IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

RP HPLC Method For The Estimation Of Riboflavin In Various Extracts Of Pumpkin: A Review

[*1] Sathiyavani.G,[2]Haridass.S, [3]Yogesh. R, [4]Durga Nandhini.S,

[5]Evangalin Preetha, [6]Dr.N.Astalakshmi, And[7] Dr.M.Surendra Kumar

[1]M.pharm ,assistant professor, senghundhar college of pharmacy,

[2,3,4,5] b.pharm final year, senghundhar college of pharmacy,

[6] M.pharm.,ph.d.,hod,dept. Of pharmaceutical chemistry, senghundhar college of pharmacy,

[7] M.pharm., ph.d., principal, senghundhar college of pharmacy,

ABSTRACT:

Riboflavin was estimated in various pumpkin extracts using the RPHPLC method and the direct comparison method in pumpkin pulp, skin, and seed using a 25x4 6mm, particle size 5-micron C18 reversed phase column and methanol, water as mobile phase. In isocratic mode, with a mobile phase flow rate of 1ml/min, and uv-visible detection at 270nm. Regression analysis revealed a strong linear relationship with r2=0.998.

KEYWORDS: Pumpkins,Pulp,Seed,Peel,Riboflavin ,RPHPLC

INTRODUCTION:

Pumpkins are a mineral powerhouse, containing calcium, iron, potassium, magnesium, phosphorus, copper, and manganese. Pumpkins contain high levels of vitamin C, vitamin E, vitamin B6, thiamin, and niacin. The purpose of this review was to look into the functional and nutraceutical constituents found in all three parts of pumpkin (peel, flesh, and seeds), as well as the health benefits of these nutrients.

Pumpkins have both male and female flowers, and honeybees play an important role in fertilisation. Pumpkins were historically pollinated by the native Squash bee Peponapis pruinose, but this bee has declined, most likely due to pesticide (imidacloprid) sensitivity **(Stephens et al., 2014)**, and most commercial plantings are now pollinated by honeybees. Inadequately pollinated pumpkins typically begin to

grow but die before reaching full maturity. Pumpkins are grown all over the world for a variety of reasons, from agricultural (such as animal feed) to commercial and ornamental sales **(Nicole BM, et al., 2002)**

They are native to Mexico's central, gulf, and northeast regions, and have been introduced to Alabama, Albania, Bangladesh, the Bahamas, India, France, and Finland, among other places

Pumpkins are a warm-weather crop that is typically planted in July. Specific conditions for growing pumpkins necessitate soil temperatures of 8 centimetres (3 in)

Deep are at least 15.5 degrees Celsius (60 degrees Fahrenheit) and soil that holds water well.

Pumpkin crops may suffer from a lack of water or cold temperatures (below 18 °C or 65 °F; frost can be harmful), as well as sandy soils with poor water retention or poorly drained soils that become waterlogged after heavy rain. Wolford and colleagues (2008)

Pumpkins, on the other hand, are quite hardy, and even if many leaves and portions of the vine are removed or damaged, the plant can quickly regenerate secondary vines to replace what was lost.

Riboflavin is a form of vitamin B. Because it is water soluble, it is not stored in the body. Vitamins that are water soluble dissolve in water. Excess vitamin excretion from the body occurs through the urine. The body stores a small amount of these vitamins. They must be taken on a regular basis in order to keep the reserve.

Because riboflavin is soluble in water, about twice as much riboflavin content is lost in cooking water when foods are boiled as when they are prepared in other ways, such as steaming or microwaving (Dainty JR, et al., 2007). They are classified as water-soluble or fat-soluble vitamins. Tarwadi, K. et al., 2002

The well-known B-complex is a member of the First group. Vitamin B2 (riboflavin) has a well-defined process in fat, carbohydrate, and respiratory protein metabolism. Riboflavin (also known as vitamin B2) is one of the water-soluble B vitamins. Riboflavin occurs naturally in some foods, is added to some foods, and is available as a dietary supplement.

This vitamin is required for the formation of two major coenzymes, flavin mononucleotide (FMN; also known as riboflavin-5'-Phosphate) and flavin adenine dinucleotide (FAD) (FAD). These Coenzymes play critical roles in energy production, cellular function, growth, and development, and fat, drug, and steroid metabolism

Up to a maximum of about 27 mg of riboflavin per meal or dose, approximately 95% of riboflavin in the form of FAD or FMN from food is bioavailable. FAD is required for the conversion of the amino acid tryptophan to niacin (also known as vitamin B3) (Food and Nutritional Board , 2012)

Milk and milk drinks, bread and bread products, mixed foods with meat as the main ingredient, ready-to-eat cereals, and mixed foods with grain as the main ingredient are the largest dietary contributors to total riboflavin intake in men and women in the United States.

Similarly, FMN is required for the conversion of vitamin B6 to Coenzyme pyridoxal 5'-phosphate. Furthermore, Riboflavin aids in the maintenance of normal levels of homocysteine, an amino acid in the blood. Eggs, organ meats (kidneys and liver), lean meats, and milk are particularly high in Riboflavin.

Because pumpkin seeds are high in macronutrients like calcium, magnesium, and phosphorus, as well as micronutrients like calcium, zinc, copper, and manganese, they can be used as supplements in a variety of foods.

Many species in the Cucurbitaceae family are consumed by humans. Cucurbita species are among the most important, with various species being prepared and eaten in a variety of ways.

Riboflavin is an essential component of coenzymes that are involved in cell growth, energy production, and the breakdown of fats, steroids, and medications.

The RDA for men and women over the age of 19 years is 1.3 mg and 1.1 mg daily, respectively. The amount increases to 1.4 mg and 1.6 mg daily during pregnancy and lactation, respectively.

Riboflavin is primarily found in meat and fortified foods, but it is also found in some nuts and green vegetables.

Pumpkin seeds are high in macronutrients such as calcium, magnesium, and phosphorus, as well as adequate amounts of micronutrients such as calcium, zinc, copper, and manganese, so they can be used as supplements in a variety of foods (Stevenson DG, et al., 2007)

As a result, pumpkin seeds can be turned into pumpkin seed flour and pumpkin seed oil. Pumpkin seed flour can be used to fortify a variety of foods because it is inexpensive, has a high nutritional value, and has exceptional sensory qualities. Pumpkin seeds contain adequate amounts of nutrients such as zinc, magnesium, potassium, phosphorous, and selenium, which act as medicine and fighting elements for diseases such as cancer, inflammation, prostate, and arthritis. (P. Maheshwari et al., 2015)

Pumpkin Peel, fruit, and seed nutritional profile:

Nutrient	Pumpkin Peel (Value/100 g)	Pumpkin Fruit (Value/100 g)	Pumpkin Seed (Value/32.25 g)
Energy	520.78 kJ	109 kJ	NR
Water	89.527 mg	91.6 g	1.69 g
Lipids	1.650 mg	0.1 g	15.82 g
Protein	14.670 mg	1.0 g	9.75 g
Ash	7.317 mg	0.8 g	1.54 g

Nutritional facts Pumpkin seeds

AMOUNT PER 100 grams

Total fat : 19g

Total Carbohydrate: 54g

Protein: 19g

Iron : 18%

Magnesium : 65%

calcium : 5%

DRUG PROFILE:

Riboflavin(Vitamin B2):

Riboflavin, also known as vitamin B2, is a vitamin that can be found in foods and is also available as a dietary supplement. It is required for the synthesis of two major Co enzymes, flavin mononucleotide and flavin adenine dinucleotide. Riboflavin, or vitamin B2, is one of eight B vitamins that are essential for human health. It is present in grains, plants, and dairy products. It is necessary for the breakdown of food components, the absorption of other nutrients, and the maintenance of tissue. Riboflavin is D-ribitol with a 7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[g]pteridin-10(2H)-yl moiety in place of the hydroxy group at position 5. It is found in milk, eggs, malted barley, liver, kidney, heart, and leafy vegetables, but yeast is the richest natural source.The free form is found only in the retina of the eye, whey, and urine; flavin mononucleotide and flavin-adenine dinucleotide are its major forms in tissues and cells. It functions as a photosynthetic agent, food colourant, E. coli metabolite, mouse metabolite, cofactor, plant metabolite, human urinary metabolite, anti-inflammatory agent, basic metabolite, and antioxidant. It contains both vitamin B2 and flavin. It is riboflavin's (1-) conjugated acid.

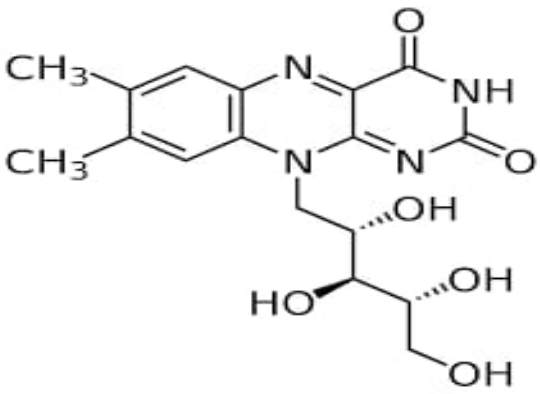
IUPAC ID : 7,8-Dimethyl -10-[(2S,3S,4R)-2,3,4,5,-tetrahydroxypentyl] benzo[g] Petridine-2,4-dione

Formula: C17H20N4O6

Molar mass: 376.36 g/mol

Melting point: 280 °C

Other names: lactochrome, lactoflavin, vitamin G



Vitamin composition of pumpkin fruit and seed.

Nutrient	Pumpkin Fruit	Pumpkin Seed	
	(mg/100 g)	(mg/100 g)	(mg/32.25 g)
Vitamin A	0.426	0.019	0.0015
Vitamin C	9.0	0.3	0.61
Vitamin B1	0.05	0.034	0.09
Vitamin B2	0.11	0.052	0.05
Vitamin B3	0.6	0.286	1.61

MATERIALS AND METHODS:

Collection Sample:

The pulp of 14 pumpkin cultivars from the Cucurbita maxima species was used in the study. 'Dolina Mogilnicy' Organic Farming Products Cooperative provided all of the cultivars. The plants were irrigated, weeded, and the soil was loosened while they were growing. The pumpkins were harvested in October 2018 and immediately transported to a laboratory for cleaning. The experimental unit consisted of two pumpkins from each variety chosen at random. All chemical analyses were done in triplicate for each pumpkin. The edible pulp was cut into pieces, freeze-dried, and then analysed. The dried pulp was kept at room temperature, away from oxygen and light.

Sample extraction:

Extraction is a critical step in sample preparation before chromatography. It entails isolating target analytes from a complex sample or a large sample volume. The procedure eliminates interfering sample components that could clog HPLC and GC columns.

Small non-polar molecules, such as many drugs, will have an affinity for the SP in RPHPLC, and separation and elution can be accomplished by following through an aqueous (polar) MP solvent using an isocratic or gradient approach.Pumpkin was chosen, and organically grown vegetables were purchased and used to extract the seeds, pulp, and peel from it.

Preparation of pumpkin skin juice:

The pumpkins were washed in cold water and their skins were manually removed after entering the laboratory. The thickness of the skin was 1.0 0.2 cm. Pumpkin skin powdered with a particle size of 2 mm after being oven-dried at 40°C.

Preparation of pumpkin peel:

After entering the laboratory, pumpkins were washed with cold water and their peels were manually removed. The peel's thickness was 1.0 0.2 cm. The pumpkin peel was dried in an oven at 40 degrees Celsius and powdered to a particle size of 2 millimetres.

Preparation of samples:

The fresh and nutritious vegetables were washed right away. Excessive water dripped from the faucet. Vegetable portions (100 g) were cut into small pieces and homogenised in a blender. Before adding 2.5 ml of 2% a-amylase, the mixture was cooled in an ice bath. Following a gentle mixing, the mixtures (seeds, pulp, and peel) were sonicated for 15 minutes and placed in a water bath for 5 minutes. After cooling, the mixture was diluted to 25 mL with deionized water. A bench top centrifuge was used to spin the resulting mixture at 2500 rpm for 15 minutes at room temperature. Before HPLC analysis, the supernatant was filtered through a 0.45 m nylon filter Disc. All samples were collected in triplicate.

Riboflavin:

One gramme of sample was weighed and placed in a graduated polypropylene centrifuge tube with a volume of 50 ml. The mixture was then treated with 17.5 ml of 0.1 N sulphuric acid. The mixture was vigorously shaken for 1 minute before being placed in boiling water for 30 minutes and shaken at 10-minute intervals. In 100 grammes of boiled, boiled, drained, and salted pumpkin, there is 0.08 mg of riboflavin, also known as vitamin B2.

This vitamin's most important role in the body is involved in unlocking energy from the nutrients we consume and allowing wounds to heal faster. Because it reduces sensitivity to painful stimuli and has anti-inflammatory properties, studies over the last decade have made the vitamin very appealing to migraine

sufferers. If you eat meat, you are unlikely to be deficient. B2 can also be obtained from yeast products, sprouts, mushrooms, broccoli, and wheat germ.

Riboflavin was prepared by dissolving 20 mg of riboflavin in 200 mL of deionized water and adding three drops of pure acetic acid glacial. It was warmed in a water bath at 80°C to dissolve the riboflavin and achieve the standard concentration of 100 g/ml.

Riboflavin, a heat-stable and water-soluble flavin from the vitamin B family, is an essential human nutrient. Riboflavin is a coenzyme precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (FAD). These coenzymes play critical roles in normal tissue respiration, pyridoxine activation, tryptophan-to-niacin conversion, fat, carbohydrate, and protein metabolism, and glutathione reductase-mediated detoxification.

Riboflavin may play a role in erythrocyte integrity. This vitamin is necessary for having healthy skin, nails, and hair.

The most notable role of this vitamin in the body is its involvement in unlocking the energy from the nutrients we consume and allowing wounds to heal faster.

Over the last decade, research has shown that this seemingly innocuous vitamin can reduce sensitivity to painful stimuli and exert anti-inflammatory effects in migraine sufferers. Deficiency is extremely rare if you eat meat. B2 can also be obtained from yeast products, sprouts, mushrooms, broccoli, and wheatgerm, in addition to meat**.**

Determination of vitamin by HPLC method:

The standards were injected onto an HPLC system using the following condition

Column : Genesis C18; 250 x 4.6mm,

Mobile phase : Methanol / water /glacial acetic acid

(65/35/0.1) Flow rate : 1 ml/min

	Pulp	**Peel**	**Seed**
Mobile phases	Methanol-water-acetic acid glacial (65:35:0.1)	Methanol-water-acetic acid glacial (65:35:0.1)	Methanol-water-acetic acid glacial (65:35:0.1)
Flow rate	1.0 ml/min	1.0 ml/min	1.0 ml/min
Detection	270 nm	270 nm	270 nm

Preparation of standard solutions:

Preliminary studies involved solvents most commonly used for the preparation of plant extracts (acetone, ethyl acetate, ethanol, methanol, water). The extraction was carried out for various concentrations of selected solvents (20%, 40%, 60%, 80%, 100%) and the extraction time (0.5, 1, 2, 3, 4 h).Tests were carried

in three temperature ranges, which are used in plant components extraction as the most effective (30, 50, and 70 ◦C). Variants with the highest DPPH radical scavenging values were selected for further stages of the study. As a result, the above factors were limited to the previously mentioned nine solvents, two times and three extraction temperatures. In order to optimize the extraction pumpkin extracts were prepared by weighing 5 g of freeze-dried pumpkin and dissolving them in 50 mL of a solvent (water, methanol, 80% water–methanol solution, ethanol, 80% water–ethanol solution, acetone, 80% water–acetone solution, ethyl acetate, 80% ethyl acetate, and water solution).Next, the whole was shaken in a water bath (SWB 22N) at 30, 50, or 70 ◦C for 1 or 2 h. The extracts were centrifuged (1500 rpm, 10 min) and filtered through paper. Next, their anti oxidative activity was assessed.

The extraction of all samples was triplicated. The most favourable extraction conditions were selected on the basis of the optimization results: 2 h and 70 ◦C, maintaining the ratio between the amount weighed and the volume of the solvent used (1:10). Extracts for the pumpkin cultivars were prepared according to these conditions to find differences in their antioxidative activity, which included determination of the ABTS and DPPH radical scavenging assay, oxygen radical absorbance capacity(ORAC), the ferric reducing antioxidant capacity assay (FRAP), and the iron chelating activity assay

RESULT AND DISCUSSION:

Calibration curves for the standards (riboflavin) were obtained using a series of concentrations ranging from 1 to 10 microgram/ml of these compounds. As shown, the regression coefficient is 0.998. The chromatograms for the standard, extract sample for pulp, peel, and seed are shown in Figs. 1,2,3,4. The samples were analysed using the same separation conditions. The target compounds were identified by comparing their retention times to those of the calibration standards. UV wavelength was discovered to be 270 nm.

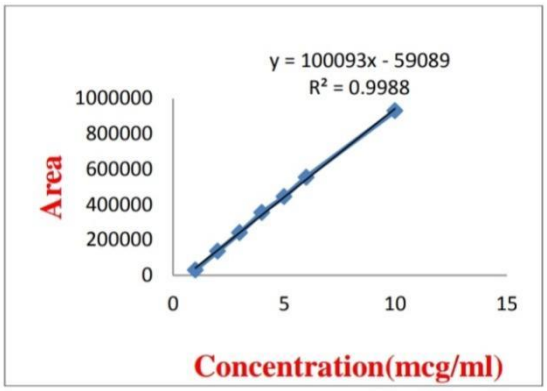


Fig 2: Calibration curve for the Std sample

The method of estimation of riboflavin was determined with clean chromatograms such as that shown below :

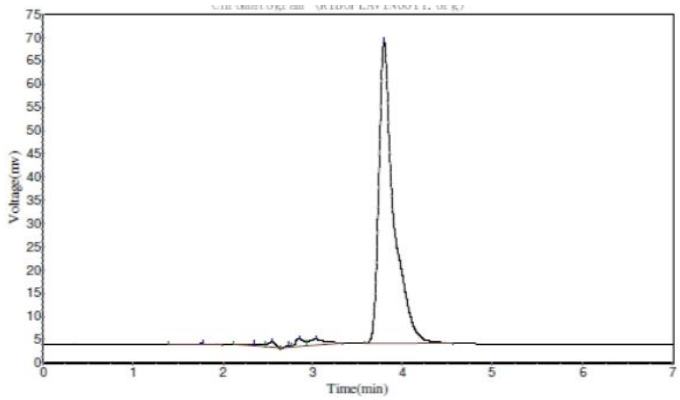


Fig 3: Chromatogram for std sample

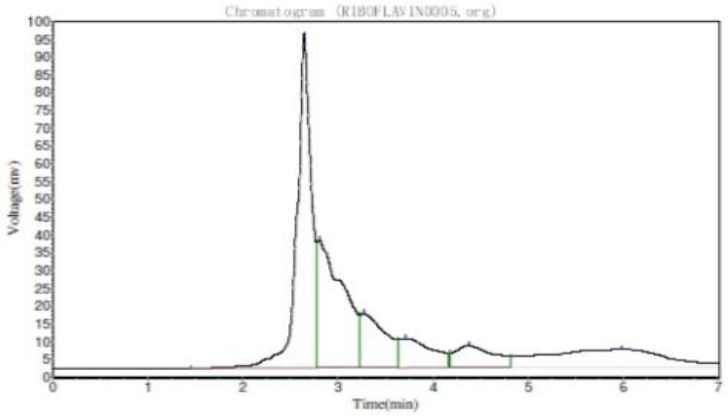


Fig 4: Chromatogram for peel extract

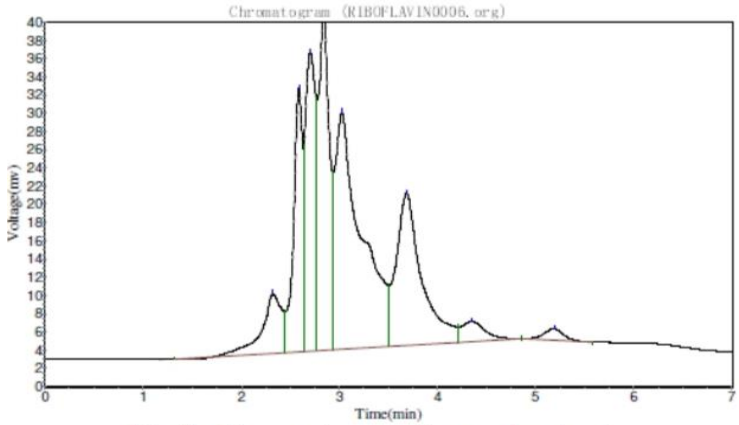


Fig 5: Chromatogram for seed extract

CALCULATION:

1. The amount of riboflavin is present in the pulp is found be:

a. Extrapolation

Definition of Extrapolation Formula. The extrapolation formula is the formula used to estimate the value of the dependent variable concerning an independent variable that shall lie in a range outside of the given data set

Extrapolation

= 6.1×25

= 152.5mg/ml

b. Direct comparison method

Test Area/Standard Area (571974/929735.688xa10) x standard concentration

= 6.1×2

= 152.5mg/ml

2. The amount of riboflavin is present in the peel is found be:

a. Extrapolation

2.3×25 =57.5mg/ml

b. Direct comparison method=TA/SA×SC

= 202085.6/929735.688×10

= 2.3×25

=57.5mg/ml

3. The amount of riboflavin is present in the seed is found be :

a. Extrapolation

3.6×25 =90mg/ml

b. Direct comparison method=TA/SA×SC

=30218.7/929735.688×10

=3.6×25

=90mg/ml

CONCLUSION

An easy, quick, precise, and accurate isocratic reversed-phase high performance liquid chromatography (HPLC) method was developed to measure the riboflavin content of pulp. The amount of pumpkin skin and seeds determined by extrapolation and direct comparison methods was 152.25 mg/ml, 57.5 mg/ml, and 90mg/ml, respectively. Separation was achieved using a 25 4.6 mm column with a particle size of 5 microns c18 reverse phase column and an isocratic elution mode and a mobile phase flow rate of 1 ml/min (phenomenex). UV visible detection was performed at 270 nm. Regression analysis revealed a strong linear association (r2 =0.998).

REFERENCE:

1. Agte V, Tarwadi K, Mengale S, Hinge A, Chiplonkar S. ―Vitamin profile of cooked foods: how healthy is the practice of ready-to-eat foods?‖ Int J Food Sci Nutr 2002;53:197-20

2. Astill, Gregory (2018). "Pumpkins: Background & Statistics". United States Department of Agriculture Economic Research Service, December 2, 2018.

3. Berger NA (1985). ―Poly (ADP-ribose) in the cellular response to DNA Damage. Radiat‖, Res. 101: 4-15.

4. Carpenter KJ, Lewin WJ (1985). ―A reexamination of the composition of Diets associated with pellagra.‖ , J. Nutr. 115: 543-552.

5. Cucurbita pepo L. Kew Science, Plants of the World, Royal Botanic Garden, UK, 8 December 2018.

6. Dainty JR, Bullock NR, Hart DJ, Hewson AT, Turner R, Finglas PM, et al. ―Quantification of the bioavailability of riboflavin from foods by use of stable-isotope labels and kinetic modeling‖. Am J Clin Nutr 2007;85:1557-64.

7. Ferriol, María; Picó, Belén (2007). "3". Handbook of Plant Breeding: Vegetables I. New York: Springer. p. 317. ISBN 978-0-387-72291-7.

8. Gregory JF, 3rd. ―Accounting for differences in the bioactivity and bioavailability of vitamers‖. Food Nutr Res 2012;56.

9. Horticulture Questions and Answers". Garden Help FAQ. Missouri Botanical Garden.

10. Institute of Medicine. ―Food and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.‖ Washington, DC: National Academy Press; 1998.

11. Maheshwari P, Prasad N, Batra E. Papitas-the underutilized byproduct and the future cash crop-a review. American International Journal of Research in Formal, Applied & Natural Sciences. 2015;15(432):31-4.

12. McCormick DB. Riboflavin. In: Erdman JW, Macdonald IA, Zeisel SH, eds. ―Present Knowledge in Nutrition‖. 10th ed. Washington, DC: Wiley-Blackwell; 2012:280-92.

13. Paris, Harry S. (1989). "Historical Records, Origins, and Development of the Edible Cultivar Groups of Cucurbita pepo(Cucurbitaceae)". Economic Botany. New York Botanical Garden Press. 43 (4): 423–443.doi:10.1007/ bf0 2935916JSTOR 4255187.

14. Pass more R, Nicole BM, Rao M, Narayana Beaton GH, de Mayer EM FAO/WHO (1974). ―Human Vitamin and Mineral Requirements, Handbook on Human Nutritional Requirements‖ Eds.,FAO Nutritional Studies WHO Monograph Series, Rome, Italy. 6: 28 FAO/WHO (2002). WHO,Rome, Italy.

15. Rivlin RS. Riboflavin. In: Coates PM, Betz JM, Blackman MR, et al., eds. ―Encyclopedia of Dietary Supplements‖. 2nd ed, London and New York: Informa Healthcare; 2010:691-9.

16. Said HM, Ross AC. Riboflavin. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. ―Modern Nutrition in Health and Disease‖, 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:325-30.

17. Stephens, James M. "Pumpkin — Cucurbita spp". University of Florida. November 23, 2014.

18. Stevenson DG, Eller FJ, Wang L, Jane JL, Wang T, Inglett GE. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. Journal of agricultural and food chemistry. 2007 May 16;55(10):4005-13.

19. The Pumpkin Patch". 2007, 19 February 2008.

20. Under son SH, Charles TJ, Nichol AD (1985). ―Thiamine deficiency at a District general hospital‖: report of five cases. Q. J. Med. 55: 15-32.

21. Wolford, Ron; Banks, Drusilla (2008). "Pumpkins and More". University of Illinois Extension, 19 Feb2008.