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Estimation of *ergosterol* in dry Fruits Collected from Different Regions of Rewa District

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ABSTRACT: The high quantities of beneficial components, particularly pectin and phenolic acids, as well as the nutritional content, convenience, and durability of dry fruits make them a popular snack. The unique physical, chemical, biological, and multifunctional capabilities of phenolic compounds are due to their basic structure, which is an scented ring with "one or more hydroxyl groups, along with various functional groups". Unlike phenolic acids, stilbenes, and their derivatives, which are not considered flavonoids, anthocyanins and flavonols are. Because of their strong antioxidant activity, which inhibits oxidative damage, the phenolic acids found in plants have a practical use. Conservation becomes a serious issue as a result. The quick spoilage of climacteric fruits is a big problem, which is why there are a lot of processing technologies for turning pulp into juice, nectar, jam, and jellies.

Keywords: Beneficial components, Dry fruits, Snack, Phenolic acids, antioxidant activity.

INTRODUCTION

Fruits, in general, and climacteric or ethylene contingent fruits in particular, are perishable due to their short shelf life after harvest, a peak in respiration, and the ability to ripen post-harvest through the production of ethylene (Ahmed *et al*., 2010). In both the initial and subsequent avoidance of NCDs, nutrition is an important factor. One of the most important dietary recommendations for preventing NCDs is to eat plenty of "fruits and vegetables". Eating three to five servings of fruits and vegetables per day can reduce your risk of non communicable diseases, according to a plethora of research. For extended periods of time, dried fruits and nuts are preferable to fresh fruits due to their longer shelf life. The export of agricultural products is crucial to the economies of emerging nations, as these exports form the backbone of their economies. "Protein, fatty acids, potassium, dietary fibers, and bioactive chemicals are all found in dried fruits and nuts, making them the ideal source of these nutrients. The third Drying is one of these methods; it is a very old method of food preservation that is still used today". The chemical composition of fruits can be altered by thermal treatment, which can increase, reduce, or have no discernible effect on the bioavailability, content, and antioxidant activity of these fruits. A concise description of the effects of various drying processes on fruits is, hence, the goal of this work. The process of drying frequently alters the product's nutritional value, texture, shape, and flavor. These changes might either be a benefit or a drawback. The study conducted by Akoy *et al.* (2008) indicates that the color of dried-up mango slices at various temperatures (60, 70, and 80 °C) is significantly influenced by the drying duration. According to Alasalva *et al.* (2013) research on Niger mangoes, dried mangoes that were pre-treated retained nutrients like vitamin C and β-carotene. This makes them not only a healthy and nutritious food ingredient, but also a key component in the creation of functional foods and dietary supplements. When compared to fresh mangoes, the "antioxidant capacity of sliced mangoes dried by various methods (freezing, microwave (120 and 350W) and hot air (60, 70, and 80 °C)) dropped by 18.4-54.6%". Surprisingly, the sample dried by microwave at 350W had the highest phenolic content, while the one dried by hot air at 80°C had the lowest.

WALNUT : A variety of names are used to describe walnuts (*Juglans regia* L.) around the globe. In Hindi it is akhrot, in Kashmiri it is doon, and in Unani it is gardgani, the most popular term. The insignificant name walnut comes from the old English word "nutu," meaning "foreign nut" (a word that is related to Welsh and Vlach). The walnut's name comes from its origins in Gaul and Italy. The walnut was originally called the "Gallic nut" (nux Gallica) in Latin. The name Jovis glans, meaning Jupiter's Acorn, is the etymological ancestor of the Latin name Juglans regia. Legend has it that during the classical golden period, the gods Jupiter and Zeus feasted on walnuts while mortals subsisted on acorns. Wallnuss, meaning "foreign nut" in German, is whence the contemporary name originates. California is the world's leading producer of walnuts, although its cultivation in Europe really took off in the 1500s. Walnuts are native to the Indian states of Darjeeling and Sikkim, which are located in the northwest Himalayan area. We don't know anything about walnuts' past from antiquity. Slate believes it predates humans based on fossil evidence,

however Alasalva and Shahidi (2013) notes its global spread from Asia Minor.

Composition and uses: The plant's nearly every part is put to good use, but the fruit and wood have seen their full potential.

Kernel: The table below shows the composition of the walnut kernel, which is the edible part and accounts for approximately half of the overall weight of the fruit (Al-Farsi and Lee 2008). The concentrated energy in walnuts comes from their high protein, fat, and mineral content. Vitamin B-6 is most abundant in it, and it also contains a substantial quantity of other B-group vitamins. A variety of names are used to describe walnuts (*Juglans regia* L.) around the globe. In Hindi it is akhrot, in Kashmiri it is doon, and in Unani it is gardgani, the most popular term. The origins of the innocuous name walnut is obscure; it originates with the Old English word "nutu," which means "foreign nut" (wealhh is similar to the words "Welsh" and "Vlach"). The walnut's name comes from its origins in Gaul and Italy. The walnut was originally called the "Gallic nut" (nux Gallica) in Latin. The name Jovis glans, meaning Jupiter's Acorn, is the etymological ancestor of the Latin name Juglans regia. Legend has it that during the classical golden period, the gods Jupiter and Zeus feasted on walnuts while mortals subsisted on acorns. Wallnuss, meaning "foreign nut" in German, is whence the contemporary name originates. California is the world's leading producer of walnuts, although its cultivation in Europe really took off in the 1500s. Walnuts are native to the Indian states of Darjeeling and Sikkim, which are located in the northwest Himalayan area. We don't know anything about walnuts' past from antiquity. Alsaif *et al.* (2007) uses fossil evidence to suggest it predates humans, although notes its global expansion from Asia Minor.

Composition and uses: The fruit and timber have been utilized to their fullest potential, but nearly every element of the plant has found some use. The edible part of a walnut, called the kernel, makes up almost half of the fruit weight; the table below shows the components of the kernel according to Alvarez-Parrilla *et al*. (2013). A concentrated source of energy, walnuts are also rich in minerals, lipids, and proteins. It is the most abundant source of vitamin B-6 and contains a considerable quantity of other B-group vitamins. Walnut, scientifically known as *Juglans regia* L., goes by many names in different parts of the globe. "Akhrot" in Hindi, "Doon" in Kashmiri, and "Gardgani" in Unani are the most popular names. Old English wealhh nutu means "foreign nut" (as in Welsh and Vlach), which is an etymologically unrelated word to the more common walnut. The walnut got its name from the fact that it originated in Gaul and Italy. Nux Gallica, meaning "Gallic nut" in Latin, was the original name for the walnut. Juglans regia is a Latin name that comes from Jovis glans, meaning Jupiter's Acorn. During the classical golden age, it was stated that the gods gorged themselves on walnuts, while mortals subsisted on acorns. But the contemporary name derives from the German word for "foreign nut," Wallnuss. Walnut orchards first sprang up in Europe in the 1500s, but today you can find them all around the globe, with California being the top producer. The northwest Himalayan area, which extends into Darjeeling and Sikkim, is where you may find walnut trees in India. In antiquity, walnut's history disappeared.

MATERIALS AND METHODS

A. Collection of dry fruits during different seasons:

Sr. No.	Name of samples	Number of samples in Summer Season	Number of samples in Winter Season	Number of samples in Rainy Season	Distributer in Rewa District
1.	Walnut	03	03	03	Kamal Kirana Store, Prakash Chowk, Rewa
2.		03	03	03	Bajrang Gupta Store, PTS, Rewa (M.P.)
3.		03	03	03	ABC Store Market, Near Kothi, Rewa (M.P)

B. Samples collection during the research

A total of nine analytical examples were prepared from walnuts; “all samples were within their expiration dates, and each lot of the exact same fruit had a similar” producing date. All collections were collected from the dry fruit distributors and were of the same publicly traded brand. This was done for the purpose of the experiment. The laboratory at Govt. Science College, Rewa (M.P.) 486001 was where all the samples were kept for future experiments. Rewa (M.P.) 486003 is the location of the Institute for Biotechnology and Microbiology Studies, which also handled some of the samples and conducted the experiments. The homogenized samples were kept in a refrigerator (5°C) until they were needed.

Processing of samples: Crushing the samples and adding one gram to ten milliliters of sterile distilled water, then vortexing to create a homogeneous suspension, were the steps taken to isolate the fungi. After homogenizing the suspension, it was diluted in a series of steps and streaked over Sabouraud's Dextrose agar (Oxoid) using a sterile cotton swab. The mixture was then incubated at 25°C for a minimum of one week. Colony forming units per gram (cfu/g) were determined by counting the colonies that developed on Sabouraud's Dextrose agar (Oxoid) plates after incubation. Both gross and microscopic features helped to identify the distinct fungal strains that were isolated.

Macroscopic characteristics: After incubation, the macroscopic features (colony color, texture,

pigmentation, and reverse) of the fungal colonies were carefully examined and documented. Using the following approaches, fungal strains were examined under a microscope.

Method of wet mounting: This method involved using "a sterile fungus needle to deposit a small piece of fungal growth into a drop of lacto phenol cotton blue (LPCB) that had been placed on a clean, grease-free slide". Using a sterile fungal needle, the growth was delicately teased.

RESULTS

Collection and processing of samples: Near the Rewa district, we gathered 90 samples of dried fruits and nuts using the convenience sampling method. The samples were placed in clean polyethylene bags and collected from approved market distributors and entire sale distributors. Before being put to use, the bags were appropriately labeled, sealed, and stored in the refrigerator. Crushing the samples and adding one gram to ten milliliters of sterile distilled water, then vortexing to create a homogeneous suspension, were the steps taken to isolate the fungi. After homogenizing the suspension, it was diluted in a series of steps and streaked over Sabouraud's Dextrose agar (Oxoid) using a sterile cotton swab. The mixture was then incubated at 25°C for a minimum of one week. Colony forming units per gram (cfu/g) were determined by counting the colonies that developed on Sabouraud's Dextrose agar (Oxoid) plates after incubation. Both gross and microscopic features helped to identify the distinct fungal strains that were isolated.

Microscopic characteristics: Using the following approaches, fungal strains were examined under a microscope. Method of wet mounting: This method involved using a sterile fungus needle to deposit a small piece of fungal growth into a sample of lacto phenol cotton blue (LPCB) that had been placed on a clean, grease-free slide. Using a sterile fungal needle, the growth was delicately teased.

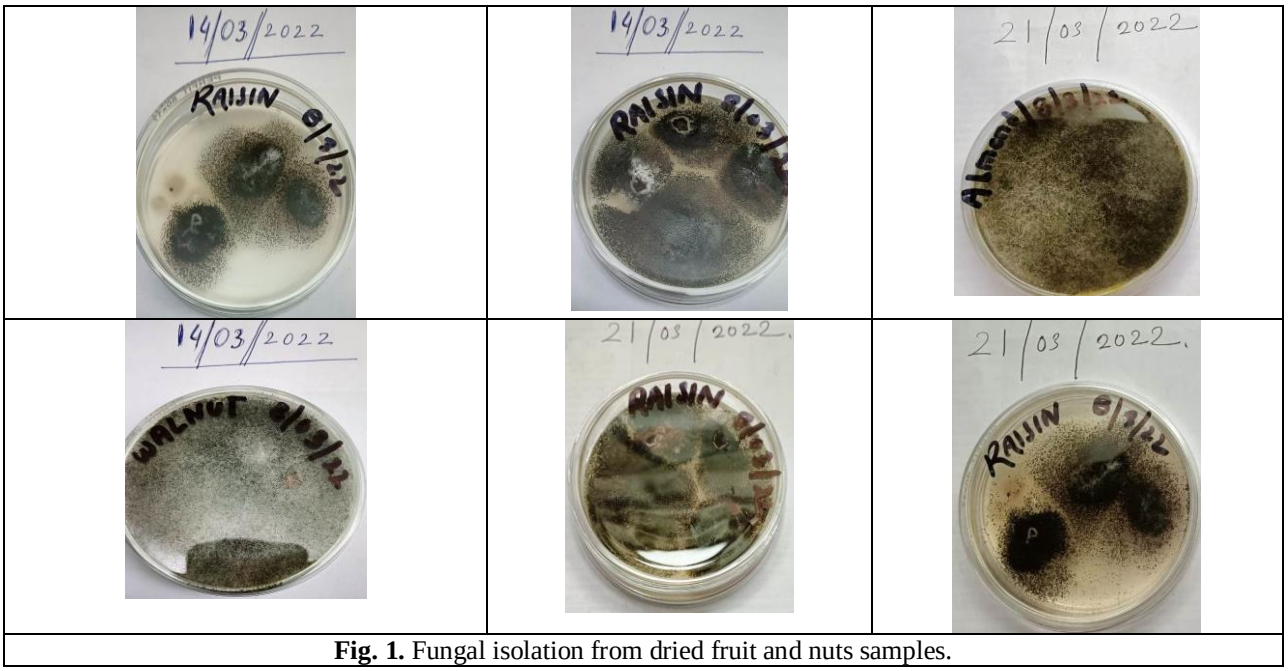

Fig. 1. Fungal isolation from dried fruit and nuts samples.

Slide culture technique: Using the slide culture method, we were able to identify the fungal strains that had proven elusive using the wet mount technique. Using this method, a sterile petri dish was placed on a bend rod and a sheet of filter paper was placed on top. After setting the grease-free slide on the glass rod, a sterile scalpel was used to cut a 1 × 1 cm block of agar off the SDA plate and transfer it to the middle of the glass slide. In the middle of the agar block, a sterile fungus needle was used to inoculate the fungal culture. The next step was to press a cover slip onto the block to make sure it stayed put. After adding 1.5 ml of sterilized distilled water to the bottom from the petri dish, it was left to incubate at ambient humidity for a period of 2 to 5 days. Anderson *et al*. (2011) first described this method, which is a sensitive and fast way to distinguish between aflatoxin-producing and non-producing Acinetobacter flavus and Acinetobacter parasiticus strains. Here, a Petri dish containing potato dextrose agar was used to cultivate a single fungus colony. Next, 1 or 2 drops of a highly concentrated ammonium hydroxide were added to the inside of the lid after inverting the plate. According to Arkoub *et al.* (2016) colonies that produce aflatoxin have their backs become plum red, while colonies that do not produce aflatoxin do not undergo any such change.

Ultra violet (UV) photography: It is a quick method for identifying aflatoxin-producing fungi. By exposing "fungal colonies cultured on Potato Dextrose agar to UV light at 365 nm", aflatoxin-producing strains may be identified using this method. When exposed to ultraviolet light, aflatoxigenic strains fluoresced, whereas negative strains did not Bays (2014).

The method's repeatability was assessed using the relative average deviation (CV, %). Tables 1 and 2 demonstrate the results of using this method to determine the total quantity of ergosterol in 10 different fungus species.

Table 1: Isolation of fungal starins from different dried fruits and nuts samples.

Sr. No.	Name of samples	Total number of fungal samples (cfu/g)	Mean	Standard Error Mean	Isolated fungal strains
1	Walnut (n=6)	36	6.000	0.258	*A. flavus, A. terrus, A. niger, A. fumigates, A. versicolor, Absidia* spp.
Total isolates		**36**			

Table 1: Contents of ergosterol in 10 fungal species.

Isolated fungal spp.	Co (ppm)	Peak-area	C (g/l)	CV (%)
Aspergillus flavus	0.866	233.389	0.006	0.148
Aspergillus niger	30.601	7717.287	0.031	0.011
Aspergillus glaucus	0.776	212.152	0.008	0.654
Aspergillus fumigatus	7.018	1780.298	0.001	0.06
Aspergillus terreus	1.390	366.428	0.009	0.010
Aspergillus versicolor	7.114	1805.774	0.001	0.015
Penicillium	4.708	1200.779	0.001	0.018
Rhizopus	5.457	1387.866	0.005	0.017
Chaetomium	7.732	1962.527	0.003	0.011
Curvularia	4.488	1145.173	0.009	0.00735

Table 2: Contents of ergosterol peroxide in 10 fungi species.

Isolated fungal spp.	Co (ppm)	Peak-area	C (g/l)	CV (%)
Aspergillus flavus	0.134	126.494	0.004	4.750
Aspergillus niger	2.370	2179.747	0.007	1.064
Aspergillus glaucus	0.032	28.783	0.003	4.851
Aspergillus fumigatus	0.030	28.578	0.003	3.672
Aspergillus terreus	0.056	54.428	0.006	2.233
Aspergillus versicolor	0.034	36.534	0.004	1.979
Penicillium	0.034	32.030	0.004	4.863
Curvularia	0.022	26.080	0.003	5.184

Physical-chemical and functional characterization of dehydrated fruits. Because there was a fluctuation of more than 10% across the different batches of the same variety of fruit, the chemical contents of the dried fruit samples are shown as a range of values. Given that each sample was boosted by different lots in addition to the inherent heterogeneity in fruit composition, this behavior was anticipated. The specified specifications, which advise a maximum moisture level of 25% for prune, apricot, walnut, almond, cashew, pine, pistachio, rasin, and packed dry date samples, were not met. Based on the moisture levels, it appears that these products could be contaminated with microbes, particularly fungi. The greatest difference among lots' moisture levels was observed in prune samples.

DISCUSSION

This research set out to identify and characterize fungal strains in dried fruits and nuts peddled by street vendors at the District Rewa market in Madhya Pradesh, India. In this work, a total of 385 fungal strains, including Aspergillus, Penicillium, Rhizopus, Candida spp., and others, were recovered from the mycological profile of dried fruits (Table 1, Fig. 1). Most often found fungus species were *Aspergillus niger* (25.8%), *Aspergillus flavus* (19.3%), and *Aspergillus fumigatus* (11.8%). Prior to HPLC analysis, the samples were methanol extracted to remove ergosterol and ergosterol peroxide. The chromatographic run was terminated after 20 minutes of utilizing a “mobile-phase gradient consisting of a mixture of MeOH and MeCN in various ratios (85:15, 80:20, 75:25, etc.) on a reversed-phase RP 18 (150 × 4.6 mm, 5 µm) column”. We began by combining 2 grams of dry powder with 24% humidity and 20 milliliters of an 85:15 (v/v) mixture of MeOH and MeCN. The mixture was then extracted by swirling it in an ultrasonic bath set at 4°C for 30 minutes. Afterwards, the concoction was spun in a centrifuge at 3,500 rpm for ten minutes. Two further extractions were performed on the residue, and the resulting extracts were mixed. The models were kept in a dark place at 4°C until they were analyzed using HPLC.

CONCLUSIONS

The study titled "Estimation of Ergosterol in Dry Fruits Collected from Different Regions of Rewa District" provides valuable insights into the fungal contamination levels in commercially distributed dry fruits within the region. By quantifying ergosterol—a biomarker for fungal presence—the research highlights the extent of contamination, which is crucial for ensuring food safety and quality. The findings underscore the necessity for stringent monitoring and control measures during the processing and storage of dry fruits to mitigate fungal growth. Implementing proper handling practices can significantly reduce contamination risks, thereby

safeguarding consumer health. Moreover, the study emphasizes the importance of regular surveillance of dry fruits in the market. Such proactive measures can help in early detection of contamination, allowing for timely interventions to prevent potential health hazards associated with fungal toxins.

CONCLUSIONS

This research serves as a foundation for developing comprehensive strategies aimed at enhancing the safety and quality of dry fruits in Rewa District. It calls for collaborative efforts among producers, distributors, and regulatory bodies to implement effective contamination control measures, ensuring the well-being of consumers.

FUTURE SCOPE

Investigating the ergosterol content in dry fruits from various regions of Rewa District offers several avenues for future research can enhance the safety, quality, and nutritional value of dry fruits in Rewa District.

REFERENCES

Ahmed, T., Sadia, H., Khalid, A., Batool, S. & Janjua, A. (2010). Report: Prunes and liver function: A clinical trial. *Pakistan Journal of Pharmaceutical Sciences*, *23*, 463–466.

Akoy, A. O. M., Hörsten, D. V. & Luecke, W. (2008) Drying kinetics and colour change of mango slices as affected by drying temperature and time. Tropentag International Conference on Competition for Resources in a Changing World: New Drive for Rural Development, Hohenheim, Germany.

Alasalvar, C. (2013). Functional characteristics of dried figs. In C. Alasalvar & F. Shahidi (Eds.), *Dried fruits: Phytochemicals and health effects* (pp. 284–299). Oxford: Wiley-Blackwell.

Al-Farsi, M. & Lee, C. Y. (2008). Nutritional and functional properties of dates: A review. *Critical Reviews in Food Science and Nutrition*, *48*, 877–887.

Alsaif, M. A., Khan, L. K., Alhamdan, A. A., Alorf, S. M., Harfi, S. H., Al-Othman, A. M. & Arif, Z. (2007). Effect of dates and gahwa (Arabian coffee) supplementation on lipids in hypercholesterolemic hamsters. *International Journal of Pharmacology*, *3*, 123–129.

Alsaif, M. A., Khan, L. K., Alhamdan, A. A., Alorf, S. M., Harfi, S. H., Al-Othman, A. M. & Arif, Z. (2007). Effect of dates and gahwa (Arabian coffee) supplementation on lipids in hypercholesterolemic hamsters. *International Journal of Pharmacology*, *3*, 123–129.

Alvarez-Parrilla, E., de la Rosa, L. A., González-Aguilar, G. A. & Ayala-Zavala, J. F. (2013). Phytochemical composition and health aspects of peach products. In C. Alasalvar & F. Shahidi (Eds.), *Dried fruits: Phytochemicals and health effects* (pp. 309– 324). Oxford: Wiley-Blackwell.

Anderson, J. A., Andersen, K. F., Heimerman, R. A., Larson, M. M., Baker, S. E., Freeman, M. R., Carughi, A. & Wilson, T. (2011). Glycaemic response of type 2 diabetics to raisins. *FASEB Journal*, *25*, 587.5.

Anderson, J. W. & Waters, A. R. (2013). Raisin consumption by humans: Effects on glycemia and insulinemia and cardiovascular risk factors. *Journal of Food Science*, *78*, A11–A17.

Arkoub, D. L., Boulekbache, M. L., Zeghichi, H. S., Bellili, S. and Boukhalfa, F. (2016) Influence of the thermal processing on the physico-chemical properties and the antioxidant activity of a Solanaceae vegetable: Eggplant. *Journal of Food Quality, 39*(3), 181-191.

Bays, H. E. (2014). Raisins compared with other snack effects on glycaemia and blood pressure: A randomized, controlled trial. *Postgraduate Medicine*, *126*, 37–43.

Bays, H.,Weiter, K. & Anderson, J. (2015). A randomized study of raisins versus alternative snacks on glycemic control and other cardiovascular risk factors in patients with type 2 diabetes mellitus. *Physician and Sportsmedicine*, *43*, 37–43.

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