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Time Course and RPM Based Studies for Its Effect on Various Proteins in Dry Fruits

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Abstract

The objective of this study was to identify the difference in protein profile of dry fruits and its concentration level in response to variance in rpm and time. Cashew, Dates and Currant were selected and prepared as smoothie using phosphate buffer saline and distilled water. Time based and varying rpm levels studies were also carried out followed by SDS-PAGE analysis to determine the protein profiling. Dry fruit protein concentration was found higher at a value of 735mg at 15000rpm for 10 minutes for cashew and value of 509mg at 10000rpm for 15 minutes for dates, while in currant it was found comparatively less with a value of 366mg concentration. SDS-PAGE analysis also revealed the difference in the purity of the bands among cashew, date and currant proteins. Prospective work might include expanding our studies on complete purification of proteins and establish the role of properties and functions of dry fruit proteins.

Keywords: Cashew, Dates, Currant, RPM, Time Course, Dry Fruits

INTRODUCTION

A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system for their interesting antioxidant activities [1]. Seeds in a fruit provides an interesting and often essential complementary means for controlling the quality and stability of numerous food products [2]. A technique has been developed for preparation of powders of fruit tissues for protein studies [3]. Proteins are extracted in buffer and studied by means of disk electrophoresis on polyacrylamide gel [4]. The resulting patterns are reproducible, and appear to be characteristic for a particular tissue of a given fruit. Hawthorn fruit extract has been shown to have many health benefits including being cardiovascular protective, hypotensive and hypercholesterolemia [5]. The protein composition of the grape berry was examined from flowering to ripeness by gel electrophoresis. A protein with an apparent molecular mass of 24 KDa, which was one of the most abundant proteins in extracts of mature berries [6]. Among all nuts, almonds and hazelnuts had the highest mean α-tocopherol content, results show the heterogenic amounts of antioxidants in nuts, which emphasises the recommendation of a mixed nuts intake [7]. Edible parts of date palm demonstrate the potential of dates as antioxidant functional food ingredients [8]. Small berries such as black currant constitute one of the important sources of potential health-promoting phytochemicals because these fruits are rich sources of compounds with high antioxidant properties [9]. Use of herbal intervention is widespread and Indian traditional dry fruit plant *Piper attenuatum*, a substitute for black pepper has been investigated for its antioxidant and anticancer activity [10].

MATERIALS AND METHODS

Sample Preparation

Cashew, Dates and Currant samples were procured from local market at Vijayawada, Andhra Pradesh. Samples were weighed each of 25g and cleaned thoroughly with autoclaved distilled water to remove contaminants and dust and were collected in zip lock covers and or made into smoothie [11] with PBS and or distilled water and collected into a sterile vial.

Determination of protein concentration

Crude extract was precipitated by adding varying concentrations of 50%, 60%, 70%, 80% and 90% ammonium sulphate [12] at 4^0C and the extract was then spin at high speed. The pellet obtained was then suspended in one ml of PBS to determine the concentration of protein. Protein concentration was determined using Bradford with bovine serum albumin as standard using Thermo UV Genesys spectrophotometer.

Time course and rpm studies

Concentration of protein was determined at various intervals with difference in course time and rpm levels [13]. After protein precipitation, extract was centrifuged at 5000, 10000 and 15000rpm for 5, 10 and 15 minutes respectively. At each end of centrifugation, the concentration of protein extract was determined using Bradford assay using bovine serum albumin as standard.

SDS-PAGE analysis of proteins

To check the purity of protein, the samples were run on 12% SDS-PAGE. Samples were layered on stacking gel surface with SDS loading dye (Merck Biosciences). A volume of 10ul sample was loaded and electrophoresis was run for 2 hours at 100V or until the gel loading dye reached to the end of gel. Gel was washed and fixed in 50%

methanol solution for few minutes and stained with Ezee blue direct stainer (Merck Biosciences) for 30 minutes. After staining, gel was analysed by white illuminator and photographed.

Table 1: Concentration of proteins in dry fruits

S.No	Common Name	Botanical Name	Bradford Assay (mg)	
			Smoothie	Salt method
1	Cashew	Anacardium occidentale	865	735
2	Dates	Phoenix dactylifera	780	509
3	Currant	Ribes nigrum	489	366

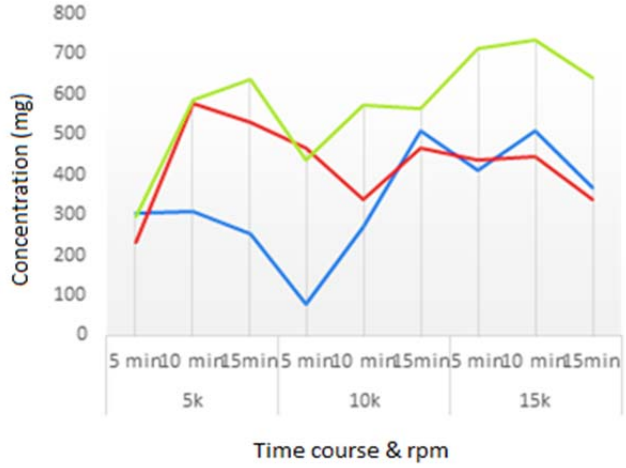

Figure 1: Time course and rpm based studies of various proteins extracted from dry fruits. Green line represents Cashew, red line denotes dates and blue line represents to currant.

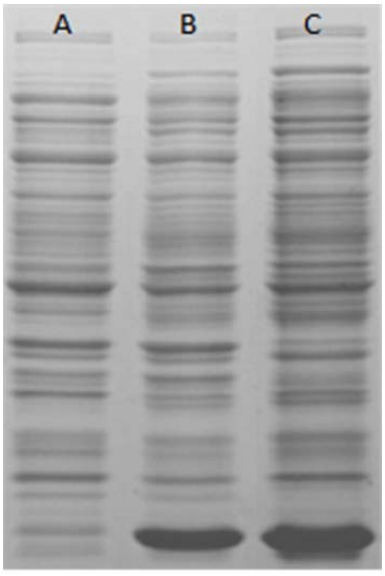

Figure 2: SDS-PAGE analysis of various proteins extracted from dry fruits

RESULTS AND DISCUSSION

Determination of protein concentration

Although the proteins in dry fruits are not purified completely, the use of ammonium sulphate made it possible to partially purify the proteins. The proximate composition analysed in various dry fruit sample were shown in Table 1 that summarizes the protein concentration done spectrophotometrically for each extract.

Time course and rpm studies

Dry fruit protein precipitated with ammonium sulphate salt solution was determined its concentration by Bradford method and as plotted in figure 2, concentration was found higher at a value of 735mg at 15000rpm for 10 minutes for cashew and value of 509mg at 10000rpm for 15 minutes for dates, while in currant it was found comparatively less with a value of 366mg concentration.

SDS-PAGE analysis of proteins

To determine the levels of proteins, samples were analysed by 12% polyacrylamide gels. The gels were scanned on a Gel scanner with white light converter (UVI-Tech, Lark Innovative) and the resulting images were analysed with UVI-Tech Software (Figure 2). In Lane A, B and C, Cashew, Dates and Currant samples were loaded to check its purity and molecular mass. Preparative PAGE separated various bands of proteins that exploit the difference in molecular mass among the various dry fruits.

CONCLUSION

The intake of protein from the dietary sources is becoming new insights in nutritional and functional foods. Though there are many nuts that are rich in proteins like apricots and raisins, the choice of our samples made an exposure to the field of nutritional facts that cashew and dates are also a good source of protein content. Use of this assay will allow researchers to obtain raw data about the protein source for quick use in molecular assays that require specialized instrumentation were time-consuming. Researchers today are emphasizing on evaluation and characterization of various fruit constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. The main aim of this work was to determine using salt method whether dry fruits have the same amount of protein population. Extraction of the bioactive constituents from plant or fruit sources has always been a challenging task for the researchers. In this present study, an attempt has been made to give an overview of certain extracts with their advantages. This analysis suggests that the cashew nuts are quiet more in amount than that of dates and currant.

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