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EFFECT OF GERMINATION ON PHYTOCHEMICAL LEVELS OF DIFFERENT TYPES OF SPROUTS OF *Vigna radiata*, *Cicer arietinum*, *Phaseolus vulgaris* AND *Vigna mungo* pulses

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ABSTRACT

The aim of this study was to investigate the phytochemical changes which occur during germination of *Vigna radiata*, *Cicer arietinum*, *Phaseolus vulgaris* and *Vigna mungo* sprouts. Among sprouts of pulses, highest protein levels were found in urad dal (*Vigna mungo)* (2083.3mg/ml), followed by moong dal (*Vigna radiata)* (1813mg/ml), rajma (*Phaseolus Vulgaris)* (1350mg/ml) and black gram (*Cicer arietinum)* (1079.2mg/ml). The urad dal (*Vigna mungo)* had highest flavonoid content (708.4 mgAAE/gm), ascorbic acid content (0.9mg/ml), phenolic content (10.432mgTAE/gm) and reducing sugars content (133.91mg/ml). The antioxidant levels in sprouts were found to be decreased in the order: rajma (*Phaseolus Vulgaris)* (1.7357mgAAE/gm), urad dal (*Vigna mungo)* (1.5821mgAAE/gm), moong dal (*Vigna radiate)* (1.5285mgAAE/gm) and black gram *(Cicer arietinum)* (1.3607mgAAE/gm. Among pulses studied, black gram (*Cicer arietinum)* was characterized by low level of proteins (1079.2mg/ml) and phenolics (5.277mgTAE/gm). The flavonoid content was least in rajma (*Phaseolus vulgaris)* (542.3 mgAAE/gm).

Keywords: Proteins, Antioxidant activity, Ascorbic acid, Flavonoid, moong dal, urad dal, chick pea, black gram

INTRODUCTION

Seed germination is defined as the natural process during which seeds take up water rapidly & swells. The seed germination starts with the activation of enzymes by imbibition of water, development of the radicle to root for absorption of water from soil and development of the plumule to give rise to seedling **[1]**. At the seed imbibition stage, activation of hydrolytic enzymes takes place which play an important role in catalysis of reserved food such as proteins, carbohydrates, lipids, hemicellulose, polyphosphates and other food materials that will be easily available for embryo to uptake **[2]**.

Pulses are the seeds of legumes that are used for human consumption which includes peas, beans, lentils, chickpea,etc. **[3]**. They are an important source of macronutrients. In addition, it also contains dietary fibers, proteins, carbohydrates, minerals & used for human and animal consumption & production of oils. The major polyphenolic compounds present in pulses are tannins, flavonoids, phenolic acids, saponins, phytic acid, lectins, protease inhibitors, amylase inhibitors, sterols, carotenoids, isoflavones, etc. **[4]**.

The sprouting of legumes increases protein and carbohydrate digestibility as these seeds have more maltose content **[5]**. It also enhances vitamin contents e.g. vitamin C, reduces the antinutritional factors and improves their overall nutritional quality.

The varous legumes which are included in the study are:-

a) ***Vigna radiata (mung dal)***:- It is a leguminous crop and also called a Green gram and mung bean. Mung bean is a resource of high-quality protein which can be consumed as whole grains, dal, or sprouted form. It contains high level of amino acids, vitamins, minerals, dietary fiber, unsaturated fatty acids, and bioactive compounds including polyphenols, polysaccharides and peptides.

b) ***Cicer arietinum (black gram)***:- It is also called chickpea and is an annual legume from the fabaceae family. Its seeds are high in protein. In addition to protein, it mainly contains minerals, polyphenols, folic acid and carbohydrates **[6, 7, 8]**.

c)***Vigna Mungo (black dal)***:-.It is a rich source of proteins, vitamins, antioxidants & phenolics. Traditionally, the seeds of *Vigna Mungo* are used as food & leaves as vegetable. It is plentiful in all essential amino acids such as histidine, tryptophan, and isoleucine. Its seed coat contains large quantities of endogenous antioxidants such as phenolic compounds.

d) ***Phaseolus vulgaris***: ***(rajma)-***It is considered as important food crop and major source of protein throughout the world. It also contains a large number of

phytochemical compounds including phenolics, flavonoids, vitexin and isovitexin etc. It also contains starch, vitamins, and fructo-oligosaccharides which have been known to protect the body from oxidative stress, heart disease, diabetes, cancer, and metabolic syndrome.

MATERIAL AND METHODS

2.1Plant material

Seeds of *Vigna radiata, Cicer arietinum, Vigna Mungo and Phaseolus vulgaris* were collected from a local mill. All these seeds were sterilized using 2% of Bavistin to avoid fungal infection. The soaked grains seeds were sown in triplicates in pots having normal soil. Watering of pots was done regularly so that the soil remained moist. Sprouting and germination were carried out in an uncontrolled temperature (average 24-25°C). The grasses were grown in the laboratory in daylight. The room was subjected to a diurnal cycle with fluctuations of natural temperature, humidity and light.

2.2 Preparation of sample

The sprouts were harvested on day 5th after sowing and all the suspended dirt particles were thoroughly removed. Nearly 1-2gm of samples were weighed and washed. The leaves were crushed using a mortar/ pestle and dissolved properly in 10ml of sterilized water to make the mixture.

2.3 Estimation of total protein content

The total protein content was determined using Bovine Serum Albumin (BSA) as a standard spectrophotometrically **[8]**. The sample extract (1ml) was added to test tubes containing 1ml of distilled water and 5ml of alkaline solution.0.5 ml of Folin-Ciocalteu solution was added to each test tube and incubated for 30 minutes at room temperature. The absorbance was measured spectrophotometrically at 750 nm. The total protein content was calculated and results were expressed in mg/ml.

2.4 Estimation of total phenol content (TPC)

The total phenol content (TPC) was determined spectrophotometrically using tannic acid as a standard **[8]**. 0.2ml of the diluted sample extract (in triplicate) was added to tubes containing 0.125ml of Folin-Ciocalteu's reagent and 2.5ml of distilled water. Then, 1.25ml of 7% sodium carbonate solution was added and incubated at room temperature for 90 minutes. The absorbance was measured at wavelength 760nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of tannic acid equivalent per g dry weight (mg TAE/g).

2.5 Determination of Total flavonoid content

Total flavonoid content was measured by the modified aluminum chloride colorimetric assay **[8]**. The reaction mixture

consisted of 1.0ml of extract and 4ml of distilled water taken in the test tube. 0.30ml of 5% sodium nitrite was added and after 5 minutes, 0.3 ml of 10% aluminum chloride was mixed. After 5 minutes, 2.0ml of 1M Sodium hydroxide was added and the final volume of the mixture was brought to 10ml with double-distilled water. The absorbance for test and standard solutions were determined against the reagent blank at wavelength 510nm with an UV/Visible spectrophotometer. The total flavonoid content was calculated from the calibration curve and was expressed as mg Ascorbic acid equivalent (AAE)/g of extract.

2.6 Determination of antioxidant power by using modified ferric ion reducing antioxidant power assay (FRAP)

The total antioxidant capacity was determined by spectrophotometry, using the modified FRAP assay **[8]**. 0.1ml of extract was taken and to it 0.9 ml of ethanol, 5.0ml of distilled water, 1.5ml of 1M HCl, 1.5ml of 1% potassium ferricyanide, 0.5ml of 1% SDS and 0.5ml of 0.2% of ferric chloride was added. This mixture was boiled in a water bath at 50°C for 20 minutes and cooled rapidly. Absorbance was measured at wavelength 750 nm to measure the reducing power of the extract. The antioxidants in samples were derived from a standard curve of ascorbic acid and were expressed as mg ascorbic acid equivalent (mgAAE)/ g.

2.7 Estimation of ascorbic acid

The Ascorbic acid was measured spectrophotometrically by 2,4-DNPH method. 0.3ml of extracts was pipetted out in test tubes **[8]**. To all the test tubes containing extract, distilled water was added to make up to 1.5ml. To all the test tubes, 0.5 ml of 2, 4- DNPH was added and after proper mixing, test tubes were incubated at 37º C for 3 hours. 3.5ml of 80% H2SO4 was added to the test tubes to dissolve the orange red osazone crystals formed and absorbance was spectrophotometrically measured at wavelength 540nm. It was expressed in mg/ml.

2.8 Estimation of Reducing sugars

Sugar acts as a reducing agent that reduces 3,5-dinitrosalicylic acid under alkaline medium to form an orange red coloured product which was measured spectrophotometrically at 540nm.1ml of extract was taken in the test tube. The distilled water was added so as to make the volume up to 3ml. 1ml of DNS reagent was added in each test tube and incubated for 15 min in a boiling water bath. The test tubes were cooled & absorbance was measured.

Statistical Analysis:-

The assays were carried out in triplicate, and the results were expressed as mean values and the standard deviation (SD). The statistical differences were done by one-way ANOVA ($p \leq 0.05$).

RESULTS AND DISCUSSION

Germination can cause changes in phytochemical compounds and their functional properties due to aerobic respiration and biochemical metabolism. Among sprouts of pulses, highest protein levels were found in urad dal (*Vigna mungo)* (2083.3mg/ml), followed by moong dal (*Vigna radiata)* (1813mg/ml), rajma (*Phaseolus Vulgaris)* (1350mg/ml) and black gram (*Cicer arietinum)* (1079.2mg/ml). *Vigna mungo* has significantly higher protein levels than *Phaseolus Vulgaris* and *Cicer arietinum* ($p \leq 0.05$). However, the protein levels were similar in *Vigna mungo* and *Vigna radiata*. It has been observed that the germination causes the activation of enzymes like protease which immediately converts protein molecules into amino acids as reported earlier in *Phaseolus Vulgaris* **[10]**. Protease also increases the protein digestibility value which is a major determinant of protein quality in foodstuffs **[11]**. The high protein contents could be due to the increased hydrolytic activities of the enzymes caused by sprouting which results in high protein contents due to the disappearance of starch **[12]**.

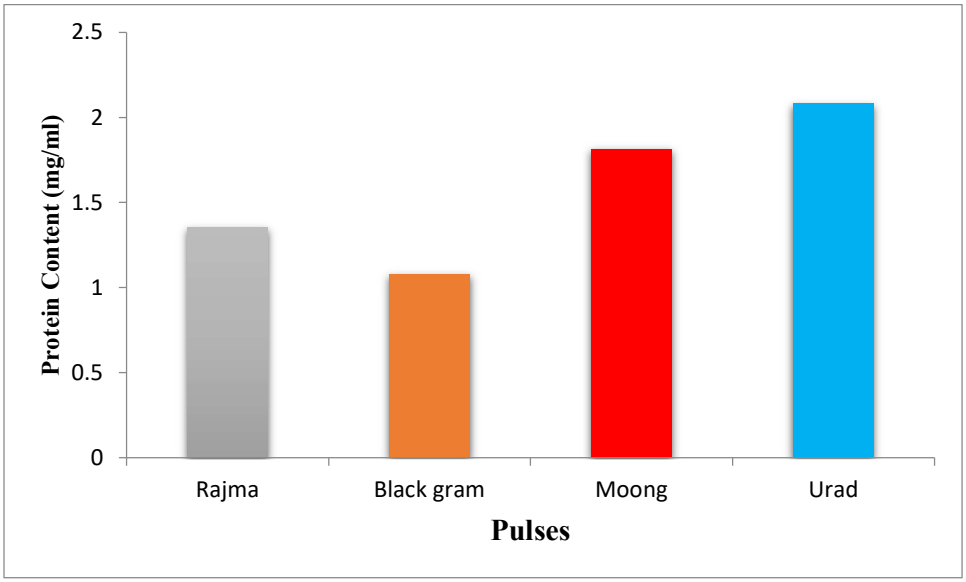

Figure 1: Graph showing the total protein content among selected pulses

The flavonoids present in pulses are involved in stress protection i.e., oxidative and temperature stress), early plant development and signaling i.e., legume nodulation. The highest flavonoid levels were found in urad dal (*Vigna mungo)* (708.4 mgAAE/gm) followed by black gram (*Cicer arietinum* 702.18 mgAAE/gm), *moong dal (Vigna radiata)* (561.9 mgAAE/gm) and rajma (*Phaseolus*

Vulgaris) (542.3 mgAAE/gm). The increased amount of flavonoids in *Cicer arietinum seeds* has also been reported by Khyade and Jagtap **[13]**. *Vigna mungo* (708.44 mgAAE/gm) has significantly higher flavonoid levels than *Phaseolus Vulgaris* (542.3 mgAAE/gm) and *Vigna radiata* (561.9 mgAAE/gm) ($p \leq 0.05$). In *Vigna radiate* various flavonoids such as flavone, isoflavone, flavonoids, and isoflavonoids are found **[14]**.

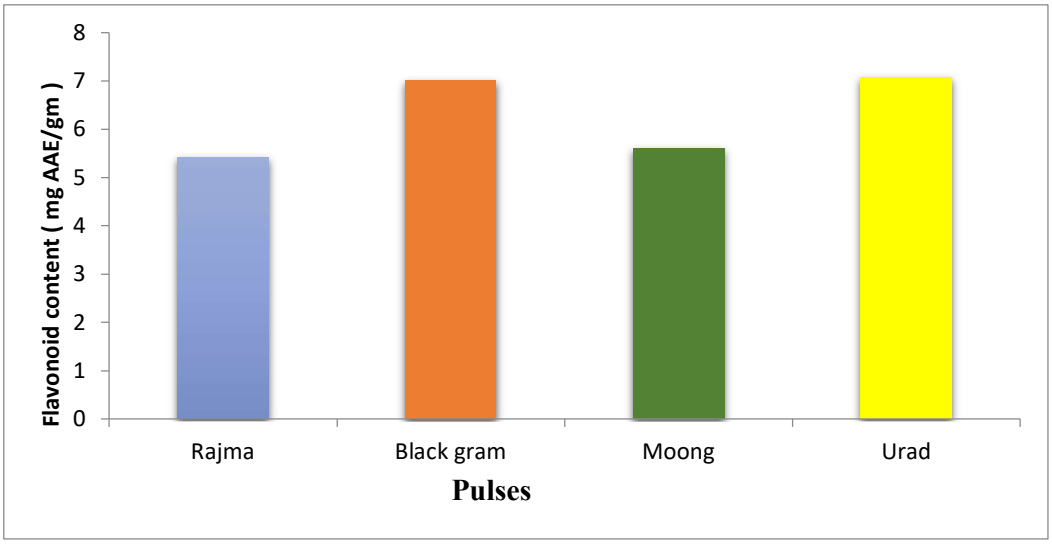

Figure 2: Graph showing the total flavonoid content among selected pulses

The major phenolics present in legumes consists of protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, vanillin, ferulic acid, sinapic acid, *p*-coumaric acid, benzoic acid, ellagic acid and cinnamic acid **[15]**. The highest levels of phenolics were found in urad dal (*Vigna mungo)* (10.432mgTAE/gm), followed by rajma (*Phaseolus Vulgaris*) (6.896mgTAE/gm), *moong dal (Vigna radiata)* (6.579mgTAE/gm) and black gram (*Cicer arietinum)* (5.277mgTAE/gm). *Vigna mungo* has significantly higher phenolics than *Phaseolus Vulgaris* and *Vigna radiata* ($p \leq 0.05$). It has been reported that Vigna *mungo* seeds have high phenolic content in as compared to Cicer *arietinum* on sprouting **[13]**. It has been found that during sprouting gentistic acid, cinnamic acid, and *p*-hydroxybenzoic acid are the major phenolic acids found in pulses **[16]**. In *Vigna radiata*, the levels of caffeic acid, ferulic acid, and shikimic acid increases during sprouting **[17]**. The water absorption by seed during sprouting hydrolyzes various enzymes to produce various phenolic compounds by strengthening the enzyme-substrate interaction. Thus, sprouts can be consumed as phenolic-rich foods and can reduce the incidence of inflammation and cancer **[18]**.

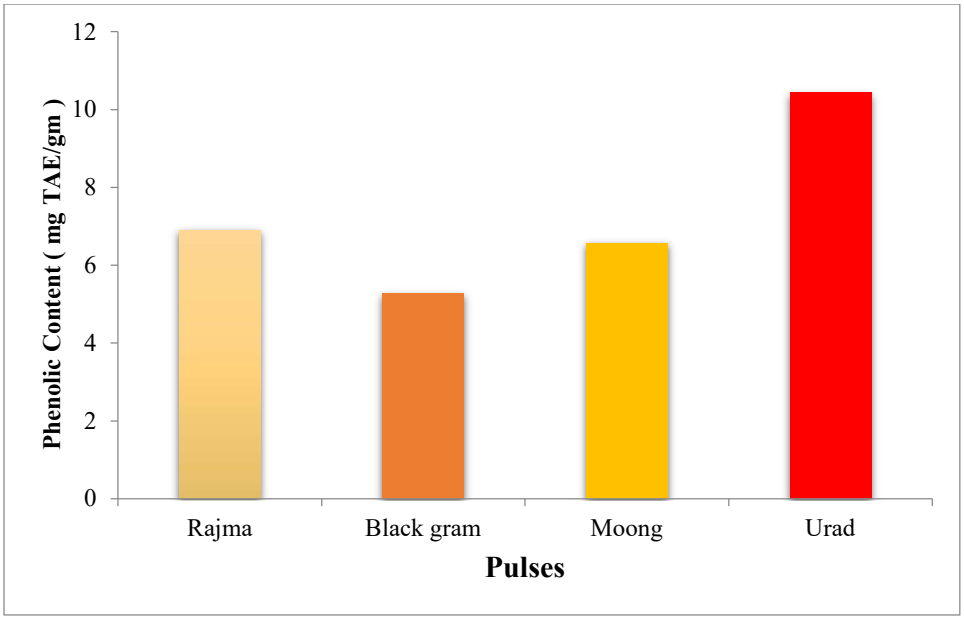

Figure 3: Graph showing the total phenolic content among selected pulses

The ascorbic acid, which is either completely absent or present in negligible amounts in dry legumes is synthesized during the germination process **[15]**. In the present study, the presence of ascorbic acid during sprouting could be due to deactivation of ascorbic acid biosynthesis by enzymes such as L-Galactono- γ-lactone dehydrogenase **[19]**. The highest ascorbic acid levels were found in urad dal (*Vigna mungo)* (0.9mg/ml) followed by rajma (*Phaseolus Vulgaris)* (0.76mg/ml), chick pea (*Cicer arietinum)* (0.7mg/ml) and moong dal (*Vigna radiata)* (0.69mg/ml). *Vigna mungo* has significantly higher ascorbic acid than *Phaseolus Vulgaris* and *Cicer arietinum* ($p \leq 0.05$). The high amount of ascorbic acid in *Vigna mungo* seeds as compared to *Cicer arietinum* has also been reported by Khyade and Jagtap **[13]**. Among Vigna *radiata* and *Vigna mungo*, Vigna *mungo* has significantly higher levels of ascorbic acid than *Vigna radiata*. ($p \leq 0.05$). The difference in level of biosynthesis of ascorbic acid with sprouting in various beans in the study might be attributed to the legume type, maturity, climatic conditions, light conditions, harvesting and storage methods **[20]**.

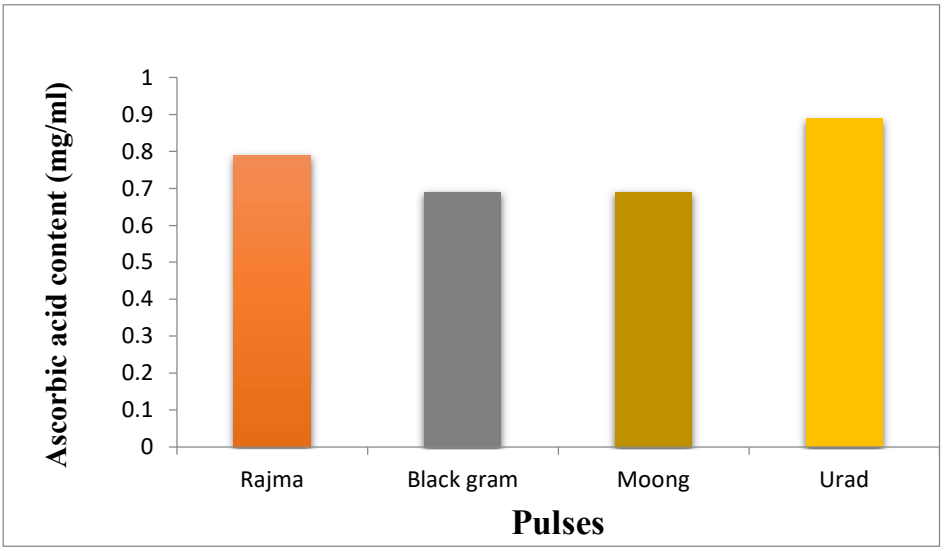

Figure 4: Graph showing the total ascorbic acid content among selected pulses

Germination leads to the enzymatic breakdown of carbohydrates into simple sugars through activation of endogenous enzymes such as α-amylase thereby improving digestibility. The highest maltose and glucose content were found in urad dal (*Vigna mungo)* (133.91mg/ml) followed by moong dal (*Vigna radiata)* (95.871mg/ml), rajma (*Phaseolus Vulgaris)* (83.56mg/ml) and black gram *(Cicer arietinum* (49.55mg/ml). The α-amylase activity breaks down complex carbohydrates to simpler sugars which were utilized by the growing seedlings in the initial stages of germination **[21]**. The increased amount of soluble sugar such as maltose and glucose could be due to the presence of invertase which hydrolyzes sucrose into glucose and fructose during germination.

Antioxidant capacity of plants is usually due to the presence of phenolic compounds consisting of phenolic acids, tannins, and flavonoids. The antioxidant levels in sprouts were found in order as rajma (*Phaseolus Vulgaris)* (1.7357mgAAE/gm), urad dal (*Vigna mungo)* (1.5821mgAAE/gm), moong dal (*Vigna radiata)* (1.5285mgAAE/gm) and black gram *(Cicer arietinum)* (1.3607mgAAE/gm). The high antioxidant level reported in *Phaseolus Vulgaris* was also observed earlier **[22]**. It has been found that antioxidant activity of legumes are correlated with phenolic acids rather than flavonoids **[23]**. In legumes, phenolic acid such as Sinapic acid contributes maximum to antioxidant activity of legume phenolics due to presence of both 3,5-dimethoxyl and 4-hydroxy groups **[9]**.

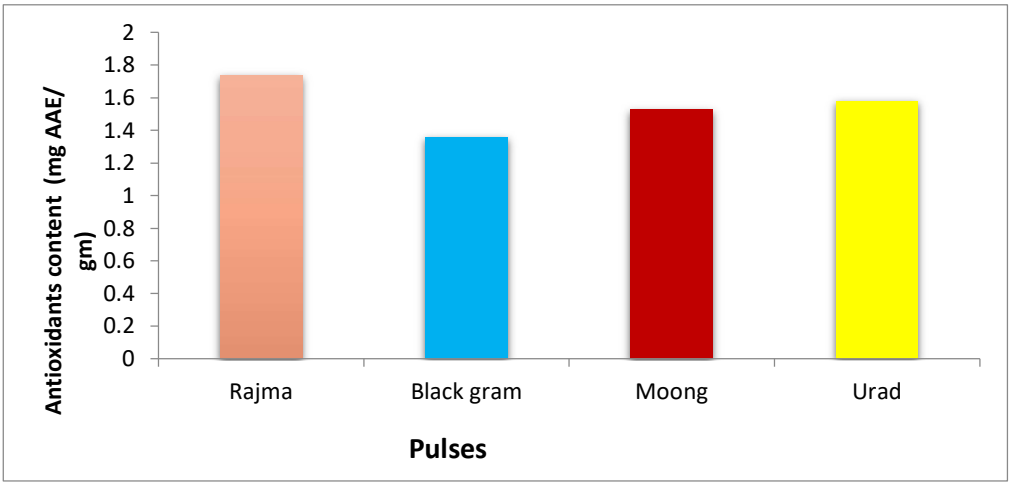

Figure 5: Graph showing the total antioxidant content among selected pulses

Since there is an increase in the nutritive value of the seeds during sprouting, it can be concluded that germination brings significant change in the micronutrient, phytonutrient content of all selected seeds. These sprouts should be considered a vital component of the economically balanced diet and can be incorporated with a variety of variations. The germination process in sprouts also reduces the glycosides saponins and sapogenin contents which cause a bitter taste in legumes and then convert them to sugar. Thus sprouts can be used in a variety of antioxidant rich food products e.g., Phaseolus *Vulgaris* sprout milk has a delicious taste and flavor resulting from the breakdown of proteins with the reducing sugars.

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