A PRELIMINARY ASSESSMENT ON THE DEVELOPMENT OF MILK BASED SORBET WITH GLUCOSINOLATES USING *RAPHANUS SATIVUS* (RADISH)

Maria Vanessa R. Co[1], Arriane Mae D. C. Lagdamen[1], Robby Raven G. Navarro[1] and Louie Ian B. Mariano, RChE, MEN[2,a]

[1]Bachelor of Science in Nutrition and Dietetics,
College of Business Administration and Accountancy
[2]Faculty Member, Natural Sciences Area, College of Liberal Arts and Sciences
[a]louieian.mariano@letran.edu.ph

ABSTRACT

This study aims to determine the glucosinolate content in Raphanus sativus, *and given the potential of a more potent glucosinolate in low temperature, this research will attempt to produce a sorbet that is fortified with radish that has the possibility of being a cancer fighting food source. The variations of glucosinolates that are present in trace amounts in* Raphanus sativus *were evaluated and identified as isothiocyanates which has a sulfur-containing compound using Clarus 500 Gas Chromatograph. Fifty random panelists were presented 2 variations of sorbet coded as Sample A and Sample B, to be evaluated using a 7-point hedonic scale. Sample A was the controlled sorbet while Sample B was the sorbet with* Radish. *The translation of scores using simple averaging and ANOVA with regards to the sorbet's appearance, taste, odor, texture, and over-all acceptability was done after the test for the purpose of statistical analysis. Based on the results, glucosinolates such as sulfur-contaning compound have been found present in* Raphanus sativus. *The structures of all of the identified glucosinolates such as isothiocyanates products of myrosinase hydrolysis were confirmed by GC/MC. Translation of scores of the sensory evaluation using simple averaging for statistical analysis of the 7-point hedonic scale shows that the appearance and texture of the control sorbet are higher than the* Radish *sorbet but they are not significantly different. While the taste, odor and general acceptability of the control is found to be significantly higher compared to the* Radish *sorbet.*

Keywords: *Raphanus sativus, Glucosinolates, Gas Chromatography, ANOVA*

INTRODUCTION

Cancer is recognized as a disease that strikes with no substantial and specific reason. Many of the causes of cancer have been identified by scientists; however the specific cause of the disease is not yet fully explored and explained. Aside from intrinsic factors like heredity, hormones and diet, scientific studies point to several extrinsic factors that contribute to the development of cancer: chemicals from smoking, exposure to radiation and infection by viruses or bacteria. Given that exposure to cancer- causing agents (carcinogens) is responsible for triggering most human cancers, people can decrease cancer risk by decreasing or avoiding exposure such agents. Thus, the first step in cancer prevention is to recognize the exposure to carcinogens that have a direct effect on carcinogenesis (National Cancer Institute).

In the Philippines, cancer ranks third among the leading causes of morbidity and mortality after communicable diseases and cardiovascular diseases (Department of Health-Health Intelligence Service or DOH-HIS, 1992, 1996). In the Philippines, 75% of all cancers occur after the age of 50 and about 3% occur at age of 14 and below. At present, most Filipino cancer patients seek medical advice only when symptoms appear or during advanced stages which results to occurrence of new cancers wherein one die annually (Ngelangel, C. A., and Wang, E. H. M., 2001).

Epidemiological studies demonstrated that vegetable consumption decreases risk of various diseases, especially cancers of various types. Among the plant groups Brassica research continues to attain greater pace for the past 15 years. Vegetables of the Brassica genus such as broccoli, cabbage, cauliflower, mustard, radish, etc have received much attention, because they are reported to have anticancer activity both *in vitro* and *in vivo* (Devi, J.R., and Thangam, E.B., 2010).

Raphanus sativus also known as Radish belongs to the Brassicaceae family and were originally cultivated in China (Delahaut, K.A., and Newenhouse, A.C., 1998). It is a quick growing cool season root vegetable (Advisory Committee on Vegetable Crops). Radish is considered a super food as it contains large amounts of enzymes that aid in fat and starch digestion as well as high levels of vitamin C, phosphorous and potassium. It also contains other phytonutrients that fight cancer. In the Philippines it is known as "*labanos*", a white pungent tuber with hairy edible leaves, which is known as a good source of iron, calcium and Vitamin B. It is a versatile and inexpensive vegetable, which is often added to stews, sautéed or made into a salad. Radishes are grown worldwide and have been shown to contain potent phytochemicals, glucosinolates, and their breakdown products such as sulphorafane (Mithen et al., 2000). Foods that contain phytochemicals or biologically active substances are recognized to have healing properties, of which plants are known to naturally contain two hundred different glucosinolates (Clarke, 2010). Plant tissues contain a variety of phytochemicals. These phytochemicals are sometimes referred to as phytonutrients.

It is also believed that phytochemicals may be effective for combating or preventing diseases due to their antioxidant effects. The prevention and treatment of cancer, diabetes, heart disease, hypertension, cataracts, and strengthening of the immune system (Polk, 1996). Certain phytochemicals have been recommended for the treatment for human beings because of their chemoprotective, antioxidant, and antibiotic properties. And one of the classes of phytochemicals is called glucosinolates, and glucosinolates are also present in a wide variety of plants.

Glucosinolates are water-soluble, stable compounds, which are converted to isothiocyanates by the enzyme, myrosinase, and co-exists within the plant tissues. These isothiocyanates are well-known for having antimicrobial, fungicidal, and pesticidal activities (Folley, 2007). Numerous studies indicate that antioxidants found at high levels in vegetables and fruits can protect the human body from free radicals and retard the progress of many chronic diseases (Aires et al., 2013).

Glucosinolates are metabolized, by an enzyme also contain within the vegetable called myrosinase, into numerous products, such as nitriles and isothiocyantes which are believed to be active phytochemicals. Isothiocyantes have the health promoting effects like cancer protection (Traka et al., 2009). These compounds have gained renewed interest in recent years due to the chemo protective properties of their major hydrolysis products particularly isothiocyanates (Gao-feng et al., 2009) which is stored in different compartments of the plant cells that separate them from other glucosinolates. When plant tissues are damaged, myrosinase rapidly hydrolyzes the glucosinolates to glucose and other unstable intermediates, which spontaneously rearrange to a variety of biologically active products (Jia et al., 2009).

Splitting of glucosinolates leads to the formation of a wide range of biologically active compounds (Bonnesen et al., 2001; Holst and Williamson, 2004) including isothiocyanates (Hennig, 2013) known to be S- and N-containing secondary metabolites that are abundantly found in plants belonging to the Brassicacaea (Cruciferae) genus and posses chemopreventive agents. (Garcia et al., 2008)

According to the Linus Pauling Institute at Oregon State University, glucosinolates contain enzymes and antioxidants that help the body to fight off breast, lung, and colon cancer, as well as esophageal, stomach, and prostate cancer. Epidemiological evidence showed that consumption of Brassica vegetables is associated with reduced risk of many kinds of cancer (Mithen et al., 2000).

Glucosinolates (GLS) are natural sulfur-containing phytochemicals that for example are synthesized in vegetables from the Brassicaceae family such as broccoli, kale, cabbage, cauliflower, Brussels sprouts, radish, turnip or mustard greens (Rechcigl, 1983). Besides their high potential as bioactive molecules, some GLS and their hydrolysis products influence the flavor of Brassica vegetables, but also the taste of the food products in which they are added they are known to have a bitter taste (Rade-Kukic, 2010). This bitter taste is the reason, why a lot of foods containing glucosinolates are less liked. Because these foods are disliked, only insufficient amounts of glucosinolates are consumed, so that these beneficial compounds cannot produce their beneficial effect. There is a need possible process for a formulation on how the perception of the bitter taste of glucosinolates is reduced. According to the article of Rade-Kukic 2010, the inventors show that a complex formation between BLG major bovine whey protein found in most of the milk, is one example of a milk protein, and GLS enables reducing the undesirable taste of GLS. The researchers used the process called electrostatic complex – any form of association between the at least one glucosinolate and at least one milk protein. The protein preferably has an overall net charge opposite to the overall net charge of the glucosinolate. The complexation mechanism may involve both, enthalpic and entropic forces. The enthalpic part in the free energy of association is linked to the pairing of the opposite charges, while the entropic contribution to the free energy may result from the release of counter ions, as well as from the loss of degrees of freedom of molecules in their bound state. The composition comprising the complexes of the present invention may have any pH, as long as the particular pH allows the formation of the complex of the present invention, in other words as long as at least one milk protein and at least one glucosinolate is oppositely charged. Compositions and/or products comprising the complexes of the present invention are preferably selected from desserts, frozen desserts, dairy products, petfood, culinary products, clinical nutrition products etc. In particular, they may include sauces, soups, mayonnaises, salad dressings, creams, ice cream, chocolate, mousses, and/or milk (Rade-kukic,2010). Consequently, the composition of the present invention may be used to improve the taste of glucosinolate containing products, in particular to mask the bitterness of glucosinolates.

In essence, cooking influences the glucosinolates and their hydrolysis products (Blazevic, 2009). Water-soluble compounds leach into the cooking water (Rosa, 1993) and myrosinase activity decreases due to thermal inactivation. The higher temperature to be induced to the radish, the more its glucosinolates decrease because of inactivation of the primary enzyme.

Given the potential of a more potent glucosinolate in low temperature, this research will attempt to produce a sorbet that is fortified with Radish. A sorbet is a frozen dessert made from sweetened water with flavoring typically fruit juice or fruit purée, wine, and or liqueur. Whereas, ice cream is based on dairy products with air continuously whipped in, sorbet has neither, which makes for a dense and extremely flavorful product. Sorbets may also contain alcohol, which lowers the freezing temperature, resulting in softer texture. Milkfat content is between 1 and 2 percent with slightly higher sweetener content than ice cream (Bender, 2005). Sorbet produced without aeration is a dispersion of ice crystals distributed randomly in a freeze-concentrated liquid phase. The viscosity of sorbet is essential for the improvement of product quality and the selection of process equipment (Arellano et al., 2013).

Food fortification refers to the addition of micronutrients to processed foods. This strategy can lead to relatively rapid improvements in the micronutrients status of a population, and at a very reasonable cost. Since the benefits are potentially large, food fortification can be a very cost-effective public health intervention. It is also necessary to have access to, and to use, fortificants that are well absorbed yet do not affect the sensory properties of foods (Allen, L., Benoist, B. D., Dary, O., and Hurrell R., 2006). The micronutrient deficiencies have persisted as public health problems affecting a significant proportion of the population, resulting in adverse physical, mental, social and economic consequences to individuals, communitires and country; and that Food Fortification, one of the impact programs of the Philippine Plan of Action for Nutrition, is the long-term solution and most cost-effective strategy in addressing micronutrient malnutrition particularly among mothers and young children (Food and Nutrition Research Institute).

The general objective of this study is to produce a fortified sorbet with Radish with the possibility of being a cancer fighting food source; with specific objectives as: to determine the presence of glucosinolate in *Raphanus sativus* which makes it a cancer fighting food source; to asses the time and peak where the sulfur containing compound appears; and to know the over-all acceptability of the sorbet with Radish in comparison with the controlled sorbet.

This study will benefit sorbet consumers because of its *Raphanus sativus* glucosinolate content which has the potential of being a cancer fighting food source.

This study is limited to the use of Radish *(Raphanus sativus)* focusing on the use of the tuber which has been grated; harvest time and other varieties of *Raphanus sativus* were not included. Moreover, determination of glucosinolate present in Radish will be part of

METHOD

Preparation of Raphanus Sativus

Radish *(Raphanus Sativus)* wasbought from the supermarket, located at SM Manila. The radish used was authenticated by National Museum: Botany Division on September 9, 2014. It was first peeled then sliced into pieces and air dried for 48 hours using a dehydrator to make sure that it will be dried fully without affecting the glucosinolate compound. After drying, the Radish was crushed using an osterizer.

Extraction of glucosinolate

At 50-60°C heat, a 95% solution of ethanol in 100 ml of water was combined with 15g of powdered Raphanus Sativus, it was continuously stirred until it mixed well. The mixture was then filtered using a whatman no. 1 filter paper, and was set aside to cool down in a room temperature at 27°C. After cooling down, the supernatant was transferred directly to a sterilized vial.

Determination of glucosinolate

The vial containing solution was brought to De La Salle University, Taft Avenue, Manila for the determination of glucosinolate from *Raphanus Sativus* using a Clarus 500 Gas Chromatograph.

Radish Sorbet

In an ice bath, 900ml buko juice, 50g coconut powder, 2 drops of pure vanilla extract, 2 cups white sugar, and 1 cup grated radish was mixed in a bowl. This is to decrease the pungent smell of the radish. Then, 1½ cup of Nestle cream was added to the mixture, stirring constantly until it thickens. It was frozen and was stirred after an hour. After, the mixture was poured in a container. The sorbet had a soft, creamy texture.

Sensory Evaluation

A total of 50 random panelists was presented with two variations of sorbet coded as Sample A and Sample B. Sample A was the controlled sorbet while Sample B was the sorbet with Radish. The sorbet was evaluated using a 7-point hedonic scale: 7 – Like very much, 6 – Like moderately, 5 – Like slightly, 4 – Neither like or dislike, 3 – Dislike slightly, 2 – Dislike moderately, and 1 – Dislike very much.

7-point scales are a little better than 5-points—but not by much. The psychometric literature suggests that having more scale points is better but there is a diminishing return after around eleven points (Nunnally 1978). Having seven points tends to be a good balance between having enough points of discrimination without having to maintain too many response options (Sauro, J., 2010).

Statistical Analysis

Simple averaging and ANOVA was used for statistical analysis with regards to the sorbet's appearance, taste, odor, texture, and over-all acceptability.

RESULTS AND DISCUSSION

Gas Chromatography – Mass Spectrometry Analysis

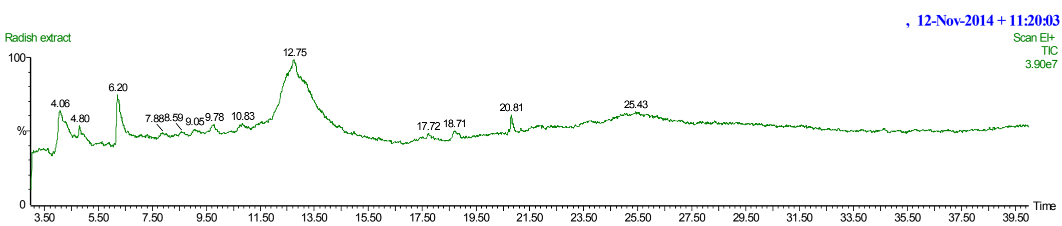

Figure 1. Gas Chromatogram of *Raphanus sativus* extract

Figure 1 shows that there are five peaks produced by the chromatogram at the time of 4.06 minutes (A), 4.48 minutes (B), 6.20 minutes (C), 12.75 minutes (D), and 20.81 minutes (E). The highest peak was produced at Peak D while, the lowest peak was at Peak B.

Glucosinolate determination

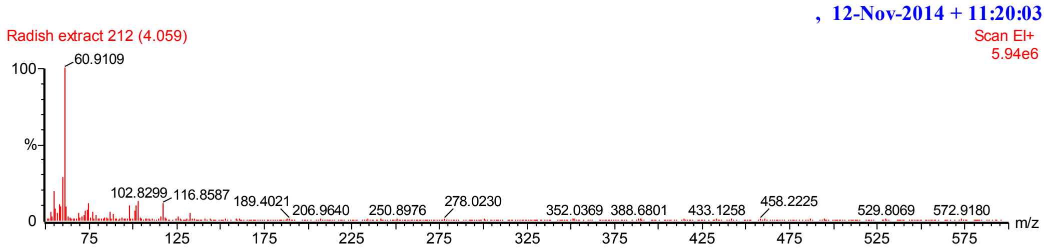

Figure 2. Mass Spectometry data and corresponding match of each Peak A

Table 1. List of compound found in Peak A

Hit	REV	for	Compound Name	M.W	Formula	CAS	Library
1	599	490	1-BUTANETHIOL, 4-(METHYLTHIO)-	136	C5H12S2	66890-94-6	NIST
2	599	490	1-BUTANETHIOL, 4-(METHYLTHIO)-	136	C5H12S2	66890-94-6	Nist
3	551	423	2-IZOPROPYL-4,6-DIMETHYL-1,3,2-OXATHIABORINANE	172	C8H17OSB	143674-23-1	NIST
4	551	423	2-IZOPROPYL-4,6-DIMETHYL-1,3,2-OXATHIABORINANE	172	C8H17OSB	143674-23-1	Nist
5	535	481	PROPANOIC ACID, 2-(ETHYLTHIO)-, ETHYL ESTER	162	C7H14O2S	20461-97-6	NIST
6	535	481	PROPANOIC ACID, 2-(ETHYLTHIO)-, ETHYL ESTER	162	C7H14O2S	20461-97-6	Nist
7	534	438	BUTANE, 2,2'-THIOBIS-	146	C8H18S	626-26-6	NIST
8	534	438	BUTANE, 2,2'-THIOBIS-	146	C8H18S	626-26-6	Nist
9	527	359	METHYL 2-O-ACETYL-.BETA.-D-XYLOPYRANOSIDE	206	C8H14O6	80973-64-4	NIST
10	527	359	METHYL 2-O-ACETYL-.BETA.-D-XYLOPYRANOSIDE	206	C8H14O6	80973-64-4	Nist
11	524	428	2-HYDROXYETHYL BUTYL SULFIDE	134	C6H14OS	5331-37-3	NIST
12	524	428	2-HYDROXYETHYL BUTYL SULFIDE	134	C6H14OS	5331-37-3	Nist
13	519	426	CYCLOPROPANECARBOXYLIC ACID, 2-PENTYL, ETHYL(-2-BUTYLTHIO) ESTE	272	C15H28O2S	900221-99-7	NIST
14	519	426	CYCLOPROPANECARBOXYLIC ACID, 2-PENTYL, ETHYL(-2-BUTYLTHIO) ESTE	272	C15H28O2S	900221-99-7	Nist
15	508	408	BUTANE, 1,4-BIS(METHYLTHIO)-	150	C6H14S2	15394-33-9	NIST
16	508	408	BUTANE, 1,4-BIS(METHYLTHIO)-	150	C6H14S2	15394-33-9	Nist
17	506	433	2,5,6-TRIMETHYL-1,3-OXATHIANE	146	C7H14OS	114376-06-6	NIST
18	506	433	2,5,6-TRIMETHYL-1,3-OXATHIANE	146	C7H14OS	114376-06-6	Nist
19	486	379	3,4-ALTROSAN	162	C6H10O5	900129-76-4	NIST
20	486	379	3,4-ALTROSAN	162	C6H10O5	900129-76-4	Nist

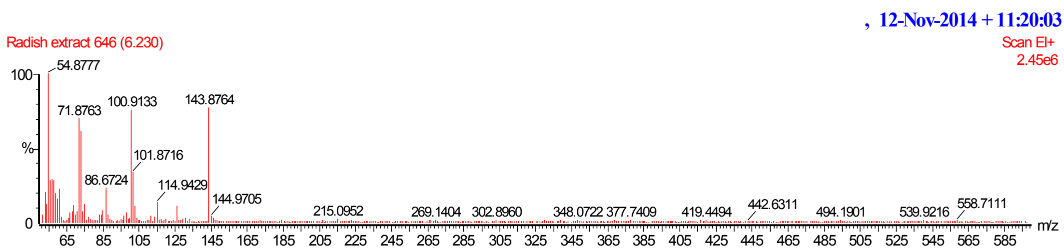

Figure 3. Mass Spectometry data and corresponding match of each Peak

Table 2. List of compound found in Peak C

Hit	REV	for	Compound Name	MW	Formula	CAS	Library
1	686	552	N-(1-METHOXYCARBONYL-1-METHYLETHYL)-4-METHYL-2-AZA-1,3-DIOXANE	203	C9H17O4N	76683-94-8	NIST
2	686	552	N-(1-METHOXYCARBONYL-1-METHYLETHYL)-4-METHYL-2-AZA-1,3-DIOXANE	203	C9H17O4N	76683-94-8	Nist
3	624	344	1,1-DIMETHYL-1-SILACYCLOBUTANE	100	C5H12Si	2295-12-7	NIST
4	624	344	1,1-DIMETHYL-1-SILACYCLOBUTANE	100	C5H12Si	2295-12-7	Nist
5	621	315	1,3-DIOXOLANE, 4,5-DIMETHYL-2-PENTADECYL-	312	C20H40O2	56599-61-2	NIST
6	621	315	1,3-DIOXOLANE, 4,5-DIMETHYL-2-PENTADECYL-	312	C20H40O2	56599-61-2	Nist
7	617	307	1,3-DIOXEPANE, 2-PENTADECYL-	312	C20H40O2	41563-29-5	NIST
8	617	307	1,3-DIOXEPANE, 2-PENTADECYL-	312	C20H40O2	41563-29-5	Nist
9	609	467	TRANS-3-METHYL-2-N-PROPYLTHIOPHANE	144	C8H16S	118068-75-0	NIST
10	609	467	TRANS-3-METHYL-2-N-PROPYLTHIOPHANE	144	C8H16S	118068-75-0	Nist
11	604	421	OCTANOIC ACID, 2-HEXYL-	228	C14H28O2	60948-91-6	NIST
12	604	421	OCTANOIC ACID, 2-HEXYL-	228	C14H28O2	60948-91-6	Nist
13	603	457	CIS-3-METHYL-2-N-PROPYLTHIOPHANE	144	C8H16S	118068-76-1	NIST
14	603	457	CIS-3-METHYL-2-N-PROPYLTHIOPHANE	144	C8H16S	118068-76-1	Nist
15	581	446	2-N-PROPYLTHIANE	144	C8H16S	17912-23-1	NIST
16	581	446	2-N-PROPYLTHIANE	144	C8H16S	17912-23-1	Nist
17	575	443	2-PROPYLOCTANOIC ACID	186	C11H22O2	31080-41-8	NIST
18	575	443	2-PROPYLOCTANOIC ACID	186	C11H22O2	31080-41-8	Nist
19	573	389	10,12-DIMETHYL-1,4,7-TRIOXA-10,12-DIAZACYCLOTETRADECAN-11-ONE	246	C11H22O4N2	83540-66-3	NIST
20	573	389	10,12-DIMETHYL-1,4,7-TRIOXA-10,12-DIAZACYCLOTETRADECAN-11-ONE	246	C11H22O4N2	83540-66-3	Nist

In Figure 2 and 3, each peak has a corresponding match has an equivalent in Mass Spectrometry data from NIST library. Each peak shows the different compounds that are possible in *Raphanus sativus* extract. Only Peak A and C contains Sulfur containing compounds that can be a possible carrier of Glucosinolate.

Table 1 shows the MS data and corresponding match of each peak from NIST library. The first peak at 4.06, these are the list of the sulfur containing compound: 1-Butanethiol, 4-(methylthio)-; Propanoic acid, 2-(ethylthio)-,ethyl ester; Butane, 2,2'-thiobis-; 2-hydroxyethyl butyl sulfide; Cyclopropanecarboxylic acid,-2-pentyl, ethyl(-2-butylthio) este; Butane, 1,4-bis(methylthio)-; and 2,5,6-trimethyl-1,3-oxathiane. These sulfur containing compound suggests the presence of glucosinolate in Radish.

Table 2 shows the MS data and corresponding match of each peak from NIST library. The first peak at 6.230, indicates the list of the sulfurs containing compound: 1,1-dimethyl-1-silacyclobutane; Trans-3-methyl-2-n propylthiophane; Cis-3-methyl-2-n-propylthiophane; and 2-n-prophylthiane. These sulfur containing compounds suggest the presence of glucosinolate in radish.

Statistical Analysis

A total of 50 random panelists were presented 2 variations of sorbet coded as Sample A and Sample B. Sample A esd the controlled sorbet while Sample B the sorbet with radish.

Table 3. Sensory Evaluation an ANOVA Treatment of Controlled and Radish Sorbet

Appearance	6.1		5.98		
	ANOVA for Appearance				
	SS	**df**	**MS**	**F**	**CV**
Between	0.36	1	0.36	0.463	3.94
Within	76.23	100	0.78		
Total	76.59	99			
*Significant at the 5% level (**α = 0.05**)					
	Control Mean		**Radish Sorbet Mean**		
Taste	6.54*		5.48*		
	ANOVA for Taste				
	SS	**df**	**MS**	**F**	**CV**
Between	28.09	1	28.09	30.74	3.93
Within	89.56	100	0.91		
Total	117.65	99			
*Significant at the 5% level (**α = 0.05**)					
	Control Mean		**Radish Sorbet Mean**		
Odor	6.22*		5.46*		
	ANOVA for Odor				
	SS	**df**	**MS**	**F**	**CV**
Between	14.44	1	14.44	14.54	3.94
Within	97.35	100	0.99		
Total	111.79	99			
*Significant at the 5% level (**α = 0.05**)					
	Control Mean		**Radish Sorbet Mean**		
Texture	5.92		5.86		
	ANOVA for Texture				
	SS	**df**	**MS**	**F**	**CV**
Between	0.090	1	0.090	0.09	3.93
Within	95.13	100	0.97		
Total	95.22	99			
*Significant at the 5% level (**α = 0.05**)					
	Control Mean		**Radish Sorbet Mean**		
Acceptability	6.3*		5.56*		
	ANOVA for Acceptability				
	SS	**df**	**MS**	**F**	**CV**
Between	13.69	1	13.69	15.48	3.94
Within	86.66	100	0.88		
Total	100.35	99			
*Significant at the 5% level (**α = 0.05**)					
* indicates statistical significance (Control VS Radish Sorbet); ($p < 0.05$)					

Results showed that there is no significant difference between the two samples with regards to appearance and texture. On the other hand, results showed that there is significant difference between the two samples with regards to taste, odor, and general acceptability.

Based on the sensory evaluation and ANOVA result, the appearance of the control is higher but they are not significantly different compared to the Radish Sorbet (RS). While for the taste and the odor the control is significantly higher compared to the Radish Sorbet (RS). The texture of the control is higher than the Radish Sorbet (RS) but they are not significantly different. And for the general acceptability the control is significantly higher compared to the Radish Sorbet (RS).

CONCLUSION

Based on the results, glucosinolates such as sulfur-contaning compounds that have been found present in *Raphanus sativus* were easily identified and have been determined in the presence of Isothiocyanates with the use of gas chromatography/MS. The structures of all of the identified glucosinolates such as isothiocyanates products of myrosinase hydrolysis were confirmed by GC/MC. And this analysis of isothiocyanates, although not applicable to all glucosinolates, was more sensitive in allowing for the detection of trace amounts of sulfur containing glucosinolates.

Translation of scores of the sensory evaluation using simple averaging for statistical analysis of the 7-point hedonic scale shows that the appearance and texture of the control sorbet are higher than the Radish sorbet but they are not significantly different. This means that the *Raphanus sativum* can be used as one of the raw materials for making sorbet. While the Taste, Odor and General Acceptability of the control were found to be significantly higher compared to the Radish sorbet.

RECOMMENDATION

To future students who will conduct a related study, the researchers recommend that they use a more effective extraction method for glucosinolate there by producing a more accurate GC-MC chromatogram.It is also recommended to lessen the amount used of the grated *Raphanus sativus* to not leave an after taste after eating the Radish sorbet. Lastly, the researchers recommend the use of a method that will quantify the glucosinolate present in *Raphanus sativus*.

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