORAC assay when different reference substances were evaluated. It could be interpreted as TEAC method is more sensitive to the presence of ascorbic acid which possibly leads to biased results. Although DPPH assay is also widely used in assessing antioxidant capacity, the spectra of carotenoids from rosehip overlaps the 515 nm wavelength measured in DPPH assay, clearly, the interference is not neglectable.

Instead of investigating different antioxidants individually, the total antioxidant capacity of raw rosehip powders was evaluated in this study. The food matrix is such a complex system with several factors that may affect results and it was not within the scope of this work to identify the potential active inhibitory components. In rosehips, the presence of pectic substances (**Figure 6, the cracks**), free sugars, organic acids and other antioxidants such as vitamin C, carotenoids and lycopenes, may affect the final results. According to Gao's research (Gao et al., 2000), phenolics are dominant phytonutrients in rosehip with much smaller amount of ascorbate and trace amount of carotenoids. Measurement of antioxidant capacity using ORAC assay have shown a correlation between the content of anthocyanin rather than the ascorbic content of small fruits (Kalt et al., 1999). In addition, the carotenoids have been reported to be good quenchers of peroxyl radicals as used in ORAC method (Prior et al., 2005) indicating that phenolic compounds seems to be mainly responsible for detected high antioxidant capacity as measured by the ORAC method.

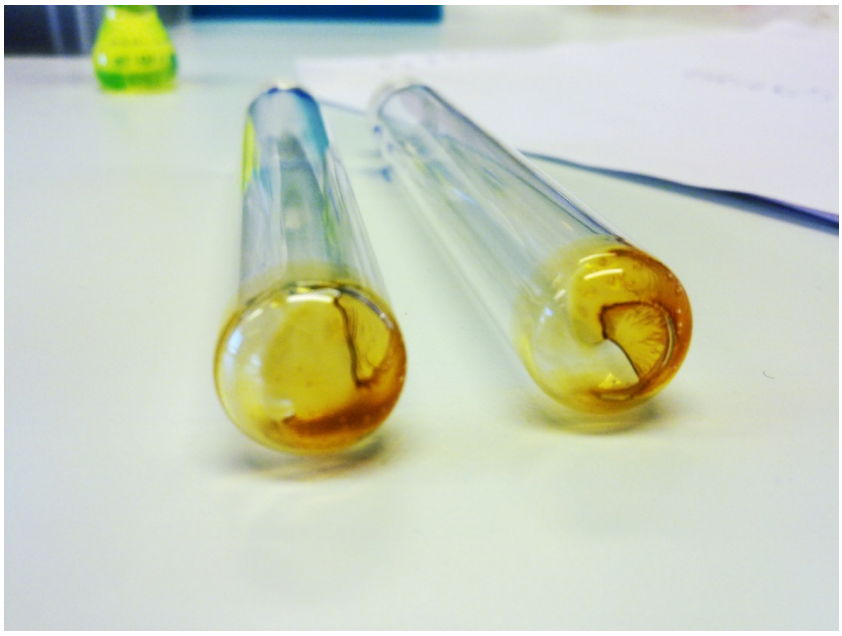


Figure 6 Picture of concentrated rosehip aqueous extracts in test tubes. The cracks observed after moisture evaporation indicated the broken parts because of gel formation.

2.7 α-Amylase inhibition and α-glucosidase inhibition assays

The number of patients with chronic metabolic disorders, especially type 2 diabetes mellitus has been growing fast in recent years and seems to continue if no proper actions are taken in time. Frequently-increased postprandial glucose level can lead to deterioration of retinal, renal, neurological and cardiovascular function, which impairing human health in the long term.

Starchy food firstly is chewed and mixed with salivary enzymes in oral cavity and then reaches to the intestine as smaller oligomers. Then α-amylase secreted by pancreas cleaves the oligomers into oligosaccharides which pass through lumen later. During the bowel movement, these oligosaccharides will be further degraded into glucose by α-glucosidases and the mucosa absorbs glucose and delivers them into blood circulation eventually. When these two enzymes activities are constantly high, the level of blood glucose will be increased rapidly after meal, which triggers the development of type 2 diabetes mellitus.

Though enzymatic inhibitors, such as acarbose (under brand name, Glucobay, Precose and Prandase) miglitol and voglibose are already available in pharmacy, they are accompanied with unpleasant side effects from undesirable fermentation of undigested starch in the colon. Accordingly, less inhibition on α-amylase and more inhibition on α-glucosidase are preferred, because it can allow large carbohydrates to be cleaved and absorbed but prevent possible side effects at the same time.

One potential approach for decreasing blood glucose response after food intake is by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase. Polyphenol-rich extracts from different resources have been claimed having the capability to inhibit both α-amylase and α-glucosidase (Iwai et al., 2006, Loizzo et al., 2007, Apostolidis et al., 2006), and several active components have been identified and linked to the inhibitory ability. Normally the overall phenolic content is not well correlated to the inhibiting effects well (Grussu et al., 2011, McDougall et al., 2005). The conclusion from some published results, tannins and ellagic acid derivatives acted as effective α-amylase inhibitors while anthocyanins has more inhibitory effect on α-glucosidase. Based on the result from additional studies, rosehip had the highest polyphenol content as well as antioxidant capacity among other soft fruits (**Table 2**). Thus the potential inhibitory effect on these enzymes by rosehip extracts was evaluated by *in vitro* assays in present study.

Table 2 Total phenolic contents and antioxidant capacity of selected fruits from additional study

Sample	Total phenolic content (GAE mg/g rosehip)	ORAC (TE µmol/g rosehip)
Lingonberry	29.3±1.5	455.0±30.8
Strawberry	31.7±0.5	245.2±52.0
Bilberry	39.2±1.7	664.6±174.3
Apple	5.2±0.1	1192.5±173.5
Rosehip	86.7±21.1	2997.9±307.8

Note: the extraction solvent used was 70% methanol, 30% milli-Q water and 1% TFA.

The ability of the rosehip extracts to inhibit α-amylase activity was performed and screened in 96-well plates. The increase in absorbance indicated a higher product formation and a decrease represented the inhibition of enzymatic activity. The absorbance was monitored as a function of time to provide a curve for the reaction.

2.8 Additional study

Total phenolic compounds contents and antioxidant capacity were tested in a number of fruits and cereals and extruded cereal product prototypes. Comparison was made to investigate the possible synergistic effects of the combinations. It was an innovative approach to improve nutritional values in food products by taking advantage of ingredients characteristics through optimal combination.

3. Materials and Methods

3.1 Raw materials and chemicals

Rosehip along with other fruits samples and cereal samples were kindly provided by Lantmännen (Sweden). Fruit samples and extruded cereals were sent to laboratory intact, thus they were grounded into powder by a coffee mixer. Rosehip samples were delivered as powder. All the samples were kept in freezer at -20°C.

α-Amylase (EC 3.2.1.1) and α-glucosidase (EC 3.2.1.20) Were purchased from Sigma Chemical Co. (St. Louis, MO). Gallic acid and fluorescein (3', 6'-dihydroxyspiro [isobenzofuran-1[3H], 9' [9H]-xanthen]-3-one) were bought from FlukaChemika (Switzerland). p-Nitrophenyl-α-D-maltopentaoside (PNPG5) was purchased from Sigma Chemical Co. Unless specially noted, all chemicals were purchased from Sigma Chemical. Co. (St. Louis, MO).

3.2 Aqueous and methanol extraction

Approximately 0.5 g sample was dissolved in either 5 ml water or 70% methanol with 1% TFA for extraction. All test tubes were vortexed for 15 sec and ultrasonicated for 5 min before putting into a shaking water bath at 37°C for 30 min. The tubes were centrifuged afterwards and the supernatants were transferred into new tubes respectively. The procedure was repeated, and the collected extracts were kept in fridge until analysis.

3.3 Total phenolics content

Both aqueous and methanol extracts of test samples were measured using the method described below. Briefly, 500 µl of both extracts, blank (milli-Q water) and standard solutions were pipetted directly into Eppendorf vials, and then 250 µl of 20% Folin-Ciocalteau reagent and 250 µl saturated Na_2CO_3 were added into each vial with a time interval of 1 min in between. 200 µl of the supernatant from vials centrifuged at a speed of 15000 g for 5 min were collected for plate reading. The wavelength used for measurement was 765 nm. A standard curve was made of gallic acid in 10% methanol in concentrations from 0.01 to 0.1 mg/ml. The results of total phenolics were presented as µg equivalents of gallic acid per gram of fresh weight of tested samples. In addition, different concentrations of sodium bicarbonate solutions were prepared to test the effect of varying concentration on the analysis of content.

3.4 ORAC assay for antioxidants

The ORAC assay was slightly modified based on a previous report (Davalos et al., 2004). Extracts and chemicals were diluted or dissolved in 75 mM phosphate buffer (pH 7.4) unless specified. AAPH (40 mM), fluorescein (1.17 mM) and Trolox (500 µM) were selected as peroxyl radical generator, fluorescein probe and standard respectively. 20 µl properly diluted sample extracts, blank (phosphate buffer) and standards were added to a 96-well plate, followed by addition of 120 µl 1.17 mM fluorescein. Blank samples were prepared by replacing extracts to phosphate buffer and a calibration curve was proposed by a series of Trolox solutions from 10-80 µM. The microplate was then incubated at 37°C for 15 min and then 60 µl 40 mM AAPH solutions were added to each vial by a multi-pipette and the measurement was started immediately. Fluorescence intensity was measured every 30 sec for 90 min at 37 °C. The final results are expressed as micromoles of Trolox equivalents (TE) per gram tested sample (µmol/g).

3.5 α-Amylase inhibition assay

Porcine pancreatic α-amylase (EC 3.2.1.1) was purchased from Sigma Chemical Co. 500 µl α-amylase solutions (0.5 mg/ml) were mixed with 500 µl sample extracts in test tubes, and then incubated the mixture at 37°C for 10 min. After pre-incubation, 500 µl starch solutions (1%) were added to each tube at an interval of 15 sec followed by exact 10 min incubation at 37°C. The enzymatic reaction was stopped by adding 1 ml dinitrosalicylic acid color reagent and the tubes were put into boiling water bath for 5 min. They were moved out and cooled down to room temperature before diluting with 10 ml milli-Q water. Pipetted 200 µl solution for plate reading and the absorbance was measure at 540 nm. The percentages of inhibitory effect were calculated using the equation below:

$$Inhibition = \frac{A_{540}^{blank} - A_{540}^{extract}}{A_{540}^{blank}} \times 100\%$$

3.6 Kinetic assay of α-amylase inhibition

All chemicals were dissolved in 50 mM HEPES (pH 7.1). The kinetics assay was performed as previously described (Funke and Melzig, 2006) with several modifications. Briefly, 50 µl 25 mM substrate solutions (PNPG5) in buffer were added to the wells in a 96-well plate. Rosehip extracts ranging from 10 µl to 100 µl were pipetted into the plate and HEPES was used to fill the total volume to 150 µl of corresponding wells. The reaction was started by rapidly adding 50 µl porcine pancreatic amylase solutions by a multi-pipette gun. The measurement was set as recording absorbance at 405 nm every 3 minutes at 37°C for 90 min in total. Blank was prepared by using HEPES instead of rosehip extracts, and acarbose was used as positive control.

3.7 α-Glucosidase inhibition assay

α-Glucosidase (EC 3.2.1.20) was purchased from Sigma Chemical Co. 50 µl rosehip aqueous extracts Were mixed with 100 µl glucosidase solution (1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) and incubated in a 96-well plate at 37°C for 10 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solutions in 0.1 M phosphate buffer (pH 6.9) were added into the wells by a multi-pipette, and the measurement was started immediately by a plate-reader at 37°C. The absorbance at 405 nm was recorded at the very beginning and every 5 min after for 30 min in total. Blank was prepared using phosphate buffer instead of rosehip extract, and acrabose was used as a positive control. The kinetics of α-glucosidase inhibition was displayed by plotting measured absorbance against elapsed time, and the inhibitory effects of first 5 min were calculated as follows:

$$Inhibition = \frac{\Delta A_{405}^{blank} - \Delta A_{405}^{extract}}{\Delta A_{405}^{blank}} \times 100\%$$

4. Results and discussion

4.1 Optimization

The addition of 1% TFA played a significant role in the retention of phenolic acids in this study. Phenolic compounds extracted with 1% TFA were higher than in samples without TFA. Removal of TFA from the extraction solution resulted in a 34% decrease in phenolic content of the samples. Barnes studied various acids for optimal extraction, and found the solvent with TFA was the best for blueberry anthocyanin extraction (Barnes et al., 2009). TFA is a strong carboxylic acid, and it is used as an ion paring agent with additional merits in improving extraction efficiency and chromatograph resolution. Its strong negative charges comes from the three fluorine atoms (**Figure 7**), thereby it can attract cations. Consequently, TFA can deplete transition metal ions because of a strong affinity for them, thus more phenols can be free from phenol-mineral complex formation. In another test, Light exposure during preparation of the samples was not found to affect the analytical results.

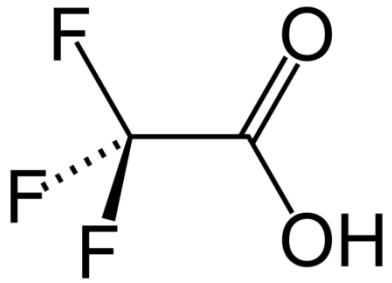


Figure 7. The molecular structure of trifluoroacetic acid (TFA)

Sodium bicarbonate solutions in different concentrations were tested individually and no significant alterations in total phenol content was found between them. The pH values of these solutions were recorded and compared, and there was no sign that the pH value would be changed considerably by increasing the concentration (**Table 3**). The slight variations in pH were probably caused by the treatments used to assist dissolution, such as mild heating and vigorous agitation, which involved more absorption of carbon dioxide from the gas phase. The obtained total phenolic contents from separate Folin-Ciocalteau test didn't show a significant variation when different Na_2CO_3 solutions were used. Our finding is consistent with previous studies by Box who reported that optimum pH for absorbance was found from each alkali tested and that rapid decreases in absorbance were noticed after exceeding an optimum pH (Box, 1983). The varied concentration of Na_2CO_3 solution had neglectable impacts on the standard curve created (**Appendix 1**). The obtained standard curve was found to become steeper with increased concentration. The slope of the standard curve affects the GAE value depending on the horizontal distance **(Figure 8)**. If the distance of two points on y-axis was fixed, the difference reflected on the x-axis from the gradual one will be larger than that from the steeper one. Thus the standard curve with a gradual slope will be more sensitive to the changes in absorbance than a steeper one, and gives higher values of GAE at the same time.

Table 3 pH values of sodium bicarbonate solution in different concentrations

Sodium bicarbonate conc. (%)	pH value
2.5	11.5±0.1
5	11.5±0.1
7.5	11.6±0.1
10	11.6±0.1
15	11.6±0.1
20	11.6±0.1
25	11.5±0.1
30	11.5

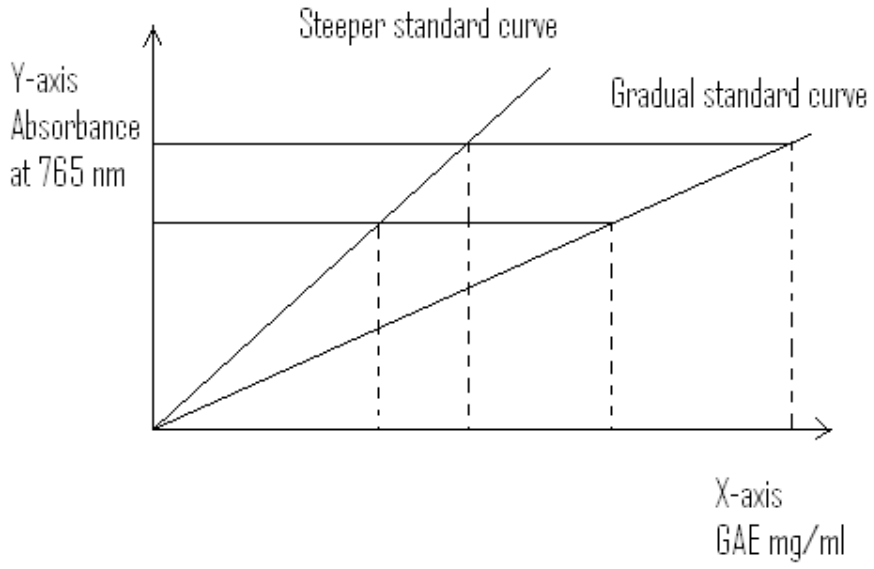


Figure 8. The difference in GAE detection between a steeper standard curve and a gradual one

Though it was attainable to get a high concentration of 30% through heating and stirring, automatic crystallizations were found in all sodium bicarbonate solutions with concentration above 15% after short storage (**Figure 9**). So there's actually no need to emphasize on the concentration to be used, as long as a defined alkaline environment was provided by this solution, which was proven to be able to enhance the stability of polyphenols. To avoid deviation between repeated tests, room-temperature saturated sodium bicarbonate solutions were prepared and used throughout this study.

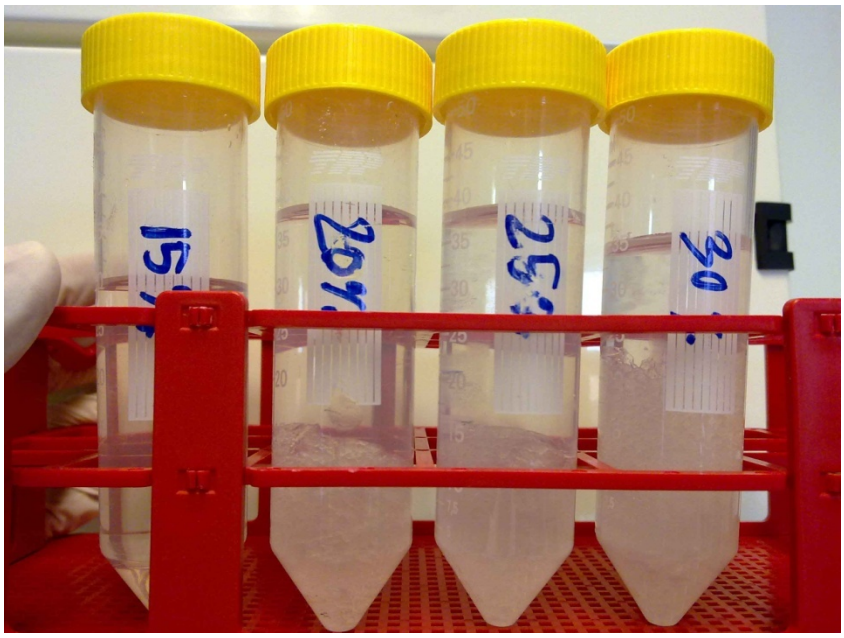


Figure 9. The natural crystallization of sodium bicarbonate solution with concentrations above 15%

4.2 Quantification of phenolic compounds and antioxidant capacity

The total phenolic content in aqueous and methanol extracts of rosehip was determined by Folin-Ciocalteau method. The total phenolic content was found to be 9 % higher in aqueous extracts (87.7 mg/g) than in methanol extract (80.4 mg/g) and seemed to be correlated with their respective ORAC values, showing a difference of 15 % (**Table 4**). Acarbose had negative ORAC values, indicating it is a prooxidant or at least without antioxidant effect.

The results are consistent with previous studies. Gao and his colleagues tested the phenolics in 18 ethanol-extracted rosehip samples and reported values ranging from 59.21 mg/g to 122.39 mg/g (Gao et al., 2000). Their samples were collected from different locations in Chile and Sweden, which proved the notion that chemical composition of a certain genus can be easily altered by external agricultural factors. Moreover, not only the phenolic compound varies greatly but also the other food constituents can result in an overestimated value. For example, in the report by Box, a wide range of aromatic, aliphatic organic and reducing inorganic compounds were speculated to result in overestimation of total phenolic content (Box, 1983).

Table 4 Total phenolic contents and antioxidant capacity of rosehips

	Extraction solvent	Total phenolic content (GAE mg/g rosehip)	ORAC (TE µmol/g rosehip)
Rosehip	100% milli-Q water	87.7±7.6	3416.8±424.2
	70% methanol 30% milli-Q water 1% TFA	80.4±5.5	2974.7±577.0

Note: The results are presented as means ± standard deviation of repeated experiments. GAE stands for gallic acid equivalent, and TE stands for Trolox equivalent.

Relative high standard deviations were found in ORAC values in the present study. It is described as an intrinsic characteristic of this method based on repeated experiments. Indeed, some improvements can be done during the preparation to minimize the high variation. Firstly, the tip of pipette need to be cleaned after transfer of diluted extracts, since only one extra drop of liquid will lead to a large difference in the end, especially after several times of dilution. Secondly, dissolution of AAPH in 37°C phosphate buffer prior to adding to the wells has been proposed to reduce the intra-assay variability effectively (Prior et al., 2003). However, the temperature difference between the outer and the inner part of the 96-wells microplate is inevitable, which reduces the reproducibility to some extent of this temperature-sensitive assay.

4.3 Inhibition on α-amylase and α-glucosidase

Aqueous rosehip extracts were assayed along with gallic acid and acarbose as positive control at 50, 100, 200, 400, 1000, 2000 and 4000 GAE μg (**Figure 10**). Rosehip amylase inhibition activity was well correlated with the amount tested, and displayed a linear correlation (R^2=0.9703). However, the amount of rosehip extracts needed to inhibit enzyme activity completely was two-fold of gallic acid used. A linear relationship for inhibition by gallic acid was only found from 1000 μg to 2000 μg, to which exerted 95% inhibition on α-amylase activity. Hypothetically, the decline in inhibition of gallic acid from 100 μg to 1000 μg proved it was a kind of competitive enzyme inhibitor. Therefore, gallic acid and the substrate compete for the active site of α-amylase, and the overall inhibitory effect was the result of a combination of dynamics between the concentrations of them and their affinity for the enzymes. Reversible inhibition caused the fluctuation in inhibition as displayed in the figure. Acarbose, known to be a strong inhibitor, suppressed 90% enzymatic activities even only the lowest amount of 50 μg was used (Nickavar et al., 2008). The inhibition mechanism is largely relying on its molecular structure (**Figure 11**), where its planar configuration simulates the flat pyran ring during starch degradation, and protonated nitrogen attracts carboxyl groups electrostatically (Brayer et al., 2000).

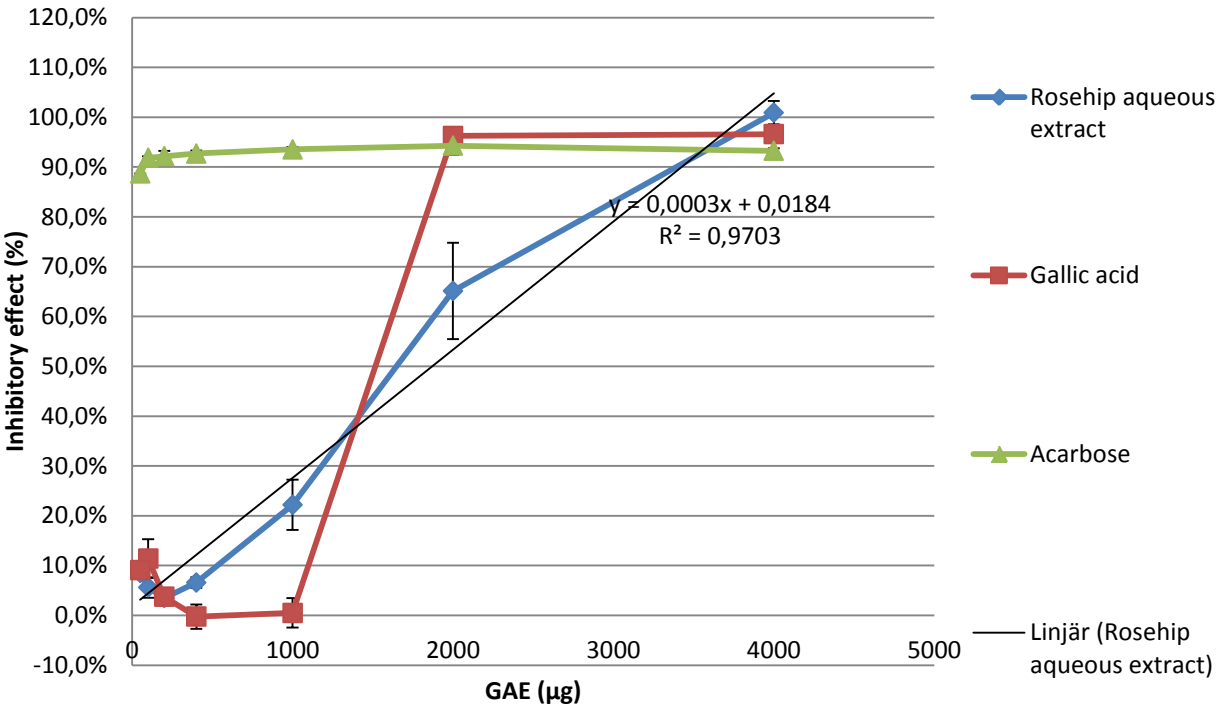


Figure 10. Inhibition on α-amylase in percentage by rosehip aqueous extracts, gallic acid and acarbose by comparison with blank. The curves were obtained by testing 50, 100, 200, 400, 1000, and 2000 and 4000 µg phenolic or equivalent. The results were presented as the means ± standard deviation from repeated experiments

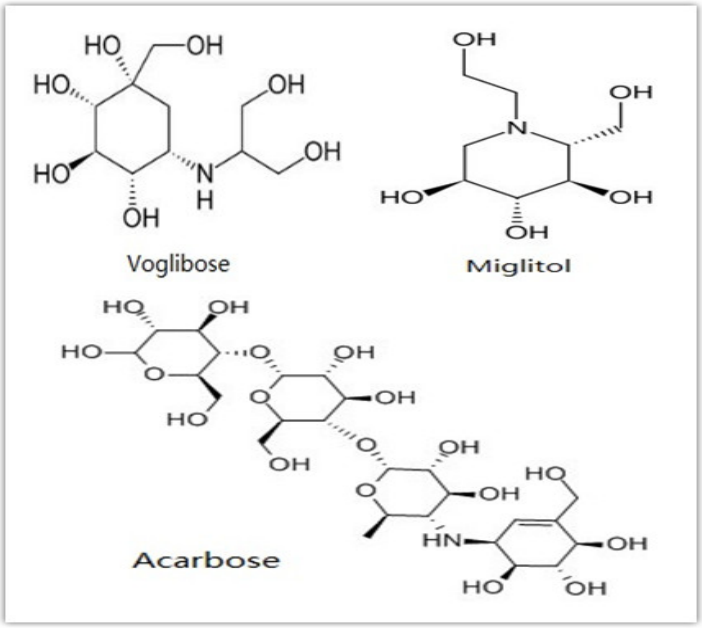


Figure 11. Chemical structures of marketed medicines targeting for type 2 diabetes mellitus management

α-Amylase inhibition kinetics was tested as well (**Appendix 2**), though it wasn't comparable with the results from direct inhibition assay for different substrates being applied. Starch was used in inhibition assay as substrate to study how much maltose was cleaved off from oligosaccharides, while kinetics utilized PNPG5 as substrate and different wavelength to detect newly-formed products. Acarbose was once again proven to be the strongest inhibitor that remaining at the bottom of the line representing absorbance, which meant nearly no products were formed from enzymatic reaction. Fluctuation in absorbance was found for gallic acid and it showed the same trend as in the inhibition assay. The rapid increase in absorbance with rosehip extracts at the lowest concentration suggested that the inhibiting effects of α-amylase would be limited at low intake. Conversely, some type of berries can completely inhibit amylase activity at lower amount as previously reported (McDougall et al., 2005). It's noteworthy that the total phenolic content in rosehips are two-fold higher than strawberry and lingonberry (our previous data), while they can only exert 100% inhibition at the amount of 1000 mg. This phenomenon gave a message that the phenol types are rather different between each other and only some particular phenols are responsible for inhibition instead all of them. In a review (McDougall et al., 2008a), tea ellagitannins, gallotannins and proanthocyanin were proposed having inhibitory effect on α-amylase. Cyanidin-3-glucoside and delphinidin-3-glucoside were studied in animal models, and they have shown as the most effective secretagogues among anthocyanins and anthocyanidins in cell insulin secretion tests (Jayaprakasam et al., 2005). Thus, further identification is needed to find out the particular phenolic acids directly involved in the inhibition.

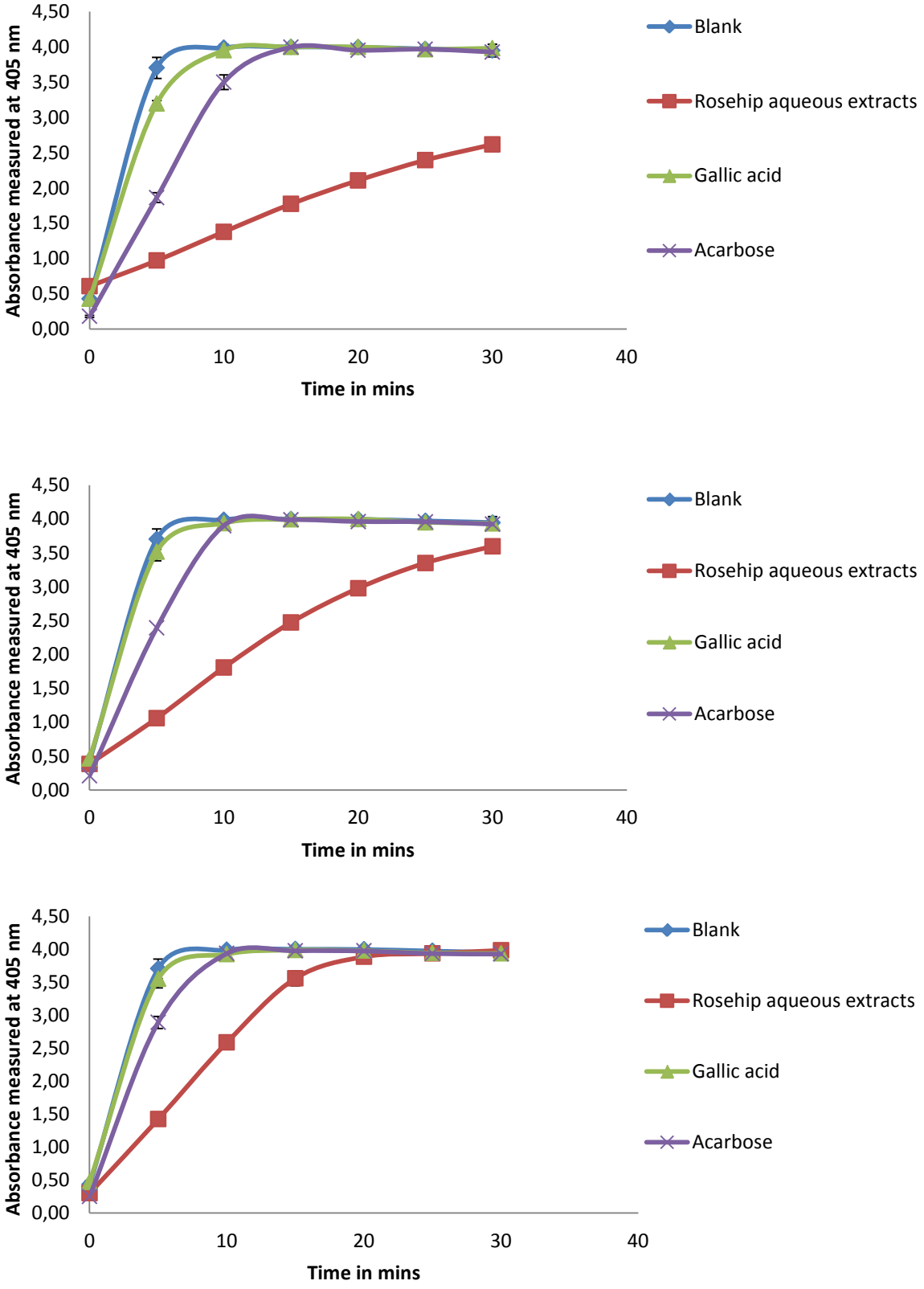


Figure 12. New product formation rates of were displayed with the presence of rosehip aqueous extracts, gallic acid and acarbose by comparison with blank. The assays were carried out using 200 µg (A), 100 µg (B) and 50 µg (C) phenolic or equivalent. The results were presented as the means ± standard deviation from repeated experiments

Aqueous rosehip extract inhibited α-glucosidase much more effective than acarbose and gallic acid. The value of absorbance measured indicated the accumulation of products formed from enzymatic reactions, and thus the slope of its curve reflected the rate of product formation. Gallic acid had very poor inhibitory effect on α-glucosidase, where the new products were formed at a similar rate to that of blank. Acarbose showed a dose-dependent effect as retarded product formation when 200 µg was tested. The inhibition caused by aqueous rosehip extract was roughly three-fold in effectiveness compared with acarbose according to kinetics graphs (**Figure 12**), and showed a clear dose-dependent pattern when different amounts were tested.

Rosehip methanol extracts were also tested (**Figure 13**) to compare with the inhibition efficacy gained from aqueous extracts. Higher inhibitions were found in methanol extracts, which can be easily interpreted as lipophilic compounds in rosehip were more effective in α-glucosidase inhibition than hydrophilic constituents. However, it could be a misleading conclusion, because organic solvents were believed to denature enzymes by damaging their hydration sphere (van Erp et al., 1991), which added up to the perceived inhibition. However, Klibanov studied the catalytic activities of different lipases in almost anhydrous organic environments (Zaks and Klibanov, 1985), and claimed some chemical reactions were found to be more compatible with non-aqueous solvents. Thus, the pure interaction between phytochemicals and extractions solvents should be taken into consideration before deciding which is more preferable in a larger scale extraction.

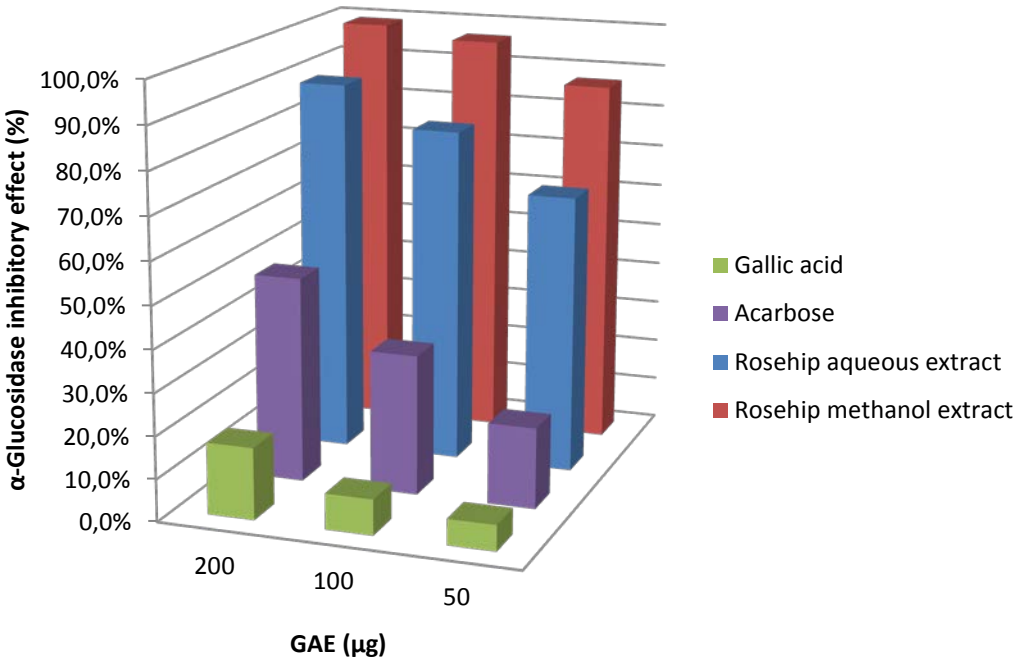


Figure 13. The comparison between inhibitory effects caused by gallic acid, acarbose and rosehip aqueous and methanol extracts. The assays were carried out using 200 µg, 100 µg and 50 µg GAE

5. Design of polyphenol-rich rosehip products

5.1 Safety issue

Safety is the priority of any food production. Rosehip in powders have been found responsible for the respiratory symptoms reported in workers handling them, thus it has been deemed as a new occupational allergen that can give rise to asthma (Kwaselow et al., 1990). But other than that, no toxicological studies have been directly linked to rosehip's chemical composition. Moreover, several toxicology studies discussing about phenolic compounds can provide us with some insight on this issue.

Yamakoshi and co-workers performed a serious of safety tests on microorganisms, animal models and human cell lines. Acute oral toxicity, reverse mutation, chromosomal aberration and micronucleus test were included in final evaluation, and the results supported the safety of foods made from grape seeds, which were rich in proanthocyanidins (Yamakoshi et al., 2002). Glei's research team studied catechin and anthocyanin-enriched functional food's toxicity on membrane integrity, proliferation inhibition, genetic damage and resistance to hydrogen peroxide by human cell lines. He concluded it was necessary to study both the complex extracts and isolated ingredients, which was critical to make a non-biased and convincing conclusion (Glei et al., 2003).

Controversial results are found as well. Rosin claimed some plant phenolics could induce mitotic conversion in *Saccharomyces cerevisiae* under alkaline condition (Rosin, 1984), and the explanation was that the autooxidation of phenolics could generate hydrogen peroxides and free radicals. In a later study, Nakayama found polyphenols could protect mammalian cells from the cytotoxicity as a result of hydrogen peroxides presence (Nakayama et al., 1992). But at the same time, he claimed phenolic acids themselves might not have protective ability while they could acquire the ability after chemical changes, for instance, esterification (Nakayama et al., 1992).

Precautious action must be taken in large-scale nutraceuticals or functional foods production. For example, ethanol is preferred in industry as solvent in most cases regarding food safety (Castañeda-Ovando et al., 2009), despite it has lower efficacy than methanol in most conditions (Albu et al., 2004). Rosehip aqueous extracts in this study were found to have higher amount of phenolics than methanol extracts as reported in herbs (Kwon et al., 2006), so it implies organic solvents are not always necessary in extraction of bioactive compounds.

5.2 Processing parameters

Thermal treatment is crucial to food production, and it is typically used to kill harmful microorganisms in raw material and to accelerate the rate of other processing steps like extrusion. It is known that both anthocyanins and β-carotene are not stable under thermal treatments and tend to degrade to some extent. Some food constituents such as lycopene

are suggested to be relatively thermal-stable (Svelander et al., 2010). However, long time heating is likely to cause degradation of folates and ascorbic acids in rosehip as previously reported (Stralsjo et al., 2003). It seems that thermal processing is detrimental to the nutrients of food products, while at the same time, thermal processing could help to enhance their nutritional values.

Mechanical and thermal treatment help to release more antioxidants by disintegrating cell structures (Dewanto et al., 2002) and hydrolyzing proteins. Hydrolysable tannins in pomegranate juice were reported to contribute to a higher antioxidant activity after thermal processing (Gil et al., 2000), and heat treatment was proposed to be used as a tool to increase antioxidant activity in certain food products (Jeong et al., 2004). Wang and co-workers found that some commercial juices had higher ORAC value than their fresh counterparts, while some were not (Wang et al., 1996). Additionally, Wu pointed out those different behaviors to processing were decided by various chemical structures of antioxidants (Wu et al., 2004a). Heat treatment can also inactivate certain inherent enzymes in fruits. For example, the denaturation of polyphenol oxidase can prevent polyphenols being oxidized to avoid food browning, which is closely related to food quality, nutritional value as well as consumer acceptability. A more detailed discussion on phenolic compounds and involved enzymes is prepared by Tomás-Barberán and Espin (Tomás-Barberán and Espín, 2001).

Storage stability was also tested by keeping rosehip aqueous extracts in fridge for five days with no additives and preservatives. The phenolic content was assayed every day during the period and the result showed no significant changes between each day (**Appendix 3**). This might imply a good stability of rosehip aqueous extract during fridge storage; however, long-term studies and other analytical methods are needed to catch the minor chemical changes that cannot be perceived by single method.

5.3 Sensory applications

Polyphenols are not only ascribed to the natural color of fruits and flowers but also are responsible for tastes, astringency and tartness in particular. Some innovative practices have been done via making breakfast cereals with anthocyanins from blueberries and grapes as natural colorants (Camire et al., 2002). Besides, rosehip extracts also have a pleasant fragrance which gives it wider usage as flavoring agents or exfoliants in soaps and other cosmetics, such as body butter and body lotion. It could be related to the presence of various volatile organic acids. In summary, rosehip is quite promising to be incorporated in food products as colorant and flavoring agents.

However, several improvements are needed before launching such kind of new product. Extrusion reduces the amount of anthocyanins, so relevant variables in industrial processing should be strictly-controlled to minimize the decrease in taste. Trace transit metals from

screw extruder intensify the darkness of the products possibly by anthocyanin-metal complexation and browning reactions, so quality inspectors should pay more attention to unusual color changes. More sugar should be added to mask the sense of tartness because normally sweetened foods are more preferred by the majority population.

Aqueous extracts of rosehip was viscous and tended to adhere to the wall of test tubes. ultrasonication was used to solve the problem in this study, but obviously, in large-scale production, more vigorous mechanical forces should be applied and a right dilution rate should be carefully-selected that can greatly reduce the viscosity.

5.4 Interactions with other nutrients

Rosehip contains various nutrients, so the interactions between them and possible synergistic effects should be scrutinized by both devoted researchers and functional food designers.

Phenolic compounds can interact with each other in either a synergistic or antagonistic way (Freeman et al., 2010), and their stability can also be greatly affected by other food constituents and food additives. Glucose, sucrose, fructose and artificial sweetener aspartame have been reported to assist black currant anthocyanin degradation (Rubinskiene et al., 2005). As the largest group of nutrients in rosehips, ascorbic acid doesn't change antioxidant capacity of phenolic compounds (Murakami et al., 2003), while it significantly accelerates anthocyanin degradation (Chaovanalikit et al., 2003) but provide a protection for folate degradation at the same time (Stralsjo et al., 2003). With more and more interests in interaction between various food ingredients, evaluation methods were developed to facilitate further research (Parker et al., 2010).

Given the health benefits of polyphenols, several prototypes of potential functional food products aiming to prevent disease have been developed. For example, rosehip drink with fermented oats was reported to increase stool volume and decrease flatulence, which was also partly linked to the addition of dietary fibers (Johansson et al., 1998). In a more recent study, phenol-enriched cheese was proposed to have ability to inhibit key enzymes related to type 2 diabetes and hypertension (Apostolidis et al., 2007). In our additional study, positive synergistic effects (retention above 100%) in both total phenolic content and antioxidant capacity were observed in several extruded products combining cereals and fruits (**Appendix 4**). It is a good attempt to product healthier food products by elegant selection and combination.

6. Conclusion

The major discovery that can be summarized from this thesis work is that rosehip extracts have mild inhibition of α-amylase and strong suppression on α-glucosidase activity. This is proposed to be somehow correlated to their high content of phenolic compounds and high antioxidant capacity.

Acarbose, the marketed drug, was analyzed as positive control and found to have strong inhibitory effects on α-amylase but not α-glucosidase, which is in accordance with side effects reported before. The standard phenol gallic acid has mediate control over these two key enzymes. Rosehip extracts are superior to both acarbose and gallic acid; the lower inhibition of α-amylase can allow the degradation of starch into smaller carbohydrates passing through gastrointestinal tract without undesirable microbial fermentation, and higher repression on α-glucosidase activity stabilizes blood sugar level by preventing rapid glucose release to bloodstream. As a result, the sudden surge of glucose level in blood stream after meal will be alleviated with little discomforts by consuming rosehip-containing products made from it. Literature reviewing also supports the possibility to launch such kinds of functional products with few controversial issues.

Isolation and identification of rosehip extracts components affecting α-amylase and α-glucosidase activities are suggested for further studies.

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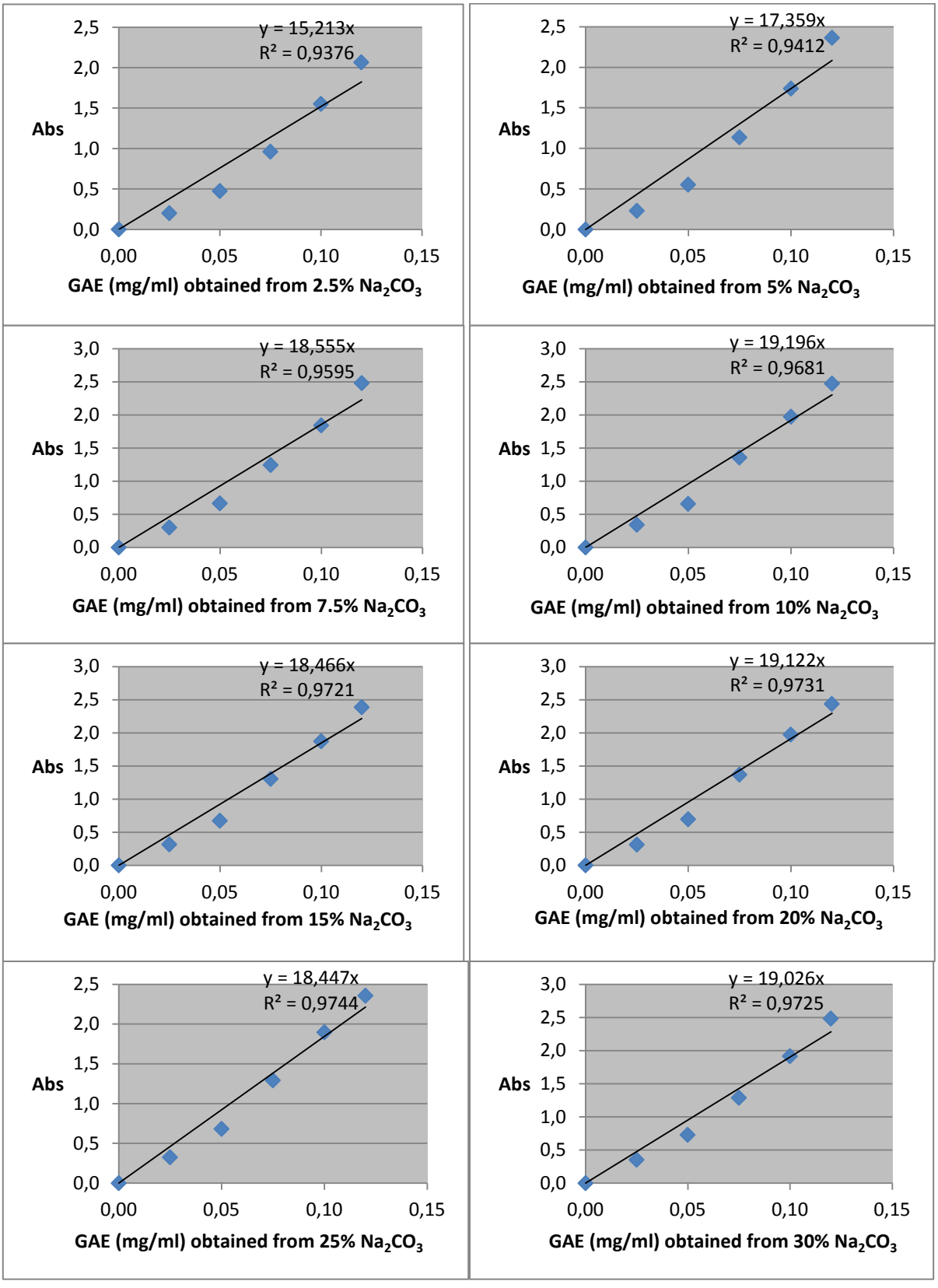
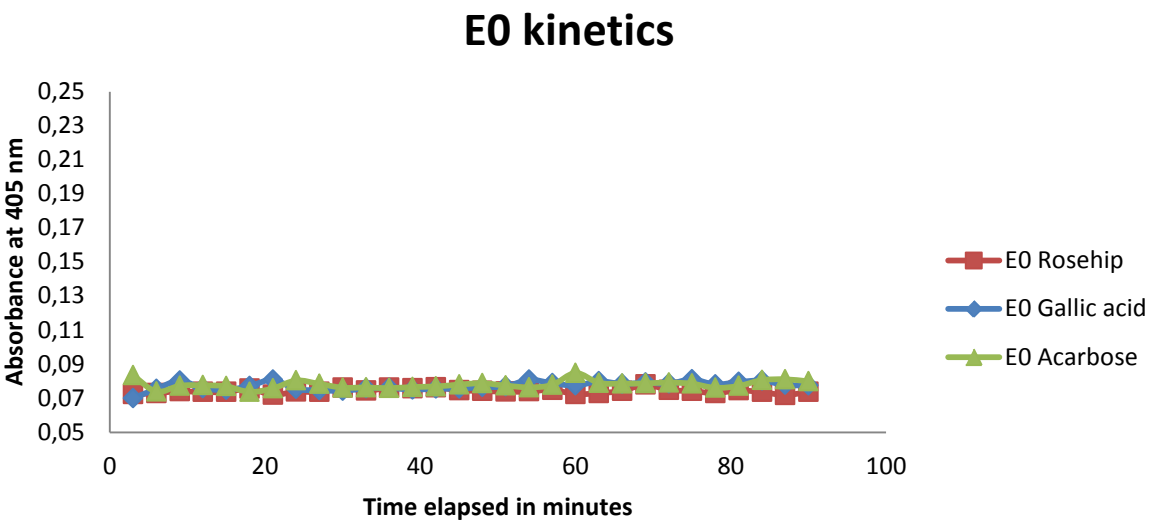
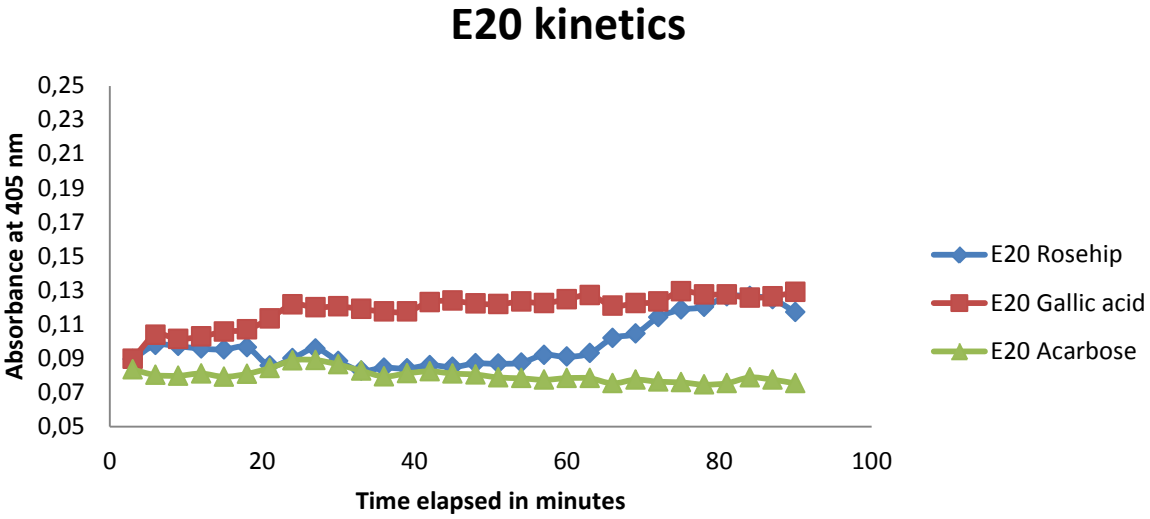
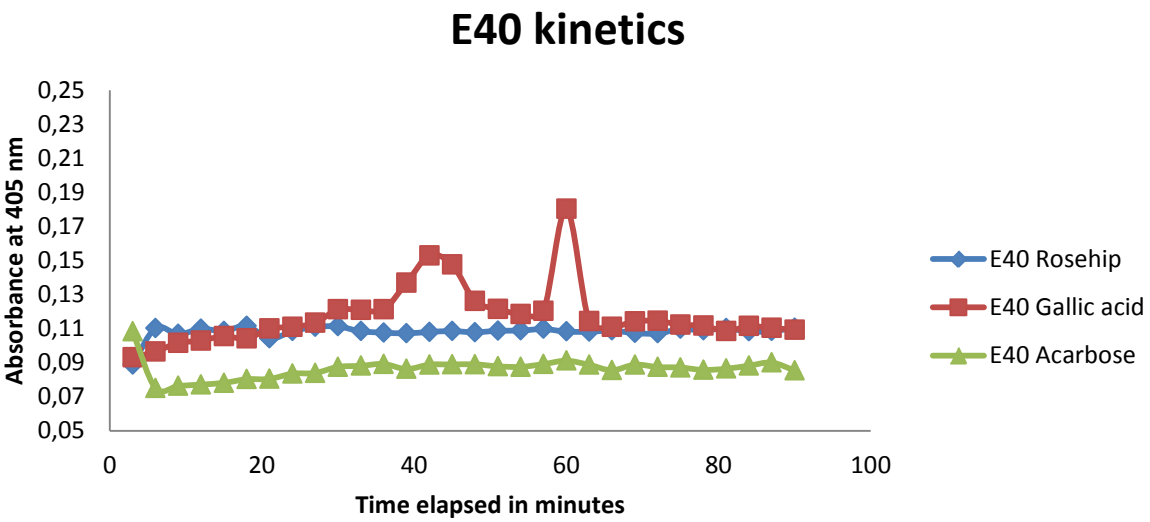
Appendix. 1 Effects of changing the concentration of Na_2CO_3 solution in Folin-Ciocalteau method on total phenolic content

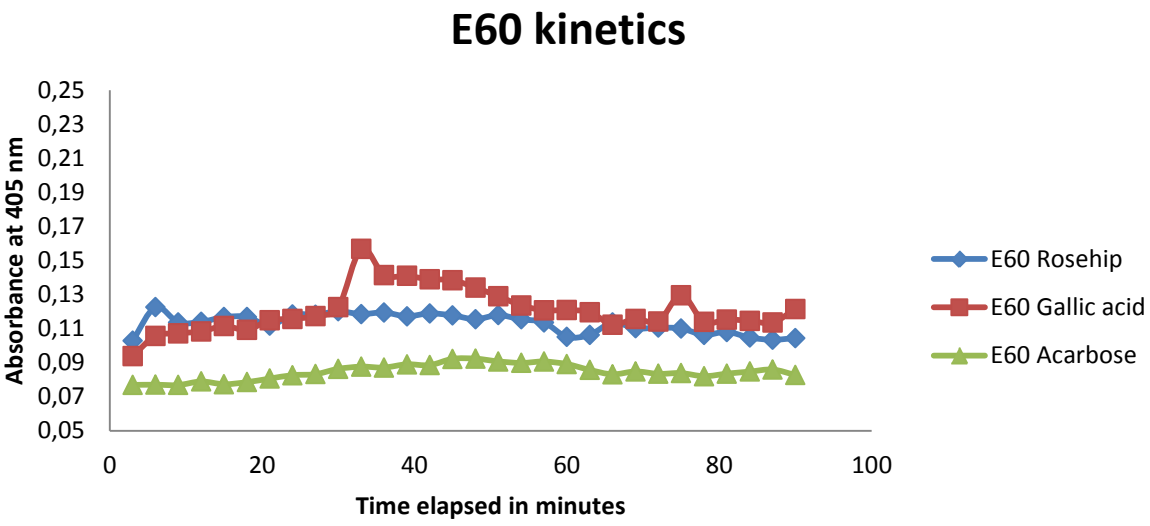


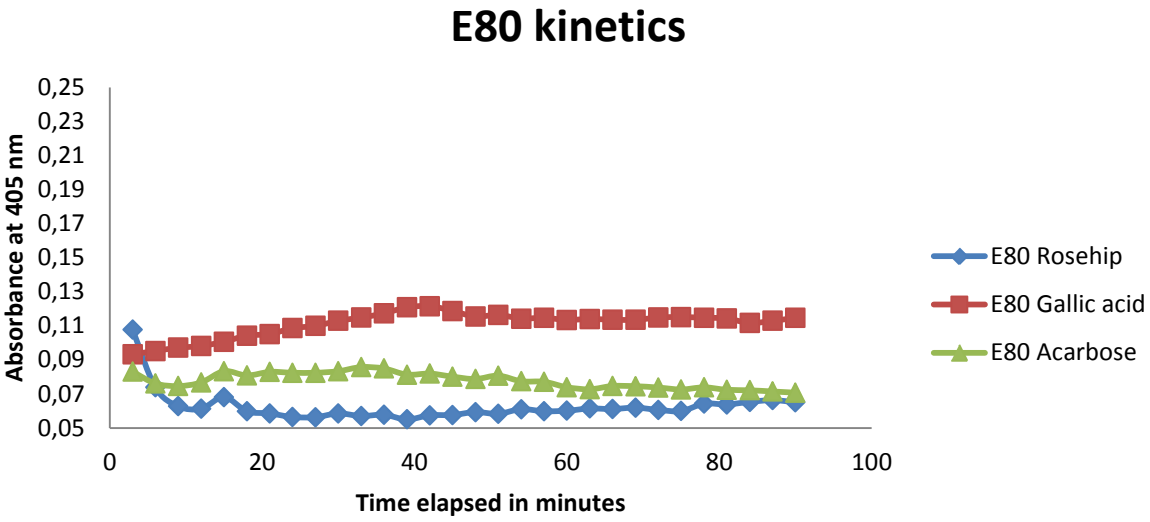
Appendix. 2 The α-amylase inhibition kinetic assay

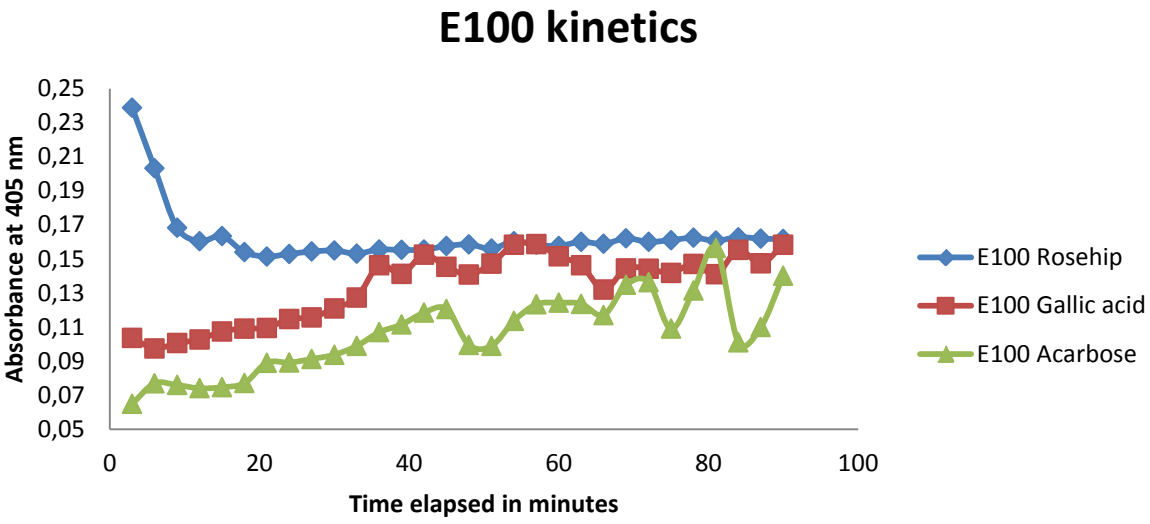






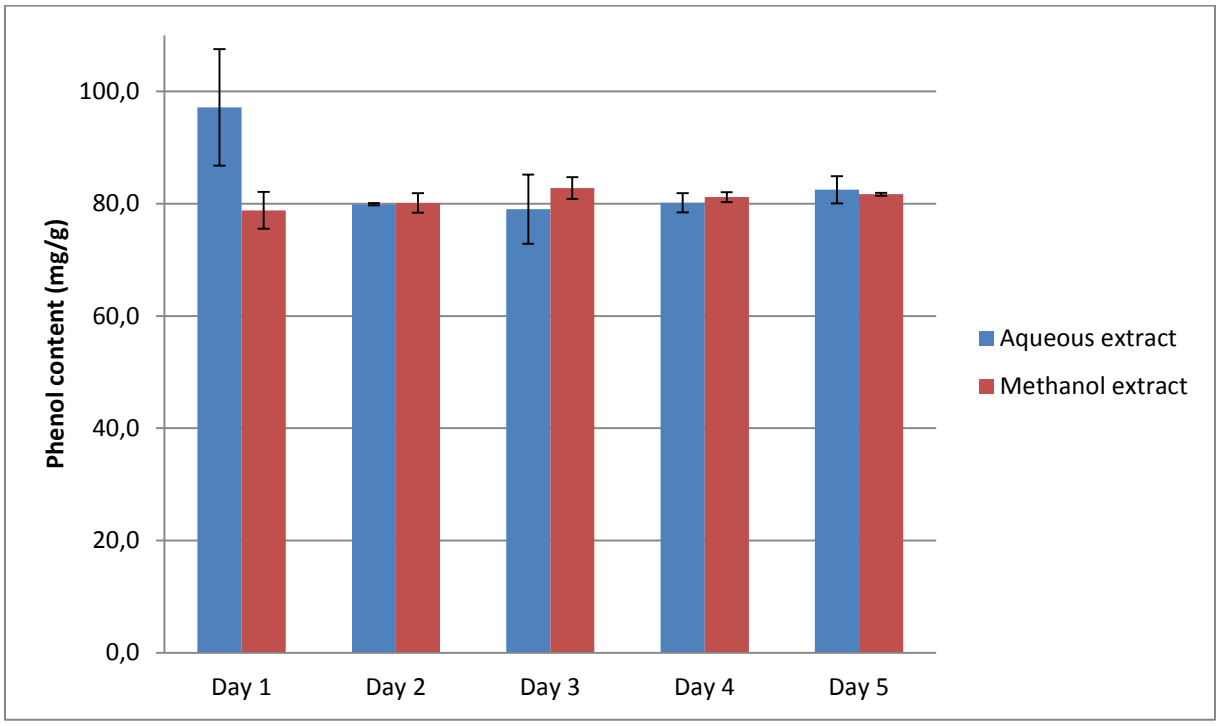






Note: E20 means 20 µl of rosehip aqueous extract was assayed, the same goes with the others.

Appendix. 3 Stability of total phenolic content by Folin-Ciocalteau method during short storage



Note: The results were presented as the means ± standard deviation from repeated experiments.

Appendix. 4 Potential synergistic effects of total phenol content and antioxidant capacity in extruded products with cereals and berries

process parameters		Total phenolics (FC)		Antioxidative capacity (ORAC)	
Products	Ingredients	Total content (mg/g dw)	Retention (%)	Total content (µmol/g dw)	Retention (%)
U1	Oat endosperm flour Apple Lingonberry	8,9±0,0	110,2%	233,7±18,9	155,6%
U2	Whole grain oat flour Apple Lingonberry	8,1±0,2	101,5%	205,9±0,9	140,6%
U3	Whole grain wheat flour Apple Lingonberry	6,3±0,1	79,8%	143,2±26,4	94,3%
U5	Wheat bran Apple Lingonberry	8,0±0,0	97,3%	242,7±10,1	146,4%
U6	Barley flour Apple Lingonberry	7,8±0,0	96,7%	222,8±21,2	148,8%
U7	Oat bran Apple Lingonberry	8,1±0,0	100,9%	187,4±37,2	126,4%
U8	Defatted whole grain oat flour Apple Lingonberry	7,2±0,1	90,1%	164,5±18,9	112,3%

Note: The results were presented as means ± standard deviation of repeated experiments. The proportion of the ingredients and the thermal treatment are not shown here.