

PHYTOCONSTITUENT CHARACTERIZATION AND APPLICATION OF *Amorphophallus paeoniifolius* IN DEVELOPMENT OF FOOD PRODUCTS

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DOCTOR OF PHILOSOPHY

By

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Nutrition plays an important role in prevention of health conditions like increased illness, reduced quality of life and premature death as well as chronic diseases. Diet low in fibre and high in calories, fats, saturated fat, cholesterol and salts are associated with increased risk of coronary heart diseases, cancer, stroke, diabetes, obesity and pregnancy problems.

India is the second most populous country in the world with an estimated 1.2 billion people and meeting the needs of this increasing population and ensuring that available produce reaches people in need are major challenges to global agriculture [1]. In spite of high economic growth and self-sufficiency in food grains production, high poverty levels, food insecurity and malnutrition persist in India. 32.7 % of the Indian population lives on less than US\$ 1.25 per day and it is estimated that an alarming 48 % of children less than 5 years of age are stunted, 43 % are underweight and 20 % are wasted, according to the National Family Health Survey (NFHS; 2005–2006) [2] .

The use of neglected and underutilized plant species by people belonging to the marginalized areas of society, brings social benefits like providing food, economic benefits (such as creating employment along the value chain) and environmental benefits (such as conserving biodiversity) [3], [4]. Root and tuber crops have tremendous importance in agricultural development as they hold important place in the dietary habits of small marginal farmers and tribal population. These crops are major calorie contributors as well as their staple food.

India holds a rich genetic diversity of widely consumed tropical root and tuber crops viz. Cassava, Sweet potato, *Colocasia* (taro), *Xanthosoma* (tannia), *Alocasia* (giant taro), *Amorphophallus* (elephant foot yam), *Cyrtosperma* (swamp taro). Aroid (*Philodendron*) family is the sixth most important root and tuber crops, and rank fourteenth among staple vegetable crops, and are thus significant in the global food system[5], [6]. All plant parts of aroids are edible and often carry a deep symbolic meaning and cultural value [7], [8].

A. paeoniifolius known as Elephant foot yam (Jimikand/ Suran) is considered as important, highly potential and nutritious tropical tuber crop of Aroid family. It has been widely used as potential natural medicinal plant in traditional Indian Ayurveda having great export potential since its commercial cultivation is not seen in other countries. The tuberous roots of the plant

posses blood purifier properties and have been used traditionally for the treatment of piles, abdominal disorders, tumours, enlargement of spleen, asthma and rheumatism [9], [10].

It is gaining wide acceptability due to its profound medicinal properties, better cooking quality, palatability and various uses in boiled or baked forms and pickles and flours. Even the stem and flowers are used as food [11].

A. paeoniifolius is an annual crop which matures and ready to harvest six- seven months after planting. Secondly, the dormancy of tubers is also a major constraint of this crop. After harvesting corms undergoes in the dormancy period and once the dormancy is broken, sprouting occurs after 3-4 months so prolonged storage is not possible. Due to these problems it is important to focus on the storage parameters. Losses due to physiological processes, rotting and pest infestation can be considerable constitute a major threat to the economic viability of these corms storage and the food security of the population concerned.

Generally vegetable contains wide variety of natural occurring enzymes and the food nutrient metabolism in vegetables is known to be under the control of these enzymes which are involved in the development of colour, flavor, aroma, texture and nutrient quality. Enzymic, chemical, physical and biological changes directly affect the deterioration of food. Enzymes like; Amylase, Cellulase, Lipase, Proteinase and respiratory enzymes control the levels of carbohydrates, lipids and proteins, while endogenous Poly phenol oxidase, Peroxidase and Catalase activities alter vegetable flavor and causes considerable economic and nutritional loss in the commercial production of fruits and vegetables [12]. After maturity many enzyme continue to act on remaining substrates accelerating the general senescence of tissue and aided further by damage during harvesting and storage [13].

Many of *A. paeoniifolius* varieties are having typical acidity which makes the crop unpopular for consumption. However the Gajendra variety of elephant foot yam developed at APAU, Hyderabad (India) is high yielding, free from acidity and is popularly grown all over India.

Amorphophallus paeoniifolius is an underutilized crop with remarkable nutritional quality and has potential to be a valuable food source for human consumption. Despite the economic importance of *A. paeoniifolius* as a food material, there is very limited scientific information on their post-harvest characteristics, which perhaps contributes for its limited improved

quality and marketing potential. To improve the use of *Amorphophallus paeoniifolius* by the food industry, much research needs to be done in order to fulfil the current limited knowledge of the functionalities and availability of acceptable food products, its nutritional components and product processing technologies also it is well known that utilization of these agro wastes provides alternative substrates and helps in solving pollution problems [14]. There is dearth in knowledge regarding the factors that impact product development with improved characteristics and longer shelf life from *A. paeoniifolius* corm and last but not the least food waste utilization also need to be explored.

The objective of this research was to evaluate and assess the complete utilization of *A. paeoniifolius* and study its potential in food industry. The utilization of *A. paeoniifolius* not only enhance the components of our vegetable basket but also helpful in increasing the beneficial effect of this corm.

The proposed thesis has 8 Chapters. In the first part a basic introduction to the subject is provided and the aim and achievements of the thesis work are clearly mentioned. Second Chapter of the thesis covers literature review, showing why *A. paeoniifolius* is selected for study, its edible, medicinal importance and uses of *Amorphophallus*; this part is published as a review paper [10]. This Chapter also describes all processing techniques used for food specially root and tubers, problems associated during processing, loss of nutritional values and intracellular structure of corm during storage, study and search for ideal processing techniques like dehydration, drying, osmo-dehydration to be used for corm *A. paeoniifolius* is mentioned. Negative impact of enzymatic browning, Purification techniques to be employed for the enzyme like Cellulase, PPO along with its thermal inactivation and thermodynamic parameters as well as enzyme inhibition studies for the purified PPO enzyme are important as it can prevent browning of the food product which needs to be developed are clearly mentioned. Chapters 3 to 7 briefly describes several aspects of Processing techniques, Development of edible products, Screening characterization of enzymes, Kinetics and Thermal inactivation of enzymes, Utilization of waste peel. Results reported in these chapters are already published [15-19] and some are communicated. Finally, the thesis is concluded in Chapter 8, where we have also discussed the scope of the future work.

The brief work done is described as follows;

“Processing of raw ingredients” from corm. Here we examined the economical techniques that can be utilized for product development and they are as mentioned below;

1. **Blanching, Dehydration:** Fresh Elephant foot yams are difficult to store and deteriorate on storage. Various drying operations like freeze drying, sun drying, oven drying has been reported for developing a stable form of dried Yam products but the effect of these food processes are not reported. These processes would also extend the potential for use of these flour and starch as added value products. In this part we have studied the viable processing techniques like Blanching, Oven Drying for flour isolation, extraction of **starch and resistant starch**.
2. **Proximate, Nutritional analysis and characterization of flour:**
Dried processed flour was checked for proximate composition and nutritional analysis to find the applicability of flour in functional food products.
3. **Extraction of Starch, resistant starch and functional characterization of starch:**
Even though *A. paeoniifolius* is being used in traditional practices since ancient time, only few studies have been reported on isolation and characterization of *A. paeoniifolius* starch. Knowledge of these functional properties could enhance the potential commercial utilization of *A. paeoniifolius* starch.

“Development of Edible Products” by utilizing *A. paeoniifolius* flour and corm. (**Bread and Osmo dehydrated Slices**)

1. **Bread formulation:** Incorporation of *A. paeoniifolius* flour into bread formulation is an attempt to improve the product nutritional quality. We have checked for four different formulations of bread and reported that wheat flour could be replaced, up to 20% (w/w), with *A. paeoniifolius* flour, to obtain consumer acceptable bread with improved nutritional value, without compromising product quality.

2. Quality characteristics of Bread:

- **Sensory evaluation:** Various sensory parameters were checked on 7 point hedonic scale to have a clear picture of consumer preference and perception of and a clear understanding of product characteristics. This analysis will increase developer confidence in product quality and help to find its marketability.
- **Nutritional analysis and shelf life prediction of Bread:** Nutritional analysis was carried out to check the enhanced nutritional value of bread with normal available breads. Shelf life prediction is important to determine the duration of product quality during storage.

3. Osmo- dehydrated Slices Osmotic dehydration is a procedure that involves immersing fruits or vegetables in a hypertonic aqueous salt or sugar solution. Here we have used different concentration of sugar solutions.

“Screening, purification and application of enzymes in the corn” was done and their potential applicability was studied.

1. **Screening, Characterization of Enzymes:** Here we have targeted polyphenol oxidase that has a role in browning, whereas cellulase, amylase and protease for softening of the vegetables. Cellulase helps to break down the cell wall in food industry. PPO could affect colour, odour and taste. The major useful factors in controlling enzyme activity i.e. temperature, pH, chemicals which can inhibit enzyme action, substrates alteration were checked.
2. **Application of Enzymes:** Juice clarification, Dough rising.

“Enzyme Kinetics and thermal inactivation of PPO”

1. **Enzyme Kinetics:** The K_m and V_{max} values of PPO were measured by evaluation of the Lineweaver–Burk ($1/V_o$ versus $1/[S]$ values) plots.

2. **Inactivation of PPO:** Here we report the thermal inactivation and thermodynamic properties of enzyme that can be utilized to inactivate the PPO enzyme so that the application of enzyme poly phenol oxidase is minimized in product development.

“Utilization of Waste Peel”

In this chapter we have screened the peel for presence of value added products like phytochemicals and enzymes. The present study summarizes *Amorphophallus paeoniifolius* peels utilization, potential to meet the industrial needs and verification of bioactive ingredients that include important constituents for pharmacological activity.

1. **Antioxidant and Phytochemical analysis:** Here we report the phyto-chemicals present and quantification of total phenol and proanthocyanidin within different extracts (aqueous, methanol, petroleum ether and chloroform) of the peel. The therapeutic potency of the peel was established by studying the anti-oxidative potential of these extracts by DPPH scavenging activity and phosphomolybdenum method.
2. **Screening and characterization of Enzymes:** Production of enzymes by the agro waste can propose alternative paths for the reuse of agro-industrial waste, as well as adding economic value to these co-products. Cellulase and Polyphenol oxidase activity is found to be present in *A. paeoniifolius* peel.

SUMMARY OF THE WORK

The present work can be summarized as:

Amorphophallus corm has been explored by many authors for their phyto-chemicals [20], [21], medicinal and insecticidal activities. This crop has the ability to provide nutrition security to the developing countries along with their medicinal aspects. Thorough screening of literature available on *Amorphophallus* depicted the fact that it is a popular remedy among the Ayurvedic and traditional practitioners for treatment of various ailments and may counter diseases like coronary heart disease, diabetes but the corm is underutilized by the general population because of acrid taste and difficulty in removing the external peel. This neglected aroid that has great potential in novel drug discovery and food applications.

We have made an attempt develop flour, starch, resistant starch and bread from *A. paeoniifolius* flour an underutilized tuber which can be cost effective as the overall acceptability is good.

The Proximate composition of flour revealed that it had 4.72% moisture, 4.0% Total ash and 2.0% acid insoluble ash. Nutritional composition of flour shows 73.48g/100 g of carbohydrate, 0.31 g /100 g of fat and cholesterol nil, 12.14 g/100 g of total dietary fiber and 45 mg/kg of gluten. These values suggest that flour is free from fat and cholesterol, high in dietary fiber and has reduced-gluten, and it suitable for most coeliacs as per Codex and FSSAI guidelines.

Starch and resistant starch was extracted from *A. paeoniifolius* flour after pretreatment for 24 hour with 0.2% NaOH. Starch has pale white colour with 10.5% yield, 70.35% water binding capacity and pH of 7.2. Starch shape was polygonal and round with average diameter of 5-20 μm as revealed by SEM analysis. XRD profile of *A. paeoniifolius* starch shows that starch has characteristic A type pattern, with three major peaks at 17.28° , 18.08° , 15.3421° , corresponding to inter planar spacing $d = 5.12 \text{ \AA}$, 4.90 \AA , and 5.77 \AA respectively.

A. paeoniifolius starch swelling power increases with increase in temperature and its light transmittance values of suspensions decreased during storage. On storage at 4°C and -80°C starch shows higher syneresis than starch stored at room temperature (30°C). 27 mg/100 g of digestible starch and 115mg/100 g of resistant starch can be obtained after enzymatic treatment. This could serve as pro-biotic in food industries, pharmaceutical companies and enhance the quality of *Amorphophallus* based food products.

We have also reported the production of bread from *A. paeoniifolius* corm flour. In this study we concluded that the shape, crust and crumb colour of the 20 % *A. paeoniifolius* breads (ApB 20) are identical to the control sample. The bitter and sour taste is slightly expressed. The biggest difference (22 %) between the samples and control bread is in the perception of typical flavour of wheat bread. The taste characteristics of the *A. paeoniifolius* flour incorporated breads are generally different from those of the control bread but it is found to be completely acceptable. Bread was shelf stable and rich in dietary fibre (2.49 g/100 g) and calcium (1158.42 mg/100gm). The results from this study show that the bread sample ApB 20

meets the RDA requirements of calcium (i.e., 1000 mg/day) for pregnant women [15]. Development of bread from underutilized *A. paeoniifolius* flour will add value to this crop. In future the type of fibre (soluble, insoluble), its prebiotic potential needs to be analysed as well as the potential medicinal value of this composite bread needs to be proved. Cost benefit analysis and relative merit of this healthy supplementation (ApB 20) needs to be evaluated using refined disability adjusted life year (DALY) frame work and detailed health data from India.

We have made osmo- dehydrated slices of *A. paeoniifolius* corm and reported that the process of osmotic dehydration decreased weight of the slices, The *Amorphophallus* slices dehydrated in the 80% sugar solution showed maximum water loss 83.63×10^2 after osmo-dehydration. The water loss and solid gain were increased by increasing concentration and temperature [16].

The stored corm extract showed the existence of cellulolytic activity in the corm as well as the peel. Enzyme was thermo stable and was stable under both acidic as well as alkaline conditions. Crude enzyme fraction from stored corm of *Amorphophallus paeoniifolius* could clarify apple juices and the clear juice obtained indicated the potential for use of crude extract of *Amorphophallus* in fruit juice processing. Cellulase activity was confirmed by Congo red plate diffusion. Optimum temperature for both corm and peel CMCase and FPase was found to be 60°C. Thermal stability studies suggested that cellulase extracted from both corm and peel retained activity even at 90°C. CMCase activity was found to be optimum at pH 10 in both corm and peel whereas FPase activity was found to be optimum at pH 5 and pH 8 in case for corm and peel respectively. Zymography studies and SDS gel electrophoresis revealed that the molecular weight of the enzyme is 66 kDa in case of corm and 43 kDa in case of peel [17], [18].

Cellulase of peel could be inhibited completely by Hg^{2+} and Mn^{2+} whereas Hg^{2+} could not effectively inhibit corm cellulase. Thermostable and pH stable cellulase from agrowaste can be of potential use in textile, dyeing industry.

Polyphenol oxidase (PPO) enzyme was extracted from *Amorphophallus* corm by acetone precipitation and ion exchange chromatography. These procedures led to 5.54 fold

purification. Enzyme has a molecular weight about 40 kDa showing optimum activity at 6 pH and 35⁰C using catechol as substrate. The inhibitory effects of L-ascorbic acid, sodium azide, NaCl, CaCl₂, ZnSO₄ and EDTA were studied and L-ascorbic acid was the most effective of them. The data obtained from our study showed that this enzyme could be useful for some industrial purposes like baking industry. PPO could also be extracted (25200 U/mg) from peel of *A. paeoniifolius* and this could raise dough effectively [22].

Thermal inactivation kinetics study of corm PPO showed that only 6.75% residual activity remained after 40 minutes incubation at 75⁰C. Based on experiment conducted in the temperature range of 55⁰ C –75⁰ C and using Arrhenius equation, the thermal inactivation of the PPO enzyme of *A. paeoniifolius* corm can be explained by the first-order model. Its k value increases as we increase the temperature. D value ranged between 418.65- 108.10 min and decreases by increase in temperature. Enzyme stability enhances with increase in D value. The activation energy (Ea) and Z values were calculated to be 64.22 KJ/mol and 34.01⁰C, respectively.

At temperatures of 55–75⁰C, the average values of ΔH^\ddagger and ΔS^\ddagger were 61.41 (kJ mol⁻¹) and -136.04 (J mol⁻¹ K⁻¹) respectively. Positive values of ΔH^\ddagger indicate the endothermic nature of the oxidation reaction [23]. ΔG increases as we increase the temperature. The Km and Vmax value for this enzyme was 17.56 mM and 0.023 U/ml/min.

Phytochemical analysis of peel revealed the presence of alkaloid, tannins, phenol, carbohydrate and fat in all extracts. Methanolic and chloroform extracts of *A. paeoniifolius* peel can be used as potential natural antioxidant. Methanol and chloroform extracts gave higher yield of phenol (4.4, 3.9) (mg/g) gallic acid per g extract powder and proanthocyanidin (2.17, 1.62) (mg/g) gallic acid/g extract powder respectively as compared to aqueous and petroleum ether extracts.

The annihilation of DPPH radical and Phosphomolybdenum assay, expressed in % inhibition, were in order AQ < PE < CF < ME and PE < AQ < ME < CF respectively. The maximum scavenging activity for methanolic extract was 50.16% and the synthetic antioxidant (BHT) was 47.4% in DPPH assay. This study confirms the peel extracts applicability to be used as a source of natural antioxidant in pharmaceutical, medical and food industry.

IMPORTANCE

Utilization of *Amorphophallus paeoniifolius* in the food industry would considerably improve the nutritional intake in the population. *Amorphophallus* (Jimikand) corm can be industrially exploited for important enzymes, phytochemicals and various innovative food products. Since the major enzyme responsible for browning can be thermally inactivated consumer acceptable innovative food products can be made from treated flour, like bread, starch, dried slices, pasta which can serve as an alternative to cereals products as well as provide high nutritional value like dietary fibers and calcium to the diet of common man. Cultivation of this sturdy plant and production of the food products would provide huge economic gains to farmers and rural communities. The present study gives the clear description of nutritional attributes of this Aroid and information can be used for making people aware of its dietetic merit.

FUTURE PROSPECTS

1. Gluten minimal bread with other gluten free other additives can be made.
2. Identification and addition of high nutrient value food source (example: high iron content and high quality proteins) that complements with *Amorphophallus paeoniifolius* flour so as to address specific nutrient deficient diet.
3. Knowledge gained from this research could be applied to develop new innovative product in Indian food industry
4. Mass cultivation of these plants can protect the minor indigenous tuber crops from extinction helping in conservation of wide biodiversity.

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2. Singh A., Wadhwa N. Antioxidative potential and phytochemical analysis of Elephant foot peel (*Amorphophallus paeoniifolius*). *National Conference on “Energy, Environment & Biotechnology Research”-NCEEER-2013th – 6th October 2013* at Mewar Institute of, Management, Ghaziabad. **Oral presentation**
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