DEVELOPMENT OF FUNCTIONAL EXTRUDED FOOD PRODUCT USING RED RICE (*ORYZA SATIVA* L.) AND PASSION FRUIT (*PASSIFLORA EDULIS* SIMS) AND ITS ANTIDIABETIC POTENTIAL

A thesis submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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JULY 2017

DEDICATED TO MY THREE BROTHERS …

DUNGJU, DUNGKEN AND DUNGTU SAMYOR

<u>Declaration by the candidate</u>

The thesis entitled "**Development of functional extruded food product using red rice (*Oryza sativa* L.) and passion fruit (*Passiflora edulis* Sims) and its antidiabetic potential**" is being submitted to School of Engineering, Tezpur University, Assam in partial fulfilment for the award of the degree of Doctor of Philosophy in the Department of Food Engineering and Technology is a record of bonafide research work accomplished by me under the supervision of **Prof. S. C. Deka**.

All helps from various sources have been duly acknowledged.

No part of the thesis has been submitted elsewhere for award of any other degree.

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(Awardee of Visitor's Best University Award, 2016 and 5th among India's Top 100 Universities, MHRD-NIRF Ranking, 2016)

CERTIFICATE OF THE SUPERVISOR

This is to certify that the thesis entitled "**Development of functional extruded food product using red rice (*Oryza sativa* L.) and passion fruit (*Passiflora edulis* Sims) and its antidiabetic potential**" submitted to the School of Engineering, Tezpur University, Assam in partial fulfillment for the award of the degree of Doctor of Philosophy in the Department of the Food Engineering and Technology is a record of bonafide research work carried out by Ms. Duyi Samyor under my supervision and guidance.

All helps received by her and from various sources have been duly acknowledged.
No part of this thesis has been submitted elsewhere for the award of any other degree.

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List of Abbreviations

L	Lenth of seed (mm)
W	Width of seed (mm)
T	Thickness of seed (mm)
S	Sphericity (%)
S_α	Surface area (mm^2)
R_α	Aspect ratio (%)
ρ_b	Bulk density (g/ml)
ρ_t	True density (g/ml)
ε	Porosity (%)
φ	Angle of repose
H	Height of the heap (mm)
D	Diameter of the heap (mm)
cP	centipoise
db	Dry basis
L*	Lightness
a*	Redness or greenness
b*	Yellowness or blueness
UA	Umling ame
LA	Lingkang ame
WR	Pungpo ame
C	Control extrudate sample
O	Optimized extrudate sample
DF	Degree of Freedom
ANOVA	Analysis of variance
CCD	Central composite design
GE	Gross energy
ME	Metabolizable energy
ATP	Adenosine-triphosphate
IRRI	International Rice Research Institute
BHT	Butylated hydroxytoluene
TCA	Trichloroacetic acid
DPPH	2, 2′-diphenyl-1-picrylhydrazyl (%)
FT-IR	Fourier transform infrared
RP-HPLC	Reverse phase- High-performance liquid chromatography
FAO	Food and Agriculture Organization
ICP-MS	Inductively coupled plasma mass spectrometry
AAS	Atomic absorption spectroscopy
PT	pasting temperature
PV	Peak Viscosity
BD	Break down
TVA	Texture profile analyser
SEM	Scanning electron microscopy
γ	Shear rate
σ_0	Yield stress
K_k	Consistency index

n_H	Flow behaviour index
K_c	Slope
K_{0c}	Intercept
K_M	Apparent viscosity
EMC	Equilibrium moisture content
M_o	Monolayer moisture content
ω	frequency
G´	Storage modulus
G´´	Loss modulus
η*	Complex viscosity
DPP-4	Dipeptidyl peptidase-4
GLP-1	Glucagon-like peptide-1
STC-1	Secretin tumour cell line-1

1.1 General Introduction

Rice (*Oryza sativa* L.) is the most important and widely consumed staple food for Asian countries. In Asia, the total rice produce is about 671 million tons [7] and almost half of the world's population consumes rice as their staple food. Rice has two species: *Oryza sativa* L. and *Oryza glaberrima* Steud. *Oryza sativa* species is originated from South-East Asia and *O. glaberrima* is native to West Africa and is grown solely in this part.[8] Although, white rice is consumed worldwide, some Asian countries also consume pigmented cultivars like black rice, purple black, red rice, and reddish brown rice etc. Pigmented rice has been consumed in glutinous rice and husked rice (4.9%) also contains colored rice cultivars. In India, the total cultivated area has been recorded as 43.77 M ha (29.4% of the global rice area) with a production of 90 million tonnes and productivity of 2.203 t ha^{-1}.[17] 'Njavara' (*O. sativa* L.) is a unique, indigenous, medicinal rice variety that matures in about 70 days' time and cultivated in Kerala, India. Njavara is of two types *viz.,*'black glumed' and 'yellow glumed' and named on the basis of the color of the outer covering of paddy. Black glumed njavara has reddish bran.[15] Although, India is an immense source of rice, there is still a great amount of rice that remains underutilized and the most underutilized rice cultivars are prevalent in some regions, e.g., south, east and the hilly tracts of the North-East. The North-East region in India is considered to be one of the richest genetic resources in the world and a potential rice-growing region with extremely diverse rice-growing conditions compared with other parts of the country. The most important indigenous rice cultivars available are pigmented rice. Some of the underutilized rice cultivars of Arunachal Pradesh, India are pigmented and nonpigmented rice and can be a source of various bioactive compounds. The pigmented rice mainly comprises various mixtures of color *viz.,* black,[3] red and dark purple rice,[4] brown,[28] dark brown.[14] A study conducted in Thai pigmented rice varieties showed anti-glycation capacity and proanthocyanidins in red rice bran also exhibited moderate chelating activity.[5] Surarit et al.[23] reported that the pigmented rice extracts are very strong intracellular candidates inside the cell-based systems. Various researchers mentioned that the lightness ($L*$), redness ($a*$) and yellowness ($b*$) values of pigmented rice are strong indicators of its bioactive components. Murdifin et al.[18] also reported that out of thirteen Indonesian pigmented rice, the darkest rice shown the lowest $L*$ value and highest $a*$ value.

Purple fruit is well known for its nutritional benefits and medicinal properties and its rind has the anti-hypertensive effect and vasodilatory effect on human body. [19, 20]

To increase the shelf life and protect the phytochemical loss of passion fruit, it needs an effective way of drying. The application of foam mat drying techniques can be an effective way to increase the shelf-life and decreases the phytochemical loss.[12]

In foam mat drying, for the porous structure of the foamed materials, mass transfer is faster, hence shorter the drying time apparently results in higher quality of dried food product.[1] Foam mat drying converts thin pulp into stable foam by whipping after the addition of edible foaming agent. Eventually, the whipping process increases surface area, and speed up the drying rates which yield dried foam mat powder of acceptable quality in reduced time span.[13]

Extrusion cooking of foods has been practised over 50 years. A twin-screw extruder mainly consists of two parallel screw shafts with the same length. The shaft which rotates in the same directions is called co-rotating and one which rotates in opposite directions called counter-rotating. Furthermore, it is subdivided into full, partial or non-intermeshing units on the basis of the relative position of the screws.[21] It is quite popular for agro-processing industries, such as pet food, cereals and snacks etc. [25]

Rheology plays a vital role in food manufacture and marketing nowadays *viz.*, design of handling systems, quality control and evaluation of sensory stimuli of viscosity.[2] It also concerned with how all food materials respond to applied forces and deformations. A flow model may be considered to be a mathematical equation that can describe rheological data, such as shear rate versus shear stress, in a basic shear diagram. It provides a convenient and concise manner of describing the data.

Fuzzy logic is a very important tool which aids in important decision-making for comparing a developed product with similar products available in the market. This evaluation will be used to determine the reasons for low and high ranking of products which was evaluated by the no. of judges. Various food products are available in the market.[9] Various researcher has reported the usefulness of fuzzy logic e.g. such as mango drinks, coffee, black rice wine etc.[16,11]

Moisture sorption isotherm (MSI) study is considered as a key tools for determining suitable packaging, storage conditions and optimized product stability.[5] According to Viswanathan et al.[24] MSI is the relationship between total moisture content and the water activity of the food at a constant temperature. Various mathematical models are also available to describe water sorption isotherms of food materials.

Diabetologists stated that there is a relationship between the intestine and insulin secretion. Dipeptidyl peptidase-4 (DPP-4) inhibitors act as a blood glucose lowering treatments of patients with type 2 diabetes mellitus. DPP-4 inhibitors stimulate GLP-1, and GIP, which enhance glucose-dependent insulin secretion and inhibit glucagon secretion.[9, 22]

1.2 Hypothesis

- Foam mat dried powder from purple passion fruit juice can be successfully achieved.
- Cereal (red rice) based extruded product incorporated with passion fruit powder by twin extruder showed a new combination of ready to eat product
- Assessment of antidiabetic property from red rice and red rice based extruded products

1.3 Technical objectives

a) To study the physicochemical analysis of pigmented and nonpigmented rice and

phytochemical analysis of purple passion fruit

b) To study the foam mat drying of purple passion fruit and characterization of the powder

c) To study the effect of extrusion cooking on the physicochemical and phytochemical properties of passion fruit powder incorporated red rice extrudates, rheology of doughs and sensory evaluation of product

d) To study the moisture sorption isotherm (MSI) and antioxidant stability of optimized product during storage

e) To study the assessment of the antidiabetic potential of red rice and rice-based products

References

[1] Brygidyr, A, M., Rzepecka, M, A. and McConnell, M, B. Characterization and drying of tomato paste foam by hot air and microwave energy. *Canadian Institute of Food Science and Technology Journal,* 10:313-319, 1977.

[2] Barnes, H, A. in "Industrial Food Processing: Experiments and Numerical Simulation", University of Plymouth, Institute of Non-Newtonian Fluid Mechanics and British Society of Rheology, 2001.

[3] Chinprahast, N., Tungsomboon, T. and Nagao, P. Antioxidant activities of Thai pigmented rice cultivars and application in sunflower oil. *International Journal of Food Science and Technology*, 51:46-53,2016.

[4] Chen, M, H., McClung, A, M. and Bergman, C, J. Bran data of total flavonoid and total phenolic contents, oxygen radical absorbance capacity and profiles of proanthocyanidins and whole grain physical traits of 32 red and purple rice varieties. *Data in Brief,* 8: 6-13,2016.

[5] Debnath, S., Hemavathya, J. and Bhat, K. K. Moisture sorption studies on onion powder. *Food Chemistry*,78:479-482, 2002.

[6] Daiponmak, W., Senakun, C. and Siriamornpun, S. Antiglycation capacity and antioxidant activities of different pigmented Thai rice. *International Journal of Food Science and Technol*ogy, 49:1805-1810, 2014.

[7] FAOSTAT. 2013. Rice production. http://faostat.org (accessed March 29, 2015).

[8] Fageria, N, K. and Baligar, V,C. Upland rice and allelopathy. *Communications in Soil Science and Plant Analysis*, 34:1311-1329, 2003.

[9] Hach, T. and Ferrannini, E. *The future of anti-diabetics in T2DM therapy*. The significance of the kidney in diabetes, 81-86, 2012.

[10] Jaya, S. and Das, H. Sensory evaluation of mango drinks using fuzzy logic. *Journal of Sensory Studies*, 18:163-176, 2003.

[11] Jha, P., Das, A,J., Dash, K, K. and Deka, S, C. Sensory evaluation of black pigmented rice (*Oryza sativa cv. Poireton*) wine fortified with probiotic Lactobacillus acidophilus ATCC 4356 and Lactobacillus sakei ATCC 15521 using fuzzy logic. *Journal of Institute of Brewing*, 121: 566-573, 2015.

[12] Kadam, D, M. and Balasubramanuan, S. Foam-mat drying of tomato juice. *Journal of Food Processing and Preservation,* 35: 488-495, 2011.

[13] Kudra, T. and Ratti, C. Foam-mat drying: energy and cost analyses. *Canadian Biosystems Engineering*, 48(3): 3.27- 3.32, 2006.

[14] Kang, K., Lim, D.H., Choi, I, H., Kang, T., Lee, K., Moon, E,Y., Yang, Y., Lee, M,S. and Lim, J,S. Vascular tube formation and angiogenesis induced by polyvinyl pyrrolidone coated silver nanoparticles. *Toxicology Letters*, 205: 227-234, 2011.

[15] Kang, M., Kim, J., Rico, C.W. and Nam, S.A. Comparative study on the physicochemical characteristics of black rice varieties. *International Journal of Food Prop*erties, 14:1241-1254, 2015.

[16] Lazim, M, A. and Suriani, M. Sensory evaluation of the selected coffee products using fuzzy approach, *The World Academy of Science*, *Engineering and Technology*, 50:717-720, 2009.

[17] Ministry of Agriculture. 2008. Government of India.www.indiastat.com Accessed 29 January 2014.

[18] Murdifin, M., Wahyudin, E., Lawrence, Manggau, M, A., Alam,G., Pakki, E., Djabir, Y., Arsyad, A. and Bahar, M, A. Antiatherogenic effect of *Mezzetia parviflora* BECC extract in high cholesterol fed rats. *Journal of Chemical and Pharmaceutical Research,* 7:499-505, 2015.

[19] Patel, S, S. Morphology and pharmacology of *Passiflora edulis*: a review*, Journal of Herbal Medicine and Toxicology,* 3:1-6, 2009.

[20] Ramaiya, S,D., Bujang, J.S., Zakaria, M,H., King,W,S. and Shaffiq, S, M,A. Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (*Passiflora*) cultivars. *Journal* of *the Science of Food and Agriculture*, 93(5):1198-205, 2013.

[21] Riaz, M, N. Introduction to extruders and their principles. *In: Extruders in food applications*, CRC Press, Boca Raton, United States of America. P 1- 23, 2000.

[22] Sharma,A., Paliwal, G., Upadhyay, N. and Tiwariet ,A. Therapeutic stimulation of GLP-1 and GIP protein with DPP-4 inhibitors for type-2 diabetes treatment. *Journal of Diabetes and Metabolic Disorders*, 14 (15): DOI 10.1186/s40200-015-0143-4,2015.

[23] Surarit, W., Jansom, C., Lerdvuthisopon, N., Kongkham, S. and Hansaku, P. Evaluation of antioxidant activities and phenolic subtype contents of ethanolic bran extracts of Thai pigmented rice varieties through chemical and cellular assays. *International Journal of Food Science and Technology,* 50:990-998, 2016.

[24] Viswanathan, R., Jayas, D, S. and Hulasare, R,B. Sorption isotherms of tomato slices and onion shreds. *Biosystems Engineering*, 86: 465-472, 2003.

[25] White, B, L., Howard, L, R. and Prior, R, L. Polyphenolic composition and antioxidant capacity of extruded cranberry pomace*, Journal of Agricultural and Food Chemistry*, 58(7): 4037-4042,2010.

[26] Xia, Q., Wang, L., Xu, C., Mei, J. and Li, Y. Effects of germination and high hydrostatic pressure processing on mineral elements, amino acids and antioxidants in vitro bioaccessibility, as well as starch digestibility in brown rice (*Oryza sativa* L.). *Food Chemistry*, 214: 533-542, 2017.

2.1 Pigmented rice

2.1.1 Nutritional composition

Rice (*Oryza sativa* L.) is a global staple food and is consumed as a whole grain. Although white rice is commonly consumed, there are several rice cultivars containing color pigments and usually known as black, purple and red rice. Pigmented rice has been reported to be an excellent source of phenolic compounds. Therefore, pigmented rice has an impact on human consumption due to its substantial antioxidant content. The North Eastern part of India, which has diverse physiographic and agro climatic landscapes, is considered as the most rice bio diversified rich region. According to Hore [69] the state of Arunachal Pradesh itself yielded around 616 germplasm collections of rice from 1987 to 2002. The most of rice cultivars grown in Arunachal Pradesh are underutilized and commonly have whitish kernels locally known as '*Pungpo ame*'. But there are also some varieties with a colored testa (black, purple or red) that give slightly colored kernels on milling.[53] The various types of rice are generally categorized on the basis of amylose content. The proportion of amylose (and amylopectin) in the starch is predominantly responsible for the different physicochemical and cooking properties of the rice kernel. The categories of rice on the basis of amylose content are high amylose, intermediate amylose, low amylose and waxy rice types.[22] Functional properties of rice such as gelatinization, rheology and gel consistency are vital for effective use of rice in food systems and depend on the amylose content. Moreover, some of the underutilize rice cultivars of Arunachal Pradesh are pigmented rice. Pigmented rice is rich source of bioactive compounds such as phenolic acid, anthocyanin, flavones, etc. All bioactive compounds are important for human health because of their pharmacological activities as radical scavengers. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds. Pigmented rice is reported to have a health-promoting potential due to its substantial antioxidant content which inhibits the formation or reduces the concentrations of reactive cell-damaging free radicals.[7, 105]

Samyor et al. (2017). Pigmented rice a potential source of bioactive compounds: a review. International Journal of Food Science and Technology. doi:10.1111/ijfs.13378. International Journal of Food Science and Technology 2017, 52, 1073–1081

Samyor et al. (2017). In: Value Addition of Underutilized Crops of India by Extrusion Cooking Technology, Innovative Food Science and Emerging Technologies, Published by Apple Academic Press, Taylor & Francis Group (In press)

Table 2.1 Types of pigmented rice

Sl. No.	Types	References
1	Purple rice	Das et al.[50]
2	Red rice	Shammugasamy et al. [132]
3	Blackish purple rice	Nam et al.[104]
4	Black rice	Jha et al.[76]

Physical, thermal and pasting properties are important to solve problems associated with the design of machines and analysis of the behavior of the product during processing. Physical properties of grain such as shape, size, volume, surface area, thousand grain weight, density, porosity and angle of repose of different grains are important parameters to determine the quality and optimum conditions for processing and safe storage.[134] The physical properties of various rice grains are also important for design and development of relevant machines and facilities for harvesting, storing, handling and processing. Amin et al.[6] stated that the bulk density, kernel density and porosity (the ratio of inter granular space to the total space occupied by the grain) can be an important parameters to size the grain hoppers and enhance the storage facilities.

(a)

(b)

(c)

(d)

Fig. 2.1 (a) Rice paddy, (b) Short kernel red rice locally called Umling ame (UA), (c) Long kernel red rice locally called Lingkang ame (LA) and (d) Short kernel white rice locally called Pungpo ame (WR)

2.1.2 Various bioactive compounds of pigmented rice

2.1.2.1 Anthocyanin

Anthocyanins are the primary functional components of pigmented rice.[45] In nature, anthocyanins are in the form of polyhydroxylated and or methoxylated heterosides which are derived from the flavylium ion or 2- phenyl benzopyrilium. Synthesis of proanthocyanidins and anthocyanins are given in Fig. 2.2. Aglycon (anthocyanidin) is found united to one or various sugars and also in return, acylated with different organic acids.[56] In rice grain, the pigmentation of pericarp is controlled by three different types of genes *viz.*, R_a, R_c, and R_d. These genes are said to be inherited monogenetically. R_a gene controls purple color and in the absence of the R_d gene, R_c gene produces brown pericarp and when R_c and R_d genes are crossed, red pericarp is produced.[144]

2.1.2.2 Proanthocyanidins

Proanthocyanidins (PAs) are high molecular weight polymers. PAs or condensed tannins are complex flavonoid polymers found in some cereals and legume seeds. It comprises of the monomeric unit flavan-3-ol (+) catechin and (−) epicatechin. The reddish colored testa is associated with the presence of a class of polymeric compounds are called the proanthocyanidins. Chen et al.[34] revealed that a 4.3-fold variation in the sum of oligomers and polymers content of total proanthocyanidins in the extractable fraction samples was obtained. Furthermore, the concentration of fractions was highly correlated with bioactive compounds like total phenolics, total proanthocyanidins, flavonoids and antiradical capacity, respectively.

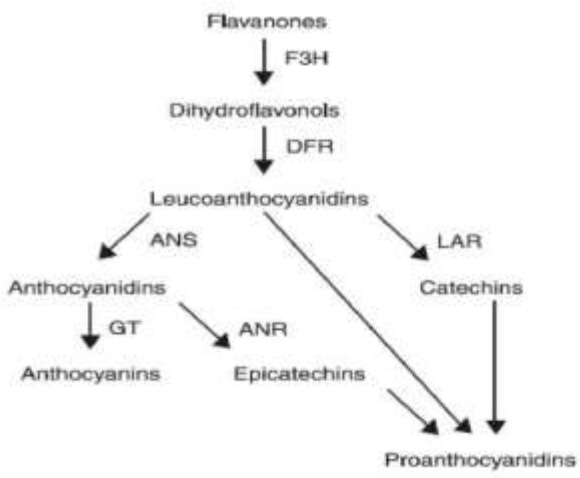


Fig.2.2 Flow chart showing the synthesis of proanthocyanidins and anthocyanins (Source; Xie and Dixon [143]),

ANS (anthocyanidin synthase); ANR (Anthocyanidin reductase); F3H (Flavones 3-hydroxylase); GT (Glucosyltransferase); DFR (Dihydroflavanol 4-reductase); LAR (Leucoanthocyanidin reductase)

2.1.2.3 Phenolic acids

Phenolic acids are generally classified into two type's *viz.,* free and bound phenolic acids.[115] Free phenolic acids are soluble in nature and bound acids are insoluble, covalently bound to structural components of cells like cellulose, hemicelluloses (e.g. arabinoxylans), lignin, pectin, and rod shaped structural proteins etc.[2] Zaupa et al.[145] stated that the main phenolic acids found in pigmented and non- pigmented rice are protocatechuic acid, synaptic acid, vanillic acid, *p*-coumaric acid, and ferulic acid. The level of soluble phenolic acids is an important parameter which provides an index of grain resistance. In cell wall especially during the structure formation, insoluble phenolics are actively involved and account for the major part of polyphenols in whole grains.[98] The amount of soluble form found are 38% [8] to 60% [99] of the total polyphenols content in light brown rice grains, and around 81% in color grains particularly in the red and black pericarp.[99] Laokuldilok et al. [90] also reported the content of insoluble and total phenolic acids in rice bran.

2.1.2.4 Flavonoids

Flavonoids are the bioactive compounds and synthesized by the phenylpropanoid metabolic pathway. Flavonoids consist of a 15-carbon skeleton which is organized in two aromatic rings (A- and B-rings) and interlinked by a three-carbon chain (structure C6-C3-C6). Flavonoids are known for their ability to donate electrons and to stop chain reactions. Flavonoids can be classified *viz.*, flavones, flavonols, flavanols (flavan-3-ols), flavanonols, isoflavones, and flavanones, which generally occur as O- or C-glycosides. Fig.2.2 shows the flavonoid structure and class of flavonoid. In pigmented rice varieties tricin appears to be the major flavonoid in the bran, covering approximately 77% of all seven flavonoids. Tricin possesses various bioactive compounds which have nutraceutical properties *viz.*, antioxidant effect, inhibition of lipid peroxidation, sparing effect on vitamin E in erythrocyte membrane, antiviral, immunomodulatory, antitubercular, antiulcerogenic, antimutagenic, mildly estrogenic, antiinflammtory and potent anticancer effects etc. [81]

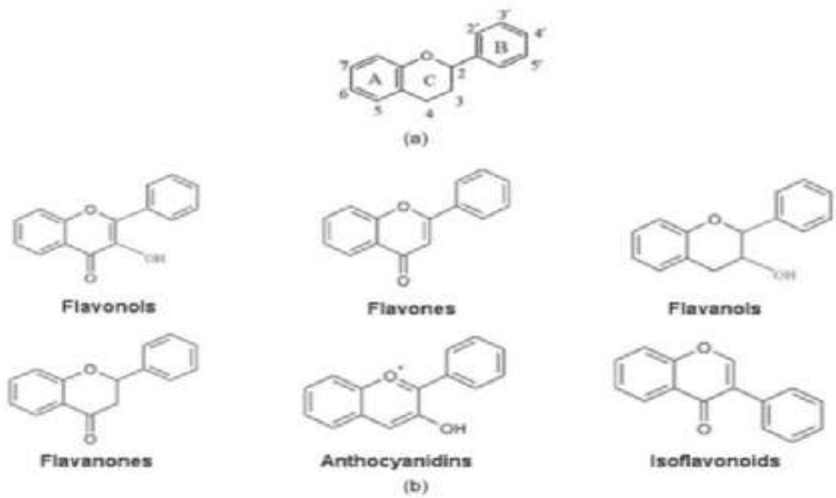


Fig.2.3 (a) Flavonoid structure and (b) Class of flavonoid (Source; Kale et al.[83])

2.1.2.5 γ – oryzanol (Steryl ferulate)

γ-oryzanol, a family of ferulic acid esters of unsaturated triterpenoid alcohols, has been characterized in rice bran. It is a class of non-saponifiable lipid and helps in the regulation of elevated LDL. Laokuldilok et al. [90] reported that γ -oryzanol and phenolic acids were the major antioxidants for pigmented rice bran. In the case of red rice bran, the γ-oryzanol contains 51.8% of the total antioxidant content. γ- oryzanol is a more potent antioxidant than α-tocopherol because of its reducing nature in cholesterol oxidation.[141] In a study conducted by Kim et al.[82] 7 Korean rice varieties *viz.,* 3 white rice (Chucheongbyeo, Kunnunbyeo, Baekjinjubyeo, 3 red rice (Hanyangjo, Chosundo, Jeokjinjubyeo) and 1 purple (Heugjinjubyeo) were obtained from International Rice Research Institute (IRRI) and γ-oryzanol contents and compositions of steryl and triterpene alcohol ferulates proportions in total γ -oryzanol (%) were studied.

2.1.2.6 Vitamin E

Vitamin E or tocols is a generic term for a group of four tocopherols (a, b, c, and d) and four tocotrienols (a, b, c, and d). Various study supported that the α-tocopherol has the highest biological activity. It is based on an amphiphilic 6-chromanol ring and a terpenoid side chain which is located at position 2 of the ring.[58] The complex form, the free hydroxyl group on the chromanol ring is responsible for the antioxidant properties. Hydrogen atom from this group can be donated to free radicals which later resulted into a resonance-stabilized vitamin E radical.

2.1.2.7 Minerals

Recently, a study reported that two black rice varieties from Korea *viz.,* Heukjinjubyeo, and Heukgwangbyeo evinced higher amount of Ca and K when compared with a white variety (Heuknambyeo).[66]

2.1.3 Health benefits of bioactive compounds

Various health beneficial bioactive compounds from pigmented rice were reported by several authors which include sterols, γ-oryzanol, tocopherols, tocotrienols, and phenolic compounds that are present in the outer layer of grains such as pericarp and aleurone.[133]

Antiatherosclerosis activities various researchers have reported that dietary supplementation of pigmented rice such as red or black rice instead of white rice induced significant increase in high-density lipoprotein (HDL) concentrations and the activity of glutathione peroxidase (GPx) in hypercholesterolemic rabbits. These activities reduced the size of atherosclerotic lesions in the hypercholesterolemic rabbits. In addition, supplementation of the outer layer fraction of black rice to these animals showed significant lowering of aortic 8-hydroxy-20-deoxyguanosine (8-OHdG) and the malondialdehyde (MDA) level of serum and aortic artery, respectively.[35, 91, 92] It was also noted that black rice outer layer extract when fed to rabbits, the level of the compounds in the serum *viz.,* aortic 8-hydroxy-20-deoxyguanosine and malondialdehyde decreased significantly in their body.[92]

2.1.3.1 Antiallergic activities

Pigmented rice evinced with a wide range of biological activity like antiallergic[140] and also black rice bran's extract showed inhibitory effects on in vitro allergic reactions.[39] Kim et al.[80] revealed about antiallergic activity study by using methanol extract of *Oryza sativa* L. subsp. Hsien Ting (OSHT), a variety of Chinese black rice. They conducted a study on Mast cells in vivo and in vitro in a murine model and tested for the inhibition of histamine release, systemic anaphylaxis and local anaphylaxis after treatment with OSHT. The results showed that OSHT (0.001–1.0 mg g^{-1} BW) had dose-dependent inhibition against systemic anaphylaxis induced by compound 48/80. Later, local anaphylaxis activated by antidinitrophenyl DNP IgE and serum histamine in rats was found. In vitro study also revealed the OSHT inhibited the histamine release from the rat peritoneal mast cells which was activated by the compound 48/80 or anti-DNP IgE in a dose-dependent manner.

2.1.3.2 Antidiabetic activities

Pre-diabetic patients have maximum chances of developing type II diabetes and other complicated health issues.[146] A study conducted by Boue et al.[13] reported that pigmented rice bran *viz.,* purple and red bran extracts, inhibited α-glucosidase activity. Furthermore, in the red rice bran extract inhibition of α-amylase activity was also found. These inhibition activity of α-amylase and α-glucosidase helped in delaying of digestion and absorption of carbohydrates. These actions ultimately lead to the suppression of postprandial hyperglycaemia in the diabetic person. Rice bran extract was studied for the stimulation of glucose uptake in 3T3-L1 adipocytes which is a key function in glucose homeostasis. The red and purple bran extracts results revealed that the basal glucose uptake increased between 2.3- and 2.7- fold and between 1.9- and 3.1-fold.

2.1.3.3 Antiinflammatory effects

Black rice is rich in cyanidin-3-b-D-glucoside (C3G) and a number of researchers have reported that C3G possesses antiinflammatory effects.[142] Limtrakul et al.[93] conducted a study on antiinflammatory effects of proanthocyanidin-rich red rice extract via the suppression of MAPK, AP-1 and NF-j B pathways in Raw 264.7 macrophages. It was found that the red rice polar extract fraction exerted anti-inflammatory activities by inhibiting the production of TNF-a, IL-6, and NO in LPS-activated macrophages whereas red rice non-polar extracts fraction had no effect on macrophages.

2.1.3.4 Anticancer activity

Various flora available in the North Eastern region of India, are considered to be a chemo preventive agents. But unfortunately, because of the lack of scientific data the information are still not clear. Many researchers have previously made in vitro and in vivo studies and elucidated that natural products are able to reduce aflatoxicosis, e.g. Korean red ginseng and neem flower Kim et al.[84] On the other hand, a local pigmented rice cultivar from Thailand called Kum Phayao was found highly cytotoxic to human hepatocellular carcinoma HepG2 cells when compared with other Northern Thai purple rice varieties.[14] Punvittayagul et al.[109] revealed that the alcoholic extract of purple rice (*O. sativa* L. var. indica) grain cultivar Kum Doisaket shown antimutagenic effects against AFB1 in the Ames test.

2.1.3.5 Antitumor Activities

In body, reactive oxygen species (ROS) are produced by some tumour promoter in the development of cancer. An in vitro studies using flow cytometry, 70% ethanol-water extracts of bran (outer layer) from seeds of five pigmented rice cultivars *viz.*, Jumlalocal-1, DZ 78, Elwee, LK1-3-6-12-1-1, and LK1A-2-12-1-1 revealed their antitumour-promoting activities by measuring the inhibition of Epstein-Barr virus early-antigen activation (EBV-EA) which was later induced by the tumor promoter 12-Otetradecanoylphorbol-13-acetate (TPA) [104] The results reported that pigmented varieties strongly inhibited phorbol ester-induced tumor promotion in marmoset lymphoblastoid cells B95-8 in vitro than non-pigmented variety.

2.1.3.6 Alleviating gallstones

Tsai et al. [135] reported that those women who are eating foods which are rich in insoluble fibre like brown rice gained more protection against gallstones. In addition, the reduction of gallstone in women by 17% was noted particularly whose diets were incorporated with brown rice.[25]

2.1.3.7 Internal rejuvenation

Pigmented rice comprises of almost all essential amino acids in proportions *viz.,* tryptophan, valine, threonine, isoleucine, lysine, leucine, phenylalanine, histidine, and methionine. Vitamins *viz.,* thiamine, riboflavin, and niacin are present in brown rice which reduces the health problems and important chemical constituents help to maintain internal fluid balance. Rice contains iron which enriches the bloodstream and phosphorus and potassium are another two important minerals which maintain internal water balance along with other nutrients. Thus, the presence of these important nutrients makes rice a vital grain in restoring the internal harmony of the human body system.[139]

2.1.3.8 Other bioactivities

Hu [67] reported that black rice pigment had significant effects on the anti-fatigue and hypoxia tolerance of mice. Anthocyanins extracted from pigmented rice such as black rice may be a strong candidate to protect mouse brain neuron against death at low concentration [46] and can be incorporated in the production of value-added products to combat certain deficiency and lifestyle diseases.

2.1.4 Passion fruit (*Passiflora edulis*)

Passion fruit (*Passiflora edulis*) is an exotic tropical fruit native to Brazil belongs to Passifloraceae family. In Latin America, the cultivation of passion fruit became dominant due to the favorable weather condition and its processed products became popular widely in European countries. In India, initially passion fruit was cultivated in South India. Cultivation extended to the North Eastern part of India due to the suitable climatic condition.[88] The genus *Passiflora* reported between 500 to 700 species. [38, 108]As per the record, *Passiflora* species are found highest in tropical regions (despite some records of species in India, China, Australia and the Pacific islands).[38,40] The most popular edible passion fruits reported are namely purple passion fruit (*Passi flora edulis* Sims), granadilla, (*Passiflora ligularis* Juss.), gulupa (*Passi flora edulis* Sims. *fo edulis*), sweet passion fruit (*Passi flora alata* Curtis),banana passion fruit (*Passi flora mollisima* (*Kunth*) Spreng.)) and yellow passion fruit (*Passi flora edulis* var. *flavicarpa* Degenerer).[42] Geographically, yellow passion fruit favored lowland tropical conditions, whilst, the purple type tends to favored by subtropical areas or at higher altitudes in the tropics. The purple passion fruit is native from southern Brazil through Paraguay to northern Argentina and yellow one perhaps from native to Amazon region of Brazil.[75] Passion fruit is consumed as fresh fruit or juice. It is popular and is very well known for its possession of exotic, flowery and fruity aroma.[44]

Fig. 2.4 Passion fruit samples (a) unripe passionfruit and (b) ripe passionfruit (c) pulp with seed (d) pulp (e) rind of fruit and (f) storage of pulp in container

Table 2.2 Fruit characteristics of some passiflora species found in India

Sl. No.	Common name	Characteristics	Reference
Passiflora edulis Sims	Purple Passion Fruit/Mountain Sweet Cup	Color: purple Shape: Round to ovoid fruit (5-8 cm long and 4-8 cm diameter) Appearance : tough, waxy smooth rind and orange yellow colored pulpy juice with pleasant and sub-acidic flavored arils	Sema and Maiti,[128]
Passiflora edulis Sims *f. flavicarpa* Degener	Yellow Passion Fruit/Golden passion fruit	Color: yellow Shape: Round to ovoid fruit, larger in size(8-10 cm long and 4-10cm diameter) Appearance: Smooth, glossy, light and airy thick (3-4mm) rind of yellow to light orange pulp having highly aromatic and acidic juice.	
Passiflora quadrangularis Medic	Giant Granadilla	Color: Greenish-white to pale yellow color Shape: Oblong-ovoid fruit of very large size (20-30 cm long and 12-15 cm diameter) Appearance: thick skin, whitish to yellowish sweet acid arils having mild flavor.	

2.1.4.1 Health benefits

Various researcher has claimed that exotic tropical fruits are abundantly rich in bioactive constituent's *viz.*, phenolic compounds, carotenoids, vitamins and fibers. Recently, surge of research has been carried out to explore recovery technologies and new applications to utilized by products of these fruits.[95] Passion fruit constitutes a powerful house of various bioactive compound like vitamins A, C and D[12] alkaloids, carotenoids and flavonoids.[38] Also found to be a good source of nicotinic acid, riboflavin and meagerly mineral. Due to presence of polyphenol, antioxidant properties like anti-inflammatory and antipyretic properties.[102, 126] Apart from its tremendous health benefits of pulp, the infusions made with the leaves is also well recognized. In the countries like America and Europe the leave is used as sedative or tranquilizer [38, 54, 114] and the tea prepared by the infusion of the leaves is reported with anti-inflammatory potential.[102]

The purple passion fruit extracts is used widely in folk medicine in South America to treat various ailments like anxiety, insomnia, bronchitis, and asthma.[148] Zibadi et al.[149] stated that the purple passion fruit peel (PFP) extract contains 3 major components: cyanidin-3*O*-glucoside, quercetin-3*O*-glucoside, and edulilic acid, and also used in treatment of hypertensive rats (modeling human essential hypertension with increased expression and activity of iNOS) results in significantly attenuated blood pressure through NO modulation. Also found reduced systolic and diastolic blood pressure in hypertensive patients.

2.1.4.2 Foam mat drying

Foam-mat drying is a simple process of drying liquid - solid foods. Addition of stabilizing agent or foaming agent *viz.*, proteins, gums and various emulsifiers (e.g., glycerol monostearate, propylene glycerol monostearate, carboxymethyl cellulose [CMC], trichlorophosphate) acceleration[15] and drying at 50-80°C [86,52] Foam mat drying is carried out at relatively low temperatures to obtain a thin porous honeycomb sheet to get a free-flowing powder. The main cause of moisture removal acceleration is larger surface area.[15] Sankat and Castaigne [130] reported that capillary diffusion is the main reason for the moisture movement or displacement within the sample during drying.

There are various drying methods such as belt/tray drying method but it changes the color of sample, protein denaturation and poor rehydration quality has reported. Freeze-drying of liquids product is popular for its excellent product quality with good rehydration and color but costly affairs. Foam mat drying is also cheaper than vacuum drying, relatively simple and cost effective.[79,129]

The various food items such as soy milk,[4] star fruit [85] cowpea,[57] apple juice,[116] mango,[121] banana,[137] tomato juice,[78] bael,[18] shrimp[4] has been used for foam mat drying. Though, there is scanty of research on foam mat drying of purple passion fruit.

2.1.4.3 Foam generating methods

According to Eisner et al.[51] in liquid sample foaminess is characterized by the volume of foam which is either bubbling air through liquid or by using any mixing method. Foam generation classifications is term as the static method and most common methods are whipping, shaking and bubbling.

2.1.4.3.1 Whipping

Whipping or beating are done by using different devices such manual and automatic blenders, vortex mixers, and homogenizers, which agitate the liquid into a gas and while vortex mixers can only be used for liquid suspension.[113] During incorporation of air foam usually increases with an increase of beating intensity.[1]
The final size of bubbles is defined by speed of the agitator, the geometry of the apparatus and rheological properties of the liquid respectively.[1]

2.1.4.3.2 Shaking

Shaking method is another foam generation method but rarely used method. More the amplitude of shaking more the formation of bubbles. Volume of foam formed is meagre.[1]

2.1.4.3.3 Bubbling

Bubbling involves injection of gas through narrow openings into a known quantity of liquid, produces uniform bubble sizes. Size of bubbles is depends on the viscosity of the liquid.[1]

2.1.5 Extrusion cooking technology

In the food industry, extrusion cooking has been continuously developed since its invention.[96,19] Olden days, the extruder was engaged in the production of macaroni and ready to eat cereal pellets only but now ingredients are transformed into various modified intermediate and finished products respectively.[28,32,127,122] Extrusion cooking techniques are preferable than other techniques due to its high productivity and significant nutrient retention, owing to the high temperature and short time required.[59] This technology also reported in the reduction of microbial contamination increase the shelf life of extrudates product which has a water activity of 0.1 to 0.4.[23]

In the recent year, high temperature-short time (HTST) extrusion cooking has been used successfully in the food industry. Products like nutritious ready-to-eat meals such as a protein-rich instant porridge were available in the markets[110,127] .Extruders come in various design depending on the utility. Two broad categories are single screw extruder and twin extruder.

2.1.6 Rheology

Rice flour is hypoallergenic, colorless, and bland taste in nature but one of the most suitable flour for the preparation of gluten-free products and properties.[62] Rheology is a science which deal with the deformation and flow of matter. Rheology has a very important role in food manufacture and marketing sector *viz.*, design of handling systems, quality control and evaluation of sensory stimuli associated with oral and non-oral evaluation of viscosity.[117] Basic concepts of stress (force per area) and strain (deformation per length) are keys to all rheological evaluations. Stress (r) is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). The direction of the force with respect to the impacted surface area determines the type of stress. Normal stress occurs when the force is directly perpendicular to a surface and can be achieved during tension or compression. Shear stress occurs when the forces act in parallel to a surface. Various food show different rheological behavior and categorized into solid and liquids stages. It basically means that food varies their characteristic in viscous and elastic behaviors commonly known as viscoelasticity of food cause by entanglement of long chain molecules with other molecules.[5]

Dynamic oscillation test is the very convenient and suitable test for rheological property of viscoelastic food material. The elastic nature is measured by dynamic storage modulus (G') which is a measure of the energy stored in the food material and recovered from it per cycle of sinusoidal deformation while viscous nature i.e. the loss modulus (G'') is a measure of the energy dissipated or lost per cycle.[138, 127]

Table 2.3 Various rheological models

Sl. No.	Model name	Equation	Reference
1	Power law	$\sigma = k(\gamma)^n$	Holdsworth[68]
2	Bingham model	$\sigma - \sigma_0 = n'\gamma$	Bingham [24]
3	Herschel –Bulky model	$\sigma = K_{H\gamma^{nH}} + \sigma_{OH}$	Barnes and Walters [26]
4	Casson model	$\sigma^{0.5} = K_{oc} + K_c(\gamma)^{0.5}$	Casson,[33]
5	Mizrahi and Berk	$\sigma^{0.5} = K_M \gamma'^{n_M} + \sigma_{OM}$	Mizrahi and Berk [103]

The Casson model is a used for structure-based model and used for food dispersions which include cooked rice flour dispersions [29] and rice starch dispersions.

Power law

Power law model describes the data of shear-thinning and shear thickening fluids or material

$$\sigma = k(\gamma)^n \qquad \text{eq. (1)}$$

where, γ is the shear rate, K is the consistency coefficient with the units and η is the viscosity of the fluid.

Bingham model

Bingham model exhibits a yield stress,

$$\sigma - \sigma_0 = n'\gamma \qquad \text{eq. (2)}$$

where η' *is the* is called the Bingham plastic viscosity, γ is the shear rate and σ_0 is the yield stress

Herschel –Bulky model

$$\sigma = K_{H\gamma^{nH} + \ \sigma_{OH}} \qquad \text{eq. (3)}$$

σ_0 is the yield stress, γ is the shear rate , KH is the consistency index , nH is the flow behavior.

Casson model

Casson model is used in characterization of various number of food dispersions

$$\sigma^{0.5} = K_{oc} + K_c(\gamma)^{0.5} \qquad \text{eq.(4)}$$

Where $(\sigma)^{0.5}$ is the square root of shear stress, γ is the shear rate , Kc is the slope and K_{0c} is intercept.

Mizrahi and Berk model

Mizrahi and Berk model is a three-parameter viscoplastic model which also exhibits yield stress (Mizrahi and Berk) [103] and eq. is given below

$$\sigma^{0.5} = K_M \gamma^{'n_M} + \sigma_{OM} \qquad \text{eq.(5)}$$

Where K_M = Apparent viscosity or consistency coefficient, σ_{OM} = Yield stress and n_M = Flow behavior index

2.1.7 Fuzzy logic tool

Value addition of underutilised crops is growing in the food markets. Incorporation of various ingredients add varieties in the items and enhance their textural or nutritional quality. Extrusion cooking , popularly known as high temperature/short time (HTST) is an important and popular food processing technique classified as a process to produce a fiber-rich product.[60] The process is not only popular for developing ready to eat food items but nutritionally to some extent may improve the bioavailability of bioactive compounds.[27]

The *fuzzy* model can be used to determine the importance of individual factors to the overall quality of a product. In the model another important parameter is ‘weighting subset’, which tailored a product for specific consumer groups or geographic regions respectively[147,138] Various food products such as mango drink[77] black rice wine[76] gluten free pasta,[125] risk dependency chain model of dairy agro-industry supply chain[124] but no study has been reported on ready to eat product develop rom underutilized crops such as red rice and purple passion fruit.

2.1.8 Moisture sorption isotherm

Water plays a crucial role in food processing and, food quality preservation depends on various aspects *viz.,* moisture content, moisture migration or moisture uptake by the material during storage. Adsorption (refers weight gain of dry material because of water intake from atmospheres of increasing relative humidity) or desorption (refers weight loss (wet material) under the same relative humidity) state of a food product depends on vapor pressure of water present in the sample and of surroundings. The state of moisture content at which vapor pressure of water present in sample equals that of the surroundings termed as equilibrium moisture content (EMC).[100] Moisture sorption isotherm (MSI) is a relationship between total moisture content and the water activity of the food at constant temperature. According to Brunauer et al.[21] stated that on the basis of the van der Waals adsorption of gases on various solid substrates, Moisture sorption isotherm adsorption can be categorized into 5 types *viz.*, Type I (Langmuir) and Type II (sigmoid shaped adsorption isotherm) and no specific name has been reported yet for others, Types III, Types IV and V. But later, Ricardo et al.[117] reported that MSI characteristics are of five types *viz.*, Type 1: Langmuir or similar isotherm, Type 2 : sigmoidal sorption isotherm, Type 3 : Flory-Huggins isotherm, Type 4 : described the adsorption of a swellable hydrophilic solid until a maximum of site hydration is reached, and type 5: Brunauer-Emmett-Teller (BET). Mostly, MSI of foods are sigmoidal in shape (non-linear) i.e. Type II isotherms. Caurie [41] stated that water content in fresh food exerts a vapour pressure near of pure water, i.e. unity. In food, this vapour pressure is maintain to attend 22% moisture content. In unity, vapour pressure will starts dropping which in dehydrated food showed sigmoidal shape of water sorption isotherms. The food products during storage and processing at different range of temperature, showed the mobility of water molecules and the dynamic equilibrium between the vapour and adsorbed phases in food. Iglesias and Chirife[71] reported that the storage of food items in different increasing temperature make it less hygroscopic. Palipane and Driscoll [111] also stated that due to higher temperatures, water molecules are activated to energy levels and break away the sorption sites causing decrease in the equilibrium moisture content.

A fundamental property of a biomaterial is the water sorption characteristics.[30] The moisture sorption isotherm (MSI) predicts the possible changes in food item and also used to find out the storage study method and selection of packaging material for food products.[16] These parameters helps to optimize or maximize retention of color, flavor, texture, nutrients, biological stability [119] and also crucial for design, modelling and optimization of different processes like aeration, drying and storage.[20]

Table 2.4 Mathematical models used to fit the equilibrium MSI of extruded products

Sl. No.	Model	Mathematical equation	Reference
1	Oswin	$M_W = A(\frac{a_w}{1-a_w})^B$	Andrade et al.[9]
2	Smith	$M_W = A + B\ln(-a_w)$	Andrade et al.[9]
3	Curie	$M_W = \exp(A + Ka_w)$	Curie [41]
4	Peleg	$M_W = Aa^c{}_w + Ba^k{}_w$	Basu et al. [16]
5	Langmuir	$\frac{1}{CM_o} = a_w(\frac{1}{M_w} - \frac{1}{M_O})$	Langmuir [94]
6	Brunauer-Emmett-Teller (BET)	$\frac{M_w}{M_0} = \frac{Ca_w}{(1-aw)[1+(C-1)a_w]}$	Brunauer et al.[22]

M_w and M_o are equilibrium and monolayer moisture content, respectively (g water/100 g dry matter); a_w is the water activity (decimal); A, B, C and M are respective model constants.

2.1.9 Dipeptidyl peptidase-4 (DPP-4) inhibitory activity and GLP-1 secretion

2.1.9.1 Diabetes mellitus

India is reported to be largest number of diabetics in the world, 3.8% in rural and 11.8% in urban adults.[123] Diabetes mellitus are of two types namely type 1 and type 2 [74,10]. Type 2 diabetes mellitus (T2DM) showed 90 % cases of diabetes (approx.) and soon going to be declared as global epidemic of the twenty-first century.[72]

2.1.9.2 Incretin System

The incretin a gastrointestinal hormones namely glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are insulinotropic i.e. stimulate insulin secretion.[63] As soon as the meal is taken GLP-1 (7-36 amide) i.e. 30 amino acid polypeptide cause the release of insulin from from β-cells of the Islets of Langerhans and inhibit glucagon release from the α-cells of the Islets of Langerhans. Half-life of glucagon-like peptide (GLP) which secreted from L- cell is 1- 1.5 minutes total but abruptly degraded by dipeptidyl peptidase-4 (DPP-IV) enzyme and get cleaved in two N-terminal residues ogGLP-1[47,31] Another intestinal hormone i.e. glucose-dependent insulinotrophic polypeptide (GIP) secreted from K cells of the duodenum released GIP after meal is taken. Half-life of GIP secreted have reported to be 5-7 minutes [101,48,120,49]

2.1.9.3 Dipeptidyl peptidase-4 (DPP-4) inhibitory activity

In record, dipeptidyl peptidase IV (DPP IV) was identified by Hopsu-Hovu and Glenner[70] as glycylproline napthylamidase. Later, in the year 1967 and 1968, it was purified from rat liver and pig kidney respectively. The first inhibitor characterization was stated in the late 1980s and 1990s.[131] DPP4 is present in various organ *viz.,* kidney, spleen, lungs, pancreas, and prostate.[61] Highly expressed at high levels on various cells such as endothelial, differentiated epithelial, and immune cells such as T cells, dendritic cells, and macrophages. Kirino et al. [87] and Andrieu[11] stated that altered expression of soluble DPP4 is commonly ascertain in various disorders such as solid tumors, autoimmune diseases, hepatitis C, type 2 diabetes (T2DM), and obesity. Pharmacological involvement and clinically approved strategies *viz.,* glucagon-like peptide-1 (GLP-1), inhibitors of dipeptidyl peptidase-4 (DPP-4) which act to halt the physiological breakdown of glucagon-like peptide-(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which is the need of hour to cease the rate of diabetic people in the world specially in the developing countries.[56,64,65,89]

2.1.9.4 GLP-1 secretion

Glucagon-like peptide (GLP-1) is a type of incretin which is secreted from enteroendocrine L cells, and also a part of lower small intestine and large intestine. GLP-1 stimulates insulin secretion in a blood glucose concentration dependent manner which results in reduction of pancreatic cell exhaustion, hypoglycemia, and weight gain etc. GLP-1 is reported with various diabetes-suppressing actions and considered as an important and effective molecule for treating type 2 diabetes.[106] Review of literature has showed various studies *viz.,* administration of lipase inhibitor (Orlistat) in type 2 diabetes patients to validate GLP-1 and GIP secretion during glucose and oil intake, showed lowered glucose tolerance,[107] whey protein stimulates the secretion of GLP-1 during mashed potato intake and helps in postprandial hyperglycemia[97] alcohol consumption after a meal helps in suppressing the increase of GLP-1 concentration[43] healthy subjects who consume sandwich (containing bread, butter, and dried meat) was stated with strong GIP secretion and effective incretin, when compared to a meal consisting of dried meat or butter,[36] increase intake of fish (polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid) in Japanese type 2 diabetes patients reported effectiveness of glycated hemoglobin reduction.[73]

2.1.9.5 Mechanism of action

In the body, DPP-IV inhibitors helps in inhibition of DPP 4 enzyme which degrade incretin GLP-1 and GIP after meal. GLP-1 and GIP increases the secretion of insulin and prevent the release of glucagon by the pancreas to maintain blood glucose in normal. Once the normal level is attained in the body, insulin released and glucagon suppression declined, prevents hypoglycemia in oral hypoglycemic agents.[112]

References

[1] Arzhavitina, A. and Steckel, H. Foams for pharmaceutical and cosmetic application. *International Journal of Pharmaceutics*, 394(3):4-7, 2010.

[2] Acosta-Estrada, B, A., Gutierrez-Uribe, J, A. and Serna-Saldivar, S, O. Bound phenolics in foods, a review. *Food Chemistry*, 152:46-55, 2013.

[3]Akintoye, O, A, A. and Oguntunde, O. Preliminary investigation on the effect of foam stabilizers on the physical characteristics and reconstitution properties of foam-mat dried soymilk. *Drying Technology*, 9(1):245-262,1991.

[4] Azizpour, M., Mohebbi, M., Khodaparast, M, H, H. and Varidi, M. Foam-mat drying of shrimp: Characterization and drying kinetics of foam. *Agricultural Engineering International*: *CIGR Journal*,15:159-165,2013.

[5] Abang, Z, D, N., Chin. N, L. and Yusof, Y, A. A Review on rheological properties and measurements of dough and gluten. *Journal of Applied Sciences*,10(20):2478-2490,2010.

[6]Amin, M, N., Hossain, M, A. and Roy, K, C. Effects of moisture content on some physical properties of lentil seeds. *Journal of Food Engineering*, 65:83-87, 2004.

[7] Adom, K, K. and Liu, R, H. Antioxidant activity of grains. *Journal of Agricultural Food Chemistry*, 50:6170-6182, 2002.

[8] Adom, K, K., Sorrells, M, E. and Liu, R, H. Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry*, 51:7825-7834, 2003.

[9] Andrade, P, R, D., Lemus, M, R. and Perez, C,C,E. Models of sorption isotherms for food: uses and limitations. *Vitae, Revista De La Facultad De Quimica Farmaceutica,* 18(3):325-334, 2011.

[10] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*,33: S62- 90,2010.

[11] Andrieu,T., Thibault, V., Malet.,I., Laporte ., L., Bauvois,B., Agut,H. and Cahour,A. Similar increased serum dipeptidyl peptidase IV activity in chronic hepatitis C and other viral infections. *Journal of Clinical Virology*, 27(1):59-68,2003.

[12] Amata, R., Otipa, M., Waiganjo, M., Wabule, M., Thuranira, E., Erbaugh, M. and Miller, S. Incidence, prevalence and severity of passion fruit fungal diseases in major production regions of Kenya. *Journal of Applied Biosciences*, 20:1146-1152, 2009.

[13] Boue, S, M., Daigle, K, W., Chen, M., Cao, H. and Heiman, M, L. Antidiabetic potential of purple and red rice (*Oryza sativa* L.) bran extracts. *Journal of Agricultural and Food Chemistry*, 64:5345-5353, 2016.

[14] Banjerdpongchai, R., Wudtiwai, B. and Sringarm, K. Cytotoxic and apoptotic-inducing effects of purple rice extracts and chemotherapeutic drugs on human cancer cell lines. *Asian Pacific Journal of Cancer Prevention*, 14:6541-6548,2013.

[15] Brygidyr, A, M., Rzepecka, M, A. and McConnell, M, B. Characterization and drying of tomato paste foam by hot air and microwave energy. *Canadian Institute Food Science and Technology Journal*, 10:313-319, 1977.

[16] Basu, S., Shivhare, U, S. and Mujumdar, A, S. Model for sorption isotherm for food: A review. *Drying Technology*, 24(8):917-930, 2006.

[17] Brennan, M, A., Monro, J, A. and Brennan, C, S. Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration. *International Journal of Food Science and Technology*, 43:2278-2288, 2008.

[18] Bag, S, K., Srivastav, P, P. and Mishra, H, N. Optimization of process parameters for foaming of bael (*Aegle marmelos* L.) fruit pulp. *Food and Bioprocess Technology*, 4:1450-1458, 2011.

[19] Bouvier, J, M. and Campanella, O, H. The Generic Extrusion Process I, in Extrusion Processing Technology, John Wiley & Sons, Ltd, Chichester, UK. doi: 10.1002/9781118541685.ch4,2014.

[20] Bala, B, K. Physical and thermal properties of malt. *Drying Technology*, 9(4):1091-1104,1991.

[21] Brunauer, S., Deming, L, S., Deming, W, E. and Troller, E. On the theory of Van der Waals adsorption of gases, *Journal of the American Chemical Society*, 62:1723-1732, 1940.

[22] Bhattacharya, K, R. and Sowbhagya, C, M. Size and Shape Classification of Rice. *Il Riso*, 29, 181-184,1982.

[23] Bordoloi, R. and Ganguly, S. Extrusion technique in food processing and a review on its various technological parameters. *Indian Journal of Science, Research and Technology*, 2(1):1-3, 2014.

[24] Bingham, E,C. Fluidity and Plasticity. New York, McGraw-Hill. p 452,1922.

[25] Babu, P, D., Subhasree, R,S., Bhakyaraj, R. and Vidhyalakshmi, R. Brown rice-beyond the color reviving a lost health Food. *American-Eurasian Journal of Sustainable Agriculture*, 2:67-72, 2009.

[26] Barnes, H, A. and Walters, K. The yield stress myth? *Rheology Acta*, 24:323-326,1985.

[27] Brennan, C., Brennan, M., Derbyshire, E. and Tiwari, B. Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science and Technology*, 22:570-575, 2011.

[28] Camire, M, E., Camire, A, L. and Krumhar, K. Chemical and nutritional changes. *Critical Reviews in Food Science and Nutrition*, 29: 35-57, 1990.

[29] Chun, S, Y. and Yoo, B. Rheological behavior of cooked rice flour dispersions in steady and dynamic shear. *Journal of Food Engineering*, 65:363-370, 2004.

[30] Chukwu, O. and Ajisegiri, E, S, A. Moisture-sorption isotherms of yam and cocoyam. *Nigerian Journal of industrial Systems Studies*, 5(2):19-24,2006.

[31] Christopher, H, S. Incretin based therapies for type 2 diabetes. *Canadian Journal of diabetes*, 32(2):131-139, 2008.

[32] Camire, M, E. Extrusion cooking. In: The Nutrition Handbook for Food Processors. 314-330. Henry, C. J, K. and Chapman, C., Eds., Woodhead Publishing Limited, Cambridge, 2003.

[33] Casson, N. A flow equation for pigmented-oil suspension of the printing ink type. In Rheology of Disperse Systems, (C.C. Mill, Ed.) 82-104, Pergamon Press, New York, 1959.

[34] Chen, M., McClung, A, M. and Bergman, C, J. Concentrations of oligomers and polymers of proanthocyanidins in red and purple rice bran and their relationships to total phenolics, flavonoids, antioxidant capacity and whole grain color. *Food Chemistry*, 208:279-287, 2016.

[35] Chen, Q., Ling, W, H., Ma, J. and Mei, J. Effects of black and red rice on the formation of aortic plaques and blood lipids in rabbits. *Journal of Hygiene Research*, 29:170-172, 2000.

[36] Carrel, G., Egli, L., Tran, C., Schneiter P., Vittorio Giusti, V., D'Alessio, D. and Tappy, L. Contributions of fat and protein to the incretin effect of a mixed meal. *American Journal of Clinical Nutrition*, 94:997-1003,2011.

[37] Coleta, M., Batista, M, T., Campos, M,G., Carvalho, R., Cotrim, M, D., Lima, T,C, M. and *Cunha, A,P.* Neuro pharmacological evaluation of the putative anxiolytic effects of *Passiflora edulis* Sims, its sub-fractions and flavonoid constituents. *Phytotherapy Research*, 20(12):1067-1073,2006.

[38] Cerqueira-Silva, C, B, M., Jesus, O, N., Santos, E, S, L., Correa, R, X. and Souza, A, P. Genetic breeding and diversity of the genus *Passiflora*: Progress and perspectives in molecular and genetic studies. *International Journal of Molecular Sciences*, 15:14122-14152, 2014.

[39] Choi, Y., Jeong, H, S. and Lee, J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chemistry*, 103:130-138, 2007.

[40] Cutri, L., Nave, N., Ami ,M, B., Chayut, N., Samach, A. and Dornelas, M, C. Evolutionary, genetic, environmental and hormonal-induced plasticity in the fate of organs arising from axillary meristems in Passiflora spp. *Mechanisms of Development*, 130(1):61-69, 2013.

[41] Caurie, M. Derivation of full range moisture isotherms. In Water Activity: Influences on Food Quality (L.B. Rockland and G.F. Stewart, eds.) pp. 63-87, Academic Press, New York, 1981.

[42] Carr, M, K,V. The water relations and irrigation requirements of passion fruit 531 (*Passiflora edulis* Sims): a review. *Experimental Agriculture*, 49:585-596, 2013.

[43] Dalgaard, M., Thomsen, C., Rasmussen, B. M., Holst, J, J. and Kjeld H. Ethanol with a mixed meal decreases the incretin levels early postprandially and increases postprandial lipemia in type 2 diabetic patients. *Metabolism,* 53:77-83, 2004.

[44] Dhawan, K., Dhawan, S. and Sharma, A. *Passi flora* : A review update. *Journal of Ethnopharmacology*, 94 (1):1-23, 2004.

[45] Delgado-Vargas, F., Jimenez, A, R. and Paredes-Lopez, O. Natural pigments: carotenoids, anthocyanins, and betalains -characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, 40:173-289, 2000.

[46] Dong, B., Xu, H., Zheng, Y, X., Xu, X., Wan, F, J. and Zhou, X, M. Effect of anthocyanin-rich extract from black rice on the survival of mouse brain neuron. *Cell Biology International*, 32: S8-S8, 2008.

[47] Drucker, D.J. Glucagon-like peptides. *Diabetes*, 47:159-169,1998.

[48] Daniel, C., Stephanie, L, L. and Robert, A, P. *International Anesthesia Research Society*, 08(6):1803, 2009.

[49] Davidson, J, A. Incorporating incretin-based therapies into clinical practice: differences between glucagon-like peptide 1 receptor agonists and dipeptidyl peptidase 4 inhibitors. *Mayo Clinic Proceedings,* 85(12): S27-S37,2010.

[50] Das, A, B., Goud, V, V. and Chandan, Das. Extraction of phenolic compounds and anthocyanin from black and purple rice bran (*Oryza sativa* L.) using ultrasound: A comparative analysis and phytochemical profiling. *Industrial Crops and Products* 95:332-341, 2017.

[51] Eisner, M, D., Jeelani, S, A, K., Bernhard, L. and Windhab, E, J. Stability of foams containing proteins, fat particles and nonionic surfactants. *Chemical Engineering Science*, 62:1974-1987, 2006.

[52] Febrianto, A., Kumalaningsih, S. and Aswari, A,W. Process engineering of drying milk
powder with foam-mat drying method, a study on the effect of the concentration and types of filler process. *Journal of Basic and Applied Scientific Research*, 2(4):3588-3592, 2012.

[53] Finocchiaro, F., Ferrari, B., Gianinetti, A., DallAsta, C., Galaverna, G., Scazzina, F. and Pellegrini, N. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Molecular Nutrition and Food Research,* 51:1006-1019, 2007.

[54] Ferreres, F., Sousa, C., Valentao, P., Andrade, P, B., Seabra, R, M. and Gil-Izquierdo, A. New C-deoxyhexosyl flavones and antioxidant properties of *Passiflora edulis* leaf extract. *Journal of Agricultural and Food Chemistry,* 55(25):10187-10193, 2007.

[55] Furukawa, T., Maekawa, M., Oki, T., Suda, I., Iida, S., Shimada, H., Takamure, I., and Kadowaki, K. The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. *The Plant Journal*, 49:91-102, 2006.

[56] Flatt, P, R., Bailey, C,J. and Green, B, D. Dipeptidyl peptidase IV (DPP IV) and related molecules in type 2 diabetes, *Frontiers in Bioscience*,13:3648-3660,2008.

[57] Falade, K,O., Adeyanju, K,I. and Uzo-peters, P, I. Foam mat drying of cowpea (*Vignaunguiculata*) using glyceryl monostearate and egg albumin as foaming agents. *European Food Research and Technology*, 217:486-491, 2003.

[58] Goufo, P. and Trindade, H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Science and Nutrition*, 2:75-104, 2014.

[59] Guy, R. *Extrusion Cooking Technologies and Application.* Boca Raton Boston New York Washington, DC,1-200,2001.

[60] Gaossong,J. and Vasanthan,T. The effect of extrusion cooking on the primary structure and water solubility of β-glucans from regular and waxy barley. *Cereal Chemistry*, 77:(396-400), 2000.

[61] Gorrell, M, D. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clinical Science*, 108:277-292,2005.

[62] Gujral, H, S. and Rosell, C, M. Improvement of the bread making quality of rice flour by glucose oxidase. *Food Research International*, 37:75-81,2004.

[63] Garber,A, J. Incretin effects on beta-cell function, replication, and mass: the human perspective. *Diabetes Care*, 34(2):S258-S263,2011.

[64] Green, B, D. and Flatt, P, R. Incretin hormone mimetics and analogues in diabetes therapeutics, *Best Practice and Research. Clinical Endocrinology and Metabolism,* 21:497-516,2007.

[65] Green, B, D., Flatt, P, R. and Bailey, C, J. Inhibition of dipeptidyl peptidase IV activity as a therapy of type 2 diabetes, *Expert Opinion on Emerging Drugs*, 11:525-539,2006.

[66] Hiemori, M., Koh, E. and Mitchell, A, E. Influence of cooking on anthocyanins in black rice (*Oryza sativa* L. *japonica var* SBR). *Journal of Agricultural and Food Chemistry*, 57:1908-1914, 2009.

[67] Hu, Q, L. A research report on the pharmacologic test of black rice pigment. *Journal of Wuhan Food Industrial Colloid*, 3:10-12, 1997.

[68] Holdsworth, S, D. Rheological models used for the prediction of the flow properties of food products: a literature review. *Transactions of the Institution of Chemical Engineers*, 71:139-179, 1993.

[69] Hore, D.K. Rice diversity collection, conservation and management in Northeastern, India. Genetic resource and crop evolution,52:1129-1140,2005.

[70] Hopsu-Havu, V, K. and Glenner ,G, G. A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-beta-naphthylamide. *Histochemistry*,7(3):197-201,1966.

[71] Iglesias, H, A. and Chirife, J. Handbook of food isotherms: Water sorption parameters for food and food components. Academic Press, New York, NY, USA, 1982.

[72] International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation, downloaded http://www.idf.org/diabetesatlas on 9 April 2017. http://www.idf.org/diabetesatlas,2013.

[73] Iwasaki, M., Hoshian, F., Tsuji, T., Hirose, N., Matsumoto, T.,, Kitatani, N., Sugawara, K., Usui, R., Kuwata, H., Sugizaki, K., Kitamoto, Y., Fujiwara, S., Watanabe, K., Hyo, T., Kurose, T., Seino, Y. and Yabe, D. Predicting efficacy of dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes: association of glycated hemoglobin reduction with serum eicosapentaenoic acid and docosahexaenoic acid levels. *Journal of Diabetes Investigation*. 3:464-467, 2012.

[74] Jain, S. and Saraf, S. Type 2 diabetes mellitus-Its global prevalence and therapeutic strategies. *Diabetes and Metabolic Syndrome*: *Clinical Research and Reviews,*4:48-56,2010.

[75] Joy, P, P. Status and prospects of passion fruit cultivation in Kerala, pineapple research station ,Vazhakulam, 686- 670, 2010.

[76] Jha, P., Das, A, J., Dash, K, K. and Deka, S, C. Sensory evaluation of black pigmented rice (*Oryza sativa cv. Poireton*) wine fortified with probiotic Lactobacillus acidophilus ATCC 4356 and Lactobacillus sakei ATCC 15521 using fuzzy. *Journal of Institute of Brewing,* 121:566-573, 2015.

[77] Jaya, S. and Das, H. Sensory evaluation of mango drinks using fuzzy logic. *Journal of Sensory Studies*, 18:163-176,2003.

[78] Kadam, D, M. and Balasubramanuan, S. Foam-mat drying of tomato juice. *Journal of Food Processing and Preservation,* 35:488-495, 2011.

[79] Kadam, D, M., Wilson, R, A. and Kaur, S. Determination of biochemical properties of foam mat dried mango powder. *International Journal of Food Science and Technology,* 45:1626-1632, 2010.

[80] Kim, H, M., Kang, C, S., Lee, E, H. and Shin, T, Y. The evaluation of the antianaphylactic effect of *Oryza sativa* L. subsp. Hsien Ting in rats. *Pharmacological Research*, 40:31-36, 1999.

[81] Kim, J, K., Lee, S,Y., Chu, S,M., Lim, S,H., Suh, S,C., Lee, Y,T., Cho, H,S. and Ha, S,H. Variation and correlation analysis of flavonoids and carotenoids in Korean pigmented rice (*Oryza sativa* L.) Cultivars. *Journal of Agricultural and Food Chemistry*, 58:12804-12809, 2010.

[82] Kim, H,W., Kim, J,B., Shanmugavelan, P., Kim, S,N., Cho, Y,S., Kim, H, R., Lee, J,T., Jeon, W,T. and Lee D, J. Evaluation of γ-oryzanol content and composition from the

grains of pigmented rice-germplasms by LC-DAD-ESI/MS. *BMC Research Notes*, 6:149,2013.

[83] Kale, S, P., Milde, L., Trapp, M, K., Frisvad, J, C., Keller, N, P. and Bok, J,W. Requirement of LaeA for secondary metabolism and sclerotial production in *Aspergillus flavus. Fungal Genetics and Biology,* 45:1422-1429, 2008.

[84] Kim, Y, S., Kim, Y, H., Noh, J, R., Cho, E, S., Park, J, H. and Son, H,Y. Protective effect of Korean red ginseng against aflatoxin B1-induced hepatotoxicity in rat. *Journal of Ginseng Research,* 35:243-249, 2011.

[85] Karim, A, A. and Wai, C, C. Characteristics of foam prepared from starfruit (*Averrhoa carambola* L.) puree by using methyl cellulose. *Food Hydrocolloid*, 13: 203-210,1999.

[86] Kandasamy, P., Varadharaju, N., Kalemullah, S. and Moitra, R. Production of papaya powder under foam-mat drying using methyl cellulose as foaming agent. *Asian Journal of Food and Agro-Industry*, 5(05):374-387, 2012.

[87] Kirino,Y., Sato, Y., Kamimoto,T., Kawazoe, K. and Minakuchi, K. Altered dipeptidyl peptidase-4 activity during the progression of hyperinsulinemic obesity and islet atrophy in spontaneously late-stage type 2 diabetic rats. *The American Journal of Physiology-Endocrinology and Metabolism*, 300(2):E372-E379, 2011.

[88] Kishore, K., Pathak, K, A., Shukla, R. and Bharali , R. Effect of storage temperature on physico-chemical and sensory attributes of purple passion fruit *(Passiflora edulis* Sims). *Journal of Food Science and Technology*, 48(4):484-488, 2011.

[89] Lovshin, J, A. and Drucker, D, J. Incretin-based therapies for type 2 diabetes mellitus, *Nature Reviews Endocrinology,* 5:262-269,2009.

[90] Laokuldilok, T., Shoemaker, C., Jongkaewwattana, S. and Tulyathan, V. Antioxidants and antioxidant activity of several pigmented rice brans. *Journal of Agricultural and Food Chemistry*, 59:193-199, 2011.

[91] Ling, W, H., Cheng, Q, X., Ma, J. and Wang, T. Red or black rice decrease atherosclerotic plaque and increase antioxidant status in rabbits. *Journal of Nutrition*, 131:1421-1426, 2001.

[92] Ling, W, H., Wang, L, L. and Ma, J. Supplementation of the black rice outer layer fraction to rabbits decreases atherosclerotic plaque formation and increases antioxidant status. *Journal of Nutrition*, 132:20-26, 2002.

[93] Limtrakul, P., Yodkeeree, S., Pitchakarn, P. and Punfa, W. Anti-inflammatory effects of proanthocyanidin-rich red rice extract via suppression of MAPK, AP-1 and NF- j B pathways in Raw 264.7 macrophages. *Nutrition Research and Practice*, 10:251-258, 2016.

[94] Langmuir, I. The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society*, 40:1361-1402,1918.

[95] Mirabella, N., Castellani, V. and Sala, S. Current options for the valorization of food manufacturing waste: A review. *Journal of Cleaner Production*, 65:28-41, 2014.

[96] Maskan, M. and Altan, A. Advances in Food Extrusion Technology. CRC Press, 2011.

[97] Ma, J., Stevens, J, E., Cukier, K., Maddox, A,F., Wishart, J,M., Jones, K,L., Clifton, P,M., Horowitz, M. and Rayner, C,K. Effects of a protein preload on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes. *Diabetes Care*, 32:1600-1602, 2009.

[98] Maisuthisakul, P. and Changchub, L. Effect of extraction on phenolic antioxidant of different Thai rice (*Oryza sativa* L.) genotypes. *International Journal of Food Properties*, 17:855-865, 2014.

[99] Mira, N, V, M., Massaretto, L, L., Pascual, C, S, C, I. and Lanfer-Marquez, U, M. Comparative study of phenolic compounds in different Brazilian rice (*Oryza sativa* L.) genotypes. *Journal of Food Composition and Analysis*, 22:405-409, 2009.

[100] Mujumdar, A,S., Devahastin, S. Fundamental principles of drying. In Mujumdar's Practical Guide to Industrial Drying, Devahastin, S., Ed., Exergex Corp: Montreal,1-22,2000.

[101] Michael, A, N., Tina, V,B., Baptist, G, W. and Alan, G. Incretin based therapies. *Diabetes Care*, 32(2):S223-S231, 2009.

[102] Montanher, A, B., Zucolotto, S, M., Schenkel, E, P. and Frode, T, S. Evidence of anti-inflammatory effects of *Passiflora edulis* in an inflammation model. *Journal of Ethnopharmacology*, 109(2):281-288, 2007.

[103] Mizrahi, S. and Berk Z.Flow behavior of concentrated orange juice: 433 Mathematical treatment. *Journal of Texture Studies*, 3:69-79,1972.

[104] Nam, S.H., Choi, S.P., Kang, M.Y., Koh, H.J., Kozukue, N. and Friedman, M. Bran extracts from pigmented rice seeds inhibit tumor promotion in lymphoblastoid B cells by phorbol ester. *Food Chemical Toxicology*, 43:741-745, 2005.

[105] Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I. and Sato, T. Polymeric procyanidins as radical-scavenging components in red-hulled rice. *Journal of Agricultural Food Chemistry*, 50:7524-7529,2002.

[106] Ovshin, J, A. and Drucker, D, J. Incretin-based therapies for type 2 diabetes mellitus. *Nature Review Endocrinology*, 5:262-269,2009.

[107] Pilichiewicz, A., O'Donovan, D., Feinle,C., Lei,Y., Wishart,J,M., Bryant,L., Meyer,J,H., Horowitz, M. and Jones, K,L. Effect of lipase inhibition on gastric emptying of, and the glycemic and incretin responses to, an oil/aqueous drink in type 2 diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism*, 88:3829-3834,2003.

[108] Pereira, A, D., Correa, R, X. and Oliveira, A, C. Molecular genetic diversity and differentiation of populations of somnus passion fruit trees (*Passi flora setacea* DC):

Implications for conservation and pre-breeding. *Biochemical Systematics and Ecology*, 59:12-21, 2015.

[109] Punvittayagul, C., Sringarm, K., Chaiyasut, C. and Wongpoomchai, R. Mutagenicity and antimutagenicity of hydrophilic and lipophilic extracts of Thai Northern purple rice. *Asian Pacific Journal of Cancer Prevention*, 15: 9517-9522, 2014.

[110] Pelembe, L, A, M., Erasmus, C. and Taylor, J, R, N. Development of a protein rich composite sorghume cowpea instant porridge by extrusion cooking process. *LWT-Food Science and Technology*, 35:120-127, 2002.

[111] Palipane, K, B. and Driscoll, R, H. Moisture sorption characteristics of in shell macadamia nuts, *Journal of Food Engineering*, 18: 63-76,1992.

[112] Patel, K, D. and Patel, G, M. Role of DPP-IV inhibitors in treatment of type II diabetes. *International Research Journal of Pharmacy*, 1(1):19-28, 2010.

[113] Presscott, J, K. and Clement, S, A. Blending, segregation and sampling. In: Encapsulated and Powdered Foods, pp. 312–317. Onwulata, C., Ed. Boca Raton, Florida: CRC Press, 2005.

[114] Petry, R. D., Reginatto, F., de-Paris, F., Gosmann, G., Salgueiro, J. B., Quevedo, J., Kapczinski, F., Ortega, G, G. and Schenkel, E, P. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. *Phytotherapy Research*, 15(2):162-164, 2001.

[115] Renger, A. and Steinhart, H. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *European Food Research and Technology*, 211:422-428, 2000.

[116] Raharitsifa, N., Genovese, D, B. and Ratti, C. Characterization of apple juice foams for foam-mat drying prepared with egg white protein and methylcellulose. *International Journal of Food Science*, 71:142-151, 2006.

[117] Rao, M, A. The structural approach to rheology of plant food dispersions. *Revista española de ciencia y tecnología de alimentos*, 32:3-17, 1992.

[118] Ricardo, D, P., M., lemusm, R, C, E. and Perez, C. Model of sorption isotherm for food: uses and limitation. Universidad de Antioquia, Medellín, Colombia, 18(3):325-334, 2011.

[119] Rizvi, S, S, H. Thermodynamic properties of foods in dehydration. In M, A, Rao. and S, S, H. Rizvi (Eds.), Engineering properties of foods (pp. 223-309). New York: Marcel Dekker, 1995.

[120] Renee, E, A., Joseph, L. and Anas Fassios, G, P. Efficacy and safety of incretin therapy in type 2 diabetes-systematic review and meta- analysis. *The Journal of the American Medical Association*, 298(2):194-206,2007.

[121] Rajkumar, P., Kailappan, R.,Viswanathan, R., Raghavan, G,S,V. and Ratti, C. Foam mat drying of alphonso mango juice. *Drying Technology*, 25:357-365, 2007.

[122] Riaz, M, N. *Introduction to extruders and their principles. In: Extruders in food applications*, CRC Press, Boca Raton, United States of America.1- 23, 2000.

[123] Reddy, K, S., Shah, B., Varghese, C. and Ramadoss, A. Chronic diseases 3. *Lancet*, 366:1746-51, 2005.

[124] Septiani, W., Marimin, Herdiyeni,Y. and Haditjaroko, L. Risk dependency chain model of dairy agro-industry supply chain using fuzzy logic approach, *Supply chain forum: an International Journal*, 218-230,2016.

[125] Sakre, N., Das, A, B. and Srivastav, P,P. Fuzzy logic approach for process optimization of Gluten-free pasta. *Journal of Food Processing and Preservation* ISSN 1745-4549,2015.

[126] Saravanan, S., Arunachalam, K. and Parimelazhagan, T. Antioxidant, analgesic, anti-inflammatory and antipyretic effects of polyphenols from Passi flora subpeltata

leaves-A promising species of passiflora. *Industrial Crops and Products,* 54:272-280, 2014.

[127] Singh J., Kaur, L. and McCarthy J, O. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications-A review, *Food Hydrocolloids*, 21:1-22,2007.

[128] Sema, A. and Maiti, C, S. Status and Prospects of Passion Fruit Industry in Northeast India. Central Institute of Horticulture, Medziphema-797 106, Nagaland, 2006.

[129] Sangamithra, A., Venkatachalam, S., John, S, G. and Kuppuswamy, K. Foam mat drying of food materials: a review, *Journal of Food Processing and Preservation*, 1745-4549,2014.

[130] Sankat, C, K. and Castaigne, F. Foaming and drying behavior of ripe bananas. *LWT-Food Science and Technology,* 37:517-525, 2004.

[131] Sebokova, E, Christ A, Boehringer, M. and Mizrahi, J. Dipeptidyl peptidase-4 inhibitors- A new approach in diabetes. *Current Topics in Medicinal Chemistry,* 7: 547-555, 2006.

[132] Shammugasamy, B., Ramakrishnan,Y., Ghazali,H,M. and Muhammad, K. Tocopherol and tocotrienol contents of different varieties of rice in Malaysia. *Journal of The Science of Food and Agriculture,*95: 672-678,2015.

[133] Samad, N. Rice bran oil prevents neuroleptic-induced extrapyramidal symptoms in rats: Possible antioxidant mechanisms. *Journal of Food Drug and Analysis*, 23:370-375, 2015.

[134] Sahay, K, M. and Singh, K, K. *Unit Operations of Agricultural Processing*, 1st Ed., Vikas Publishing House Pvt. Ltd., New Delhi, India, 1994.

[135] Tsai, C, J., Leitzmann, M, F., Willett, W, C. and Giovannucci, E, L. Long-term intake of dietary fiber and decreased risk of cholecystectomy in women. *American Journal of Gastroenterology,* 99:1364-1370, 2004.

[136] Tronsmo, K, M., Magnus, E, M., Baardseth, P., Schofield, J, D., Aamodt, A. and Faergestad, E, M. Comparison of small and large deformation rheological properties of wheat dough and gluten. *Cereal Chemistry*, 80(5):587-595, 2003.

[137] Thuwapanichayanan, R., Prachayawarakorn, S. and Soponronnarit, S. Drying characteristics and quality of banana foam mat. *Journal of Food Engineering*, 86(4):573-583, 2008.

[138] Uprit, S. and Mishra, H, N. Fuzzy multi attribute decision making approach and comparison of soya fortified paneer. *Journal of Sensory Studies*, I7(2):163-176,2002.

[139] Umadevi, M., Pushpa, R., Sampathkumar, K,P. and Bhowmik, D. Rice-Traditional medicinal plant in India. *Journal of Pharmacognosy and Phytochemistry*, 1:6-12, 2012.

[140] Wang, Q., Han, P., Zhang, M., Xia, M., Zhu, H., Ma, J., Hou, M., Tang, Z. and Ling ,W. Supplementation of black rice pigment fraction improves antioxidant and anti-inflammatory status in patients with coronary heart disease. *Asia Pacific Journal of Clinical Nutrition*, 16: 295-301, 2007.

[141] Xu, Z., Hua, N. and Godbar, J, S. Antioxidant activity of tocopherols, tocotrienols and γ-oryzanol component from rice bran against cholesterol oxidation accelerated by 2,20 - azobis (2- methylpropioamidine) dihydrochloride. *Journal of Agricultural and Food Chemistry*, 49:2077-2081, 2001.

[142] Xia, X., Ling, W., Ma, J., Xia, M., Hou, M., Wang, Q., Zhu, H. and Tang Z. An anthocyanin-rich extract from black rice enhances atherosclerotic plaque stabilization in apolipoprotein E-deficient mice. *Journal of Nutrition*, 136:2220-2225, 2006.

[143] Xie, D, Y. and Dixon, R, A. Proanthocyanidin biosynthesis -still more questions than answers? *Phytochemistry*, 66:2127-2144, 2005.

[144] Zhang, M, W., Guo, B, J., Zhang, R.F.,Chi, J, W., We, Z,C., Xu., Z, H., Zhang,Y. and Tang, X, J. Separation, purification and identification of antioxidant compositions in black rice. *Agricultural Science in China*, 5:431-440, 2006.

[145] Zaupa, M., Calani, L., Rio, D, D., Brighenti, F. and Pellegrini, N. Characterization of total antioxidant capacity and (poly) phenolic compounds of differently pigmented rice varieties and their changes during domestic cooking. *Food Chemistry*, 187:338-347, 2015.

[146] Zimmet, P., Alberti, K, G, M, M. and Shaw, J. Global and societal implications of the diabetes epidemic. *Nature*, 414:782-787, 2001.

[147] Zhang, Q. and Litchfield, J, B. Applying fuzzy mathematics to product development and comparison. *Food Technology*, 45(7):108-115,1991.

[148] Zibadi, S. and Watson R, R. Passion fruit (*Passiflora edulis*): composition, efficacy and safety (Review). *Evidence-Based Integrative Medicine*; 3:183-7, 2004.

[149] Zibadi, S., Farid, R., Moriguchi, S., Lu, Y., Foo, L,Y., Moslemzadeh, T,P., Ulreich, J, B. and Watson, R, R. Oral administration of purple passion fruit peel extract attenuates blood pressure in female spontaneously hypertensive rats and humans. *Nutrition Research*, 27:408-16, 2007.

The Chapter 3 has been discussed under two sub-heads as follows:

A) Physicochemical analysis of pigmented and nonpigmented rice cultivars

3.1 Introduction

Rice (*Oryza sativa* L.) is considered as major staple food worldwide. The Ayurvedic Treatise (Indian Materia Medica) records showed that some Indian rice has medicinal properties.[17] Northeastern region of India namely, Assam, Arunachal Pradesh and Manipur grow pigmented rice cultivars. In Arunachal Pradesh, red rice is grown abundantly in some part of the state but it is still considered as underutilized rice cultivars. The categories of rice on the basis of amylose content are high amylose, intermediate amylose, low amylose, and waxy rice types.[10]

Physical properties of different grains are important parameters to determine the quality and conditions for processing and safe storage.[48] Amin et al.[3] also stated that the parameters like bulk density, kernel density and porosity reveal important information to design grain hopper. During heating, starch-rich cereal flour undergo starch gelatinization by melting starch crystallites and produce viscous mass. These thermal properties should be known for novel food development such as tortillas, beverage, pudding and gluten-free snacks.

Pigmented rice are good source of antioxidant compounds *viz.,* flavonoid, anthocyanin, phytic acid, proanthocyanidin, tocopherols, tocotrienols, γ-oryzanol, and phenolic compounds respectively.[12]

Therefore, the present study was conducted to investigate physicochemical and phytochemical analysis of two pigmented (Lingkang ame:LA and Umling ame:UA), and one non-pigmented rice (Pungpo ame:WR) rice cultivars of Arunachal Pradesh, India. Also, investigate about the pasting and thermal properties of flour of three rice was carried out.

Samyor et al. (2016). Phytochemical and antioxidant profile ofpigmented and non-pigmented rice cultivars of Arunachal Pradesh, India, International Journal of Food Properties, 19, 1104–1114.

Samyor et al. (2016).Evaluation of physical, thermal, pasting characteristics and mineral profile of pigmented and non -pigmented rice cultivars. Journal of Food Processing and Preservation, 40, 174–182.

3.2 Materials and methods

3.2.1 Materials

Two red rice (*Oryza sativa* L.) cultivars locally known as Lingkang ame (LA: long grain) and Umling ame (UA: short grain) and one white rice known as Pungpo ame (WR: short grain) were purchased from the farmers of Yingkiong, Upper Siang district and Manigong circle, West Siang district of Arunachal Pradesh, India. All rice samples were cleaned and ground, and the samples were packed in polyethylene bags and stored in ambient temperature.

3.2.2 Proximate compositions

The moisture, ash, total carbohydrate, crude protein, crude fat, and amylose contents were analyzed using standard methods.[5]

3.2.3 Physical properties of rice

3.2.3.1 Moisture and ash content

The moisture and as content (%) of samples were carried out by using standard methods.[5]

3.2.3.2 Axial dimensions

The axial dimensions, *viz.*, length, breadth and thickness, were measured for rice grain with the help of a vernier calliper with an accuracy of 0.01 mm. The geometric mean diameter of grain (*Dg*) and equivalent diameter (*Dp*) of all the three rice grain were calculated using the following expression.[42]

$$D_g = (LWT)^{\frac{1}{3}}$$

Where, *L* is the length, *W* is the width and *T* is the thickness of grain in millimeters.

$$D_P = \left[L\frac{(W+T)^2}{4}\right]^{1/3}$$

The sphericity (Sp) defined as the ratio of the surface area of sphere having the same volume as that of the grain to the surface area of the grain. The sphericity of three rice cultivars was calculated using the following formula.[42]

$$Sp = \frac{(LDT)^{1/3}}{L}$$

Aspect ratio of rice cultivars were (R_a) calculated using following formula [41]

$$\mathrm{R_a} = \frac{\mathrm{W}}{\mathrm{L}}$$

Where W and L are width and length of rice grain, respectively;

Grain volume (V) and surface area (S) was also calculated according to Jain and Bal [30]

$$V = \pi \frac{B^2 L^2}{6(2L-B)}$$

$$S = \frac{\pi B L^2}{(2L-B)}$$

$$B = (WT)^{1/2}$$

To evaluate thousand kernel weights, the grains were measured by counting 100 seeds and then weighing in an electronic balance to an accuracy of 0.001. It was multiplied with 10 to give mass of 1000 kernels.

3.2.3.3 Bulk and true densities

Bulk density was calculated using mass/volume relationship. It was determined by method of Gupta and Das[20] were a cylindrical container of 500 mL in volume was filled with the grain from a height of 150 mm at a constant rate and then weighing the contents. The true density was define as the ratio between the mass of grain and the true volume of the grain, was determined using the kerosene displacement method.[48]

3.2.3.4 Porosity

Porosity is the percentage of void space in the bulk grain which is not occupied by the grain.[58] Porosity was calculated using the relationship between the bulk density and true density as shown in the eq.

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_t} \times 100$$

Where ρ_t is true density and ρ_b is bulk density of the grain (g/ cm^3).

3.2.3.5 Angle of repose

Angle of repose was measured as described by Jain and Bal.[30] A Plywood box measuring 300mm×300mm×300mm with a removable front panel was filled with grains at the desired moisture content and the front panel was quickly removed. Grains were allowed to flow to their natural slope. The angle of repose was calculated from measurements of grain free surface depths at the end of the box and midway along the sloped surface and horizontal distance from the end of the box to its midpoint.

3.2.3.6 Color

The color of the rice cultivars were measured according to Saikia et al.[55] Hunter Colorimeter (Color Lab Ultra scan Vis) was used to measure the color of the rice cultivars. The $L*$, $a*$ *and* $b*$ values were recorded as the mean of three replicates. Hue angle and chroma of all the three rice cultivars were also determined using the following equations.

$$Chroma = [a^{*2} + b^{*2}]^{1/2}$$

$$Hue\ angle = \tan^{-1}(b^{*}/a^{*})$$

3.2.4 Mineral profile

The mineral profiles of the rice cultivars were determined by AOAC.[5]

3.2.5 Pasting properties

Pasting properties of the whole rice flour samples were measured using a rapid visco analyzer (RVA starch master 2 pulverisette instrument). 2 g of sample was taken for viscosity profiles. Rice flour suspensions were prepared using 25 ml distilled water. The sample holding temperature was initially at 50˚C to 95˚C in 3:45 min, a second holding phase was at 95˚C for 2:40 min, a cooling phase from 95˚C to 50˚C in 4 min and a final holding phase at 50˚C for 1 min. The pasting point (PP), corresponding gelatinization temperature (GT), peak viscosity (PV), hot paste viscosity (HPV), cold paste viscosity (CPV), breakdown (BD), and total setback (SBt) were recorded. HPV is the minimum viscosity at 95˚C and CPV is the final viscosity at 50˚C. BD = PV–HPV and SBt = CPV–HPV.[61]

3.2.6 Cooking characteristics

3.2.6.1 Optimal cooking time

Optimal cooking time of three different rice flours was determined using the AACC approved method.[5]

3.2.6.2 Water uptake ratio

Water uptake ratio of different rice sample was described by the method of Thomas et al.[57] 2 grams of rice samples were added into 20 ml of distilled water and cooked in a boiling water bath for a minimum cooking time. The remaining water contents in the samples were drained out. The adhering superficial water present on cooked rice was further removed by pressing the samples between filter papers. Cooked samples were weighed and the water uptake ratio was calculated.

3.2.7 Texture profile analysis (TPA)

The fresh cooked rice sample was taken directly to perform texture profile analysis. Hardness of cooked rice were measured using a texture analyzer as the modified method described by Tian et al.[59] Briefly, the cooked rice sample was put on the sample table at the centre of the probe in a flat form. A load cell of 50 kg was used. Then the cooked rice sample was compressed using a 2.5 mm diameter cylindrical probe at a test speed of 0.5 mm/s and a control force of 10 g. The deformation level was 60% of original. This process was repeated for three times for each sample.

3.2.8 Differential scanning calorimeter (DSC) analysis

Thermal analysis was performed with a DSC 60 SHIMADZU instrument. About 5 mg of the sample was used in each experiment with measured amounts of water, hermetically sealed, and kept it for equilibrium (~1hr) prior to analysis. Heating was carried out from 30 to 350˚C at the rate of 10˚C/min. The sample was placed in the silver cup, covered with the silver lid, and sealed very carefully with the sealer supplied by the manufacturers. Another empty cup was used after sealing as before, making this the reference (air). The DSC instrument's software was used to calculate the onset (T_o), endset

(T_p), conclusion temperature (T_c) and gelatinization temperature range (R) was calculated as $2\times(T_p - T_o)$ as described by Krueger et al.[34]

3.2.9 X-Ray diffraction (XRD) analysis of rice cultivars

The X-ray diffraction analysis was carried out for three rice flour samples to obtain the X-ray diffraction (XRD) pattern using an X-ray diffractometer (D8 Focus, Bruker AXS,Germany) with acceleration potential of 35 kV with 25 mA current. The scans adjusted for Bragg angle were in the range of 7 to 80˚ on a 2θ scale with a step-size of 0.05. Software used was XRD Commander and Diffrac. EVA. The total area under the curve and the area under each prominent peak was determined using OriginPro 8.0 software and percentage crystallinity formula was given below

% Crystallinity = (Area under peaks/total area)× 100

3.2.10 Fourier transform infrared (FT-IR) spectra of different compounds

Fourier transform infrared (FT-IR) spectra were used for detecting functional groups present in the three different rice cultivars from the state of Arunachal Pradesh. The rice grain was ground into flour and mixed with KBr (spectroscopic grade) powder. Mixer was pressed into pellets for FT-IR measurement in the frequency ranging from 4000 to 400 cm^{-1} and spectra of the materials were obtained at a resolution of 8 cm^{-1}.[35]

3.2.11 Phytochemicals and antioxidant activities

3.2.11.1 Sample extraction

Sample preparation was done according to the method describe by Atala et al.[6] Three rice cultivars (10 g each) were powdered in a grinder. The ground sample was extracted with 100 mL of extraction solvent (75:25 v/v, acetone: water). Extracts were shaken in a water bath at 25˚C for 90 min, and centrifuged in refrigerated condition (SIGMA Laborzentrifugen, 3-18 KS, Osterode, Germany) at 950 g for 15 min. Supernatant was stored at -20˚C for further analysis.

3.2.11.2 Total phenolics content

Modified version of the Folin-Ciocalteu assay described by Slinkard and Singleton [46] was used to determine the total phenolic content in the extracts from the three different rice cultivars. Gallic acid was used for preparation of standard curve at various concentrations. Independently extract (20 µL each), gallic acid, and blank were prepared and mixed with 1.58 mL distilled water. Folin-Ciocalteu reagent (100 µL) was added to 300 µL of sodium carbonate within 8 min. The samples were vortexed immediately and incubated for 30 min at 40˚C. The absorbance was measured at 765 nm in ultraviolet-visible (UV-VIS) spectrophotometer (Spectrascan UV-2600, Thermo Fisher Scientific, Nasik, India). The phenolic content was expressed in mg GAE/100 g.

3.2.11.3 Total monomeric anthocyanins

The monomeric anthocyanin content of extracted solutions were determined using the pH differential method [21] Absorbance was measured at 515 and 700 nm. Anthocyanin was calculated as cyanidin-3-glucoside using a molar extinction coefficient of 26,900 and a molecular weight of 449.2.

$$A=(A_{515}-A_{700})pH1-(A_{515}-A_{700})pH4.5$$

$$\text{Anthocyanin content (mg/L)}=\frac{(A\times MW\times DF)}{\varepsilon\times L}\times 1000$$

Where, DF was dilution factor, MW cyanidin-3-glucoside molecular weight (449.2) and ε molar absorptivity (26,900). All measurements were done in duplicates.

3.2.11.4 DPPH scavenging activity

DPPH radical-scavenging activity of rice extracts were evaluated according to Brand-Williams [11] method. Briefly, extracts (100 µL) were taken and added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). After 30 min of incubation period, absorbance reading was taken at 517 nm using a spectrophotometer (Chemito, Spectrascan UV 2600, double beam UV-VIS Spectrophotometer Thermo Scientific). The percentage of radical-scavenging activity was calculated using the formula:

$$\text{Radical scavanging activity (\%)}=\frac{A_o-A_s}{A_o}\times 100$$

Where, A_o is absorbance of control blank, and A_s is absorbance of sample extract.

3.2.11.5 Metal chelating activity

Metal chelating activity was done as per method described by Dinis.[16] Ferric chloride (50 µL of 2 mM) was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) and 0.2 ml of 5 mM ferrozine solution was added. The mixture was vigorously shaken and kept at room temperature for 10 min. The absorbance reading was taken at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as $[(A_o- A_s)/A_s] \times 100$, where A_0 was the absorbance of the control and A_s was the absorbance of the extract. EDTA was used as standard.

3.2.12 Rverse phase-High-performance liquid chromatography (RP-HPLC) analysis of phenolic compounds

3.2.12.1 Sample preparation

Sample (50 g) was mixed with 0.5 g ascorbic acid and added with 100 ml of 80% methanol followed by filtration (Whatman no.2). The excess amount of the methanol and water was evaporated. Sample was washed in a separating funnel with hexane to remove carotenoid and other nonpolar compounds. Volume was made up to 50 mL using distilled water and pH was adjusted to 7.0 and 10 mL sample were taken for further analysis.

3.2.12.2 Detection

A HPLC system (Ultimate 3000 Liquid Chromatography Systems) with an ultimate 3000 variable wavelength UV detector at 215 nm and Ultimate 3000 pump were used for analysis. The column was Acclaim 120 C18 column (5 µm, 120Å) with a size of 4.6×250 mm. The HPLC analysis was performed with 20 µl of sample injected into the column. The solvent system was eluent A-acidified water pH adjusted to 2.64 with the dil. hydrochloric acid and eluent B–acidified water: acetonitrile (20:80). A constant flow rate of 1.5 mL/min with a gradient run was maintained. The quantification of polyphenolic compounds was quantified using the calibration curves of their respective standards. The software chameleon ver. 6.80 was used for analyzing data.

3.2.13 Statistical analysis

Experiments were carried out in triplicates and presented as mean ± standard deviation of mean using SPSS version 16. The data were statistically analyzed by Duncan's multiple range tests at 5% significance level. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for statistical analysis.

3.3 Results and discussion

3.3.1 Nutritive quality

The data presented in Table 3.1 shows that the LA cultivar content significantly ($p \leq 0.05$) high amount of moisture (11.13 %) than UA and WR cultivar. Ash content was found highest in the UA 1.33 %, followed by LA 0.97 % and lowest in the WR 0.93 %. Fat content was significantly ($p \leq 0.05$) high in UA 2.60 % followed by LA (1.80%) and WR (1.77%). Very low amylose content (%) was observed in three rice were LA (6.46±0.026), UA (11.86±0.04) and WR 5.30±0.04 respectively. According to IRRI, rice varieties were classified into five groups as per their amylose content: waxy (1–2%), very low (2–9%), low (10–20%), intermediate (20–25%), and high (25–33%).

Table 3.1 Proximate composition of rice cultivars

Parameters	LA	UA	WR
Moisture (% w.b)	11.13 ± 0.02^{a}	11.01 ± 0.005^{b}	11.01 ± 0.005^{b}
Ash (% d.b)	0.97 ± 0.01^{b}	1.33 ± 0.57^{c}	0.93 ± 0.05^{a}
Fat (% d.b)	1.80 ± 0.20^{b}	2.60 ± 0.19^{c}	1.77 ± 0.06^{a}
Protein (% d.b)	1.95 ± 0.02^{c}	0.24 ± 0.03^{b}	0.12 ± 0.02^{a}
Carbohydrate (% d.b)	79.01 ± 0.16^{b}	81.37 ± 0.93^{c}	76.44 ± 0.59^{a}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

Values expressed as mean ± SD (n=3)

3.3.2 Physical properties

Axial dimensions (mm), geometric mean diameter (mm), sphericity index (%), moisture content (%) and ash content (%) of three rice cultivars were varied significantly ($p \leq 0.05$). Moisture and ash content ranged from 11.00 to 11.50 % (db) and 0.93 to 1.33% (db) respectively. Physical properties of three rice cultivars are presented in Table 3.2. Average length (l) of rice grain varied from (UA) 5.37±0.24 mm to (LA) 6.80±0.34 mm while the average breadth/width ranged from (UA) 3.47±0.58 to (WR) 3.81±0.02. Therefore, it is significantly ($p \leq 0.05$) longer than the two other grain samples. Equivalent diameters of three samples were varied from (UA) 4.10±0.01 to (WR) 4.56±0.04. Sphericity (%) of grains ranges from (LA) 0.66±0.01 to (UA) 0.76±0.03 %. The bulk and true density of three different rice cultivars were in the ranged of (UA) 0.37±0.01 to (WR) 0.37±0.04 g/cm^3 and (UA) 1.06 ± 0.11 to (WR) 1.56±0.11 g/cm^3 respectively. Bulk density among the three cultivars were differ significantly ($p \leq 0.05$). Angle of repose of different grains varied significantly ranged from (UA) 39.19±0.66˚ to (LA) 43.23±0.13˚. Angle of repose value ranged from WR (41.98˚) to LA (43.23˚) shows a good flowability and handling properties. The result was supported by previous literature which confirmed that a material with an angle of repose between 40˚ and 45˚ are free-flowing and powders with repose angles above 50˚ are very cohesive and could cause handling problems.[4] Porosity of rice samples were varied from (UA) 65.76 to (WR) 75.91 (%).Weight of 1000 grain (g) ranged from (LA) 18.67±0.03g to (WR) 22.04±0.02g. Aspect ratio of three samples were varied between (UA) 0.93±0.01 to (LA) 1.03±0.01. Surface areas (mm^2) were ranged from (UA) 45.32±4.08 to (WR) 54.99±2.02. Grain volume (mm^3) of the three different samples was ranged from (UA) 24.99±1.86 to (WR) 35.65 ±1.01. Weight of 1000 grains (g) LA, UA and WR were ranged from 18.67-22.04g. The bulk and true density of three different rice cultivars (LA, UA and WR) showed no significant differences between the cultivars at the 0.05% probability. Thousand grain weights (g), angle of repose and porosity of LA, UA and WR were varied significantly. It was revealed that all the rice variety was a good source mineral as a micronutrient.

L, a*, b** values of three different rice cultivars were shown in the Table.3.2 L* values of the rice flours which indicates whiteness /lightness, varied from 79.83 ±0.05 (WR) to 44.52 ±0.17 (LA). The reason may be because of (WR) has whitish bran than other two grains. The positive a* values for redness ranged from (WR) 0.351 to (UA) 4.192. (UA) have highest a* values, it may be due to more reddish external layers color than other two rice variety. The yellowness b* value was in the ranged from (UA) 5.98 to (WR) 9.06. Chroma of three different samples were ranged from (LA) 7.26±0.05 to 9.12±0.06 and hue angle varied from (UA) 54.59±0.53 to (WR) 87.62 ±0.13. *L*, a*, b** color parameters varied from sample to sample. UA cultivar exhibited higher degree of redness and yellowness than the LA and WR. Mineral profiles of the rice cultivars were analyzed.

Table 3.2 Physical properties of raw rice cultivars

Properties	**LA**	**UA**	**WR**
Length (mm)	6.60±0.34^{b}	5.37±0.24^{a}	6.73±0.30^{c}
Breadth (mm)	3.73±0.11^{b}	3.47±0.58^{a}	3.83±0.02^{c}
L/B	1.76±0.01	1.54±0.02	1.75±0.01
Equivalent diameter (mm)	4.50±0.04^{b}	4.10±0.01^{a}	4.56±0.04^{c}
Sphericity (%)	0.66±0.01^{a}	0.76±0.03^{c}	0.67±0.03^{b}
Bulk density (g/cm^{3})	0.372±0.01^{a}	0.371±0.03^{a}	0.378±0.04^{a}
True density (g/cm^{3})	1.26±0.05^{b}	1.06±0.11^{a}	1.56±0.11^{c}
Porosity (%)	71.03±0.82^{b}	65.76±0.85^{a}	75.91±0.08^{c}
Angle of repose (deg.)	43.23±0.13^{c}	39.19±0.66^{a}	41.98±0.10^{b}
Weight of 1000 grain (g)	18.67±0.03^{a}	20.96±0.03^{b}	22.04±0.02^{c}
Aspect ratio	1.03±0.01^{b}	0.93±0.01^{a}	1.02±0.02^{b}
Surface area (mm^{2})	53.54±2.23^{b}	45.32±4.08^{a}	54.99±2.02^{c}
Grain volume (mm^{3})	34.12±1.27^{b}	24.99±1.86^{a}	35.65±1.01^{c}
Color			
Chroma	7.26±0.05^{a}	8.22±0.10^{b}	9.12±0.06^{c}
*L**	44.52±0.17^{a}	59.25±0.17^{b}	79.83±0.05^{c}
*a**	3.51±0.02^{b}	4.19±0.09^{c}	0.35±0.10^{a}
*b**	7.50 ±0.15^{b}	5.98±0.29^{a}	9.06±0.12^{c}
Chroma $[a^{*2}+b^{*2}]^{1/2}$	8.28±0.05^{a}	7.30±0.10^{b}	9.06±0.06^{c}
Hue angle $\tan^{-1}(b^*/a^*)$	64.85±0.08^{b}	54.84±0.53^{a}	87.78±0.13^{c}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

3.3.3 Mineral profile

The concentration of elements such as Al, Ca, Cu, Cr, Fe, K, Mg, Mn, Mo, Na and Zn found in rice samples are shown in Table 3.3. Out of eleven minerals, Al (0.97 mg/100g), Cr (0.19mg/100g), Mg (54.25mg/100mg), K (29.00 mg/100g), Zn (0.65 mg/100g), Na (19.54 mg/100g) and Ca (9.55 mg/100g) concentration were observed highest in (LA) Mo, Fe, Mn and Cu concentration were recorded highest in UA (Table 3.3). The mean values for most elements were consistent and similar to the result published previously.[49]

Table 3.3 Mineral profiles of three rice cultivars determined by atomic absorption spectroscopy (AAS)

Mineral (mg/100g)	LA	UA	WR
Al	0.97	0.57	0.74
Cr	0.19	0.16	0.16
Mg	54.25	52.39	45.91
Mo	0.09	0.04	0.02
Ca	9.55	5.74	8.60
Fe	1.45	1.72	0.25
K	29.00	21.73	19.48
Zn	0.65	0.46	0.47
Cu	0.27	0.36	0.27
Na	19.54	16.60	13.72
Mn	0.25	0.33	0.24

3.3.4 Pasting properties

Pasting properties of LA, UA and WR flours are shown in Fig 3.1 and different viscogram data of rice cultivars were reflected in the Table 3.4. Among three rice cultivars UA (90.5± 0.25) showed significantly ($p \leq 0.05$) higher pasting temperature than LA, (83.7˚C ± 0.32) and WR (77.7˚C ± 0.18) which indicates the minimum temperature needed to cook the rice flour. Previously, Huaisan et al.[25] reported that in the rice starch, pasting temperature (PT) was ranged from 79.1˚C to 79.5˚C. Peak viscosity (PV) is the maximum viscosity attained by gelatinized mixture during heating in water i.e. water holding capacity of the mixture. PV was observed highest in WR (2767.6± 1.5 cP) followed by LA (1804±0.57cP) and UA (1601±0.57cP). It may be because of the higher damage caused to starch during dry grinding process. Final viscosity shows the ability of starch to form viscous paste. Final viscosity of samples was varied significantly, ranged from (LA) 2709 to (UA) 3477 cP. Variation in final viscosity might be due to the variation in amylose molecules and its amount.[40] Breakdown of any mixture can be depending on temperature, degree of mixing and shear stress. The breakdown viscosity of the flour samples were varied significantly ($p \leq 0.05$) from (UA) 17 to (LA) 43. Higher the breakdown in viscosity, lower the ability of starch sample to withstand heating and shear stress during cooking.[2] Break down value indicates the heat stability of starch at 95˚C. Therefore, low BD value indicates thermal stability.[37] In UA, break down (cP) results shows the lowest value i.e 17 cp. Therefore, it can be concluded that UA can be an ideal sample to withstand the heating and shear stress during cooking. Setback viscosity results in rearrangement of amylose molecules that have been leached out from the swollen starch granules during cooling.[32] It is a measure of gelling ability or retrogradation tendency of the starch. Setback viscosity of three rice cultivars were ranged from (WR) 591 to (UA) 1876 cp. The paste properties of the mixture can provide information about the organoleptic and functional properties of rice and thus influence the type of formulations rice flour can be used in the future.[1] Guha et al.[19] reported that the viscosity of a completely gelatinized starch slurry decreases during heating. Inglett et al.[27] also stated in their study that the pasting curves of the quinoa, oat products and their composites shows dissimilar patterns. Initially, pasting viscosity shows high (~223 RVU) and sharp viscosity around (~20 RVU min $^{-1}$) at initial 11-min heating period at ~90˚C followed by a rapid decrease in viscosity (69 RVU min $^{-1}$) to ~20 RVU during continued heating to 95˚C at 15 min. According to Tester and Morrison [56] pasting enhance the changes that occur after gelatinization due to further heating and leads to further swelling of granules, leaching of molecular components from the granules and finally, disruption of granules especially with the application of shear forces. Ding et al.[15] also stated that the change of swelling power of ozone-treated waxy rice starch was due to data of molecular size distribution, which further explained the change of pasting property.

Table 3. 4 Viscography parameters of three rice flour

Pasting properties	**LA**	**UA**	**WR**
Pasting temperature(°C)	83.70 ± 0.32^{b}	90.5± 0.25^{c}	77.7± 0.18^{a}
Peak Viscosity(cP)	1804±0.57^{b}	1601±0.57^{a}	2767.6± 1.5^{c}
Hold viscosity (cP)	1762.30± 1.50^{b}	1583.3±0.57^{a}	2743.3±0.57^{c}
Final Viscosity (cP)	2709± 1.54^{a}	3476.6± 1.52^{c}	3361±1.73^{b}
Break Down (cP)	43± 1.52^{c}	17±1.00^{a}	26±1.00^{b}
Set Back (cP)	905±0.57^{b}	1876±0.57^{c}	591±0.57^{a}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

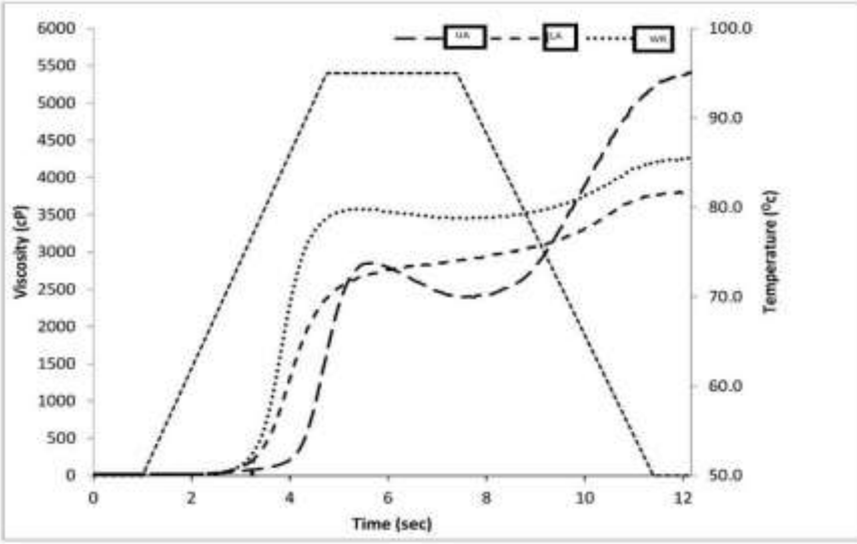


Fig.3.1 Viscosity profile of three different rice cultivars

3.3.5 Cooking characteristics

The rice cultivars shows significant ($p \leq 0.05$) different in optimal cooking time (min), ranged from (WR) 21.3- (UA) 31.16 minutes (Table.3.5). UA shows the highest cooking time (31.16) min than LA (26.48min) and WR (21.3min). It may be due to the variation in amylose content and size of rice sample. Hogan and Plank [24] suggested that the hydration characteristics of rice influenced by variety and size of grain. Water uptake properties of rice grain directly relate with cooking time of the grain. Arns et al.[8] reported that the longer cooking time shows higher internal restructuring of the grain with increasing time of heat–moisture treatment, requires longer time and a greater amount of water for their cooking. Highest water uptake properties was found in UA (3.65±0.01) and hence, optimal cooking time (31.16±0.01 mins) was also highest in UA. Sozer et al.[52] also reported longer the optimal cooking time higher the water uptake capacity.

Table 3.5 Cooking characteristics of three rice grain samples

Properties	LA	UA	WR
Optimal cooking time (min)	26.48±0.02^{b}	31.16±0.14^{c}	21.3±0.01^{a}
Water uptake ratio (%)	3.06±0.01^{b}	3.65±0.03^{c}	2.66±0.02^{a}

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range test.

3.3.6 Texture profile analysis (TPA)

Texture profile analyser (TPA) of cooked rice was carried out for three different samples. The data (Table 3.6) revealed that the cooked UA (3.55±0.84g) rice had higher hardness value than the cooked LA (2.13±0.198g) and WR (3.52 ± 0.97g). It may be attributed to the presence of higher amount of amylose in UA variety than other two varieties. In a previous study, Yu et al.[60] stated that the hardness was positively correlated with the amylose contained in rice grain. Rice with higher amylose content was liable to leach more into the cooking water and formed a coating on rice grain, which increase the hardness.[39] In a study conducted by Katekhong and Charoenrein [33] stated that the increasing ageing duration and the number of freeze-thaw cycles, leads to the significant ($P \leq 0.05$) decreases in stickiness of the cooked rice. According to the Chrastil [13] and Chrastil and Zarins [14] stated about the possible factors contributing to the harder texture of cooked rice. During rice grain ageing number of disulphide bonds and the high molecular weight peptide subunit of the storage protein oryzenin increases. Apparently, disulphide linkage might retard water uptake by granules. Texture profile such as hardness (g), springiness (length/length) and cohesiveness of cooked rice had significant variation.

Amylose, on the other hand, is easier to retrograde and increased the hardness of the cooked rice in a short period. Springiness (length/length) is a measure of how much the gel structure is broken down by initial compression. Springiness value of three rice samples ranged from (LA) 0.37±0.10 to (WR) 0.38 ±0.02 (length/length) and cohesiveness from (LA) 0.17±0.17 to (UA) 0.23±0.03 were found to be non-significant. These changes may be due to the variation in amylose and amylopectin in rice cultivars, responsible for variation in gel network formed in rice during temperature cooking.

Although, there was less difference in springiness in three rice cultivars but UA (0.39±0.84) possess higher gel structure than others. Huang et al.[26] also discussed that a high springiness appears as a gel structure is broken into few large pieces during the first TPA compression, whereas low springiness results from a gel breaking into many small pieces.

Table 3.6 Texture profile analysis (TPA) of cooked rice

Properties	LA	UA	WR
Hardness (g)	2.13± 0.19^a	3.55± 0.84^b	3.52± 0.97^b

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range tests.

3.3.7 Differential scanning calorimetry (DSC) analysis

Fig.3.2 showed the differential scanning calorimetry (DSC) thermograms of three rice cultivars. The thermal properties (melting point) of cooked rice were studied using DSC and summarized in Table 3.7. For endothermic enthalpy, heating was carried out from 30 to 350˚C at the rate of 10˚C/min. Among the three rice flour the onset (T_o), peak (T_p) and endset (T_c) value were highest in the UA (77.12, 104.61 and 140˚C) and lowest was found in the WR cultivar (67.27, 93.53 and 117.92˚C) as shown in the Table 3.7. The reason of high onset temperature in the UA may be because of bran layer losses during hulling which contained bran oil. Previously the authors have reported that defatting marginally increased the melting points.[50] Among three samples, highest reaction rate occur in the (UA) 104.61˚C and lowest in (WR) 93.53˚C. Differences in the range of T_o, T_p and T_c in three rice cultivars may be attributed by differences in amylose content, starch structure, amylose to amylopectin ratio, the degrees of heterogeneity of crystallites within granules, and the content of amylose-lipid complex.[31] Moreover, high amylose starches with longer average chain exhibit higher transition temperatures. For wheat, rice and maize previous researcher [53] was reported the onset (T_o), peak (T_p) and conclusion (T_c) temperatures as 54, 69 and 86˚C (wheat) 66, 82 and 100˚C (rice) and 67, 78 and 95˚C (maize) respectively. Sodhi et al. [51] also reported the transition temperatures of Basmati cultivars varied between 66.25-74.70˚C. In thermal properties onset (T_o), peak (T_p) and endset (T_c) value of UA cultivar were shown higher than WR and LA. The above results could provide important information for the utilization of rice cultivars and to develop new food product. In thermal properties onset (T_o), peak (T_p) and endset (T_c) value of UA cultivar were shown higher than WR and LA.

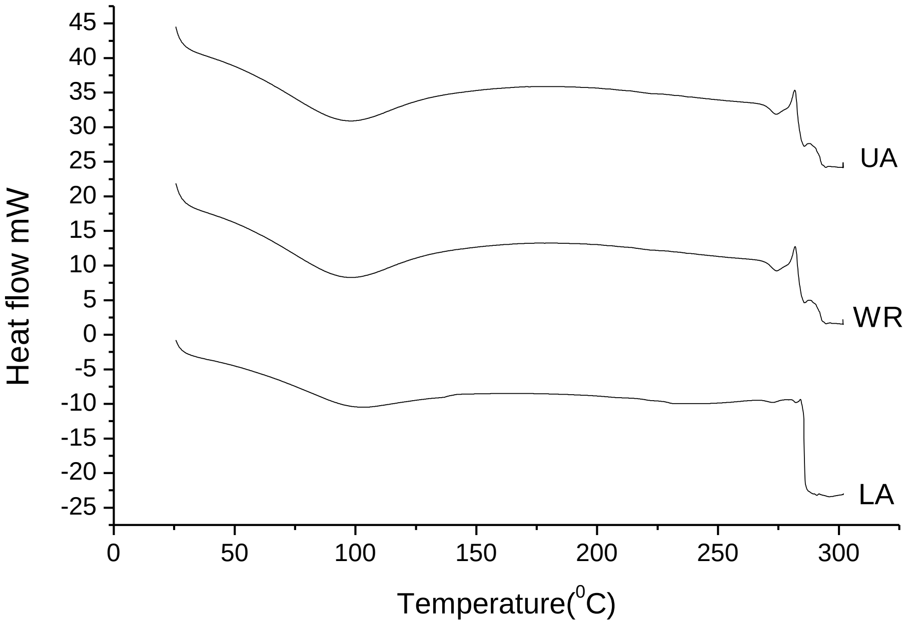


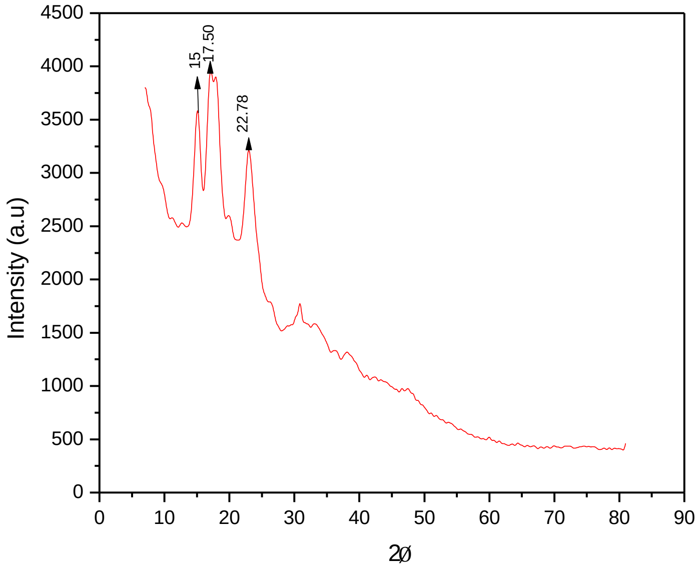
Fig 3.2. Differential scanning calorimetry (DSC) graphs of three rice cultivars

Table 3.7 DSC thermograms of rice flours

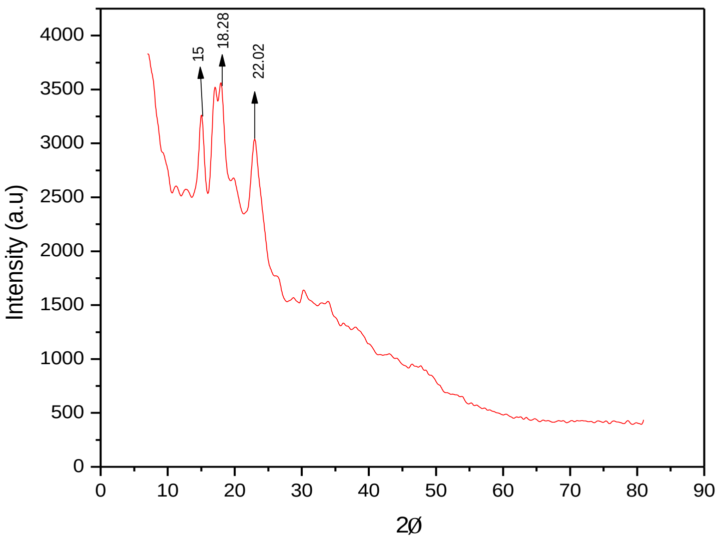
Types	**Gelatinization temperature (˚C)**			
	(*T_O*)	**(*Tp*)**	**(*Tc*)**	**R**
LA	68.51± 0.02[b]	97.70±0.20[b]	123.91± .23[b]	58.38±0.17[c]
UA	77.12± 0.02[c]	104.61±0.33[c]	141.66±1.52[c]	54.8± 0.16[b]
WR	67.27±0.015[a]	93.53± 0.60[a]	117.92± .03[a]	52.71±0.21[a]

Means with different letters in the same row indicate that there is significant difference between samples ($p \leq 0.05$) from Duncan's multiple range tests. T_o: onset temperature; T_p: peak temperature; T_c: *endset temperature.*

3.3.8 X-Ray Diffraction (XRD) analysis of rice flour



(LA)



(UA)

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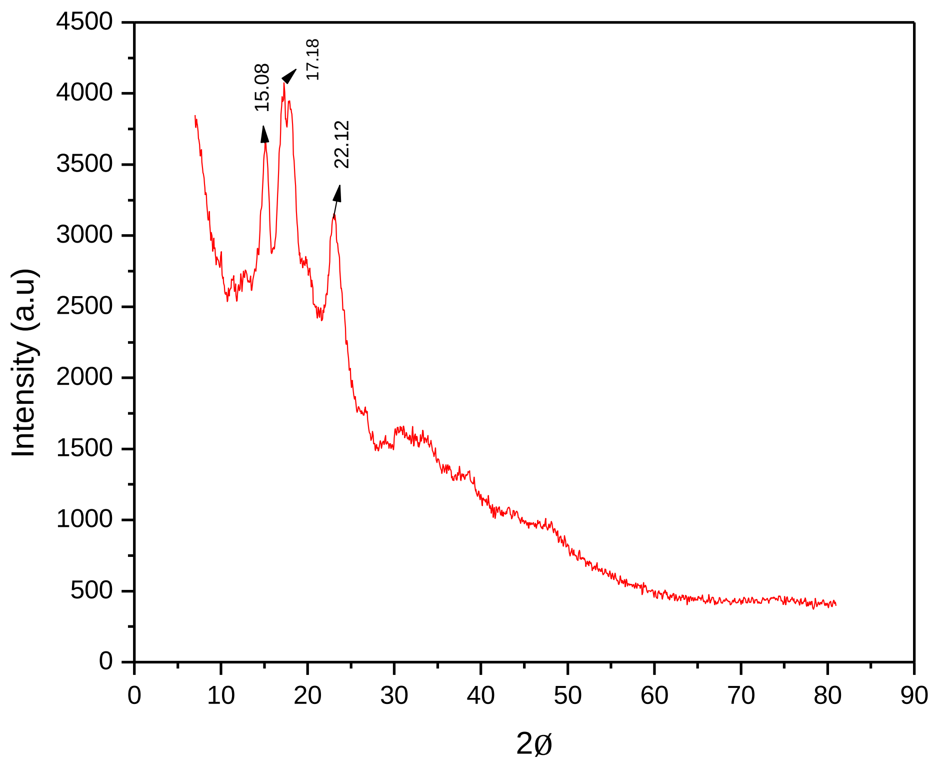


(WR)

Fig. 3.3. X-ray diffraction of three rice flour a) LA b) UA and c) WR

In the Fig. 3.3., the rice flour of three sample revealed the presence of A type pattern in the granular level. The angles obtained were in the ranged of 15.02-23 θ which usually present in the cereal crop. The % crsytallinity of the LA, UA and WR were 61.16 ,70.39 and 59.78 %.

3.3.9 Phytochemicals and antioxidant activities

3.3.9.1 Total phenolic content

Total phenolic content and the anthocyanin content of three cultivars are shown in Table 3.8. The phenolic compound and anthocyanin content of three rice cultivars varied significantly. The phenolic contents in the white rice (WR) and the two red types rice (LA) and (UA) ranged from 142-349.30 mg GAE/100 g and anthocyanin content ranged from 1.34-12.79 mg cyanidin-3-glucoside Eq/100g respectively.

3.3.9.2 Chelating activity

It was observed from the Table 3.8 that UA (77.53 µg/mL) has the highest iron chelating activity than LA (72.33 µg/mL) and WR (58.92 µg/mL). It might be due to the presence of higher amount of phenolic compounds which reacted with iron and disrupted the red color complex formation. Therefore, measurement of color reduction, allows the estimation of the chelating activity of the sample.[9] Chelating activity of rice extracts depends upon concentration of extract. From the Fig. 3.3(b) it was also observed that there was strong correlation between iron chelating activity and concentration of UA and LA sample. In general ferrozine can quantitatively form complexes with Fe^{2+} but for presence of phenolic compounds which act as chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased with the sample concentrations.[43] The UA cultivar showed significantly ($p \leq 0.05$) the high amount of chelating activity compared to LA and WR rice cultivars.

3.3.9.3 DPPH scavenging activities

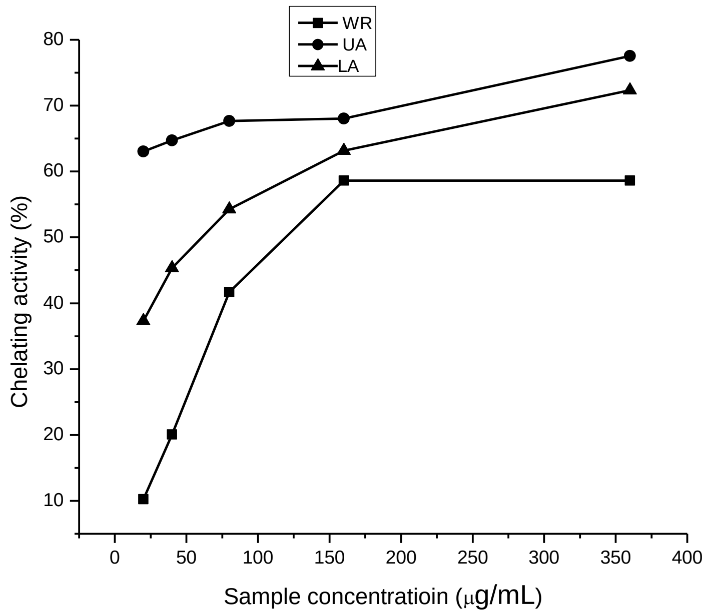
It was observed from Table 3.8, the DPPH scavenging activity was highest in UA (88.48 %) followed by LA (76.97%) and lowest in PA (68.87 %). It may be attributed to the presence of higher amount of phenolic compounds. Fig 3.3. Shows that DPPH scavenging activities of all the three samples were strongly dependent (R^2 value of LA 0.86, UA 0.71 and PA 0.93) on the concentration of the sample. It could be due to marked effect of phenolic compounds of pigmented rice on DPPH scavenging activity. The phenolic compounds react with deep violet color solution of DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) and convert it to 2, 2-diphenyl-1-picrylhydrazine with decolonization and measurement of color reduction allows to estimate the DPPH scavenging activity of the samples.[11] Similar type of result has been observed by numbers of researchers.[29, 23]

Table 3.8 Antioxidant activity of three rice cultivars

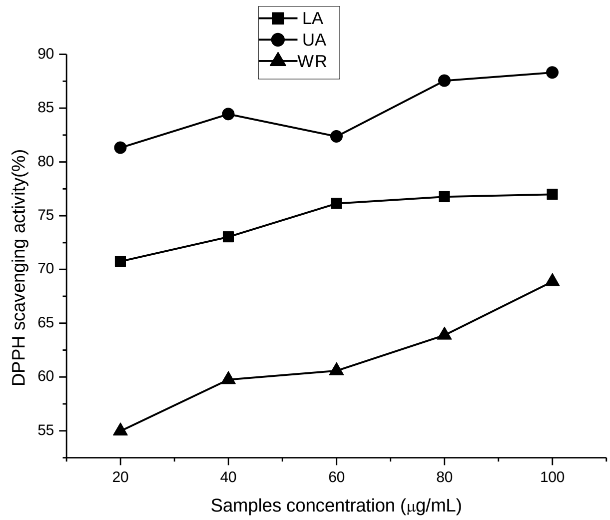
Concentration (μg/mL)	**LA**	**UA**	**WR**
Metal chelating activity			
20	37.31±0.01[b]	63.04 ±0.01c	10.23±0.02[a]
40	45.35± 0.01[b]	64.72±0.02[c]	20.07± 0.01[a]
80	54.24± 0.02[b]	67.60±0.08[c]	41.73±0.01[a]
160	63.17± 0.02[b]	68.04±0.03[c]	58.58±0.03[a]
360	72.33± 0.02[b]	77.53±0.01[c]	58.92±0.2[a]
DPPH scavenging activity			
20	70.63±0.13[b]	81.97±0.27[c]	54.97±0.15[a]
40	73.32± 0.10[b]	84.78±0.13[c]	59.62±0.07[a]
60	76.14± 0.04 [b]	82.36 ±0.19[c]	60.15±0.15[a]
80	76.76±0.001[b]	87.55±0.1[c]	63.296±0.3[a]
100	76.97 ±0.15[b]	88.48±0.11[c]	68.87±0.15[a]

Values expressed as mean ± SD (n=3)

Means with different letters in the same row indicate that there is significant difference between samples (*p* ≤0.05) from Duncan's multiple range test.



(a)



(b)

Fig.3.4 Scavenging capacity of three (LA, UA and WR) rice cultivars (a) Chelating activity and (c) DPPH activity

3.3.9.4 Fourier transform infrared (FT-IR) spectra analysis

The Fourier transform infra-red spectrum of three rice cultivars are shown in Fig.3.4 (a, b and c). It was observed from the figures that the broad band of LA varied from 1455.51 to 3643.40 cm^{-1}, UA 1650.63 to 3751.16 cm^{-1} and WR 1655.63 to 3874.61 cm^{-1}. Intense characteristic peak at approximately in the region of 3600 to 3700 cm^{-1} and 3200–3500 cm^{-1} have been seen in all the three rice cultivars. Broad band at 3610–3640 cm^{-1} and 3200–3500 cm^{-1} is attributed to –OH stretching vibrations, which represents possibly the presence of phenolic OH.[45] On the other hand, a weak C–H stretching band at around 2891 to 2937 cm^{-1} was observed in the rice cultivars, which represents the asymmetrical and symmetrical stretching vibration of hydroxyl group, respectively. The weak C–H stretching band in FT-IR spectrum was the plausible reason for the presence of phenolic acid in the rice cultivars.[7, 38] The presence of carbonyl (C=O) linkages were confirmed from the peaks at 1076 and 1162 cm^{-1}. The FT-IR band of C=O stretching was mainly due to the presence of carboxyl (-C=O) group of phenolic compounds of the pigmented rice cultivars. The bands approximately in the regions of 3400, 2930 and 1650 cm^{-1} are characteristic of a carbohydrate ring.[54]

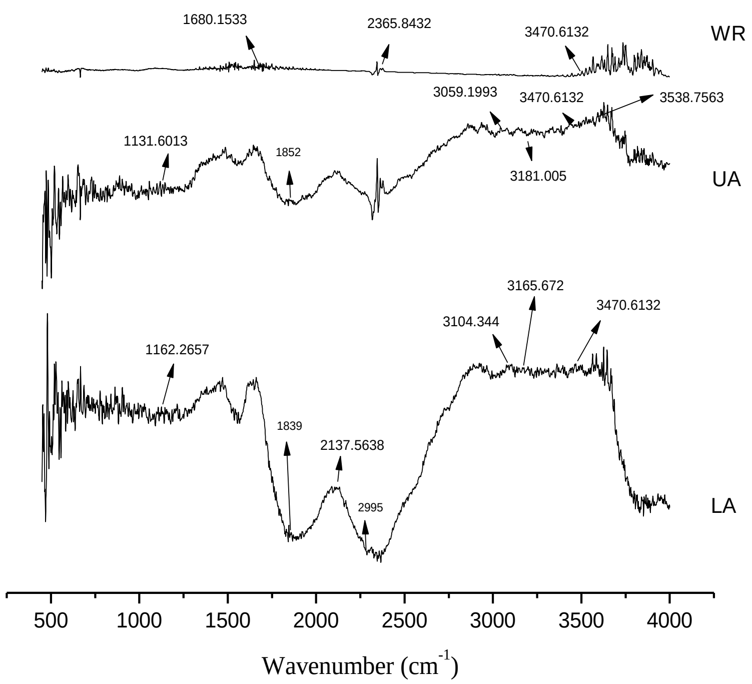


Fig. 3.5 Typical FTIR spectra of (a) LA (b) UA and (c)

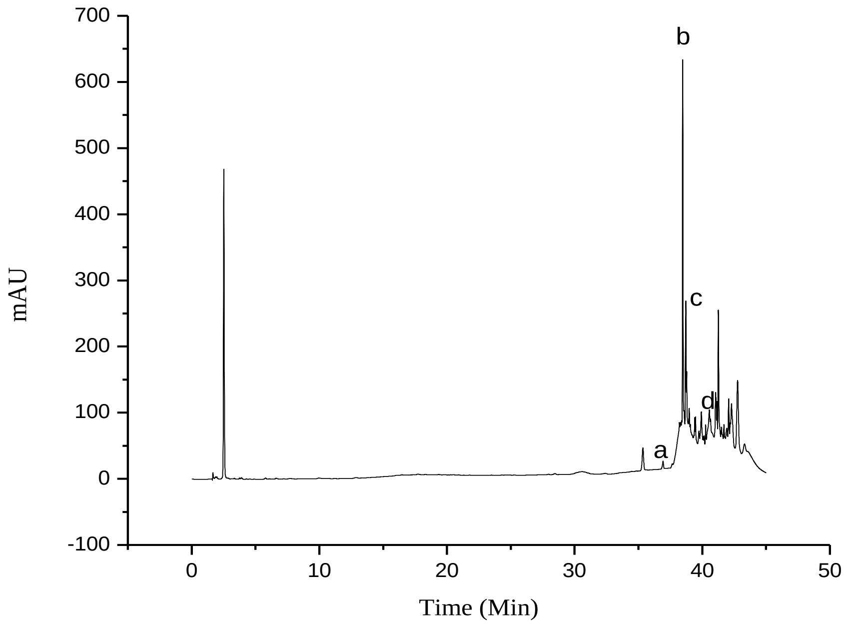
3.3.9.5 Reverse Phase-High-performance liquid chromatography (RP-HPLC) analysis

The identification of phenolic compounds by RP-HPLC revealed differences in the, phenolic fraction profile among the rice cultivars (Fig. 3.5a, b and c). The distribution of phenolic acids in all samples is illustrated in Table 4. The main phenolic acids identified in all three cultivars were salicylic acid, apigenin and quinic acid. The salicylic acid was present at 302.06 ±0.03, 44.50 ± 0.01 and 231.94±0.02 mg/L level in UA, LA and WR respectively. The highest amount apigenin was detected in UA (7.03±0.01 mg/L) followed by LA (0.42±0.02 mg/L) and WR (0.49±0.01 mg/L). Quinic acid was detected in all the three rice cultivars in high amount. It was found that UA, LA and WR had 255.46±0.01, 611.46 ± 0.01 and 133.92±0.02 mg/L of quinic acid respectively. Quercetin was only detected in LA (33.27±0.01 mg/L) cultivar and there was no detectable amount of ferulic, gallic and caffeic acid in LA. UA rice cultivar contained detectable amount of gallic acid (0.98±0.04mg/L) and ferulic acid (17.21±0.02 mg/L) whereas white rice cultivars contained only ferulic acid (15.18±0.01 mg/L) and very less amount of caffeic acid (1.83±0.02 mg/L). The concentration of total phenolics in the cultivars is associated with the antioxidant activities [28,36] which has potential benefits such as reduction of oxidative stress, cardiovascular problems, blood and lipids related diseases.[36] The rice cultivars used in the present study have various types of phenolic acid content. Salicylic, caffeic, quinic, apigenin, ferulic, gallic and quercetin acid were identified in pigmented cultivars whereas the last two acids were not detected in white rice.

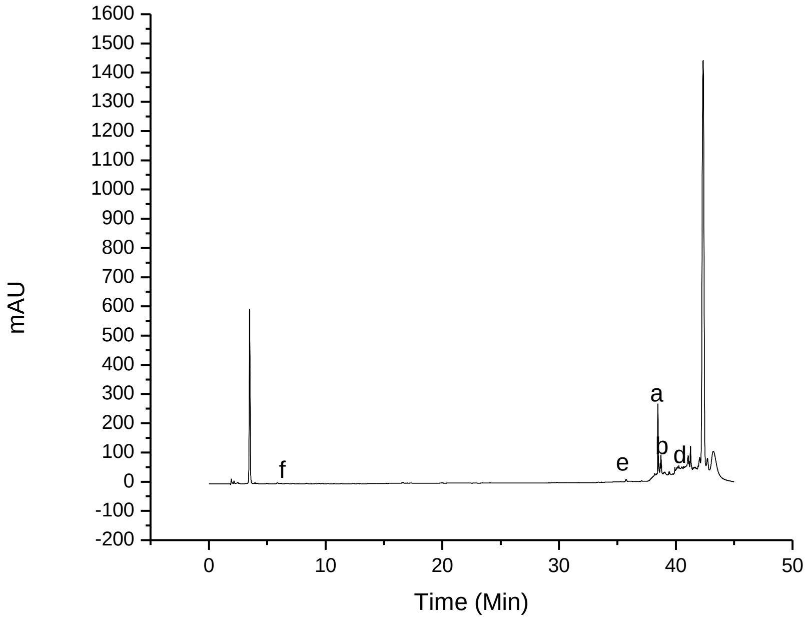
Table 3.9 Quantification of predominant phenolic acids present in three rice cultivars

Phenolic acid (mg/L)	**LA**	**UA**	**WR**
Quinic acid (a)	611.46 ± 0.01^{c}	255.46±0.01^{b}	133.92±0.02^{a}
Salicylic acid (b)	44.50±0.01^{a}	302.06±0.03 ^{c}	231.94±0.02^{b}
Quercetin (c)	33.27±0.01	ND	ND
Apigenin (d)	0.42±0.02^{a}	7.03±0.01^{b}	0.49±0.01^{a}
Gallic acid (f)	ND	0.98±0.04	ND
Caffeic acid (g)	ND	ND	1.83±0.02
Ferulic acid (e)	ND	17.21±0.02	15.18±0.01

Note: N.D (not detected)

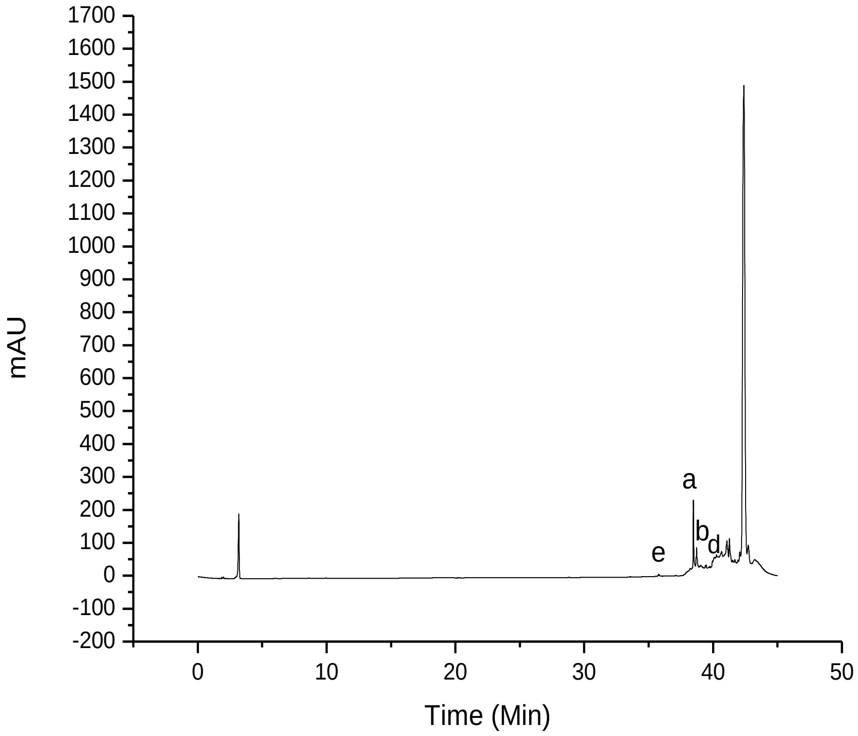


(a)



(b)

Contd.



(c)

Fig. 3.6 RP-HPLC chromatograms of (a) LA (b) UA and (c) WR

3.4 Conclusion

The results of the proximate composition of the pigmented rice cultivars differed significantly over the non-pigmented rice. Physical and thermal attributes varied with difference in cultivars. Moisture and ash content of different rice cultivars varied significantly ($P \leq 0.05$) from 11 to 11.50% (d.b.) and from 0.93 to 1.33% (d.b.). The bulk and true density of the three different rice cultivars (LA, UA and WR) showed no significant differences between the cultivars at the 0.05% probability. Thousand grain weights (g), angle of repose and porosity of LA, UA and WR varied significantly. L, a^* and b^* color parameters varied from sample to sample. UA cultivar exhibited higher degree of redness and yellowness than LA and WR. Mineral profiles of the rice cultivars were analyzed. It was revealed that all of the rice varieties were a good source of mineral as a micronutrient. Texture profile such as hardness (g), springiness (length/length) and cohesiveness of cooked rice had significant variation. In thermal properties, T_o, T_p and T_c values of UA cultivar were shown to be higher than WR and LA. In addition, pigmented rice cultivars evinced substantial amount of phenolic compounds (UA-349.3 ± 0.02 and LA-262.30 ± 0.01 mg GAE/100 g) compared to white rice (142.90 ± 0.01 mg GAE/100 g) and revealed high antioxidant activity. The pigmented cultivars also showed a higher amount of anthocyanin content in UA (12.79 ± 0.001) and LA (11.47 ± 0.001 mg/100 g) compared to WR (1.34 ± 0.001) further reinforced its potential for high value addition. FT-IR and RP-HPLC analysis of the rice cultivars deciphered the presence of seven phenolic compounds viz., quinic, salicylic, quercetin, apigenin, ferulic, gallic, and caffic acid which are paramount for functional foods. It is prudent to summarize that these pigmented untapped rice cultivars of Arunachal Pradesh, India have enormous potential in the field of the pharmaceutical industry vis-a-vis its health benefits. The above results could provide important information for the utilization of rice cultivars and to develop new food products. The results of the present study clearly suggest that the pigmented rice cultivars of Arunachal Pradesh, India are rich in phytochemicals. These novel cultivars can undoubtedly be used in preparation of functional foods and therefore it elucidates that these cultivars has enormous potentials as nutraceuticals and can be exploited industrially

References

[1] Acquaah, S,G., Manful, J,T., Ndindeng, S,A. and Tchatcha, D,A. Effects of soaking and steaming regimes on the quality of artisanal parboiled rice. *Journal of Food Processing and Preservation,* doi:10.1111/jfpp.12474, 2015.

[2] Adebowale, A, A., Sanmi, L, O. and Awonorin, S,O. Effect of texture modifiers on the physicochemical and sensory properties of dried fufu. *Food Science and Technology International,* 5:373-382, 2005.

[3] Amin, M, N., Hossain, M,A. and Roy, K,C. Effects of moisture content on some physical properties of lentil seeds. *Journal of Food Engineering,* 65:83-87,2004.

[4] Antequera, M,V,V., Ruiz, A,M., Perales, M,C,M., Munoz, N,M. and Ballesteros, M,R,J,C. Evaluation of an adequate method of estimating flowability according to powder characteristics. *International Journal of Pharmaceutics*,103:155-161,1994.

[5] AOAC, *Official Methods of Analysis*, 17th Ed., Association of official analytical chemists, Gaithersburg, MD,2000.

[6] Atala, E., Vásquez, L., Speisky, H., Lissi, E. and Lopez-Alarcon, C. Ascorbic acid contribution to ORAC values in berry extracts: An Evaluation by the ORAC-Pyrogallol red methodology. *Food Chemistry,* 113:331-335, 2009.

[7] Ascough, P,L., Bird, M.I., Francis, S,M. and Lebl, T. Alkali extraction of archaeological and geological charcoal: evidence for diagenetic degradation and formation of humic acids. *Journal of Archaeological Science,*38(1):69-78,2006.

[8] Arns, B., Paraginski, R,T., Bartz, J., Schiavon, R,A., Elias, M,C., Zavareze, E,R. and Dias, A,R,G. The effects of heat-moisture treatment of rice grains before parboiling on viscosity profile and physicochemical properties. *International Journal of Food Science and Technology,* 49:1939-1945, 2014.

[9] Amma, K,P,P., Sasidharan, I., Sreekumar, M,M., Sumathykutty, A,M. and Arumughan, C. Total antioxidant capacity and change in phytochemicals of four major varieties of cardamom oils during decortication. *International Journal of Food Properties*,18:1317-1325,2015.

[10] Bhattacharya, K, R. and Sowbhagya, C, M. Size and Shape Classification of Rice. *Il Riso*, 29:181-184, 1982.

[11] Brand-Williams, W., Cuvelier, M, E. and Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28:25-30, 1995.

[12] Butsat, S. and Siriamornpun, S. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chemistry*, 119: 606-613, 2010.

[13] Chrastil, J. Chemical and physicochemical changes of rice during storage at different temperatures. *Journal of Cereal Science*, 11:71-85, 1990.

[14] Chrastil, J. and Zarins, Z, M. Influence of storage on peptide subunit composition of rice oryzenin. *Journal of Agricultural and Food Chemistry*, 40:927-930, 1992.

[15] Ding,W.,Wang, Y., Zhang,W., Shi, Y. and Wang, D. Effect of ozone treatment on physicochemical properties of waxy rice flour and waxy rice starch. *International Journal of Food Science and Technology*, 50:744-749, 2015.

[16] Dinis, T, C, P., Madeira, V, M, C. and Almeida, L, M. Action of phenolic derivatives (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics Journal*, 315:161-169,1994.

[17] Das, G, K. and Oudhia, P. Rice as a medicinal plant in Chattisgarh, India (p. 46). PGR News letter, issue 122, Bioversity and FAO,2003.

[18] Finocchiaro, F., Ferrari, B., Gianinetti, A., DallAsta, C., Galaverna, G., Scazzina, F. and Pellegrini, N. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Molecular Nutrition and Food Research*, 51:1006-1019,2007.

[19] Guha, M., Ali, S.Z. and Bhattacharya, S. Effect of barrel temperature and screw speed on rapid viscoanalyser pasting behaviour of rice extrudate. *International Journal of Food Science and Technology*, 3:259-266, 1998.

[20] Gupta, R, K. and Das, S, K. Physical properties of sunflower seeds. *Journal of Agricultural Engineering Research,* 66: 1-8, 1977.

[21] Giusti, M,M. and Wrolstad, R,E. *Characterization and measurement of anthocyanins by UV–Visible spectroscopy*. In Current Protocols Food Analytical Chemistry; Wrolstad, R.E.; Ed.; John Wiley and Sons: New York, NY,2001.

[22] Hore, D,K. Rice diversity collection, conservation, and management in North-Eastern India. *Genetic Resources and Crop Evolution*, 52:1129-1140, 2005.

[23] Hua, D., Zhang, D., Huang, B., Yi, P. and Yan, C. Structural characterization and DPPH radical scavenging activity of a polysaccharide from guara fruits. *Carbohydrate Polymer*, 103:143-147,2014.

[24] Hogan, J,T. and plank, R,W. Hydration characteristics of rice as influenced by variety and drying method. *Cereal Chemistry,* 35:469-482, 1958.

[25] Huaisan, K., Uriyapongson, J., Rayas-Duarte, P., Ali, I. and Srijesdarute, V. Effect of food additives on rheological and textural properties of frozen high amylose rice starch gels. *International Journal of Food Properties*, 12:145-161, 2009.

[26] Huang, M., Kennedy, J, F., Li, B., Xu, X. and Xie, B, J. Characters of rice starch gel modified by 1gellan, carrageenan, and glucomannan: A texture profile analysis study. *Carbohydrate Polymer*, 69:411-418, 2007.

[27] Inglett, G,E., Chen, D. and Liu, S,X. Pasting and rheological properties of quinoa-oat composites. *International Journal of Food Science and Technology*, 50:878-88, 2015.

[28] Itani, T. and Ogawa, M. History and recent trends of red rice in Japan. *Japanese Journal of Crop Science*,73(2):137-147,2004.

[29] IRRI. Rice quality characteristics of milled rice. http://www.irri.org. (accessed 25 Nov 2015).

[30] Jain, R, K. and Bal, S. Properties of pearl millet. *Journal of Agricultural Engineering Research*, 66:85-91,1997.

[31] Kang, H,J., Hwang, I,K., Kim, K,S. and Choi, H, C. Comparison of the physicochemical properties and ultrastructure of Japonica and Indica rice grains. *Journal of Agricultural and Food Chemistry*, 54:4833-4838, 2006.

[32] Karim, A., Toon, L., Lee, V., Ong, W., Fazilah, A. and Noda, T. Effects of phosphorus contents on the gelatinization and retrogradation of potato starch. *Journal of Food Science*, 72:132-138, 2007.

[33] Katekhong,W. and Charoenrein, S. Effect of rice ageing and freeze-thaw cycle on textural properties of cooked rice (*Oryza sativa* L.) cv. Khao Dawk Mali 105. *International Journal of Food Science and Technology*, 49:2283-2289, 2014.

[34] Krueger, B, R., Knutson, C.A., Inglett, G, E. and Walke, C, E. A differential scanning calorimetry study on the effect of annealing on gelatinization behavior of corn starch. *Journal of Food Science*, 52:715-718,1987.

[35] Kumar, C, G., Joo, H, S., Chio, J, W., Koo, Y, M. and Chang, C,S. Purification and characterization of an extracellular polysaccharide from *Haloalkalophilic Bacillus* sp. I-450. *Enzyme Microbial Technology*, 34:673-681,2004.

[36] Katalinic, V., Mozina, S, S., Generalic, I., Skroza, D., Ljubenkov, I. and Klancnik, A. Phenolic profile, antioxidant capacity, and antimicrobial activity of leaf extracts from six *Vitis Vinifera* L. varieties. *International Journal of Food Properties*, 16:45-60, 2013.

[37] Lee, I., We, G, J., Kim, D, E., Cho, Y, S., Yoon, M, R., Shin, M. and Ko, S. Classification of rice cultivars based on cluster analysis of hydration and pasting properties of their starches. *LWT-Food Science and Technology*, 48:164-168, 2012.

[38] Lee, F,Y., Htar, T,T. and Akowua, G, A. ATR-FTIR and spectrometric methods for the assay of crocin in commercial saffron spices (*Crocus Savitus* L.). *International Journal of Food Properties*, 18:1773-1783,2015.

[39] Leelayuthsoontorn, P. and Thipayarat, A. Textural and morphological changes of jasmine rice under various elevated cooking conditions. *Food Chemistry*, 96:606-613, 2006.

[40] Miles, M, J., Morris, V, J., Orford, P, D. and ring, S,G.The role of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research*, 135:271-281, 1985.

[41] Mohsenin, N, N. *Physical Properties of Plant and Animal Materials*, Gordon and Breach Science Publishers, New York,1980.

[42] Mohsenin, N, N. *Physical Properties of Plant and Animal Materials*, 2nd Ed., Gordon and Breach Science Publishers, New York,1986.

[43] Muller, L., Frohlich, K. and Bohm, V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (TEAC), DPPH assay, and peroxyl radical scavenging assay. *Food Chemistry*,129(1):139-148, 2011.

[44] Oki, T., Masuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I. and Sato, T. Polymeric procyanidins as radical-scavenging components in red-hulled rice. *Journal of Agricultural Food Chemistry*, 50:7524-7529,2002.

[45] Park, Y, S., Kim, S, J. and Chang, H,I. Isolation of anthocyanin from black rice (Heugjinjubyeo) and Screening of Its antioxidant activities. *Korean Journal of Microbiology and Biotechnology*, 36, 55-60,2008.

[46] Slinkard, S. and Singleton, V, L. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28:49-55, 1977.

[47] Sahay, K, M. and Singh, K, K. Unit Operations of agricultural processing, 1st Ed., Vikas Publishing House Pvt. Ltd., New Delhi, India,1994.

[48] Selvi, K, C., Pinar, Y. and Yesiloglu, E. Some physical properties of linseed. *Biosystems Engineering*, 95:607-612, 2006.

[49] Shen, F., Wu, J., Ying, Y., Li, B. and Jiang, T. Differentiation of Chinese rice wines from different wineries based on mineral elemental fingerprinting. *Food Chemistry,* 141:4026-4030, 2013.

[50] Singh, V., Okadome, H., Toyoshima, H., Isobe, S. and Ohtsubo, K. Thermal and physicochemical properties of rice grain, flour and starch. *Journal of Agricultural and Food Chemistry,* 48:2639-2647,2000.

[51] Sodhi, N, S., Singh, N., Arora, M. and Singh, J. Changes in physico-chemical, thermal, cooking and textural properties of rice during aging. *Journal of Food Processing and Preservationa*, 27:387-400,2003.

[52] Sozer, N., Dalgic, A, C. and Kaya, A. Thermal, textural and cooking properties of spaghetti enriched with resistant starch. *Journal of Food Engineering*, 81:476-484, 2007.

[53] Stevens, D, J. and Elton, G, A, H. Thermal properties of the starch water system. Part 1. Measurement of heat of gelatinization by differential scanning calorimetry. *Starch/Stärke,* 23: 8-11,1971.

[54] Santhiya, D., Subramanian, S. and Natarajan, K, A, J. Surface chemical studies on sphalerite and galena using extracellular polysaccharides isolated from *Bacillus Polymyxa*. *Journal of Colloid and Interface Science,* 256:237-248, 2002.

[55] Saikia,S., Dutta,H., Saikia,D. and Mahanta,C,L. Quality characterization and estimation of phytochemicals content and antioxidant capacity of aromatic pigmented and nonpigmented rice varieties, *Food Research International*, 46:334-340,2012.

[56] Tester, R, F. and Morrison,W, R. Swelling and gelatinization of cereal starches. Effects of amylopectins, amylose, and lipids. *Cereal Chemistry*, 67:551-557,1990.

[57] Thomas, R.,Wan-Nadiah,W,A. and Bhat, R. Physiochemical properties, proximate composition, and cooking qualities of locally grown and imported rice varieties marketed in Penang, Malaysia. *International Food Research Journal*, 20:345-1351,2013.

[58] Thompson, R, A. and Issac, G,W. Porosity determination of grains and seeds with air comparison pycnometer. *Transactions of the ASABE,* 10:693-696,1976.

[59] Tian, Y., Jin, Z., Deng, L., Xu, X. and Zhao, J. Study on the effect of vitamin D3-bcyclodextrin inclusion compound on the quality improvement in extruded nutrition-fortified rice. *Fermentation in the Food Industries*, 33:52-55, 2007.

[60] Yu, S, F., Ma, Y. and Sun, D, W. Impact of amylose content on starch retrogradation and texture of cooked milled rice during storage. *Journal of Cereal Science*, 50:139-144, 2009.

[61] Zhou, Z., Robards, K., Helliwell, S. and Blanchard, C. Effect of rice storage on pasting properties of rice flour. *Food Research International*, 36:625-634, 2003.

(B) Physicochemical and phytochemical analysis of purple passion fruit

3.5 Introduction

The genus *Passiflora* L. (family Passifloraceae) is predominant in Brazil.[10] Passion fruit (*Passiflora edulis*) is an exotic fruit i.e. restricted fruits usage in limited or particular geographic areas[14,1] is oval shape and size about 6-12 cm belongs to Passifloraceae family. Among the different species, two well-known edible passion fruit species are purple (*Passiflora edulis* Sims) and yellow (*Passiflora edulis* f. *flavicarpa* Deg.). Also, possibilities of natural hybrid between purple and another related species.[2] Yellow passion fruit mostly grown in lowland tropical conditions and purple type are found more in the higher altitudes in the tropics. The purple passion fruit is originally from southern Brazil through Paraguay to northern Argentina and yellow one perhaps from Amazon region of Brazil.[9] Purple fruit is well known for its nutritional benefits and medicinal properties and its rind have the anti-hypertensive effect and vasodilatory effect on human body.[15,16] Therefore, the present chapter deals with the physiochemical and phytochemical analysis of purple passion fruit (*Passiflora edulis* Sims).

3.6 Material and methods

3.6.1 Raw material

Fully ripe purple passion fruit was purchased from the local market of village Sinchung, West Kameng District, Arunachal Pradesh, in the month of July-September. The fruit pulp with seed was squeezed by using muslin cloth. The pH of fruit pulp was measured by pH meter and the total soluble solids (TSS) content was recorded with the help of hand refractometer in the laboratory condition (0-32˚ Brix). The pulp was collected was store at -20˚C for further analysis.

Samyor et al. (2017). Effect of foam mat drying on physicochemical and phytochemical properties of passion fruit powder. International Journal of Food Properties (Under Review)

3.6.2 The color measurement

The color measurement of pulp was measured by using COLOR measurement spectrophotometer (Ultra Scan VIS, Hunter Lab, a41-1013-504, Reston, VA) (method described in section 3.2.3.7)

3.6.3 Phytochemical analysis

3.6.3.1 Ascorbic acid (vitamin C) determination

Ascorbic acid (vitamin C) was determined according to Sadasivam and Theymoli[17]. The volumetric method was used for determination of ascorbic acid in passion fruit. Sample (1g) was weighed and taken for preparation of extract and ascorbic acid solution was used as standard.

$$\text{Ascorbic acid} = \frac{0.5}{V_1} \times \frac{V_2}{15} \times \frac{100}{s} \times 100$$

Where, V_1 is the mL of solution taken for estimation, V_2 is the volume made up, S is the weight of sample.

3.6.3.2 Total phenolic content

Determination of total phenolic content of passion fruit pulp was carried out by Folin–Ciocalteu assay[18] (method described in section 3.2.4.2).

3.6.3.3 DPPH scavenging activity

DPPH radical scavenging activity of the fruit pulp was measured according to the method of Brand-Williams et al.[4] (method described in section 3.2.4.4).

3.6.3.4 Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis

Fruit pulp sample (0.5 mL) was placed over on a multi-bounce Zn Se crystal of ATR-FTIR to identify the functional groups.[23] The IR- absorption spectra of pulp were obtained using a FT-IR spectrometer (Nicolet Impact 410, Thermoscientific, USA) equipped with KBr optics and a DTGS detector and the frequency ranged from 4000 to 400 cm^{-1}.

3.6.3.5 Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of phenolic acids

3.6.3.5.1 Sample preparation

For phenolic acid analysis, 50 ml of fruit pulp and 0.5 g ascorbic acid were mixed together. In the mixture 100 ml of 80% methanol was added and filtrated through WhatmanNo.2. (method was described previously in the section 3.2.4.7)

3.6.3.6 Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of (±) α-tocopherol, D-α-tocotrienol and β-carotene

3.6.3.6.1 Sample preparation

The (±)-α-tocopherol, D-α-tocotrienol and β- carotene were estimated by the method described by Aguilar-Garcia et al.[3] with slight modification. Passion fruit pulp (50 ml) was extracted twice with 6mL of methanol. Then, the extract was centrifuged for 10 min at 825g. The supernatant was collected and evaporated to 4 mL and volume made up to 5.0mL with methanol in a volumetric flask. This solution was filtered with Whatman ™ No. 1 and then, filtered through GD/X sterile 0.45 µm CA filter media of 25 mm before being subjected to HPLC analysis.

3.6.3.6.2 Detection

For detection of (±)-α-tocopherol, D- α- tocotrienol and β- carotene, the same RP-HPLC as used for phenolic, was used with UV Detector at 292 and 325nm. The C18, 5.0 µm (4.6 mm x 250mm) column was used to separate the compounds. The mobile phase was a mixture of methanol and acetonitrile (20:80 v/v) at a flow rate of 0.8 mL/min with isocratic mode. The software empower 2 was used for analyzing data.

3.6.4 Statistical analysis

Microsoft office excel 2013 was used for average and standard deviation calculation. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for graphs.

3.7 Results and discussion

3.7.1 Moisture content (%), pH and total soluble solid (˚Brix)

Moisture content (%), pH and total soluble solid (˚Brix) value of pulp were 82.25 ± 0.01%, 3.91 ± 0.2 and 16.85 ± 0.4 respectively. Soluble solids referred to the sugars and acids combined with minute amount of dissolved vitamins, proteins, pigments, phenolics, and minerals.[21, 22,6,11,12] TSS is a great indicator to study quality parameters to indicate sweetness of post harvested horticultural crops in laboratories for research and development purpose, also in food industry to determine marketing standards. In the Table 3.10, the color measurement and phytochemical composition were presented. *L** value represent whiteness/lightness of the pulp sample which was 36.68±2.02, the lower value of *a** value is indicates redness part 3.79± 0.61 and *b** value observed was 19.82 ±1 which indicates the yellowness. Chroma and hue were 0.19±0.01and 0.19±0.01.The term chroma (C_{ab}), or saturation index represent the quantitative attribute of colorfulness of sample which is proportional to its intensity whereas Hue (h_{ab}) is a qualitative indicator.[5]

Table 3.10 Color measurement and phytochemical compositions of pulp

Parameters	**Passion fruit pulp**
*L**	36.68±2.02
*a**	3.79± 0.61
*b**	19.82 ±1.44
Chroma $(a^{*2}+b^{*2})^{1/2}$	20.17±0.02
Hue angle $\tan^{-1}(b^*/a^*)$	79.15±0.01
Vitamin C(mg/100g)	39.85.19± 0.01
Total phenolic content(mg GAE/100g)	206.29 ±0.10
DPPH scavenging activity (%)	70.53±0.03

3.7.2 Phytochemical analysis

3.7.2.1 Vitamin C (mg/100g)

Vitamin C content of purple passion fruit pulp was 39.85.19 ± 0.01 (mg/100g). Valente et al.[24] reported that in purple passion fruit from Colombia ascorbic acid content was 36.3

mg/100g. Ramaiya et al.[16] stated that *passiflora edulis* (purple) fruit collected from Malaysia showed ascorbic acid of 0.32 ± 0.72 g/kg i.e. 32mg/100g. Therefore, from the study ascorbic acid content can be differ from plant origins.

3.7.2.2 Total phenolic content (mg GAE/100g)

Total phenolic content can be calculated by using Folin–Ciocalteu reagent (FCR) method were the reduction of the reagent by phenolic compounds present in the sample can be observed. Blue complex obtained can be measured at 765nm against gallic acid as a standard. In the passion fruit pulp total phenolic content (mg GAE/100g) determined was 206.29 ±0.10 (mg GAE/100g).

3.7.2.3 DPPH scavenging activity (%)

DPPH is a stable free radical which is widely used in research laboratory to analyses the ability of exotic fruit different solvents extracts to act as free radical scavengers or hydrogen donors, which predicts the antioxidant activity of a sample. In passion fruit pulp, DPPH scavenging activity (%) at 517 nm obtained was 70.53 %.

3.7.2.4 ATR-FT-IR analysis

ATR-FT-IR analysis of pulp has been illustrated in Fig.3.7 and spectral stretching ranging from 625 to 3312 cm^{-1}. The absorption bands appeared were 625, 1039.54, 1270, 1375, 1658, 2122 and 3312 cm^{-1}. A very sharp band observed in 1658 cm^{-1}. Among all bands, 3312 cm^{-1} was the broadest band. Spectral peak at 1057 cm^{-1} is sucrose. The medium peak ranged from 1250 to 1020 cm^{-1} denotes C–N stretch bond indicating aliphatic amines.[13] A stretching characteristic bands at 3420 cm^{-1} and 2937 cm^{-1} were due to the O–H stretching band.[20]

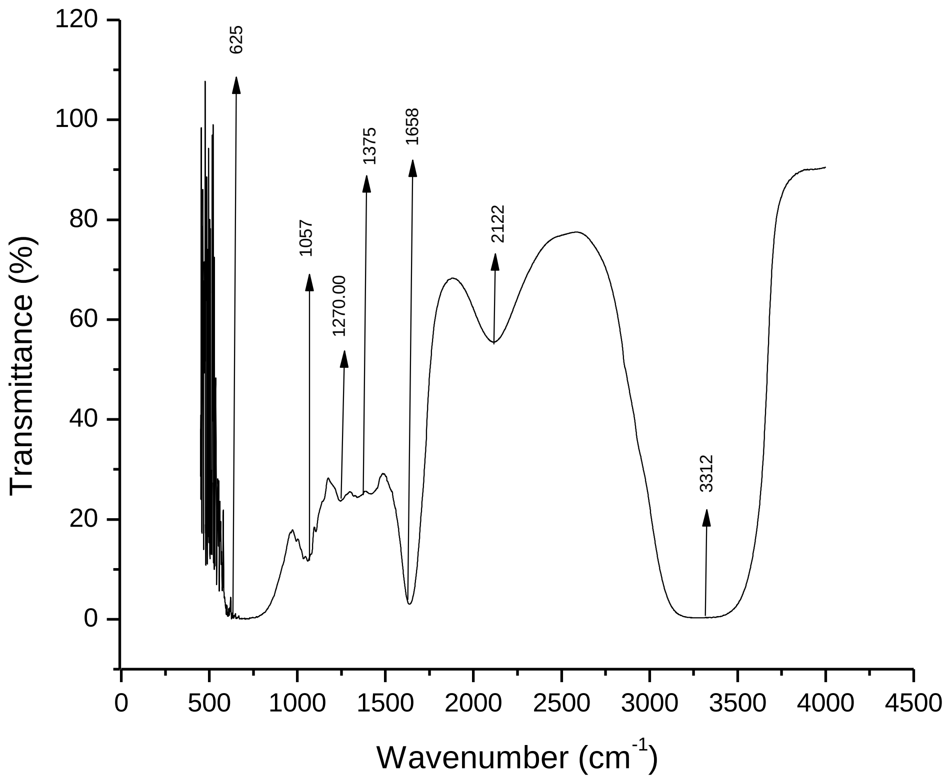


Fig.3.7. ATR-FT-IR spectra of pulp

3.7.2.5 RP-HPLC analysis

The vitamins and phenolic acids from pulp were identified and quantified by RP-HPLC (Table 3.11). The β-carotene, (±)-α-tocopherol and D-α-tocotrienol were detected at the retention times of 3, 3.5 and 3.8 min, respectively. The β-carotene, (±)-α-tocopherol and D-α-tocotrienol content in pulp were 11.79, 171.10 and 27.19 mg/100g. Cavalcante et al.[8] stated that compounds like carotene and vitamin accumulation in passion fruit are contributed by various internal as well as external factors *viz.*, maturity stage, cultivation system etc. Six phenolic acids were prominently observed in the raw pulp extract (Table 3.11). The pulp sample reveal highest content of vanillic acid 873.75 mg/100g.

Table 3.11 Quantification of vitamins and phenolic acids of passion fruit pulp

Phytochemicals (mg/100g)	**Retention time (min)**	**Passion fruit pulp**
Vitamin		
1. β-carotene	3.00	11.79
2. (±)-α-tocopherol	3.50	171.10
3. D-α-tocotrienol	3.80	27.19
Phenolic acid		
1. Caffeic acid	14.66	ND
2. (±) Catechin hydrate	13.00	ND
3. Chlorogenic acid	13.80	789.00
4. *p*- Coumeric acid	17.25	268.75
5. Transferulic acid	14.36	766.26
6. 4-Hydroxybenzoic acid	18.03	ND
7. Syringic acid	15.06	643.46
8. Sinapic acid	17.80	630.00
9. Vanillic acid	14.96	873.75

Note: ND (not detected)

3.8 Conclusion

In the present chapter physiochemical and phytochemical analysis of purple passion fruit (*Passiflora edulis* Sims) content were revealed. The pH and total soluble solids (˚Brix) of fruit pulpmeasured by pH meter and hand refractometer were 3.91 ± 0.2 and 16.85 ± 0.4. Color measurement showed that *b** which indicates yellowness were higher than *a** (redness). Phytochemical analysis *viz.,* DPPH scavenging activity (%), total phenolic content (mg GAE/100g) and vitamin C (mg/100g) presented good value of antioxidant property. The vitamins and phenolic acids from pulp were identified and quantified by RP-HPLC respectively. Some phenolic acid were found in good amount. These data can be useful for further in-depth study in the field of passion fruit utilization in food industries.

References

[1] Aparecida de Assis, S., Vellosa, J, C, R., Brunetti, I, L., Khalil, N, M., Leite, K, M, S. and C., Martins, A, B, G. and Oliveira, O, M, M, de F. Antioxidant activity, ascorbic acid and total phenol of exotic fruits occurring in Brazil. *International Journal of Food Sciences and Nutrition*, 60:439-448,2009.

[2] Akamine, E,K. and Girolami, G. Pollination and fruit set in the yellow passion fruit. *Hawaii Agriculture Statistical Bulletin*, 59:1-44,1959.

[3] Aguilar-Garcia, C., Gavino, G., Baragano-Mosqueda, M., Hevia, P. and Gavino, V, C. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chemistry*, 102:1228-1232,2007.

[4] Brand-Williams, W., Cuvelier, M, E. and Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology*, 28:25-30,1995.

[5] CIE. Recommendations on uniform color spaces, color-difference equations, psychometric color terms. CIE Publication No.15 (E-1.3.1) 1971, Supplement 2, 1978.

[6] Chope, G, A., Terry, L, A. and White, P, J. Effect of controlled atmosphere storage on abscisic acid concentration and other biochemical attributes of onion bulbs. *Postharvest Biology and Technology*, 39, 233-242, 2006.

[7] Canini, A., Daniela, A., Guiseppe, A. and Peitro, T. Gas chromatography-mass spectrometry analysis of phenolic compounds from Carica papaya L. Leaf. *Journal of Food Composition and Analysis*, 20, 584-590,2007.

[8] Cavalcante, L,F., Dias, T, J., Nascimento, R. and Freire, J, L, O. Chlorophyll andcarotenoids in yellow passion fruit plants irrigated with saline water on soil with bovine biofertilizer. *RevistaBrasileira de Fruticultura, E.*699-705,2011.

[9] Joy, P, P. Status and prospects of passion fruit cultivation in Kerala, Pineapple Research Station, Vazhakulam, 686- 670, 2010.

[10] Knight, R,J. and Winters, H,F. Pollination and fruit set of yellow passion fruit in southern Florida. *Proceedings* of the *Florida* State *Horticultural Society*, 75:412-418, 1962.

[11] Kader, A, A. Fruit maturity, ripening and quality relationships. *Acta Horticulturae*, 485:203-208,1999.

[12] Kader, A, A. Flavor quality of fruits and vegetables. *Journal of Science and Food Agriculture*, 88:1863-1868,2008.

[13] Nair,R.,Venkatesh, S., Athmaselvi, K,A. and Thakur, S. Rapid estimation and quantification of sucrose content in fruit pulps using Fourier transform infrared–attenuated total reflectance (FTIR–ATR) spectroscopy. *Food Measure*, 10:24-31, 2016.

[14] Oliveira, A, L., Lopes, R, B., Cabral, F, A. and Eberlin, M, N. Volatile compounds from pitanga fruit (*Eugenia uni flora* L.). *Food Chemistry*, 99:1-5, 2006.

[15] Patel, S, S. Morphology and pharmacology of *Passiflora edulis*: a review*, Journal of Herbal Medicine and Toxicology*, 3:1-6,2009.

[16] Ramaiya, D,S., Bujang,J,S., Zakaria, M,H., King,W,S. and Sahrir,M,A,S. Sugars, ascorbic acid, total phenolic content and total antioxidant activity in passion fruit (*Passiflora*) cultivars, *Journal of Science and Food Agriculture*, 93:1198-1205,2013.

[17] Sadasivam, S. and Theymoli, B. In: Practical Manual in Biochemistry, Tamil Nadu Agricultural University, Coimbatore p.14,1987.

[18] Slinkard, S. and Singleton, V, L. Total phenol analysis: automation and comparison with manual methods. *The American Journal of Enology and Viticulture*, 28:49-55,1977.

[19] Sivagurunathan,S., Sivasankari,S. and Muthukkaruppan, S, M. Determination of phenolic compounds in red variety of cashew apple (*Anacardiumoccidentale l.*) by GC-MS. *Journal of Basic Applied Biology*,5(1and 2):227-231,2011.

[20] Santhiya, D., Subramanian, S. and Natarajan, K, A, J. Surface chemical studies on sphalerite and galena using extracellular polysaccharides isolated from Bacillus polymyxa. *Journal of Colloid Interface Science*,256:237-248,2002.

[21] Tadeo, J,L., Ortiz, J,M. and Estelles, A. Sugar changes in clementine and orange Fruit during ripening. *Journal of Horticulture Science*, 62:531-537,1987.

[22] Ito,T., Sasaki,K., Yoshida,Y. Changes in respiration rate, saccharide and organic acid content during the development and ripening of mango fruit (*Mangifera indica* L.'Irwin') cultivated in plastic house. *Journal of the Japanese Society for Horticultural Science*, 66:629-635,1997.

[23] Vardin., H., Tay, A., Ozen, B. and Mauer, L. Authentication of pomegranate pulp concentrate using FTIR spectroscopy and chemometrics. *Food Chemistry*, 108:742-748, 2008.

[24] Valente, A., Albuquerque, T. G., Sanches-Silva, A. and Costa, H. S. Ascorbic acid content in exotic fruits: a contribution to produce quality data for food composition databases. *Food Research International*, 44:2237-2242,2011.

[25] Zubair, M., Anwar, F. and Shahid, S, A. Effect of extraction solvents on phenolics and antioxidant activity of selected varieties of Pakistani Rice (*Oryza sativa*). *International Journal of Agriculture and Biology*,14:935-940,2012.

4.1 Introduction

Fruits or plant-based foods are known for its bioactive phytochemicals activities that may provide desirable health benefits to reduce the risk promoting effects of chronic diseases.[25] Short life in terms of perishability is the main problem challenging fruit and vegetable production. One of the major postharvest spoilages occur because of the fast degradation of quality resulting in large wastage.[10] Foam mat drying is relatively low cost drying technique than freeze and spray drying. The application of foam mat drying technique can be an effective way to increase the shelf-life and decreases the phytochemical loss also allows the dehydration of difficult- to-dry, heat-sensitive, high sugar content and viscous foods.[18,19] In foam mat drying, for the porous structure of the foamed materials, mass transfer is faster, hence shorter the drying time apparently results in higher quality of dried food product.[6] Various food items such as soy milk[3], star fruit[23], cowpea[14], guava juice[11], apple pulp[27], mango[28], banana [36], tomato pulp [21], bael[7], shrimp [2] has been used for foam mat drying.

In food macromolecules, carbohydrates (methyl cellulose, carboxy methyl cellulose etc.) and proteins (egg albumin, soy protein, whey protein etc.) dominate the foams and emulsions area. Modified carbohydrates i.e. cellulose derivatives usage were reported in artificial creams and propylene glycol alginate in salad dressings[33] and increasing the stability of whipped cream through enhanced viscosity that prevented drainage during the storage period.[34] Methyl cellulose is derived by etherification of alkaline cellulose with methyl chloride to form the cellulose ethers. Upon heating, formation of completely reversible gels is the reason for film formation in fried foods, foam stabilisation, and stabilisation of fruit pie filling during baking.[16] Also the surfactancy of methyl cellulose improves the whippability of cake batters where a portion of egg whites was replaced by the methyl cellulose.[19, 37] Some studies revealed that methyl cellulose as a foaming agent in papaya, yellow variety of passion fruit and gac fruit. [4, 5, 22] Karim and Wai [23] also stated that methyl cellulose was used as foaming agent to study the characteristics of foam prepared from starfruit *(Averrhoa carambola* L.) puree.

Samyor et al. (2017). Effect of foam mat drying on physicochemical and phytochemical properties of passion fruit powder. International Journal of Food Properties (Under Review)

There is scanty of research on foam mat drying of purple passion fruit and less detail report on the phytochemical properties of foam mat dried passion fruit powder. Therefore, in the present study foam mat drying of purple passion fruit has been carried out. Methyl cellulose was used as foam stabilizer. The process was modelled using response surface methodology (RSM) and predicted using artificial neural network (ANN). Quantification of vitamins and phenolic acids of passion fruit pulp and powder were investigated.

4.2 Materials and methods

4.2.1 Plant material

Purple passion fruit cultivar at ripen stage were purchased from the local market of West Kameng District, Arunachal Pradesh, in the month of July-September. Subtropical areas or at higher altitudes in the tropics are favourable to cultivate the purple colour species of passion fruit. The Fruits were washed and graded manually. Pulp was squeezed out and stored in -20°C for future analysis.

4.2.2 Chemicals

Standard for phenolic acids *viz.,* ferulic , sinapic, syringic, hydroxybenzoic, *p*-coumeric, vanillic, caffeic, catechin and chlorogenic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA).Vitamin standards *viz*., β-carotene, (±)-α-tocopherol and D-α-tocotrienol were also purchased from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA).

4.2.3 Foam mat drying

Foam mat drying of passion fruit pulp was done in varying temperature, methyl cellulose concentration and whipping time. The methyl cellulose was used as foam stabilizer with varied range (1-3%). The passion fruit pulp and methyl cellulose mixture was whipped in a kitchen blender to incorporate air in the mixture. The foamed pulp concentrate was spread on a tray with 3mm of thickness and kept in dryer for drying at temperature 40-60°C. Foamed samples was monitored every 30 min for its moisture loss by weighing the sample plates using an electric balance with an accuracy of ±0.01 g. When the final moisture content reached 6.5% (d.b), drying was terminated.[28] After drying, the powder was vacuum packed and kept for further studies.[24]

4.2.4 Analysis

4.2.4.1 Vitamin C

Vitamin C was determined according to Sadasivam and Theymoli [29] (method described in section 3.6.3.1).

4.2.4.2 Total phenolic content

Determination of total phenolic content of passion fruit powder was carried out by Folin-Ciocalteu assay Slinkard and Singleton [30] (method described previously in section 3.2.4.2).

4.2.4.3 DPPH radical scavenging activity

DPPH radical scavenging activity of the fruit powder was measured according to the method of Brand-Williams et al.[9] (method described in chapter 3A, section 3.2.4.4).

4.2.4.4 Hygroscopicity

Hygroscopicity of foam mat powder was determined as described by Jaya and Das.[20] Briefly, 0.5 g sample was put in the pre-weighed petri dish and placed in a hermetically sealed glass desiccator, containing salt solution of Na Cl (75% RH) and stored at 20°C. At a specific time, interval weight gain was observed until, constant value was determined.

$$\text{Hygroscopicity}(\%) = \left(\frac{b+H}{a-H}\right)\times 100$$

Where H is the initial water content of the sample (0.5g), *b* (g) is the weight increase and *a* (g) is the initial sample weight.

4.2.5 Experimental design

The central composite design (CCD) followed by response surface methodology (RSM) was used to optimise experimental conditions of foam mat drying. The process was optimized in terms of whipping time (min), methyl cellulose (%) and temperature (°C). The ranges of experimental parameters were selected based on preliminary trials. Coded value of independent are shown in Table 4.1.

Table 4.1 Coded levels of independent variables for the central composite design (CCD)

Variables	Coded value	Level		
		-α	0	+α
Whipping time (min)	x_1	1	3	5
Methyl cellulose (%)	x_2	1	1.5	3
Temperature (˚C)	x_3	40	50	60

After data analysis, a second order polynomial equation was developed as follows:

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x^2{}_{ii} + \sum_{i=1}^{n} \sum_{j=i+1}^{m} \beta_{ij} x_i x_J$$

The coefficient of the polynomial equation were β_0 (constant), β_i (linear effects), β_{ii} (quadratic effects) and β_{ij} (interaction effects). x_i and x_j are the coded levels of independent variables *i* and *j* in the equation given.

4.2.6 Artificial neural networks (ANN)

ANN is used to predict the relationship between input and output parameters. Three independent variables, i.e., whipping time (min), methyl cellulose (%) and temperature (˚C) were in the input layer and output layer was vitamin C (mg/100g), total phenolic content (mg GAE/100g) and hygroscopicity (%). Neural network was trained using a single hidden layer with 3-x-1 topology where x was the number of neurons in hidden layer. To determine the optimum number of neurons in hidden layer, x was varied from 1 to 20. The experimental data was used to train the neural network. Total 20 data points were distributed into three sets: training (14 points), validation (3 points) and testing (3 points). The best training performance of the neural network was based on minimization of root mean square error (RMSE) and highest regression coefficient (R^2).

4.2.7 Phytochemical analysis

4.2.7.1 Fourier transform infrared (FT-IR) analysis

Foam mat dried passion fruit powder (2mg) and 50 mg desiccated KBr powders were thoroughly mixed in a mortar and pestle before pressing into a thin pellet (method described in section 3.2.4.6).

4.2.7.2 Reverse Phase-High-performance liquid chromatography (RP-HPLC) analysis of phenolic acids

For phenolic acid analysis, 50g of sample and 0.5 g ascorbic acid were mixed together (method described previously in the (method described in section 3.2.4.7)

4.2.7.3 Reverse Phase-High-performance liquid chromatography (RP-HPLC) analysis of β-carotene, (±) α-tocopherol and D-α-tocotrienol

The β- carotene, (±)-α-tocopherol and D-α-tocotrienol were estimated by the method described by Aguilar-Garcia et al.[1] with slight modification. Passion fruit foam mat powder (50g) was used for extraction (method described in section 3.6.3.6.).

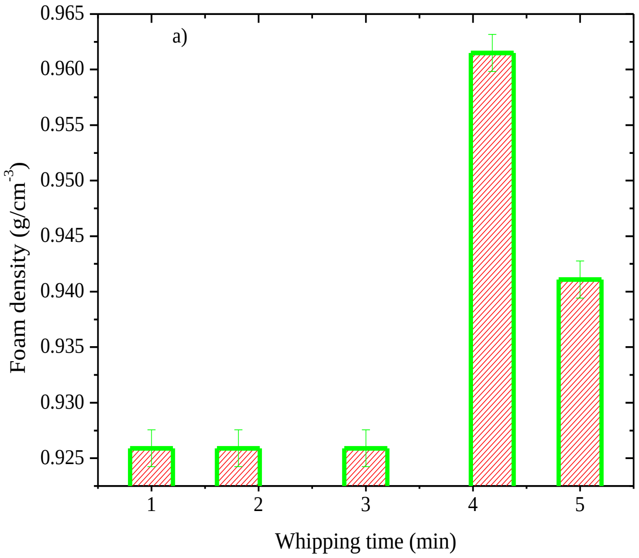
4.2.8 Statistical analysis

The experimental design was done by central composite design (CCD) followed by response surface methodology using design expert 7.0 software. Microsoft office excel 2007 was used for average and standard deviation calculation. The Origin 8.5 (Origin Lab Corporation, Northampton, USA) software was used for graphs.

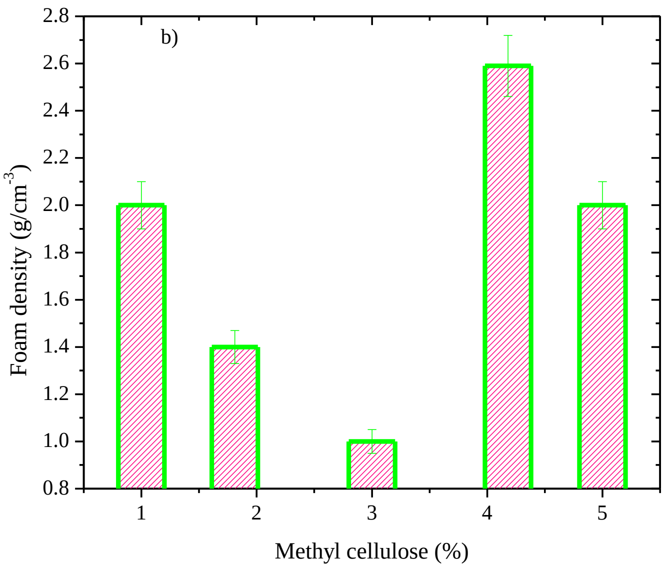
4.3 Results and discussion

4.3.1 Preliminary trial

A preliminary trial was conducted to identify the effect of whipping time and methyl cellulose on foam density of passion fruit pulp. Density of foam was varied from 0.92-0.98 g/cm^3.The Fig.4.1 (a) and (b) illustrated the effect of whipping time and methyl cellulose on foam density (FD). From Fig.4.1a, it was observed that the FD of the mixture was constant and minimum, up to 3 min of whipping and the highest FD was observed 4 min whipping. Raharitsifa et al.[27] reported that foams density decrease with increase in whipping time up to certain point and thereafter FD increase may be due to excessive whipping (overbeating) and leads to a collapse of foam. Air incorporated during whipping is inversely proportional to foam density.[14] During foam mat drying of bael Bag et al.[7] reported that the foam density of stabilized foams from bael was increased after 2 min of whipping. From Fig.4.1 (b), it was observed that for the increase of methyl cellulose concentration in fruit pulp, FD decreased proportionally up to a certain value and the lowest FD (0.9615 g/cm3) was observed at 3% methyl cellulose. Similar type of result was also observed by Bag et al.[7] during foam mat drying of bael fruit.



(a)



(b)

Fig. 4.1 Effect of (a) whipping time (WT min) and (b) methyl cellulose (MC%) on foam density of passion fruit pulp.

4.3.2 Model fitting

The CCD data was analysed using multiple regression analysis as shown in Table 4.2 and the correlation between the independent variables of foam mat drying *viz*., whipping time (1-5 min), methyl cellulose (1-3%) and temperature (40-60°C) and dependent variables such as vitamin C, TPC and hygroscopicity, were developed. After the analysis, a second order polynomial relationship was developed between dependent and independent variable. Significance test of regression model, individual model coefficients and lack of fit were carried out as shown in Table 4.2. Results showed that the models developed for responses were highly significant ($p \leq 0.05$). In order to evaluate the model adequacy, correlation of determination (R^2) of vitamin C (0.90), TPC (0.86) and hygroscopicity (0.80) were evaluated. Analysis of variance showed that R^2 of the models was higher than 85 % and lack of fit was insignificant which showed interaction among the responses and predicting implied model is adequately accurate.

Table 4.2 Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Source	**Vitamin C**		**Total phenolic content**		**Hygroscopicity**	
	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***P*-value**
Model	5.79	0.0006	11.13	0.0004	3.29	0.0006
x_1 (Whipping time)	9.81	0.0106	5.04	0.0486	1.71	0.2198
x_2 (Methyl cellulose)	6.97	0.0247	7.77	0.0192	0.059	0.0138
x_3 (Temperature)	14.20	0.0037	66.33	< 0.0001	6.42	0.0297
x_1x_2	0.92	0.3595	1.37	0.2696	2.09	0.1792
x_1x_3	0.29	0.6010	1.21	0.2976	0.066	0.8029
x_2x_3	0.026	0.8754	0.11	0.7518	5.077E-003	0.9446
x_1^2	16.14	0.0025	9.87	0.0105	11.04	0.0077
x_2^2	1.75	0.2157	10.22	0.0095	2.80	0.1255
x_3^2	1.42	0.2608	0.11	0.7502	3.55	0.0891
Lack-of-fit	4.26	0.0689	0.54	0.7428	0.73	0.629
R^2	0.90		0.86		0.80	

4.3.2.1 Response surface analysis of vitamin C

Equation below showed the relationship between vitamin C and methyl cellulose concentration (%), whipping time (min) and temperature (˚C). The develop model was analysed in term regression of determination (R^2) and lack of fit. From Table 4.2, it was observed that the regression of determination (R^2) of vitamin C was significantly high (R^2=90) and lack of fit was insignificant which elucidated that model had efficacy to represent the relationship between vitamin C and methyl cellulose concentration (%), whipping time (min) and temperature (˚C).

Vitamin C (mg/100g)

$$= 33.92 - 1.44x_1 + 1.21 \times x_2 - 1.73 \times x_3 + 0.58 \times x_1x_2 - 0.32 \times x_1x_3 + 0.097 \times x_2x_3 - 1.80x^2{}_1 - 0.59 \times x_2{}^2 + 0.53 \times x_3{}^2$$

From Fig. 4.2a, it was observed that up to 2 % of methyl cellulose concentration resulted in increases in the vitamin C content and further increase in its concentration revealed a decrease pattern of vitamin C in foam mat dried passion fruit powder (Fig.4.2a). The increase in vitamin C content may be due to the hydrocolloid activity of methyl cellulose on vitamin C during drying. For the presence of methyl cellulose in the aqueous medium, water activity of the medium decrease which might reduce the extent of the hydration reaction [15] and increase the stability of Vitamin C.

For Fig. 4.2a, it was also observed that for increase of whipping time up to 3 min the vitamin C content of the powder increased and later it decreased drastically (3 to 5 min). The increase in vitamin C content may be due to the release of vitamin C from cell during mixing. However, further increase in whipping time showed decrease in vitamin C and may be attributed to the structural break down and oxidative degradation. Temperature evinced significant effect on the vitamin C content of sample (Fig.4.2b). As the temperature increased from 40°C to 60°C, the vitamin C content of foam mat dried powder decreases and it can be ascribed to heat liability of vitamin C[13]. Increase in whipping time (1-3 min) revealed that vitamin C increases but further increases from 3 to 5 min, decreases the vitamin C content (Fig. 4.2.b).

4.3.2.2 Response surface analysis of total phenolic content

The quadratic equation below showed the relationship between total phenolic content and whipping time (min), methyl cellulose (%) and temperature ($^{\circ}$C) in passion fruit powder. The correlation coefficient (R^2 0.86) and lack of fit (0.44) of developed model was elucidated that the develop model has efficacy to represent the relationship between and independent parameters.

Total phenolic content (mg GAE/100g)

$$= 242.18 + 9.51x_1 - 11.81\times x_2 - 34.51\times x_3 - 6.47\times x_1x_2 + 6.08\times x_1x_3 + 1.80\times x_2x_3 - 12.96\times x_1^2 - 13.18x_2^2 - 1.35\times x_3^2$$

Fig.4.2(c), revealed the steady increase in the total phenolic content up to 2 % of methyl cellulose and thereafter showed decrease in the TPC over the time. The increase in TPC may be due to the stabilizing effect of methyl cellulose, based on electrostatic interactions between the phenolic compounds and the dissociated carboxylic groups of the colloids.[17] Further, increase in methyl cellulose beyond 2 %, there was a drastic decrease in TPC in passion fruit powder as shown in Fig 4.2c. The TPC of foam mat dried powder was increased with increase in whipping time up to 3 min and thereafter the trend showed a decrease pattern (Fig 4.2c).The increase in TPC may be due to the release of TPC due to the cell lysis during whipping. However, further increase in whipping time showed decreased pattern of TPC, due to the structural break down and oxidative degradation of TPC. The foam mat drying temperature also affected the TPC in foam mat dried powder. The TPC of the foam mat dried powder decreased drastically with the increase of drying temperature from 40-60˚C (Fig 4.2d).The decrease in TPC for increase in temperature was obvious because of heat sensitive and prone to oxidation which cause structural distraction thereby decrease the TPC in powder.[32] As the whipping time increases, TPC also increase up to 3 min (Fig 4.2.d). Later, the TPC content slightly decreased with the increase in whipping time (3-5min).

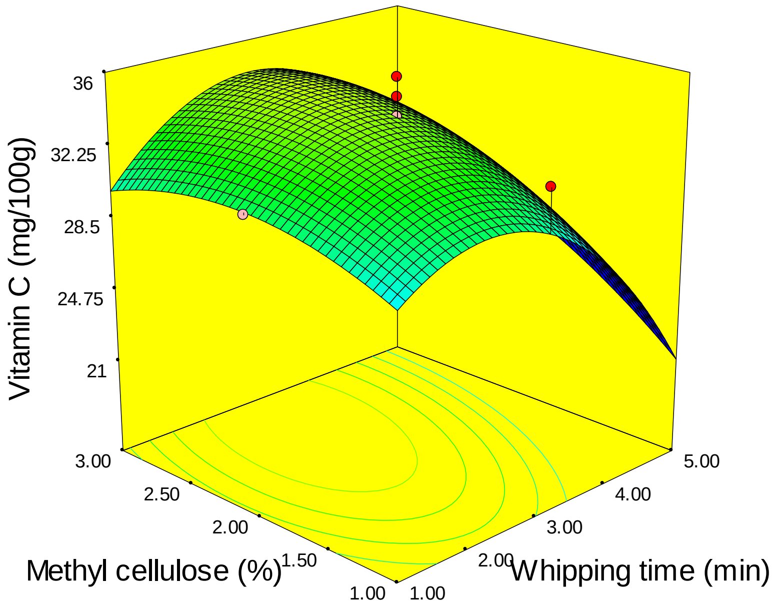
4.3.2.3 Response surface analysis of hygroscopicity

The mathematical relationship between whipping time (min), methyl cellulose (%) and temperature (˚C) with hygroscopicity (%) of foam mat powder were represented by equation below. The correlation coefficient (R^2) of the developed model was recorded as 0.80.

Hygroscopicity (%)

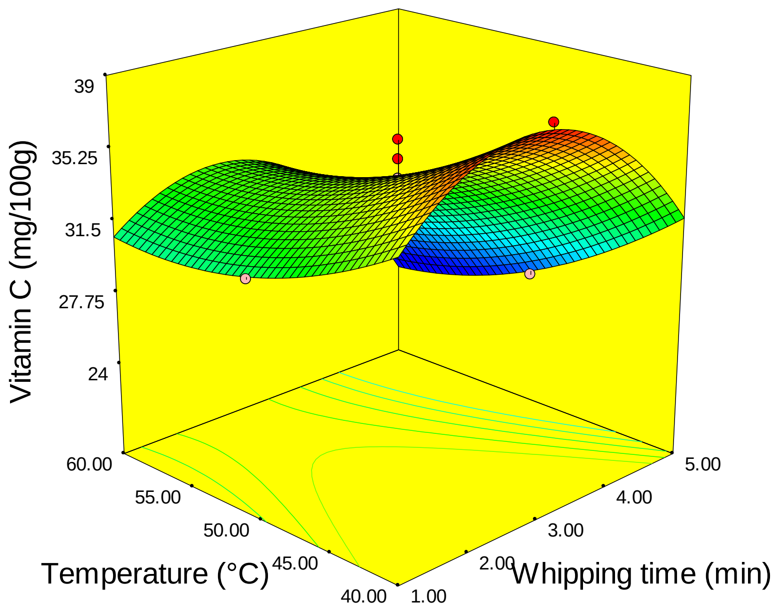
$$= 23.86 - 1.95 \times x_1 + 0.36 \times x_2 - 3.78 \times x_3 + 2.82 \times x_1 x_2 - 0.50 x_1 x_3 - 0.14 \times x_2 x_3 + 4.83 \times x_1^2 - 2.43 x_2^2 - 2.74 \times x_3^2$$

The Fig. (4.2e-f) showed the 3D-graphical relationship between whipping time (min), methyl cellulose (%) and temperature (˚C) with hygroscopicity (%). From the Fig 4.2e, it was illustrated that with the increase of the concentration of methyl cellulose up to a certain level (2%) there was simultaneous increase in hygroscopicity (41.51%) and later decreased marginally. The increase in hygrocopicity of powder can be accredited to the increase in available hydroxyl groups in the amorphous regions of the substrate as well as the surface crystalline regions and therefore, it can easily absorb the moisture from the atmosphere. Later the decrease in hygroscopicity may be due to the presence of excessive amount of methyl cellulose which slowly decreases the affinity to adsorb water. Moreover, methyl cellulose is a material with low hygroscopicity, therefore higher amount methyl cellulose may decrease the hygroscopicity.[35] Fig 4.2e illustrated the effect of whipping time on hygroscopicity of powder. With increase of whipping time up to 3 min the hygroscopicity (22.33%) decreased drastically and after that a reverse trend was observed. Fig 4.2f, illustrated that as the temperature increased (40-60°C), hygroscopicity also increased. Results revealed that higher drying temperature has the credibility to lower the moisture content and thereby increase the hygroscopicity. This is related to the water gradient between the product and the surrounding air and evinces moist powder



Vitamin C (mg/100g)
36
32.25
28.5
24.75
21
3.00
2.50
2.00
1.50
1.00
Methyl cellulose (%)
1.00
2.00
3.00
4.00
5.00
Whipping time (min)

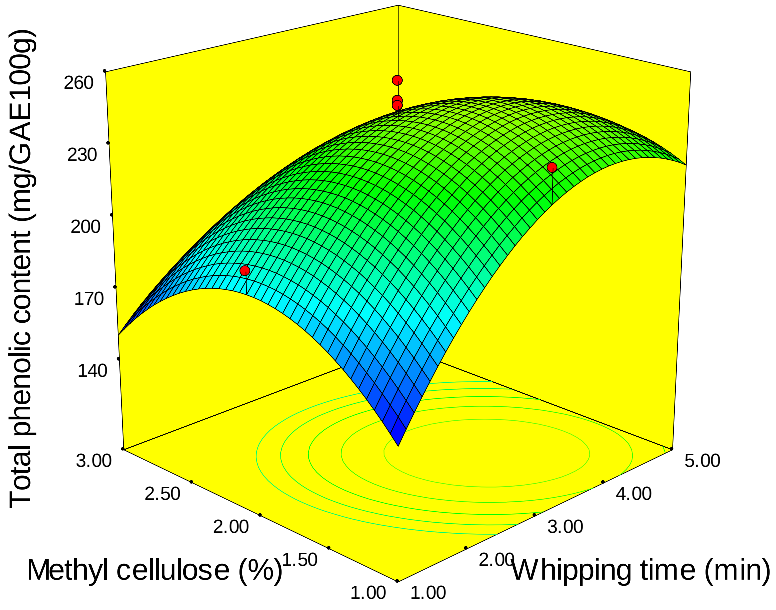
(a)



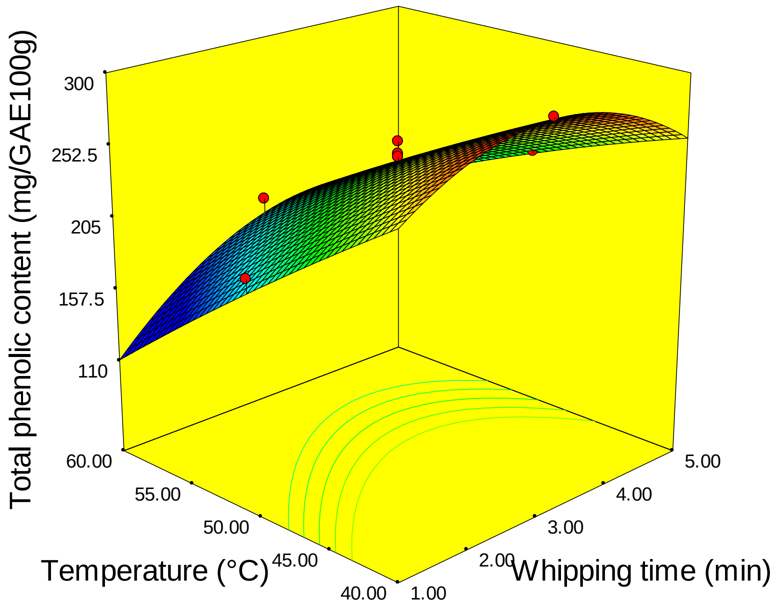
Vitamin C (mg/100g)
39
35.25
31.5
27.75
24
60.00
55.00
50.00
45.00
40.00
Temperature (°C)
1.00
2.00
3.00
4.00
5.00
Whipping time (min)

(b)

Contd.

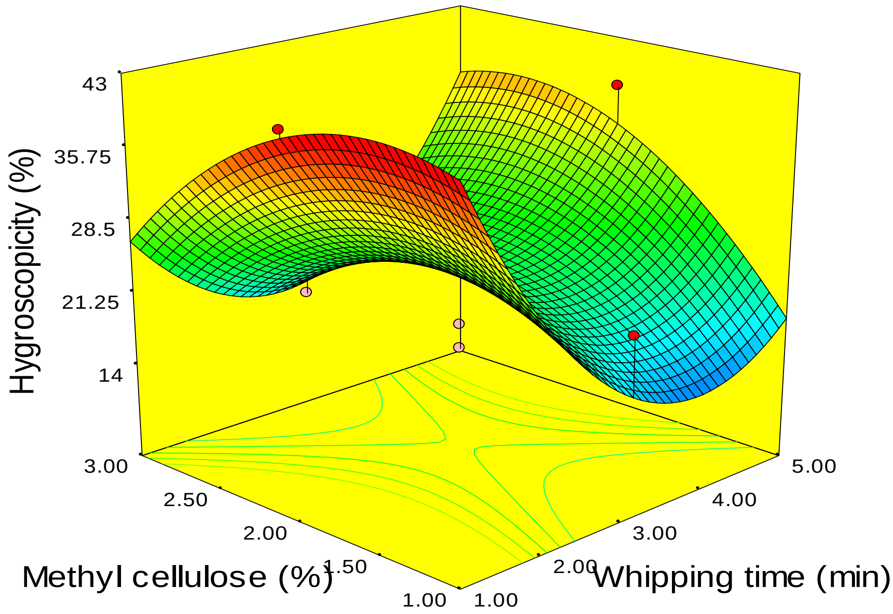

Total phenolic content (mg/GAE100g)
260
230
200
170
140
3.00
2.50
2.00
1.50
1.00
Methyl cellulose (%)
1.00
2.00
3.00
4.00
5.00
Whipping time (min)

(c)

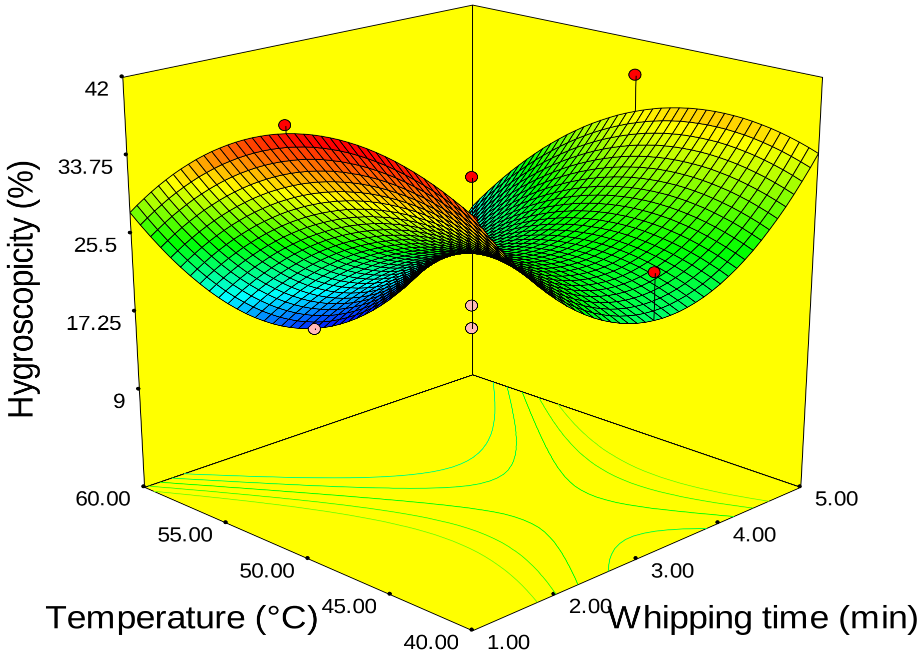

Total phenolic content (mg/GAE100g)
300
252.5
205
157.5
110
60.00
55.00
50.00
45.00
40.00
Temperature (°C)
1.00
2.00
3.00
4.00
5.00
Whipping time (min)

(d)

Contd.



(e)



(f)

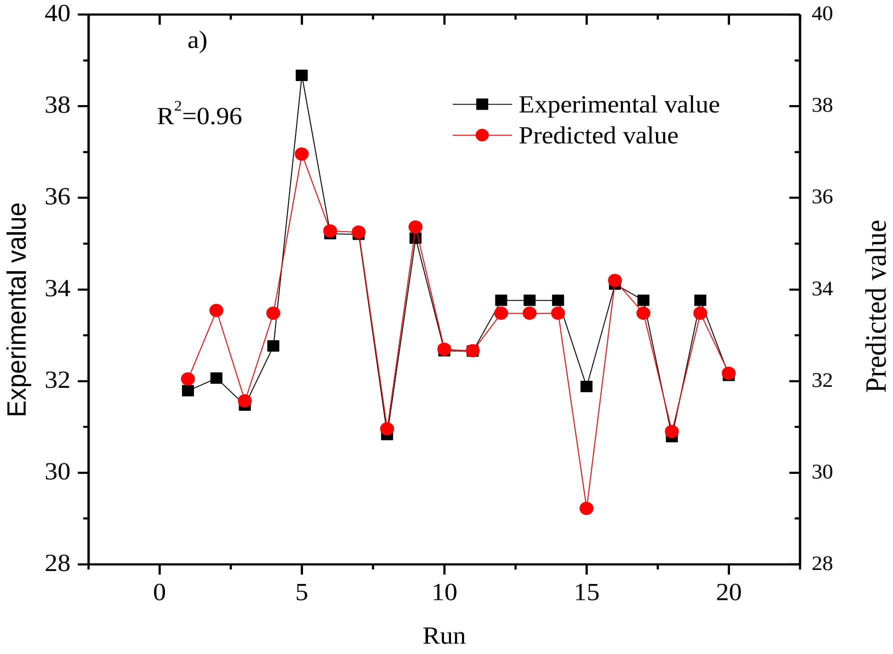
Fig.4.2 Response surface 3D graph on;(a-b) effect of methyl cellulose, whipping time and temperature on vitamin c content, (c-d) effect of methyl cellulose, whipping time and temperature on total phenolic content, and (e-f) effect of methyl cellulose, whipping time and temperature on hygroscopicity of powder

4.3.3 Optimization and validation of foam mat drying parameter

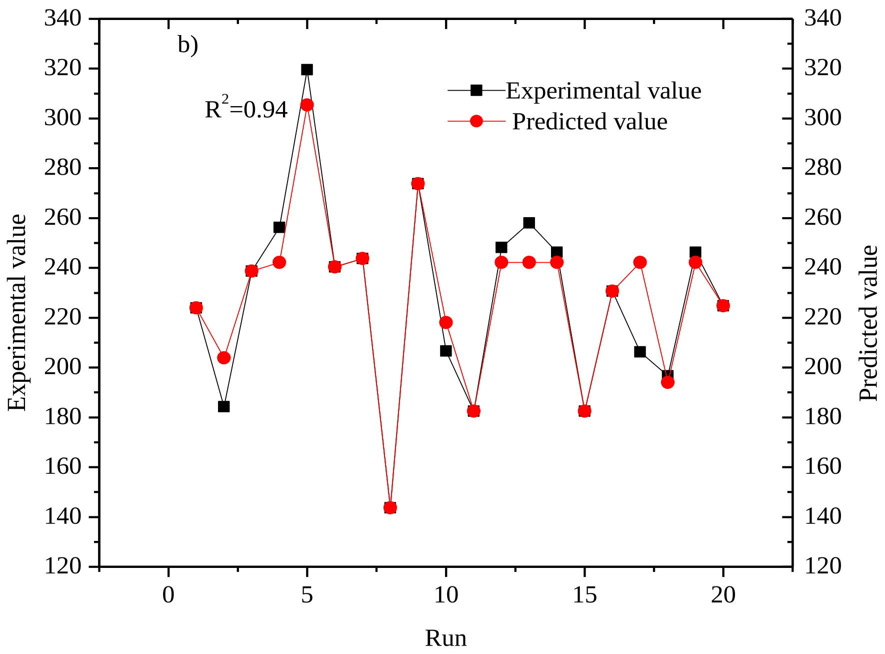
Since the foam mat concentrate is an intermediate product of foam mat drying process, therefore in the present study the effect of whipping time and methyl cellulose on foam density was not considered in the optimization process. However, initial trial was conducted to identify the effect of whipping time and methyl cellulose on foam density. In the optimization step, after response surface analysis the foam mat drying process was optimized on the basis of desirability. The optimum condition was selected on the basis of the highest TPC, vitamin C and lowest hygroscopysity. The optimum foam mat drying conditions were, whipping time 2.78 min, methyl cellulose 2.58 %, and temperature 44.05˚C. At the optimum condition the predicted value of response was vitamin C 34.67mg/100g, TPC 258.12 mg/100g and hygroscopicity 21.12%. After optimization, the process parameters were validated. During validation, the experiment was conducted in optimized condition and observed that experimental value of total phenolic content (255.87 mg GAE /100g), vitamin C (33.28mg/100g) and hygroscopicity (21.98 %) which was not differed significantly with the predicted data. The result has the evidence to support that the developed model can efficiently optimize the process.

4.3.4 Artificial-neural-network modeling

Artificial neural networks (ANN), is basically computational models based on biological neural processes which predict models. ANN is a non-linear mathematical tool comprises of inter-connected adaptive processing elements ‘‘neurons” that are actually grouped in input, hidden and output layers, which eventually send messages to other.[18,8] The data generated from experimental design planned through CCD was used for ANN model. ANN model was developed using multi-layer perceptron with logistic sigmoidal function. The CCD data was categories as training (14), testing (3) and validation (3), to measure the performance of the develop ANN. Coefficient of determination (R^2) was used to determine the efficacy of developed ANN model. For TPC, the best ANN model was obtained with one hidden layer and ten hidden neurons with (R^2=0.96) for vitamin c, TPC (R^2=0.94) and hygroscopicity (R^2=0.89). A comparison between RSM and ANN model is presented in Fig 4.3. The co-efficient of determination (R^2) for vitamin C, total phenolic content and hygoscopicity values were higher than RSM which inferred that ANN has the higher ability to predict the experimental outcome than the RSM models.

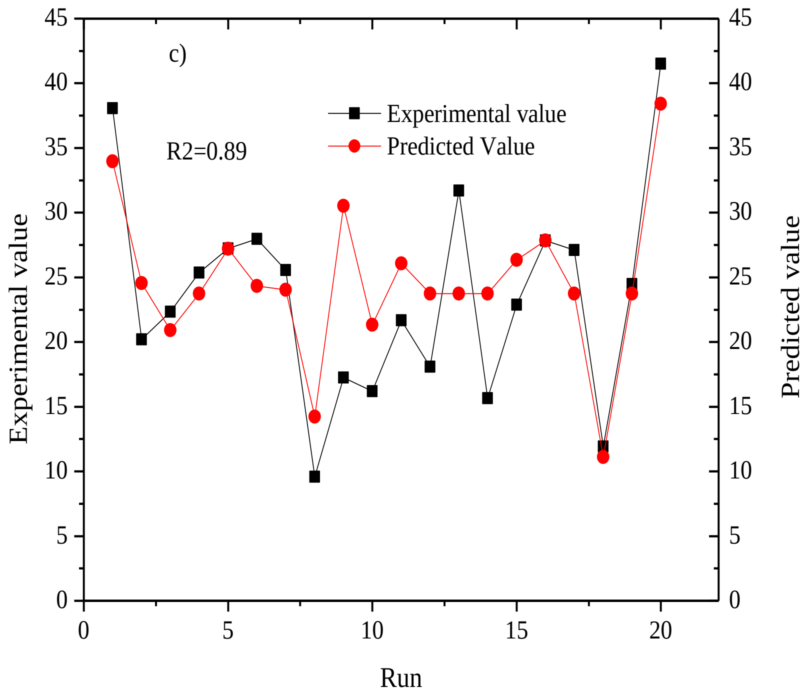


(a)



(b)

Contd.



(c)

Fig. 4.3 Comparison between experimental and predicted value obtained from RSM and ANN modelling (a) Vitamin C, (b) TPC and (c) Hygroscopicity

4.3.5 Physical and chemical compositions of powder

The physical and chemical properties of foam mat dried powder are shown in Table 4.3. The *L**, *a**, and *b** values of the foam mat dried powder differed significantly with respect to fruit pulp and the powder had more *L**, *a**, *b** values over the passion fruit pulp (Table 3.10). The DPPH scavenging activity and vitamin C content decreased in powder (60.53±0.21% and 35.19± 0.20 mg/100g) (Table 4.3) than the raw passion fruit pulp (70.53±0.03 %) and (60.53±0.21 mg/100g) (Table 3.10), whereas the TPC content of passion fruit powder (210.11±0.23mg GAE/100g) increased over raw fruit pulp (206.29 ±0.10 mg GAE/100g)(Table 4.3)

Table 4.3 Physical and chemical compositions of powder

Parameters	**Foam mat dried powder**
Moisture content (%)	6.52± 0.02
*L**	38.13 ±1.17
*a**	5.97 ±0.39
*b**	22.65 ± 0.39
Chroma(a*2+b*2)1/2	23.42± 0.02
Hue tan^{-1}(b*/a*)	75.21± 0.02
Vitamin C(mg/100g)	35.19± 0.20
Total phenolic content(mg GAE/100g)	210.11± 0.23
DPPH scavenging activity (%)	60.53±0.21

Values expressed as mean ± SD (n=3)

4.3.6 Phytochemical analysis

4.3.6.1 FT-IR analysis

FT-IR analysis of foam mat dried passion fruit powder was depicted (Fig.4.4) spectral band from 664 to 3226 cm^{-1}. A sharp peak showed in 3226 cm^{-1}. Band of C=O stretching was mainly due to the presence of carboxyl (-C=O) group of phenolic compounds. Some weak peaks were found in 644, 857.81 and 2297.48 cm^{-1} bands. A stretching characteristic peak at approximately 3226 cm^{-1} and 2937 cm^{-1} were due to the O–H stretching band. The C=O carbonyl group characteristic peak was observed at 1650 cm^{-1}vibration.[31]

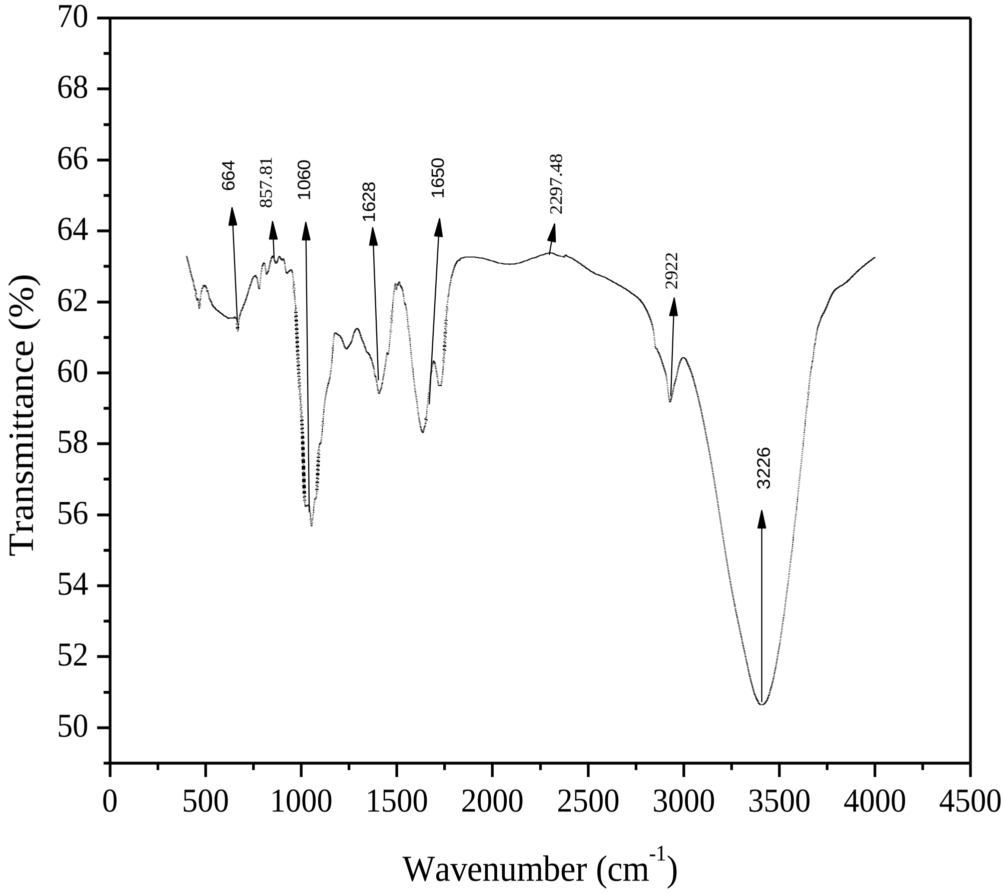


Fig. 4.4 FT-IR spectra of passion fruit powder

4.3.6.2 RP-HPLC

The vitamins and phenolic acids of foam mat powder were identified and quantified by RP-HPLC (Table 4.4). The β-carotene, (±)-α-tocopherol and D-α-tocotrienol were detected at the retention times of 3, 3.5 and 3.8 min, respectively. Cavalcante et al. (2011) stated that compounds like carotene and vitamin accumulation in passion fruit are contributed by various internal as well as external factors *viz.*, maturity stage, cultivation system etc. The vitamin content in the foam mat dried powder were illustrated in Table 4.4. Six phenolic acids were prominently observed even after foam mat drying of pulp. The foam mat dried powder was predominant with chlorogenic (790.33mg/100g), trans-ferulic acid (342mg/100g) and vanillic acid (893.87mg/100g).

Table 4.4 Quantification of vitamins and phenolic acids of passion fruit pulp and foam mat powder

Phytochemicals (mg/100g)	**Retention time (min)**	**Foam mat dried powder**
Vitamin		
1. β-carotene	3.00	13.26
2. (±)-α-tocopherol	3.50	15.20
3. D-α-tocotrienol	3.80	11.98
Phenolic Acid		
1. Caffeic acid	14.66	NA
2. (±) Catechin hydrate	13.00	NA
3. Chlorogenic acid	13.80	790.33
4. *p*- Coumeric acid	17.25	266.25
5. Transferulic acid	14.36	342.00
6. 4-Hydroxybenzoic acid	18.03	NA
7. Syringic acid	15.06	639.60
8. Sinapic acid	17.80	523.33
9. Vanillic acid	14.96	893.87

4.4 Conclusion

The foam mat drying of passion fruit pulp was successfully carried our using CCD followed by response surface methodology. The optimum process conditions of foam mat drying of passion fruit pulp were WT 2.58 min, MC 2.58 %, and temperature 44.05˚C. ANN model was successfully applied to predict the experimental outcome. In the RP-HPLC, three vitamins *viz.*, β-carotene, (±)-α-tocopherol and D-α-tocotrienol and five phenolic acids *viz.*, chlorogenic acid, trans-ferulic acid, syringic acid, sinapic acid and vanillic acid were detected and quantified. RP-HPLC of vitamins and phenolic compounds revealed that even after foam mat drying, compounds were present in the powder. The present chapter showed that foam mat dried powder from purple passion fruit can be used as an important ingredient in developing functional foods and has potential applicability in food industry. In addition, the foam mat drying is a low cost processing technique compared to spray and freeze drying and can easily be carried out in many in post harvested crops to study the various food properties.

References

[1] Aguilar-Garcia, C., Gavino, G., Baragano-Mosqueda, M., Hevia, P. and Gavino, V, C. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chemistry*, 102:1228-1232, 2007.

[2] Azizpour, M., Mohebbi, M., Khodaparast, M, H, H. and Varidi, M.Foam-mat drying of shrimp: Characterization and drying kinetics of foam. *Agricultural Engineering International*: *CIGR Journal*, 15:159-165,2013.

[3] Akintoye, O.A.A. and Oguntunde.O. Preliminary investigation on the effect of foam stabilizers on the physical characteristics and reconstitution properties of foam-mat dried soymilk. *Drying Technology*,*9* (1): 245-262,1991.

[4] Ambekar, S,A., Gokhale, S,V. and Lele, S, S. Process optimization for foam mat-tray drying of *passiflora edulis* Flavicarpa pulp and characterization of the dried powder. *International Journal of Food Engineering*, 9 (4): 433-443, 2013.

[5] Auisakchaiyoung, T. and Rojanakorn, T. Effect of foam-mat drying conditions on quality of dried Gac fruit (*Momordica cochinchinensis*) aril. *International Food Research Journal*, 22(5):2025-2031,2015.

[6] Brygidyr, A, M., Rzepecka, M, A. and McConnell, M, B. Characterization and drying of tomato paste foam by hot air and microwave energy. *Canadian Institute Food Science and Technology Journal*, 10:313-319, 1977.

[7] Bag, S, K., Srivastav, P, P. and Mishra, H, N. Optimization of process parameters for foaming of bael (*Aegle marmelos* L.) Fruit Pulp. *Food* and *Bioprocess Technology*, 4:1450-1458, 2011.

[8] Basheer, I, A. and Hajmeer, M. Artificial neural networks: fundamentals, computing, design, and application. *Journal of Microbiology and Methods*, 43:3-31, 2000.

[9] Brand-Williams, W., Cuvelier, M, E. and Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology*, 28:25-30, 1995.

[10] Basseto, E., Jacomino, A, P., Pinheiro, A, L. and Kluge, R, A. Delay of ripening of “pedro sato” guava with 1-methylcyclopropene. *Postharvest Biology and Technology*, 35:303-308, 2005.

[11] Chopda, C, A. and Barrett, D, M. Optimization of guava juice and powder production. *Journal of Food Processing and Preservation*, 25:411-430, 2001.

[12] Cavalcante, L. F., Dias, T. J., Nascimento, R. and Freire, J. L. O. Chlorophyll and carotenoids in yellow passion fruit plants irrigated with saline water on soil with bovine biofertilizer. *Revista Brasileira de Fruticultura*, E, 699-705, 2011.

.

[13] Egerg, K., Arson, A. and Etok, F. Semi-Automated Method for the Fluorometric Determination of Total Vitamin C in Food Product. *Journal- Association Official Analytical Chemists*, 60 (1):126-131,1977.

[14] Falade, K, O., Adeyanju, K,I. and Uzo-peters, P, I. Foam mat drying of cowpea (*Vignaunguiculata*) using glyceryl monostearate and egg albumin as foaming agents. *European Food Research and Technology*, 217:486-491, 2003.

[15] Gögüs, F., Düzdemir, C. and Eren, S. Effects of some hydrocolloids and water activity on nonenzymic browning of concentrated orange pulp. *Food/Nahrung*, 44(6):438-442, 2000.

[16] Grover, J. A. In M. Glicksman (Ed.), (pp. 121). *Food hydrocolloids*, 3. Florida: CRC Press, 1984.

[17] Hubbermann, E, M., Heins, A., Stöckmann, H. and Schwarz, K. Influence of acids, salt, sugars and hydrocolloids on the colour stability of anthocyanin rich black currant and elderberry concentrates. *European Food Research and Technology*, 223:83-90,2006.

[18] Hagan, M., Howard,T.,Demuth,B. and Beale, M. Neural Network Design, first ed., Boston, Thomson Learning,2002.

[19] Hardy, Z. and Jideani, V.A. Foam-mat drying technology: A review. Critical reviews in food science and nutrition, 57(12):2560-2572,2017.

[20] Jaya, S. and Das, H. Effect of maltodextrin, glycerol monostearate and tricalcium phosphate on vacuum dried mango powder properties. *Journal of Food Engineering*, 63(2): 125-134, 2004.

[21] Kadam, D, M. and Balasubramanuan, S. Foam-mat drying of tomato pulp. *Journal of Food Process and Preservation*, 35:488-495, 2011.

[22] Kandasamy,P., Varadharaju,N., Kalemullah,S. and Moitra, R. Production of papaya powder under foam-mat drying using methyl cellulose as foaming agent. *Asian Journal of Food and Agro-Industry*, 5(05):374-387,2012.

[23] Karim, A, A. and Wai, C, C. Characteristics of foam prepared from starfruit (***Averrhoa carambola L.***) puree by using methyl cellulose. *Food Hydrocolloids*, 13(3):203-210,1999.

[24] Kadam, D, M., Wilson, R, A. and Kaur, S.Determination of biochemical properties of foam-mat dried mango powder. *International Journal of Food Science and Technology*, 45:1626-1632,2010.

[25] Liu, R,H. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition*,134: 3479S-85S,2004.

[26] Morgan, A., Graham, R., Ginnette, L. and Williams, G. Recent developments in foam-mat drying. *Food Technology*, 15:37-39,1961.

[27] Raharitsifa, N., Genovese, D, B. and Ratti, C. Characterization of apple pulp foams for foam-mat drying prepared with egg white protein and methylcellulose. *International Journal of Food Science*, 71:142-151,2006.

[28] Rajkumar, P., Kailappan, R., Viswanathan, R., Raghavan, G,S,V. and Ratti, C. Foam mat drying of alphonso mango pulp. *Drying Technology*, 25:357-365, 2007.

[29] Sadasivam, S. and Theymoli, B. In: Practical Manual in Biochemistry, Tamil Nadu Agricultural University, Coimbatore p.14,1987.

[30] Slinkard, S. and Singleton, V, L. Total phenol analysis: automation and comparison with manual methods. *The American Journal of Enology and Viticulture,* 28:49-55,1977.

[31] Santhiya, D., Subramanian, S. and Natarajan, K, A, J. Surface chemical studies on sphalerite and galena using extracellular polysaccharides isolated from *Bacillus polymyxa*. *Journal of Colloid and Interface Science,* 256:237-248, 2002.

[32] Sharma, K., Ko, E, Y., Assefa, A, D., Ha, S., Nile, S, H., Lee, E, T. and Park, S, W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food Drug and Analysis*, 23:243-252, 2015.

[33] Stainsby. G., Stainsby, J.R. and Mitchell, D.A. Ledward (Eds.), Functional properties of food macromolecules, Elsevier Applied Science, London (1989), p. 315

[34] Stanley, D, W., Goff, H, D. and Smith, S. K. Texture-structure relationships in foamed dairy emulsions. *Food Research International*, 29(1):1-13, 1996.

[35] Tonon, R, V., Brabet, C. and Hubinger, M, D. Influence of process conditions on the physicochemical properties of açai (*Euterpe oleraceae* Mart.) powder produced by spray drying. *Journal of Food Engineering*, 88(3):411-418, 2008.

[36] Thuwapanichayanan, R., Prachayawarakorn, S. and Soponronnarit, S. Drying characteristics and quality of banana foam mat. *Journal of Food Engineering*, 86:573-583,2008.

[37] Yildiz, F. Glycobiology of foods. In: Advances in Food Biochemistry. pp 27–45. CRC Press, Taylor and Francis Group, London, New York, 2010.

The Chapter 5 has been discussed under three sub-heads which are as follows;

A) Effect of extrusion parameters on the physicochemical and phytochemical properties of passion fruit powder incorporated red rice product

5.1 Introduction

Rice is the second largest produced cereal worldwide, after wheat. It is the main source for carbohydrate and a minor source of protein, vitamins and minerals. However, epidemiological studies shown that whole rice grains contain high amount of fiber and phytochemicals *viz*., tocopherols, tocotrienols, oryzanols, vitamin B complex and other phenolic compounds.

In the recent decade a considerable approach has been taken on conversion of fruit into useful products with high shelf life. The reason behind this, fruits are most perishable in nature and increasing disposal thereby loss of valuable substances e.g. dietary fiber, lycopene, antioxidants. Few studies on fruit based food product with long shelf life are *viz.,* extruded orange pomace [16] apple spray dried powder, banana spray dried powder, strawberry spray dried powder,[26] bilberry based food.[15] Most of the attempted was made to develop a snack/breakfast cereal. The passion fruit from North-East India has not been utilized to develop a food product as snack/breakfast cereal.

Extrusion cooking is most widely used techniques for the production of snack, breakfast cereals and texturized vegetable protein.[11,6,21] Extrusion is a continuous cooking and shaping process used in the food induction. Extrusion technology merge various factors *viz.,* temperature, moisture, shearing, and mixing, to produce fruit based snacks/breakfast cereal foods. Therefore, to get a desire quality extruded product, indeed there is need of process optimization for extrusion cooking. Response surface methodology (RSM) is a widely used tool for optimization of design process and efficient mathematical and statistical techniques for analysis of empirical models which can describe the effect of independent variables and their interactions on responses.[29]

Samyor et al. (2017). Effect of extrusion cooking on the physicochemical and phytochemical properties of passion fruit powder incorporated red rice extrudates. Food Chemistry **(**Under Review)

On the other hand, the effect of extrusion cooking on physicochemical and phytochemicals properties in terms total phenolic content, phenolic acid, anthocyanin and antioxidant activity of passion fruit powder incorporated red rice based extrudate is not well established. Thus, there is a need of extensive research on physicochemical and phytochemical properties of extrudate from passion fruit powder incorporated red rice.

Therefore, in the present study the extrusion processing of passion fruit powder incorporated red rice was optimized using central composite design. The effect of extrusion cooking on the phytochemical properties such as total phenolic content, phenolic acid, anthocyanin and antioxidant activity was also investigated. In addition to that the thermal, crystallinity and morphological properties of extrudate were characterized.

5.2 Material and Methods

5. 2.1 Raw material

Red rice was collected from the Manigong sub division of West Siang district, Arunachal Pradesh, India. The rice was milled into flour and kept in an air tight container for further used. Ripen passion fruit (*Passiflora edulis* Sims) was also collected in the month of July-September from the Singchung circle of West Kameng district, Arunachal Pradesh. Passion fruit pulp was obtained by squeezing the fruit and the pulp was made into powder using foam mat drying (mentioned in the chapter 4.2.3).

5.2.2 Chemicals

The phenolic acids namely ferulic, sinapic, syringic, hydroxybenzoic, *p*-coumeric, vanillic, caffeic, catechin and chlorogenic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The amberlite XAD7N resin was also purchased from Sigma-Aldrich Chemical Co. Vitamin standards namely β- carotene, (±)-α-tocopherol and D- α- tocotrienol and anthocyanin namely cynanidine-3-glucoside (C-3-G) and peonidin-3-D-glucoside (P-3-G)were purchase from Sigma-Aldrich.

5.2.3 Extrusion experiments

Extrusion experiments for the production of extruded product from red rice and passion fruit powder were carried by twin extruder with co- rotating screw (Model, FUE-1F, Flytech Engineering, Chennai, India) according to central composite design (Design-Expert software version 7., U.S.A) which gave 30 experimental runs. Red rice flour (100g) was kept for the base material. The independent variables were temperature (80-150˚), screw speed (200-400rpm), feed moisture content (20-30 %) and amount passion fruit powder (0-15%) (Table 5.1) and dependent variables were expansion ratio (ER) (%), water absorption index (WAI), total phenolic acid (TPC) and *2*, 2´-diphenyl-1-picrylhydrazyl (DPPH). After the blending the samples were kept in a LDPE zipper pouch to attain equilibrium. The feed rate was maintained constant at 17 kg/h using a volumetric gravity feeder.

The experimental data were analyzed and fitted to a second order polynomial equation as follows

$$y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x^2{}_{ii} + \sum_{i=1}^{3} \sum_{j-i+1}^{4} \beta_{ij} x_i x_j$$

y is the predicted response, coefficient of the polynomial equation were β_0 (constant), β_i (linear effects), β_{ii} (quadratic effects) and β_{ij} (interaction effects). x_i and x_j are the coded levels of independent variables i and j in the equation given.

.

Table 5.1 Independent variable values of the extrusion process and their corresponding level

Variables	Coded value	Level				
		-α	-1	0	+1	+α
Temperature (˚C)	x_1	80	97.5	115	132.5	150
Screw speed (rpm)	x_2	200	250	300	350	400
Feed moisture content (%)	x_3	20	22.5	25	27.5	30
Passion fruit powder (%)	x_4	0	3.75	7.5	11.25	15

5.2.3.1 Expansion ratio

Expansion ratio of the extrudate was determined according to Ding et al.[10] Vernier calliper was used to measure the cross-sectional diameter of the extrudate. The expansion ratio was calculated as the cross-sectional diameter of the extrudate divided by the cross-sectional diameter of the die opening and the values were obtained from 10 random samples for each extrusion condition.

$$\text{Expansion ratio}=\frac{\text{Extrudate diameter}}{\text{Die diameter}}$$

5.2.3.2 Water absorption index

Water absorption index (WAI) was determined using the method described by Anderson et al.[1] Briefly, 2 g of the grounded extrudates was dispersed in 25 mL distilled water in a centrifuge tube and sample was kept in water bath for 30 min at 30˚C. Later, centrifuge tube was kept in a centrifuge at 3000 g for 10 min. The supernatant was poured carefully into a dish. WAI was calculated as the ratio of mass of the precipitate to the mass of the original sample dry weight.

$$\text{Water absorption index}=\frac{\text{Mass of the precipitate}}{\text{Mass of the original sample }\left(\text{dry weight}\right)}$$

5.2.3.3 Total phenolic content

Determination of total phenolic content of grounded extrudate was carried out by Folin–Ciocalteu assay [31] (method previously described in section 3.2.4.2).

5.2.3.4 DPPH radical scavenging activity

DPPH radical scavenging ability of the grounded extrudates was measured according to the method of Brand-William et al. [7] (method described in section 3.2.4.4).

5.2.4 Fourier transform infrared spectroscopy (FT-IR) analysis

Fourier transform infrared spectroscopy (FT-IR, Nicolet impact 410, Thermo scientific, United Kingdom) analysis was carried out to detect functional group present in extruded products (method described previously in section 3.2.4.6).

5.2.5 Differential scanning calorimeter (DSC) analysis

Thermal analysis of red rice (C) and extruded red rice with passion fruit powder (O) were carried out by differential scanning calorimeter, (DSC-60 SHIMADZU, instrument, TA-60WS, Japan) (method described previously in section 3.2.3.10).

5.2.6 X-Ray diffraction (XRD)

The method used was described in 3.2.9.

5.2.7 Morphological structure analysis by scanning electron microscopy (SEM)

Extruded samples were investigated for morphological structure (JEOL JSM-6390LV, SEM, Oxford) after undergone extrusion processing. Samples were placed carefully in a metal stud with double-sided tape, using a sputter gold coater. Morphological structure of extrudates graphs were observed at a magnification of 100X and 25000 X with accelerating voltage of 10 kV.

5.2.8 Phytochemical profiling

The phytochemical profiling of red rice (C) and red rice incorporated with passion fruit extrudate (O) were carried out in terms of β- carotene (±)-α-tocopherol and D-α-tocotrienol, cynanidin-3-glucoside (C-3-G) and peonidin-3-d- glucoside (P-3-G) content using RP-HPLC (Water Corporation, USA) with UV Detector.

5.2.8.1 Determination of β- carotene (±)-α-tocopherol and D-α-tocotrienol

The β-carotene, (±)-α-tocopherol and D-α-tocotrienol of extrudates were determined according to the modified method of Aguilar-Garcia et al. [2] (method was described previously in the section 3.6.3.6)

5.2.8.2 Determination of cynanidin-3-glucoside (C-3-G) and peonidin-3-d- glucoside (P-3-G)

5.2.8.2.1 Purification of sample

Samples (3g) were weighed and extracted with 70% ethanol. Purification of anthocyanin compound was done using activated amberlite XAD7N resin as described by Fu et al.[12] with slight modification. The resins were kept soaked in 95% ethanol for 24 h and thoroughly washed by deionized water. Then the resins were treated by 1 mol/L HCl and Na OH solutions to remove any monomers trapped inside. Activated amberlite XAD7N resin (0.5 g) was added to a 250 mL conical flask with 40 mL of extract, shaken thoroughly at 45 rpm at 25˚C for 6 h. After adsorption, the anthocyanin was desorbed in ethanol (95%) solution for 12hr at 150 rpm. The extracts were filtered and stored in refrigerator at 4˚C for 2 days to initiate precipitation of large molecules and centrifuged at 12000 g (5˚C) for 20 min. The upper layer was concentrated and filtered through 0.45 µL syringe filter before being injected to HPLC. The HPLC pumps (LC-10AT, Shimadzu) and column were connected with a dual wavelength UV/VIS detector (SPD-10A, Shimadzu) for analysis. HPLC analysis was carried out at 25˚C with C18 column (4.6 mm x 250mm) at 530nm. The solvents mixture used were water, methanol and formic acid (75:20:5 v/v) as a mobile phase with isocratic elution at 0.5 mL/min flow rate.

5.2.9 Statistical analysis

Statistical analysis was conducted using a Design-Expert version 7.00 (Stat-Ease Inc., Minneapolis, USA). Statistical significance of the dependent and independent terms was analyzed by analysis of variance (ANOVA) for each response.

5.3 Results and discussion

5.3.1 Model fitting

The analysis of variance (ANOVA) of the experimental data were presented in Table 5.2. The Table shows the F and p values of linear, quadratic and interaction term. It was observed that the independent parameters *viz.*, temperature, screw speed, feed moisture content and amount of passion fruit powder had significant ($P \leq 0.05$) effect on dependent parameters. The regression coefficient value for expansion ratio (%) (R^2=0.90), water absorption index (%) (R^2=0.88) total phenolic content (mg GAE/100g) (R^2=0.84) and DPPH scavenging activity (%) (R^2=0.87) were good which imply that the second order quadratic model is most suitable and the developed model can efficiently depict the relationship between dependent and independent parameters.

Table 5.2 Analysis of variance (ANOVA) for the fitted quadratic polynomial model

Source	**ER**		**WAI**		**TPC**		**DPPH**	
	F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
Model	10.79	< 0.0001	8.00	0.0001	5.99	0.0007	7.78	0.0002
x_1	115.79	< 0.0001	5.55	0.0325	8.19	0.0119	71.95	< 0.0001
x_2	4.64	0.0478	40.14	< 0.0001	4.74	0.0459	5.01	0.0407
x_3	0.44	0.5172	42.59	< 0.0001	2.90	0.1092	2.05	0.1732
x_4	0.68	0.4237	0.047	0.8305	5.56	0.0323	7.69	0.0142
x_1x_2	1.75	0.2054	0.36	0.5571	0.31	0.5835	4.48	0.0515
x_1x_3	0.016	0.9010	0.79	0.3893	0.51	0.4880	2.12	0.1658
x_1x_4	1.40	0.2545	0.18	0.6746	1.46	0.2454	0.033	0.8588
x_2x_3	0.30	0.5892	0.93	0.3504	1.25	0.2816	0.58	0.4585
x_2x_4	0.45	0.5121	0.31	0.5885	13.26	0.0024	0.43	0.5198
x_3x_4	3.28	0.0902	0.57	0.4627	4.18	0.0588	9.581E-003	0.9233
x_1^2	6.70	0.0206	0.12	0.7302	17.40	0.0008	12.31	0.0032
x_2^2	0.74	0.4022	0.43	0.5197	20.08	0.0004	1.24	0.2996
x_3^2	2.00	0.1781	11.37	0.0042	7.26	0.0167	4.53	0.0425
x_4^2	6.09	0.0261	3.77	0.0712	14.60	0.0017	0.37	0.2507
Lack of fit	2.01	0.2283	01.39	0.3758	1.99	0.2318	3.07	0.1136
R^2	0.90		0.88		0.84		0.87	
Adjusted R^2	0.82		0.77		0.70		0.76	

5.3.1.1 Response surface analysis of expansion ratio

The expansion ratio (ER) of the extruded product was significantly affected by the independent parameters of extrusion cooking. Table 5.2, illustrated that the regression coefficient (R^2) value (R^2=0.90) was quite high which implies that the developed equation can efficiently represent the relationship of expansion ratio and independent parameters. The Fig 5.1(a), depicted the relationship between screw speed (rpm), temperature (˚C) and moisture content (%) on the expansion ratio of extrudates (%).

$$ER = 9.42 + 2.34x_1 + 0.47x_2 - 0.14x_3 - 0.18x_4 + 0.35x_1x_2 + 0.034x_1x_3 - 0.33x_1x_4$$
$$-0.15x_2x_3 - 0.19x_2x_3 - 0.51x_3x_4 + 0.52x_1^2$$
$$+0.17x_2^2 + 0.29x_3^2 - 0.50x^2_4$$

From the Fig 5.1a, it was observed that for the increase of screw speed there was a decrease in ER until 300 rpm, however, with further increases in screw speed slight increase in ER was observed. For the increase in barrel temperature ER of product increased significantly (Fig 5.1a). As the temperature increased from 80 to 150˚C proportionally the expansion ratio also increased from 0-20 % and similarly types of results were reported by previous worker.[10] Ding et al.[10] suggested that an increase in the barrel temperature may increase the degree of superheating of water in the extruder that enhance the bubble formation in finish product and thereby increased in expansion ratio. The effects of moisture content on the ER of extruded product has been presented in Fig 5.1b. As the moisture content of the extrudates increased (20-25%) there was a slight decrease in expansion ratio (Fig 5.1b). But later further increase of moisture content showed increased in ER of the products. Although increase of passion fruit powder in dough increased in expansion ratio, but it was found not significant (Fig 5.1b).

5.3.1.2 Response surface analysis of water absorption index

After multiple regression analysis of water absorption index of the finished product and experimental data of independent variable, a second order polynomial equation with high regression coefficient value (R^2=0.88) was predicted. The lack of fit of the developed model was also insignificant (Table 5.2), which implies that the developed model can efficiently explain the relationship of water absorption index and independent parameters.

$$WAI = 2.10 + 0.18x_1 - 0.48x_2 + 0.50x_3 + 0.017x_4 + 0.056x_1x_2 + 0.083x_1x_3 + 0.042x_1x_4 - 0.090x_2x_3 - 0.054x_2x_4 - 0.074x_3x_4 - 0.025x_1^2 + 0.047x_2^2 - 0.24x_3^2 + 0.14x_4^2$$

The effect of screw speed and barrel temperature on water solubility index are presented in Fig. 5.1c. It can be inferred from the Figure that the increase of screw speed (200-400rpm) leads to a sharp decrease in the water absorption index (%) whereas increased temperature (80-150˚C) showed abrupt increases in the water absorption index (0.9-4.3). The similar types of result were reported by several workers during the extrusion of legumes [24] and rice [4] respectively. At higher screw speed the length of polymeric chain reduces due to shear, hence, the water solubility index of extrudate decreases. The results are in agreement with the observation reported by Alam et al.[4] Various researchers have reported the phenomenon of starch granule disrupted in high temperature processing thereby increased dextrinization which enhanced the water binding capacity of finish product and hence increased WAI.[4,18] These finding was agreement with findings during extrusion of carrot pomace and rice flour.[18]

In Fig 5.1.d, the effect feed moisture content showed an increased in WAI upto the moiture content of 25 %, further increases in moisture content showed slight decrease in WAI. Previous researchers Singh et al.[32] and Chakraborty et al.[8] reported that in higher feed moisture content, viscosity of starch in the flour might be decreased and allows the starch molecules to move freely and thereby enhancing the penetration of heat, results greater gelatinization and increases WAI of the finished product. WAI decreased with increase in moisture content, which may be attributed to the reduction of elasticity of dough through plasticization of melt at higher moisture content.[10] With increase in passion fruit powder up to 7.5%, WAI of finished product slightly decreased however, after 7.5% of passion fruit there was further increase of WAI of the product was observed (Fig 5.1f). Similar type of result was observed by Jones et al.[17] during the extrusion of rice pea blends. The results indicated that the presence of more passion fruit powder in the mixture reduced the availability for gelatinization of the starch granules, thus reduced viscosity and WAI because of the replacement of the starch by fiber component.

5.3.1.3 Response surface analysis of total phenolic content

The effect of extrusion parameters on the total phenolic content of the extruded product was mathematically expressed using the following model. It was observed that the regression coefficient value (R^2=0.84) of TPC for the product was quite high and lack of fit was insignificant which implies that the developed second degree polynomial equation can efficiently be used to predict the relationship (Table 5.2).

$$TPC = +119.75 - 7.36x_1 - 5.6x_2 + 4.38x_3 + 6.26x_4 - 1.77x_1x_2 + 2.25x_1x_3$$
$$+4.01x_1x_4 - 3.53x_2x_3 - 12.06x_2x_4 - 6.77x_3x_4 - 9.97x_1^2$$
$$-10.71x_2^2 - 6.44x_3^2 - 9.13x_4^2$$

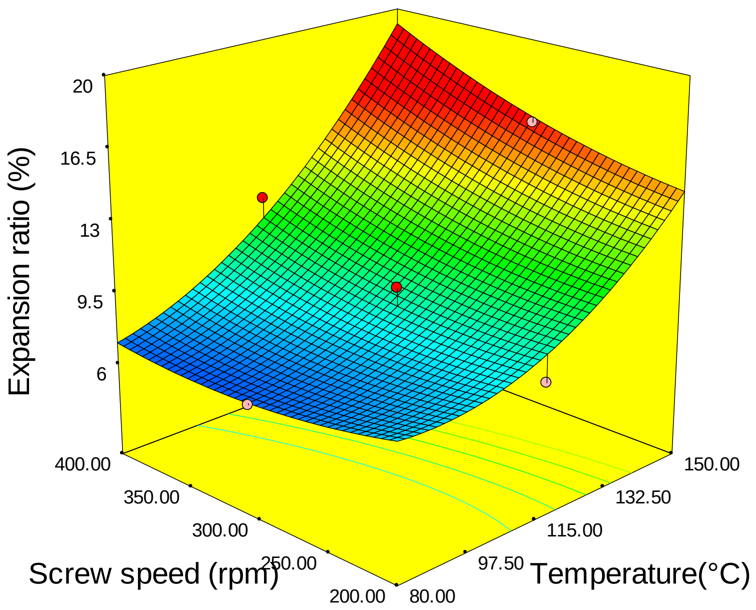
The effect of process variables *viz.,* screw speed (rpm) and temperature (˚C) on total phenolic content (mg/100g) is been illustrated in Fig 5.1.e, As the screw speed increased until 300 rpm, the total phenolic content also increased but later the trend showed decreasing pattern with increasing screw speed. The increase in phenolic content in the finished product may be due to the release of bound phenolic compound from the rice at high screw speed for the breakdown of endosperm.[13] The further increase in screw speed during extrusion showed a decreasing pattern of TPC content in finished product. The decrease in TPC may be ascribed either to the disintegration of phenolic compounds as a result of high shear during extrusion or to the change in molecular arrangement of phenolic compounds. Temperature variable also showed the same pattern with screw speed. With increase in temperature from 80-150˚C, the TPC also increased till 110˚C, later the TPC was decreased with the increasing temperature (Fig 5.1.f). The increase in TPC during extrusion could be due to the damage of cell structures and facilitate the release of bioactive compounds from the matrix, thus enhance the extractability of bound phenolics in the materials.[30] The decrease in the phenolic content after 110˚C may be due to the heat labile nature and lesser resistant to thermal processing.[35] Due to increase in temperature either decomposition or alteration of the molecular structure of phenolic compounds took place. This led to a reduced chemical reactivity due to a certain degree of polymerization in the sample.[3,5,23,28] In the Fig 5.1.h, TPC increase with increase in moisture content (20-25 %), and later it showed a negligible decrease in the TPC when moisture content further increased up to 30 %. This was supported by a study conducted by Dlamini et al.[9] The higher moisture content probably upgraded phenolic and tannin polymerization, which affect extractability of polyphenols *viz.,* phenols and tannins, and degrade antioxidant activity. In the Fig 5.1i, the TPC showed an increase pattern in the finished product, as the passion fruit ranged increased to7.5 %, beyond that the TPC content remained constant with the further increase in passion fruit powder. Increase in the TPC amount may be attributed to high phytochemical content of passion fruit and during extrusion, it may realese fron the cell and inceased the TPC content.

5.3.1.4 Response surface analysis of DPPH scavenging activity

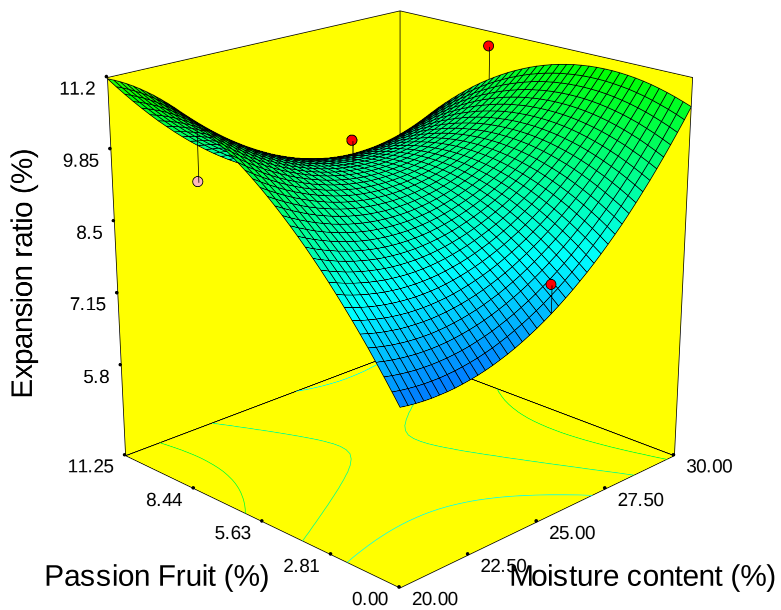
The second order polynomial relationship between DPPH scavenging activity and independent parameters. The Table 5.2, shows that the regression coefficient value (R^2=0.87) was quite high and the lack of fit of the model was insignificant which implies that the developed model can efficiently depict the relationship.

$$DPPH\ scavenging\ activity = 37.88 - 6.85x_1 - 1.81x_2 - 1.15x_3 + 2.31x_4 - 2.10x_1x_2$$
$$-1.45x_1x_3 - 0.19x_1x_4 + 0.75x_2x_3 - 0.69x_2x_4 - 0.10x_3x_4 - 2.63x_1^2$$
$$-0.81x_2^2 - 1.66x_3^2 - 0.90x_4^2$$

Screw speed showed significant effect on DPPH scavenging activity, as the screw speed increased from 200-300 rpm, DPPH scavenging activity also increased (Fig 5.1g). Further increase in screw speed, DPPH scavenging activity decreased. The increases in scavanging activity of the extrudat product has been widely reported.[28] The DPPH scavenging activity is directly depended upon the TPC of the sample. Therefore change in TPC content in the extrudate product due to screw speed proportional changed the DPPH scavging activity. As the temperature increased from 80-110˚C, DPPH scavenging activity increased and beyond that a decrease pattern in scavenging activity was observed till 150˚C (Fig 5.1g). Similar type of obseravtion was reported by Sharma et al.[28] The increase in scavenging activity may be due to the increase in TPC in the product for the lysis of cell structure or Maillard reaction. The decrease in scavenging activity may be due to the decrease in TPC during higher temperature extrusion.[5] Moisture content showed a significant effect on the DPPH content. As the moisture content increased from 20-30 %, DPPH scavenging activity also increases which was similar to the behavior of TPC in product. Passion fruit powder has showed a significant effect on DPPH content, as the temperature increased from 0-7.5% the DPPH content increases and beyond that there was a slight decrease in scavenging activity (Fig 5.1h). This observation was quite similar as observed in TPC in the product.

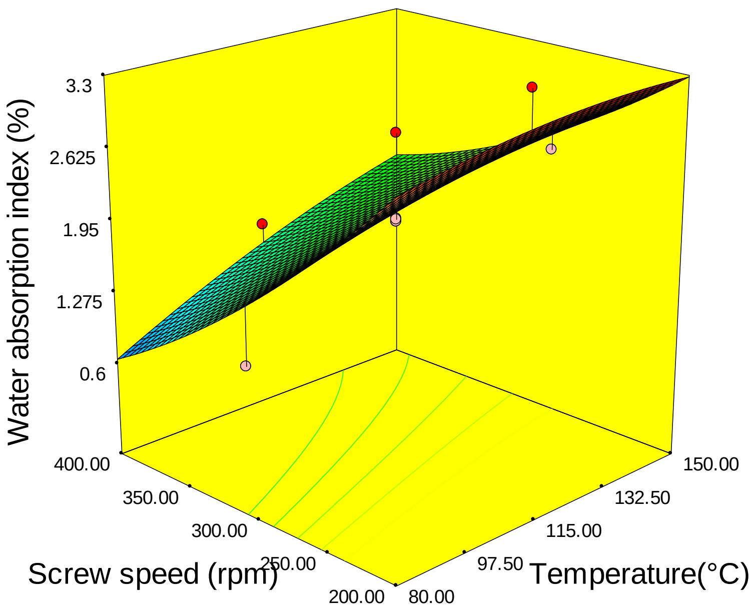

Expansion ratio (%)
20
16.5
13
9.5
6
400.00
350.00
300.00
250.00
200.00
Screw speed (rpm)
80.00
97.50
115.00
132.50
150.00
Temperature(°C)

(a)

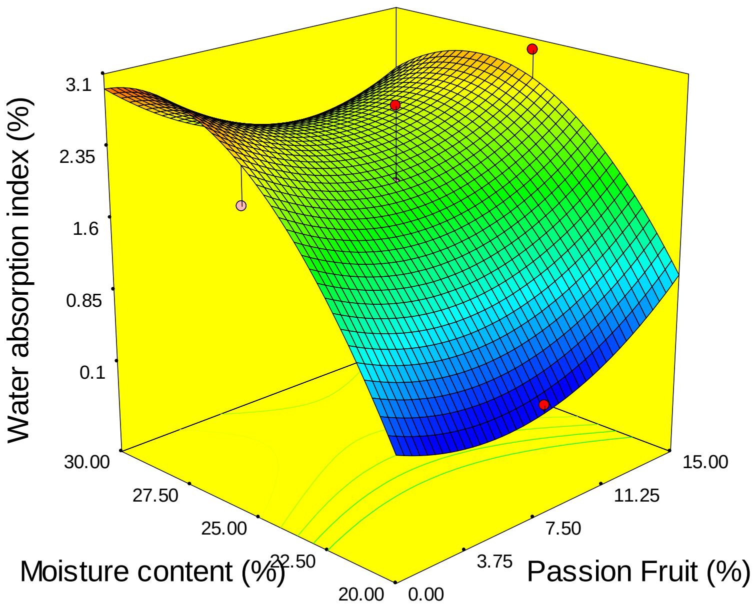

Expansion ratio (%)
11.2
9.85
8.5
7.15
5.8
11.25
8.44
5.63
2.81
0.00
Passion Fruit (%)
20.00
22.50
25.00
27.50
30.00
Moisture content (%)

(b)

Contd.

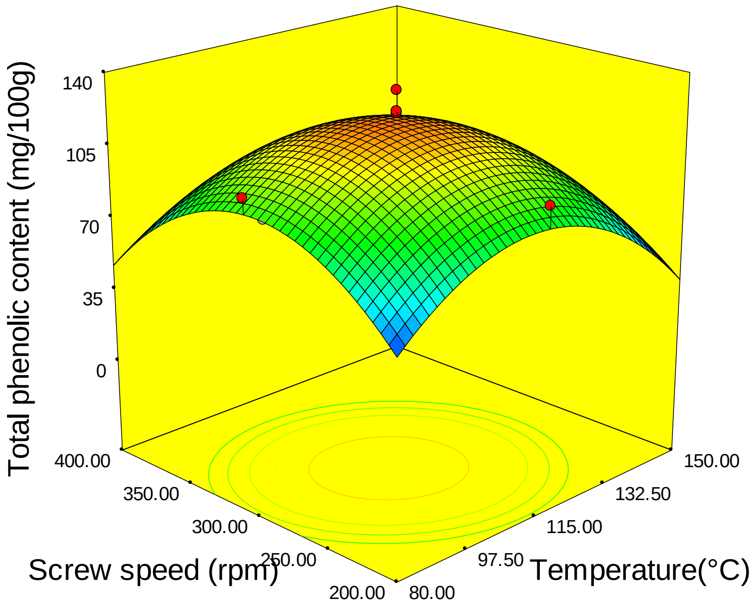

Water absorption index (%)
3.3
2.625
1.95
1.275
0.6
400.00
350.00
300.00
250.00
200.00
Screw speed (rpm)
80.00
97.50
115.00
132.50
150.00
Temperature(°C)

(c)

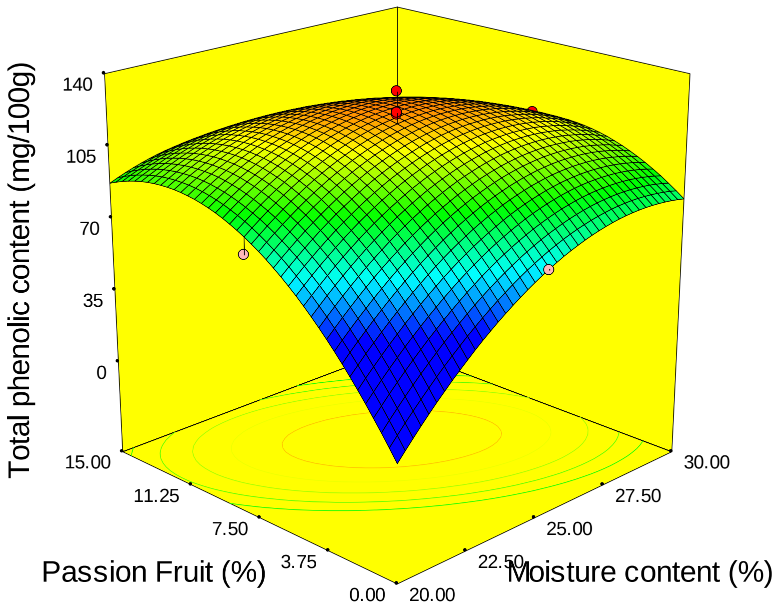

Water absorption index (%)
3.1
2.35
1.6
0.85
0.1
30.00
27.50
25.00
22.50
20.00
Moisture content (%)
0.00
3.75
7.50
11.25
15.00
Passion Fruit (%)

(d)

Contd.

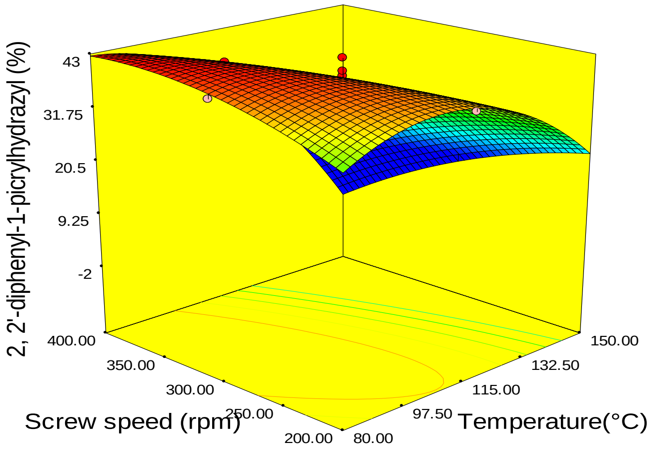

Total phenolic content (mg/100g)
140
105
70
35
0
400.00
350.00
300.00
250.00
200.00
Screw speed (rpm)
80.00
97.50
115.00
132.50
150.00
Temperature(°C)

(e)

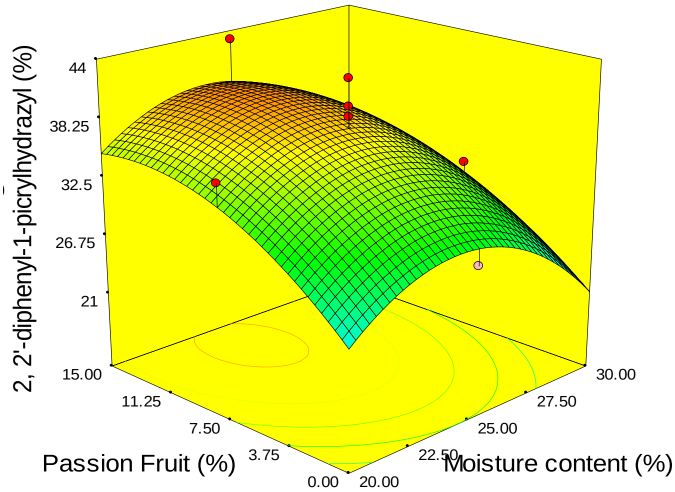

Total phenolic content (mg/100g)
140
105
70
35
0
15.00
11.25
7.50
3.75
0.00
Passion Fruit (%)
20.00
22.50
25.00
27.50
30.00
Moisture content (%)

(f)

Contd.



(g)



(h)

Fig. 5.1 Response surface 3D graphs on; (a-b) Effect of screw speed (rpm), temperature (˚C) and moisture content (%) on expansion ratio (%); (c-d) Effect of screw speed (rpm), temperature and moisture content (%) on water absorption index (%); (e-f) Effect of screw speed (rpm); total phenolic content (mg/100g); (g-h) Effect of screw speed (rpm) and temperature (˚C), moisture content and temperature (˚C) on DPPH (%).

5.3.2 Optimization and validation

After the response surface analysis, the extrusion process was optimized. The process was optimized in terms of maximum desirability value. The highest desirability value was 0.906 and the extrusion conditions were temperature 97.50˚C, screw speed 250 rpm, feed moisture content 25.20% and passion fruit powder 11.25 %. At the optimum condition, the predicted values of response were ER 8.05, WAI 2.77, TPC 129.492 mg GAE/100g and DPPH scavenging activity 65.79%. After optimization, the responses were validated. During validation, the experiment was conducted at optimized condition and observed experimental values of ER (7.08 %), WAI (2.18), TPC (130.10 mg GAE /100g) and DPPH scavenging activity (63.01%) and did not differ significantly.

5.3.3 Characterization of extrudates

5.3.3.1 Fourier transform infrared spectroscopy (FT-IR) analysis

In FT-IR spectrum of optimized sample (Fig. 5.2) was compared with the control sample. The figure shows various peaks in different wavelengths ranges from 562.60.70-3978.34 cm^{-1} and predicts about major functional groups. The band peaks found approximately in the region of 826.60 cm^{-1} indicated α-glycosidic linkages of the glycosyl residues [33] which confirmed the samples were carbohydrate in nature. The frequency of the vibration at 1498 cm^{-1} corresponds to COOH group. The small peaks at around 2322 cm^{-1} were the characteristics of –NH and NH+ group, respectively. There were numbers of sharp peaks in between 3350.00 to 3821.87 in both the samples. Various researchers have suggested that these broad bands observed from 3610–3640 cm^{-1} and 3200–3500 cm^{-1} is attributed to –OH stretching vibrations and hence, indicates the presence of phenolic OH.[25] The products showed that even after the extrusion technology the wavelengths predicted the presence of various functional groups and was present in both samples.

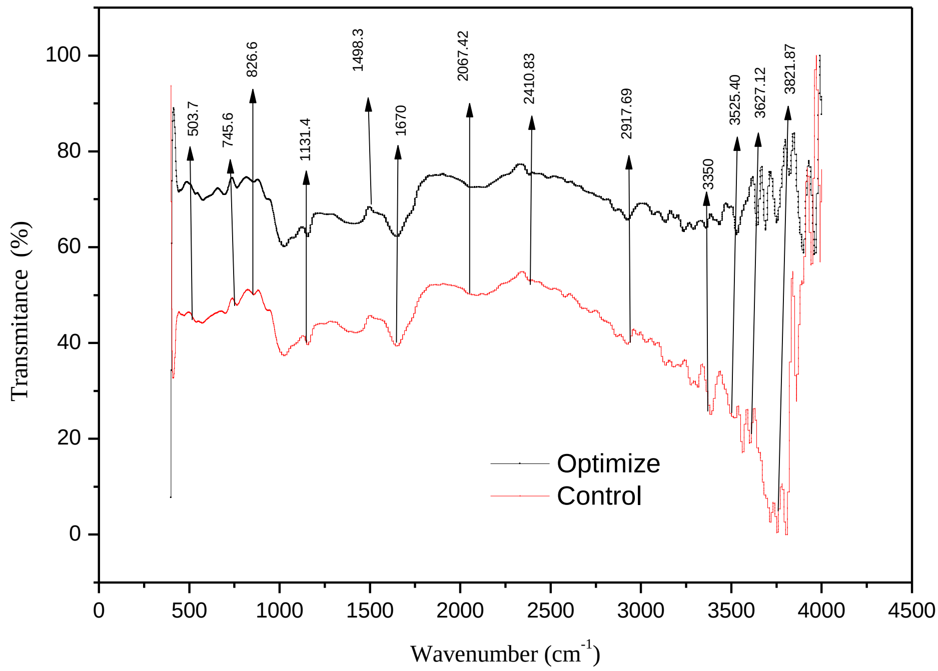


Fig.5.2 FT-IR spectra of control (C) and optimized (O) extrudate

5.3.3.2 Differential scanning calorimetry (DSC) of sample

The thermal properties of the optimized product were analyzed using differential scanning calorimetry (DSC). DSC thermograph showed an endothermic behavior of extruded product (Fig 5.3) Thermal properties of extruded products was presented at Table 5.3 The onset (61.31± 0.71) and peak temperature (62.810 ±0.1˚C) of control sample was higher than the onset (45.77±0.21˚C) and peak temperature (50.53±0.37˚C) of optimized sample. But end set temperature (84.43± 0.39) of optimized sample showed higher range than control sample (65.55±0.38). Kaur et al.[19] reported that this type of phenomena in cereal flour may be attributed to the extrusion technology that helps the starch to become pre gelatinized. Loss of birefringence, which later indicates the disorder of the starch molecules was also found. Therefore, extruded products flours attain gelatinization temperature at lower temperature as compared to the raw cereal flours.

Table 5.3 DSC thermograms of rice flours

Types	Gelatinization (˚C)		
	T_0	*Tp*	*Tc*
C (control)	61.31± 0.71	62.81 ±0.1	65.55 ±0.38
O (optimized)	45.77±0.21	50.53±0.37	84.43± 0.39

Note: T_c, endset temperature; T_o, onset temperature; *Tp*, peak temperature

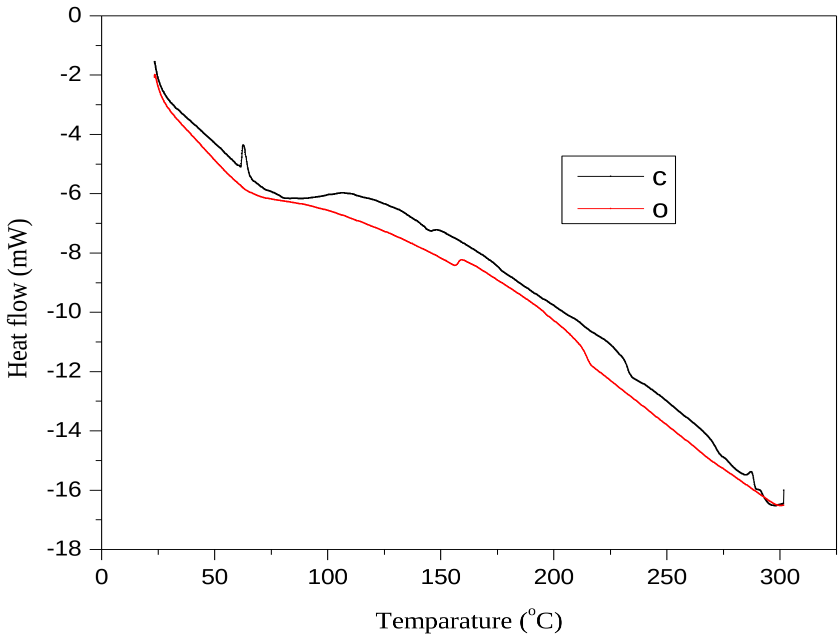
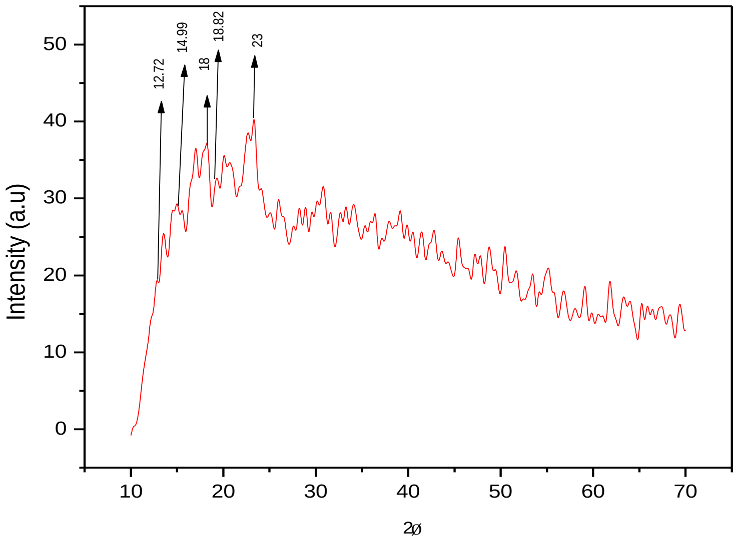


Fig.5.3 DSC graph of control (C) and optimized (O) extrudate

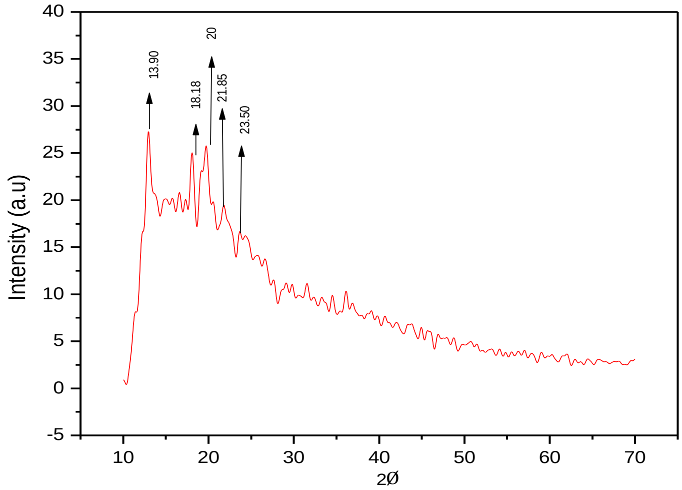
5.3.3.3 X-ray diffraction (XRD) pattern of sample

X-ray diffraction pattern provide "fingerprint" information of the crystal structure within starch of grains.[37] From the Fig 5.4, the XRD analysis of samples revealed strong peaks of control sample at 2θ *viz.*, 12.72,18 and 18.82 and for optimized sample were 18.18,20 and 23.50. According to various researchers, peaks obtained at 15 and 18 confirmed the presence A-type which are the characteristic of starch present in cereals and also supported by Taylor et al.[34] Weak peaks were also observed at 15, 18.82, 19.82 and 23.5. Moorthy [22] stated that peak at 2∅ = 15 and 23.5 showed mixed pattern and 18 possess C type pattern.[36]

Both the sample shows similar types of peaks which indicates the presence of A-type starch in red rice and passion fruit powder. Gat and Ananthanarayan [14] stated that during extrusion process loss of granular structure of pregelatinized rice flour and give mixed pattern of starch. % Crystallinity of control extrudates were 56.22 % and optimized was 65 %.



(a)



(b)

Fig.5.4 XRD graph of control (C) and optimized (O) extrudate

5.3.3.4 Morphological analysis of sample

Scanning electron microscopy (SEM) images of the surface and cross sections of two extruded products at three magnifications 100X and 2500X. Fig 5.5, revealed that control sample (C) had smoother surface than the optimized (O) sample. It could be seen from the figure that the internal structures of the product were affected by the addition passion fruit powder. The control sample was characterized as having a continuous structure that appeared smoother and aggregated. Marked changes were observed by incorporating passion fruit powder where surface became scratched, cracked, and rougher. The optimized extruded product had large numbers of flattened and sheared granules than the control product.

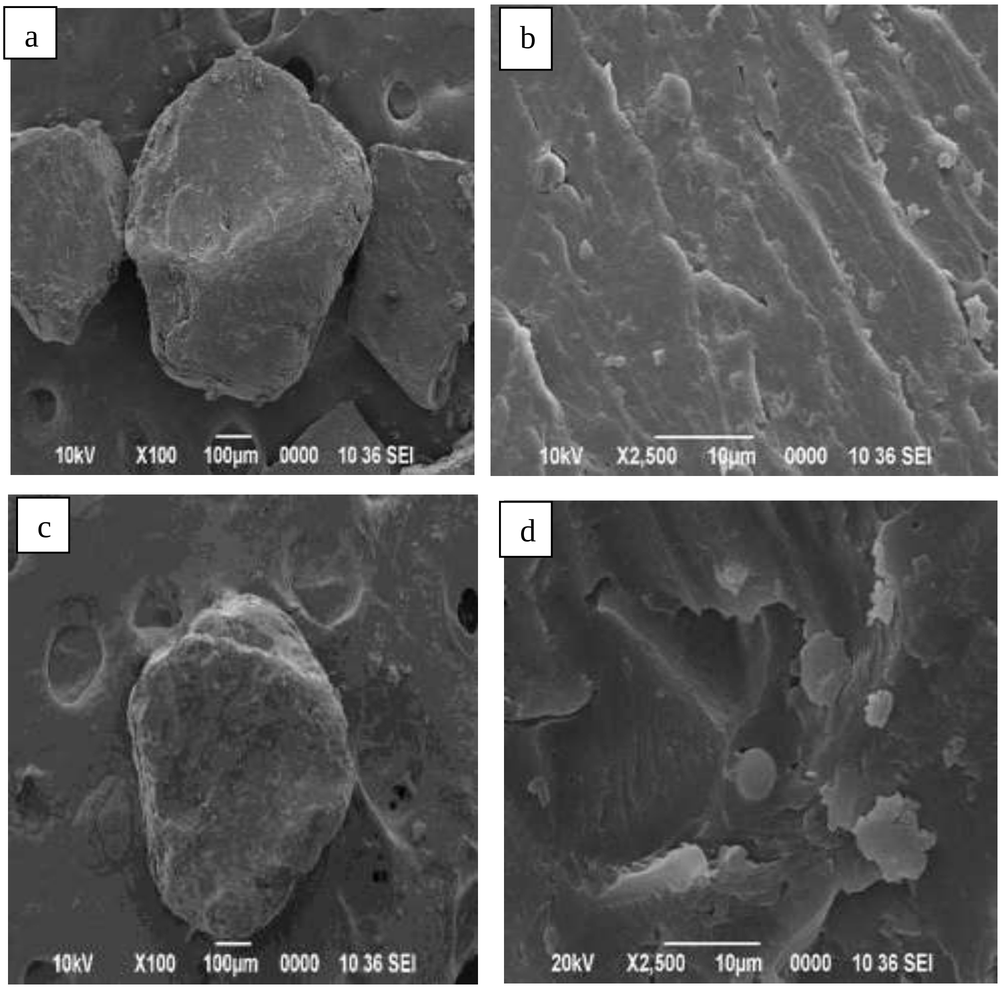


Fig 5.5 SEM of control (a and b) and optimized (c and d) sample at 100 and 2500 magnification

5.3.3.5 Phytochemical profile of sample

The phytochemical properties of control sample and optimized sample were analyzed by quantification of compounds *viz.*, vitamins and anthocyanin. In the Table 5.4, among three vitamins, optimized product (red rice incorporated with passion fruit foam mat powder) sample showed higher content of (±)-α-tocopherol and D-α-tocopherol than control. This may be due to addition of foam mat fruit powder which retain more bioactive compounds than the normal drying process. Anthocynanin *viz.*, cynanidine-3-glucoside (C-3-G) and peonidin-3-D-glucoside (P-3-G) content in control and optimized samples showed very less amount of both anthocyanin compound. The reduction may be due to the extrusion processing on the red rice. Literature revealed that the unprocessed red-pigmented rice varieties contained 19.36-37.00 mg/kg vitamin E with an average of 29.77 mg/kg.[22] Laokuldilok et al.[20] stated that aromatic red rice content very important anthocyanin compounds like cyanidin-3-glucoside (179.0±77 µg/g) and peonidin-3-glucoside (9.1±1.4 µg/g).

Table 5.4 Quantification of compounds vitamins and anthocyanin of control (C) and optimized (O) extrudate

Compounds (mg/L)	Retention time (min)	Sample	
		C (control)	O (optimized)
Vitamins			
β-Carotene	3.00	1.33310	1.31090
(±)-α-tocopherol	3.50	3.99810	9.57140
D-α-tocopherol	3.80	3.99690	9.03690
Anthocyanin			
Cynanidine-3-glucoside (C-3-G)	11.00	0.00003	0.000003
Peonidin-3-D-glucoside(P-3-G)	13.00	0.0093	0.00546

5.4 Conclusion

Extrusion cooking of passion fruit powder incorporated rice based extrudate was optimized successfully using CCD. In this study it has been observed that the incorporation of passion fruit powders has an impact on the physical and phytochemical characteristics of extrudates. The optimal extrusion process condition was found to be temperature 97.50˚C, screw speed 250 rpm, feed moisture content 25.20% and passion fruit powder 11.25 %. After optimization of extrusion process, the optimum product was compared with control sample in terms of physicochemical and phytochemical properties. The thermal and morphological properties of product showed that the incorporation of passion fruit powder has a significant effect.

References

[1] Anderson, R, A., Conway, H,F. and Griffin, E,L. Gelatinization of corn grits by roll and extrusion cooking. *Cereal Science Today*, 1:4-12, 1969.

[2] Aguilar-Garcia, C., Gavino, G., Baragano-Mosqueda, M., Hevia, P. and Gavino, V, C. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. *Food Chemistry*, 102:1228-1232, 2007.

[3] Altan, A., McCarthy, K, L. and Maskan, M. Effect of extrusion process on antioxidant activity, total phenolics and ß-glucan content of extrudates developed from barley-fruit and vegetable by-products. *International Journal of Food Science and Technology*, 44:1263-1271,2009.

[4] Alam, M,S., Pathania, S. and Sharma, A. Optimization of the extrusion process for development of high fibre soybean-rice ready-to-eat snacks using carrot pomace and cauliflower trimmings. *LWT - Food Science and Technology*, 74:135-144, 2006.

[5] Brennan, C., Brennan, M.,Derbyshire, E. and Tiwari, B, K. Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science and Technology*, 22(10):570-575, 2011.

[6] Bouvier, J,M. Breakfast cereals In: Guy, R. (Ed.), Extrusion Cooking: Technologies and Applications. Woodhead, England, pp. 133-160,2001.

[7] Brand-William,W., Cuvelier, M,E. and Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie*, 28:25-30,1995.

[8] Chakraborty, S, K., Singh, D,S., Kumbhar ,B,K. and Chakraborty, S. Millet legume blended extrudates characteristics and process optimizationusing RSM. *Food and Bioproducts Processing*, 89:492-499,2011.

[9] Dlamini, N, R., Taylor, J, R,N. and Rooney, L, W. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry*, 105:1412-1419, 2007.

[10] Ding, Q, Ainsworth, P., Plunkett, A., Tucker, G. and Marson, H. The effect of extrusion conditions on the functional and physical properties of wheat-based expanded snacks. *Journal of Food Engineering*, 73(2):142-148, 2006.

[11] Fellows, P. Food Processing Technology: Theory and Practice. Ellis Horwood, New York. 608p,2000.

[12] Fu, Y,J., Zu, Y,G., Liu, W., Hou, C,L., Chen, L,Y., Li, S,M., Shi, X,G. and Tong, M, H. Preparative separation of vitexin and isovitexin from pigeonpea extracts with macroporous resins. *Journal of Chromatograpy A*, 1139 (2):206-213, 2007.

[13] Gujral, H,S., Sharma, P., Kumar, A. and Singh, B.Total phenolic content and antioxidant activity of extruded brown rice. *International Journal of Food Properties*, 15(2):301-311, 2012.

[14] Gat,Y and Ananthanarayan, L. Effect of extrusion process parameters and pregelatinized rice flour on physicochemical properties of ready-to-eat expanded snacks. *Journal of Food Science and Technology*, 52(5): 2634-2645, 2015.

[15] Hirth, M., Leiter, A., Beck, S, M. and Heike P,S. Effect of extrusion cooking process parameters on the retention of bilberry anthocyanins in starch based food. *Journal of Food Engineering*, 125:139-146, 2014.

[16] Huang, Ya-Ling. and Ma, Ya-Sheng.The effect of extrusion processing on the physiochemical properties of extruded orange pomace. *Food Chemistry*, 192:363-369, 2016.

[17] Jones, D., Chinnaswamy, R., Tan, Y. and Hanna, M. Physicochemical properties of ready-to-eat breakfast cereals. *Cereal Food World*, 45:164-168, 2000.

[18] Kumar, N., Sarkar, B, C. and Sharma, H, K. Development and characterization of extruded product using carrot pomace and rice flour. *African Journal of Food Science*, 4(11):703-717,2010.

[19] Kaur,A., Shevkani, K., Katyal, M., Singh,N., Ahlawat, A, K. Singh, A, M. Physicochemical and rheological properties of starch and flour from different durum wheat varieties and their relationships with noodle quality. *Journal of Food Science and Technology*, 53(4):2127-2138, 2016.

[20] Laokuldilok, T., shoemaker, C, F., Jongkaewwattana, S. and Tulyathan, V. Antioxidants and antioxidant activity of several pigmented rice brans. *Journal of Agricultural Food Chemistry,* 59, 193-199,2011.

[21] Lobato, L, P., Anibal, D., Lazaretti, M, M. and Grossmann, M, V, E. Extruded puffed functional ingredient with oat bran and soy flour. *LWT-Food Science and Technology*, 44:933-939,2011.

[22] Moorthy, S, N. Physicochemical and functional properties of tropical tuber starches: A review. *Starch/Stärke*, 54:559-592,2002.

[23] Nayak, B., Berrios, J, De. J., Powers, J,R. and Tang, J. Effect of extrusion on the antioxidant capacity and color attributes of expanded extrudates prepared from purple potato and yellow pea flour mixes. *Journal of Food Science*, 76(6):874-883,2011.

[24] Oikonomou, N, A. and Krokida, M, K. Water absorption index and water solubility index prediction for extruded food products. *International Journal of Food Properties,* 15(1):157-168, 2012.

[25] Park, Y,S., Kim, S,J. and Chang, H, I. Isolation of anthocyanin from black rice (Heugjinjubyeo) and screening of its antioxidant activities. *Korean Journal of Microbiology and Biotechnology*, 36:55-6,2008.

[26] Potter, R., Stojceska, V. and Plunkett, A. The use of fruit powders in extruded snacks suitable for children's diets. *LWT - Food Science and Technology,* 51(2):537-544, 2013.

[27]Shammugasamy,B.,Ramakrishnan,Y.,Ghazali,H,M.andMuhammad,K.Tocopherol and tocotrienol contents of different varieties of rice in Malaysia. *Journal of the Science* of *Food and Agriculture*, 95: 672-678,2015.

[28] Sharma, P., Gujral, H, S. and Singh, B. Antioxidant activity of barley as affected by extrusion cooking. *Food Chemistry,* 131(4): 1406-1413, 2012.

[29] Sharma, P., Ramchiary, M., Samyor, D. and Das, A, B. Study on the phytochemical properties of pineapple fruit leather processed by extrusion cooking. *LWT - Food Science and Technology*, *72*:534-543, 2016.

[30] Shih, M., Kuo, C. and Chiang, W. Effects of drying and extrusion on colour, chemical composition, antioxidant activities and mitogenic response of spleen lymphocytes of sweet potatoes. *Food Chemistry*, *117*(1): 114-121, 2009.

[31] Slinkard, K. and Singleton,V, L. Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28:49-55,1977.

[32] Singh, B., Sekhon, K,S.,& Singh, N. (2007). Effects of moisture, temperature and level of pea grits on extrusion behaviour and product characteristics of rice. *Food Chemistry*, 100,198–202.

[33] Tsumuraya, Y. and Misaki, A. Structure of the water-insoluble α-D-glucan of *Streptocossus salivarius* HHT. *Carbohydrate Research*, 74:217-225,1979.

[34] Taylor, N, W., Zobel, H, F., White, M. and Senti, F, R. Deuterium exchange in starches and amylose. *Journal of Physics and Chemistry*, 65:1816-1820, 1961.

[35] Zielinski, H., Kozlowska, H. and Lewczuk, B. Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Science and Emerging Technology*, 2:159-169,2001.

[36] Zobel, H, F. Molecules to granules: A comprehensive starch review**.** *Starch / Starke*, 40(2):44-50,1998.

[37] Zeng, J., Li, G., Gao, H. and Ru, Z. Comparison of A and B starch granules from three wheat varieties, *Molecules*, 16:10570-10591, 2011.

B) Rheological properties of gluten free dough

5.5 Introduction

Rheology explains about the flow and deformation of food. Rheology plays a vital role in food manufacture and marketing nowadays *viz.,* design of handling systems, quality control and evaluation of sensory stimuli of viscosity.[3] It also concerned with how all food materials respond to applied forces and deformations. Basic concepts of stress (force per area) and strain (deformation per length) are keys to all rheological evaluations. Stress (r) is always a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). The direction of the force with respect to the impacted surface area determines the type of stress. Normal stress occurs when the force is directly perpendicular to a surface and can be achieved during tension or compression. Shear stress occurs when the forces act in parallel to a surface. Various food show different rheological behavior and categorized into solid and liquids stages. It basically means that food varies their characteristic in viscous and elastic behaviors commonly known as viscoelasticity of food cause by entanglement of long chain molecules with other molecules.[1]

Nowadays, the use of additives has become a common practice in the cereal processing industry. Additives in dough help to improve dough handling properties, increase nutritional quality and extend the shelf-life of stored product. However, few study has been conducted on addition of fruit powder in rice base food product [10] and scanty of research on the incorporation of passion fruit powder in rice dough are available. The acceptability of a food product for consumer mainly depend upon structure, mouthfeel, acceptability and shelf-life of the product. All these parameters are directly or indirectly related with rheological properties of dough and is pertinent to investigate the role of different rheological parameters of passion fruit incorporated rice dough.

Extruded foods products behave as non-Newtonian Fluids and their viscosity can be predicted by the Power Law model.[7] The Power law (Ostwald) and Bingham models [19, 2] reported to have adjustable parameters and most widely used. Rheological properties of fluid foods are complex and dependable on composition, shear rate, duration of shearing, and previous thermal and shear histories.[14, 22]

Samyor et al. (2017). Effect of passion fruit powder on rheological properties of gluten free red rice dough. Journal of Texture Studies (Under Review).

Therefore, the effect of passion fruit foam mat powder on rheological properties of red rice dough using shear stress (Pa) versus shear rate (s^{-1}) data was carried out. Different Flow models were used to describe shear rate (γ) versus shear stress (σ) data.

5.6 Materials and methods

5.6.1 Materials

Red rice was collected from Arunachal Pradesh, India and moisture content of grain was maintained around 12 % and stored in air tight container. Rice flour was prepared by milling in a lab grain mill. Passion fruit powder was obtained from foam mat drying.

5.6.2 Sample preparation

For rheological study, weighted amount of red rice flour was taken in a bowl and mixed with 11,25 % (w/w) passion fruit powder. The control sample was only red rice. The moisture content of the dough was maintained 35%.

5.6.3 Shear rheological study

For the shear rheological study, a rotational rheometer (Antron Paar, Physical MCR 301, and PP-50) equipped with a plate and plate geometry was used. The plate system was used with a diameter of 50 mm and a gap between plates of 0.1 mm. Sample (1g) was placed between the plates and edges of samples were carefully trimmed out with a spatula. The models were listed in the Table 5.5. Following were the rheological models used :

5.6.3.1 Power law

Power law model describes the data of shear-thinning and shear thickening fluids

$$\sigma = k(\gamma)^n \tag{1}$$

Where, γ is the shear rate, k =Apparent viscosity or consistency index and η = Flow behavior index

5.6.3.2 Bingham model

Bingham model gained popularity due to its simplicity.[16] The model exhibits a yield which also known as viscoplastic models.[4] Yield stress are very important rheological parameter to validate processing performance of sample. There is a certain level of internal structure which has to be overcome to initiate the flow.[21]

$$\sigma - \sigma_0 = n'\gamma \tag{2}$$

Where *η´ is* called the Bingham plastic viscosity or flow behavior index, γ is the shear rate and σ_0 is the yield stress.

5.6.3.3 Herschel –Bulkley model

Yield stress occurrence considered to be an engineering reality and in many food products it plays a major role.

$$\sigma = K_k \gamma^{'n_H} + \sigma_{OH} \tag{3}$$

Where K_k = Consistency index, γ is the shear rate, n_H = Flow behavior index, σ_{OH} = Yield stress.[13]

5.6.3.4 Casson model

Casson model is a structure based model which is popularly used for food dispersions.[6]

$$\sigma^{0.5} = K_{oc} + K_c(\gamma)^{0.5} \tag{4}$$

Where $(\sigma)^{0.5}$ is the square root of shear stress, γ is the shear rate, K_c is the slope and K_{0c} is intercept. The Casson model is also used for cooked rice flour dispersions.[9]

5.6.3.5 Mizrahi and Berk model

Mizrahi and Berk model is a three-parameter viscoplastic model[11] which also exhibits yield stress [17], and eq. is given below.

$$\sigma^{0.5} = K_M \gamma^{'n_M} + \sigma_{OM} \tag{5}$$

Where K_M = Apparent viscosity or consistency coefficient, σ_{OM} = Yield stress and n_M = Flow behavior index

5.6.4 Dynamic rheological properties

The dynamic rheological properties of samples were assessed using a rotational rheometer (Antron Paar, Physical MCR 301, PP 50) equipped with a plate and plate geometry. The plate system was used with a diameter of 50 mm and 0.1 mm gap was maintained between plates. The sample was placed between the plates and edges of samples were carefully trimmed out with a spatula. Before testing, dough was rested at room temperature for 20 min for the relaxation of the residual stresses.[8] A thin layer of silicon oil was used on the exposed surface of the sample to prevent drying during testing. Frequency sweep tests (mechanical spectra) from 1 to 200 W/s were performed at 30˚C. For analysis, rice dough samples were used on the plate and excess sample was removed carefully by using a sharp razor blade. The oscillatory rheology depends upon (G') and viscous or loss modulus (G'') data and replicates of each measurement were done. The storage modulus (G'), loss modulus (G''), complex viscosity (η*) and dynamic viscosity (η') were calculated for each samples and the data were analyzed using Rheoplus Version 3.61 software.

Table 5.5 Different models for rheological study

Sl. No.	Model name	Equation	Constant models
1	Power law	$\sigma = k(\gamma^{'})^{n}$	k =Apparent viscosity or consistency index η = Flow behaviour index
2	Bingham	$\sigma - \sigma_0 = n'\gamma^{'}$	σ_0 = Yield stress $n^{'}$ = Flow behaviour index
3	Herschel – Bulky	$\sigma = K_k \gamma^{'n_H} + \sigma_{OH}$	K_k = Consistency index n_H = Flow behaviour index σ_{OH} = Yield stress
4	Casson	$\sigma^{0.5} = K_{oc} + K_c(\gamma^{'})^{0.5}$	K_{oc} = Yield stress K_c =Consistency coefficient
5	Mizrahi and Berk	$\sigma^{0.5} = K_M \gamma^{'n_M} + \sigma_{OM}$	K_M = Apparent viscosity or consistency coefficient σ_{OM} = Yield stress n_M = Flow index

Where σ = shear stress (Pa), y = shear rate (s^{-1}), *K= consistency coefficient, n=flow behaviour index,* σ_0 =yield stress (Pa)

5.6.5 Statistical analysis

The data were fitted in different rheological model by using Matlab R 2008a. Origin 8.5 software was used to plot the graphs.

5.7 Results and discussion

Table 5.6 Effect of passion fruit powder on steady shear rheological properties of dough

Models	**Models constant**	**R ^{2}**	**Adjusted R^2**	**RMSE**	**Constant models**	**R ^{2}**	**Adjusted R^2**	**RMSE**
	Control				Optimized dough			
Power	k = 518 n = 0.23	0.46	0.46	73.13	K =1394, n= 0.29	0.34	0.34	291.50
Bingham	σ_0 =377.50 n'=3.10	0.80	0.79	42.91	σ_0 = 1053 n'= 11.29	0.82	0.81	148.00
Herschel – Bulkley	K_k = -5.73 n_H =0.47 σ_{OH} =398	0.81	0.81	43.83	K_k = -7.85 n_H = 0.89 σ_{OH} = 1026	0.83	0.82	149.40
Casson	K_{oc} =506.70 K_c = 1.17	0.75	0.75	49.37	K_{oc} =38.76 K_c = -2.95	0.68	0.68	201.10
Mizrahi and Berk	K_M = -0.05 n_M =0.46 σ_{OM} =382.59	0.83	0.83	40.68	K_M = 0.006 n_M = 0.88 σ_{OM} =982.82	0.87	0.86	129.8

5.7.1 Steady shear rheological analysis

The flow behavior of red rice dough and passion fruit powder incorporated red rice dough were investigated. The shear stress (Pa) versus shear rate (s^{-1}) data obtained for red rice dough and optimized rice dough with incorporate passion fruit powder (Table 5.6) was fitted to Power, Bingham, Herchel–Bulkley, Casson, and Mizrahi and Berk models. These rheological models were compared among each other depending on R^2 and root mean square error (RMSE). The higher R^2 with lower RMSE showed the best fitted model. For both the samples Herschel-Bulky and Mizrahi and Berk model showed the highest R^2 in (Table 5.6) similar type of result was observed for mango pulp [5] and for brown flour from Indica rice.[23] For the Herschel - Bulkley and Mizrahi and Berk model the yield stress of control sample was 398 and 382.59 Pa, respectively. However, the yield stress of optimized sample was 1026 and 982.82 Pa, obtained from Herschel–Bulky and Mizrahi and Berk model, respectively. Therefore, it can be inferred from the yield stress data that incorporation of passion fruit powder in red rice flour increased the yield stress. Flow index, n, for control sample was 0.46 to 0.47 for Herschel-Bulky and Mizrahi and Berk model, respectively (Table 5.6). For the optimized sample the flow index values varied from 0.88-0.89. The flow index parameter for both the red rice flour were below one (<1) and it can be inferred that the rice flour and passion fruit incorporated rice flour were pseudoplastic and shear thinning in nature [5] and therefore, incorporation of powder showed significant effect on the dough rheology.

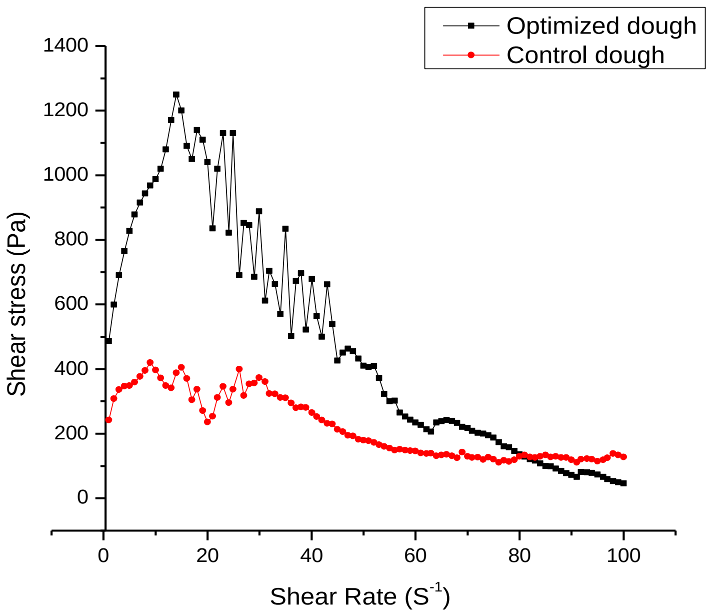


Fig 5.6 Shear stress vs. shear rate of (a) control and (b) optimized product

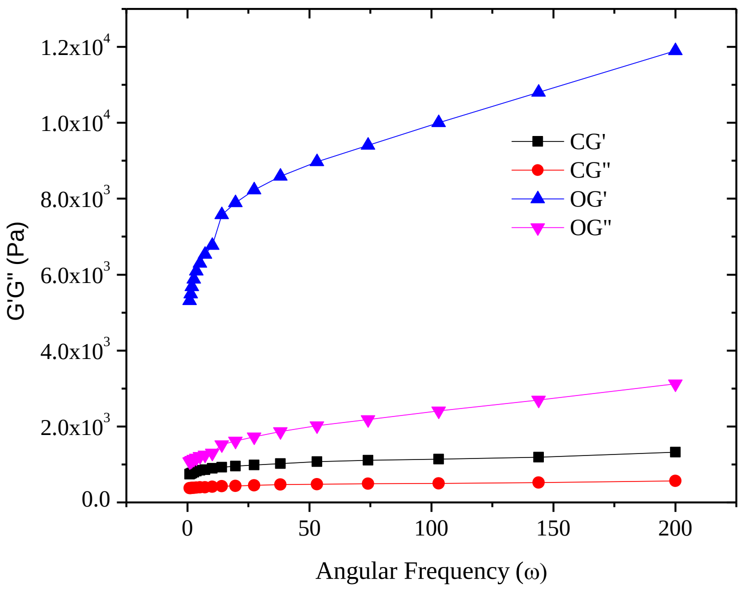


Fig 5.7 Angular frequency (ω) vs. storage modulus (G') and loss modulus (G'')

CG´- storage modulus of rice flour

OG´´- storage modulus of rice flour + passion fruit powder

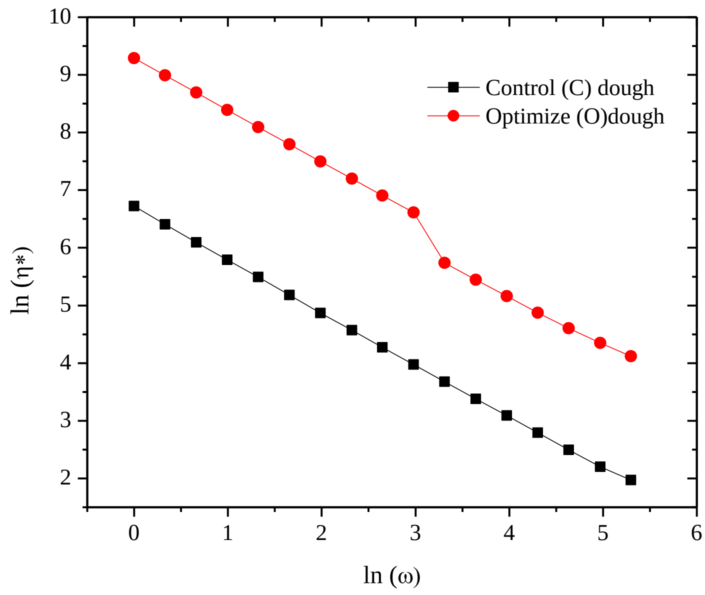


Fig. 5.8 Angular frequency (ω) vs. complex modulus (η*)

5.7.2 Dynamic oscillatory rheology

The effect of passion fruit powder on the rheological response functions (storage modulus, loss modulus, and complex viscosity) is illustrated in Fig.5.7 and 5.8. The dynamic *G´* (storage modulus) and *G´´*(loss modulus) parameters which are function of frequency (ω) for the samples were measured to provide information on changes of biopolymer structure.[18] The dynamic *G´* is a measure of the energy stored and recovered from material (per cycle of sinusoidal deformation) whereas *G''* is a measure of the energy dissipated (lost per cycle).[20] η* is a measure of the overall resistant of flow (Pa.S).[24] The storage *(G')* and loss *(G'')* modulus of red rice dough and rice dough with passion fruit powder as a function of frequency (ω) shown in Fig.5.8 Oscillation rheology experiment allows continuous measurement of storage and loss modules during frequency sweep testing of rice dough. Incorporation of passion fruit powder in rice dough led to a remarkable change in oscillatory rheological behaviours of rice dough. Storage moduli of rice dough *(G´)* and rice dough with passion fruit powder *(G')* were plotted against the angular frequency ranges from 0 to 200 Ws^{-1} (Fig.5.7). The storage modulus *(G´)* and loss modulus *(G´´)* of both the samples exhibited linear viscoelastic behaviour and revealed that lower dependence of moduli on frequency. *G´* was higher than *G´´* (Fig.5.8) and elucidated a prominent solid like behaviour (viscoelastic rather than elastoviscous) of these rice dough sample.[12] It may be attributed to the gelatinization of rice starch that enhances the overall pasting capacity of the starches and thereby increased the elastic properties of rice flour. A sharp increase in *G'* was observed for rice dough with passion fruit powder (Fig.5.7) over red rice flour and suggested more prominent solid like behaviour of rice flour with passion fruit powder than red rice flour only. Thereby, it revealed that incorporation of passion fruit powder had a significant effect on dough rheology of rice flour. It may be due to the reinforcement effect of passion fruit powder on the three-dimensional gel network of red rice dough and increased the elasticity of dough. According to fractal scaling theory, the elastic properties of a network of close-packed particle flocs is dependent on the strength of the interfloc links.[15] Therefore, the increase in *G´* might be attributed to the more integration of the intermolecular bond between rice flour and passion fruit powder, formed during dough mixing. Fig.5.8 illustrated complex viscosity (η*) of rice dough and rice dough with passion fruit powder when and plotted against ω and observed that the magnitudes of η*of dough were parallel to each other and decayed linearly with increase in frequency which revealed the frequency dependence of complex viscosity (η*) for both the samples. These results showed that the elastic properties of rice dough decreased at higher frequency due to the decrease in the rate of chain rearrangement and inclined tends to form a more ordered structure or crystalline structure.

5.8 Conclusion

Rheological studies of red rice based dough showed a different behaviour. The incorporation of powder showed significant effect on the dough rheology. The flow behavior of red rice and passion fruit incorporated red rice doughs can be explained by the Mizrahi and Berk model (R^2=0.83) and (R^2=0.87). The incorporation of powder showed significant effect on the dough rheology. The storage modulus (G´) and loss modulus (G´´) of both the samples revealed a linear viscoelastic behaviour and showed lower dependence of moduli on frequency. Complex viscosity (η*) of rice dough (C) and rice dough with passion fruit powder (O) stated that the elastic properties of rice dough were decreased at higher frequency due to the decrease in the rate of chain rearrangement.

References

[1] Abang Zaidel, D. N., Chin.N, L. and Y. A. Yusof. A review on rheological properties and measurements of dough and gluten. *Journal of Applied Science,* 10:2478-2490, 2010.

[2] Bingham, E.C. Fluidity and Plasticity. New York, McGraw-Hill. p 452,1922.

[3] Barnes, H, A., Hutton, J.F. and Walters, K. An introduction to rheology, Elsevier Science Publishing Company Inc. New York,1989.

[4] Bird, R, B, D., Gance, B. and Yarusso, J. The rheological and flow of viscoplastic material. *Reviews in Chemical Engineering*, 1:1-70,1982.

[5] Bhattacharya, S. and Rastogi, N. K. Rheological properties of enzyme-treated mango pulp. *Journal of Food Engineering*, 36(3):249-262,1998.

[6] Casson, N. A flown equation for pigmented –oil suspension of the printing ink type. In : Mill, C.C.(editor). Rheologyy of dispersed Systems. Pergamon press, New York. pp 84-104,1959.

[7] Carvalho, C.W.P., Ascheri, J.L.R. and Mitchell, J.R. Capillary rheometry of maize and wheat: effect of sugar and temperature. *Alimentaria*, 356:109-118, 2004.

[8] Campanella, O.H. and Peleg, M. Squeezing flow viscosimetry of peanut butter. *Journal of Food Science*, 52:180-184,1987.

[9] Chun, S.Y. and Yoo, B. Rheological behavior of cooked rice flour dispersions in steady and dynamic shear. *Journal of Food Engineering*, 65:363-370,2004.

[10] Chopda, C. A. and Barrett, D. M. Optimization of guava juice and powder production. *Journal of Food processing and Preservation*, 25(6):411-430, 2001.

[11] de Vicente, J., Klingenberg, D. J. and Hidalgo-Alvarez, R. "Magneto rheological fluids: A review," *Soft Matter* ,7:3701-3710,2011.

[12] Dixit, Y. and Bhattacharya, S. Rheological and sensory behaviour of rice flour dough: effect of selected additives in relation to dough flattening. *Journal of Food Science and Technology*, 52(8):4852-4862,2015.

[13] Herschel, W. H. and Bulkley, R. Konsistenzmessungen von gummi-benzollösungen. *Colloid and Polymer Science,* 39(4):291-300,1926.

[14] Halmos, A,L. Liquid foodstuffs exhibiting yield stress and shear-degradability. *Journal of Texture Studies,* 12:39-46,1981.

[15] Ikeda, S., Foegeding, E. A. and Hagiwara, T. Rheological study on the fractal nature of the protein gel structure. *Langmuir*, 15(25):8584-8589,1999.

[16] Kelessidis, V. C. and Maglione. R. "Modeling rheological behavior of bentonite suspensions as Casson and Robertson-Stiff fluids using Newtonian and true shear rates in Couetteviscometry," *Powder Technology*, 168:134-147,2006.

[17] Mizrahi, S. and Berk, Z. Flow behavior of concentrated orange juice: 433 Mathematical treatments. *Journal of Texture Studies*, 3:69-79,1972.

[18] Rao, M.A. and Tattiyakul, J. Granule size and rheological behavior of heated tapioca starch dispersions. *Carbohydrate Polymers*, 38:123-132,1999.

[19] Reiner, M. *Kolloid*-Zeitschrift *und Zeitschrift für Polymere,*39:80-87,1962.

[20] Singh, J., Kaur, L. and McCarthy, O,J. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications a review. *Food Hydrocolloid*, 21,1-22,2007.

[21] Semancik, J. R. Yield stress measurements using controlled stress rheometry. TA Instruments Publication RH-058,1997.

[22] Steffe, J.F. Rheological Methods in Food Process Engineering. second ed., Freeman Press, East Lansing, USA, ISBN: 0-9632036-1-4,1996.

[23] Ye, L., Wang, C., Wang, S., Zhou, S. and Liu, X. Thermal and rheological properties of brown flour from Indica rice. *Journal of Cereal Science*, 70, 270-274, 2016.

[24] Yoo, Kim, C. and Yoo, B. Steady and dynamic shear rheology of rice starch-galactomannan mixtures. *Starch/Stärke,* 57,310-318,2005.

C) Sensory evaluation of red rice based extruded products by fuzzy logic tool

5.9 Introduction

In urban and semi urban population, the concept of snacks are very popular and liked by consumers irrespective of ages.[14] Extrusion processing plays a vital role in the production of snack items. The attributes of extrudates *viz.*, crispiness, appearance, taste, size, and various shape attract the customers. The choice of addition of ingredients define the final products quality. The starchy materials for instant corn starch, flour, or grits, rice products etc. produce the best extruded products due to their superior expansion characteristics.[1, 3, 6, 9, 12] Nevertheless, cereal grains are low in protein with a very low biological value which may be due to meagre essential amino acid contents.[11] Nutritious snack items can be prepared by incorporation of legumes, vegetables and fruits into the formulation.[14]

Linguistic decision can be analyze and manage consistently the uncertainty and vagueness of the information during sensory evaluation.[10] Jaya and Das[4] stated that fuzzy logic is an important decision-making tool for comparing a developed product with similar products available in the market. Fuzzy logic tool is used for sensory evaluation of various products *viz.*, mango drinks,[4] coffee,[7] black rice wine fortified [5], dahi powder[13] etc. Therefore, in the present study a sensory evaluation was conducted between a red rice extruded products (C) and red rice extruded products incorporated with passion fruit powder (O) by using fuzzy logic.

5.10 Material and methods

5.10.1 Raw materials

Extruded products were developed from combination of red rice (*Oryza sativa* L.) flour and foam mat passion fruit powder. The product development was done by twin extruder with co- rotating screw (Model FUE-1F, Flytech Engineering, Chennai, India).Control extrudate was prepared from red rice dough and optimized extrudate was prepared by the combination of red rice and passion fruit foam mat dried powder (detail has been elaborated in chapter 5A).

5.10.2 Sensory evaluation of extrudates

The sensory evaluation of two extruded products (C) and (O) were carried out. The total number of panel members were 30 including faculties and research scholars of the Department of Food Engineering and Technology, Tezpur University, Assam. During the evaluation, panel members were been told about the terminology, definition of quality attributes, method of scoring used in sheet and also given instructions to rinse and swallow water between samples of testing.[4]

5.10.3 Fuzzy analysis

Fuzzy is a powerful tool of linguistic data which showed inference regarding acceptance, rejection, ranking, strong and weak attributes of food. Ranking of the extruded products was carried out using triangular fuzzy membership distribution function, explained by Das[2] and sensory scores of the samples were acquired using fuzzy scores given by the 30 judges, converted to triplets and further used for estimation of similarity values used for ranking of sample.[8]

Product.........breakfast cereal item ....Made on....................... Tested on..................

Please rate the samples for quality attributes by putting (√) mark against the appropriate grade

	Quality attributes	**Poor**	**Fair**	**Medium**	**Good**	**Excellent**
Appearance(A)	Control					
	Optimized					
Colour (C)	Control					
	Optimized					
Taste (X)	Control					
	Optimized					
Texture (T)	Control					
	Optimized					
Mouthfeel (M)	Control					
	Optimized					

Please indicate the weightage you would like to assign for each quality attributes by putting (√) mark against the appropriate choice

Quality attributes	**Not important**	**Somewhat important**	**Important**	**Highly important**	**Extremely important**
Appearance					
Colour					
Taste					
Texture					
Mouth feel					

Comments if any

Signature of the evaluator

Fig. 5.9 Fuzzy logic score card for evaluation of extruded products

5.11 Results and discussion

Sensory evaluation of samples were carried out successfully by using fuzzy logic tool. It tackles the evaluation process mathematically. Table 5.7, presents the sum of the number of judges with different preferences and quality attributes were appearance, color, taste, texture and mouthfeel. Two extruded products (control and optimized) were denoted as SM1 and SM2, respectively.

Table 5.7 Sum of sensory scores for the quality attributes of extruded samples

Quality attributes		**Poor/not satisfactory**	**Fair**	**Medium**	**Good**	**Excellent**	**Total**
Appearance	C	10	11	9	0	0	30
	O	11	15	3	1	0	30
Color	C	15	8	5	2	0	30
	O	16	7	5	2	0	30
Taste	C	5	6	9	10	0	30
	O	6	7	8	9	0	30
Texture	C	3	6	12	7	2	30
	O	3	4	14	7	2	30
Mouthfeel	C	3	7	10	10	0	30
	O	2	5	11	12	0	30

Table 5.8 Preferences to the importance of quality attributes of the extrudates

Sensory response / **Attributes**	**Not important**	**Somewhat important**	**Important**	**Highly important**	**Extremely important**
Appearance (A)	0	1	12	10	7
Color (C)	0	7	17	2	4
Taste (X)	0	12	13	3	2
Texture (T)	0	4	16	6	4
Mouthfeel (M)	0	10	12	4	4

5.11.1 Triplets associated with sensory scales

In triplets associated with sensory scales, “triplet,” terms were used for set of three numbers. The scale is a user-defined scale and subjective in nature. Ranking of the (C) and (O) samples were carried out using triangular fuzzy membership distribution function (Lazim and Suriani)[7]. The 5-point sensory scale were ‘Not satisfactory/Not at all important (0,0,25), Fair/Somewhat important (25,25,25), Medium/Important (50,25,25), Good/Highly important (75,25,25) and Excellent/Extremely important (100,25,0)’.[4,2] The Matlab 7.6 (The Math Works Inc., Natick, MA) was used for fuzzy logic evaluation.

5.11.2 Triplets for sensory quality of samples

The triplets corresponding of control (C) and optimized (O) to a particular quality attributes can be obtained from the sum of score obtained for each of the sensory scale and the number of judges. Triplets numbers for sensory quality of the extruded samples were calculated for

$$\text{appearance (S1A)} = \frac{n_1(0\ 0\ 25)+n_2(25\ 25\ 25)+n_3(50\ 25\ 25)+n_4(75\ 25\ 25)+n_5(100\ 25\ 0)}{n_1+n_2+n_3+n_4+n_5} \quad (1)$$

Where, n_1 (10), n_2 (11).n_3 (9).n_4 (0) and n_5 10 at the numerator and denominator denote the number of judges conducted the evaluation. After calculation, the three values in the matrix represent the distribution function of judges' preference on the sensory scale for extruded products. Furthermore, the values of the triplets for color (SMI_A), taste ($SM1_X$), texture ($SM1_T$) and mouthfeel ($SM1_M$) were obtained as follows:

$$SM1_A = \frac{10(0,0,25)+11(25,25,25)+9(50,25,25)+0(75,25,25)+0(100,25,0)}{10+11+9+0+0}$$

$SM1_A$ (20.0000 15.8333 25.0000)
$SM1_C$ (19.1667 11.6667 25.0000) (2)
$SM1_X$ (41.6667 20.0000 25.0000)
$SM1_T$ (50.8333 22.5000 23.3333)
$SM1_M$ (52.5000 23.3333 25.0000)

Similarly, for optimized (O) product the values of the triplets for appearance ($SM2_A$), color ($SM2_A$), taste ($SM2_X$), texture ($SM2_T$) and mouthfeel ($SM2_M$) were obtained and are mentioned below:

$SM2_A$ (24.1667 16.6666 25.0000)
$SM2_C$ (20.0000 12.5000 25.0000) (3)
$SM2_X$ (45.0000 20.8333 25.0000)
$SM2_T$ (49.1666 22.5000 23.3333)
$SM2_M$ (47.5000 22.5000 25.0000)

5.11.3 Triplets for judges' preference to the importance of quality attribute

In this step, the most important attributes i.e. appearance, colour, taste, texture and mouthfeel of extrudate products will be identified on the basis of scores. Triplets for individual preference to the importance of attributes was calculated from the data (1) the sum of sensory scores (Table 5.6), (2) triplets associated with the sensory scales and (3) number of the panelists. For the control sample (C) the triplicates of attributes were calculated as follows;

$$Q1A = \frac{0(0,0,25)+1(25,25,25)+12(50,25,25)+10(75,25,25)+7(100,25,0)}{(0+1+12+10+7)} \quad (4)$$

$Q1_A$ (69.1666 25.0000 19.1666)

$Q1_C$ (52.5000 25.0000 21.6666)

$Q1_X$ (45.8333 25.0000 23.3333)

$Q1_T$ (58.3333 25.0000 21.6666)

$Q1_M$ (51.6666 25.0000 21.6666)

5.11.4 Overall sensory scores of the extruded samples triplets

Here, triplets obtained from (Eq. 2) were multiplied with triplets obtained from (Eq.4) to get an overall sensory score of each extrudate sample. The following eq. were obtained from multiplication of triplets (a, b, c) and (d, e, f):

(a, b, c) × (d, e, f) = (a × da × e + d × ba × f + d × c) (5)

Where, values of a and d ranged between 0 and 100, product a × d ranged between 0 and 1,000. Value of the first digit of the overall sensory ranged between 0 and 40,000. Therefore, value of the first digit of overall sensory score between 0 and 100 is necessary. Eq.4 were reduced by the factor $1/Q_{sum}$, where Q_{sum} is the sum of the first digit of the triplets of products

(control and optimized). "Relative weightage of the quality attribute" for appearance is defined as

$QA_{rel} = QA/Q_{sum}$,

Colour: $QC_{rel} = QC/Q_{sum}$,

Taste: $QX_{rel} = QX/Q_{sum}$,

Texture: $QT_{rel} = QT/Q_{sum}$ and

Mouthfeel: $QM_{rel} = QM/Q_{sum}$.

Therefore, Q_{sum} was calculated from Eq.4

Q_{sum} = (69.16 +52.50+45.83+58.33+51.66)

= 277.5

After Q_{sum}, the triplicates for relative weightage of quality attributes *viz.*, appearance (QA_{rel}) was further as

$$QA_{rel} = \frac{QC}{Q_{sum}} \tag{6}$$

= (69.16/277.5 25/277.5 19.16/277.5)

$QA_{re\,l}$ = (0.2492 0.0901 0.0691)

QC_{rel} = (0.1892 0.0901 0.0781) (7)

QX_{rel} = (0.1652 0.0901 0.0841)

QT_{rel} = (0.2102 0.0901 0.0781)

QM_{rel} = (0.1862 0.0901 0.0781)

Now, using Eq.5, overall sensory score SOC (control extrudate) can be obtained as,

$$SO_C = S1A*QA_{rel} + S1C*QC_{rel} + SIX*QX_{rel} + SIT*QT_{rel} + S1M*QM_{rel}$$

= (36.4196 35.6225 39.2157) (8)

$$SO_O = S2A*QA_{rel} + S2C*QC_{rel} + S2X*QX_{rel} + S2T*QT_{rel} + S2M*QM_{rel}$$

= (35.9543 35.1245 39.1030) (9)

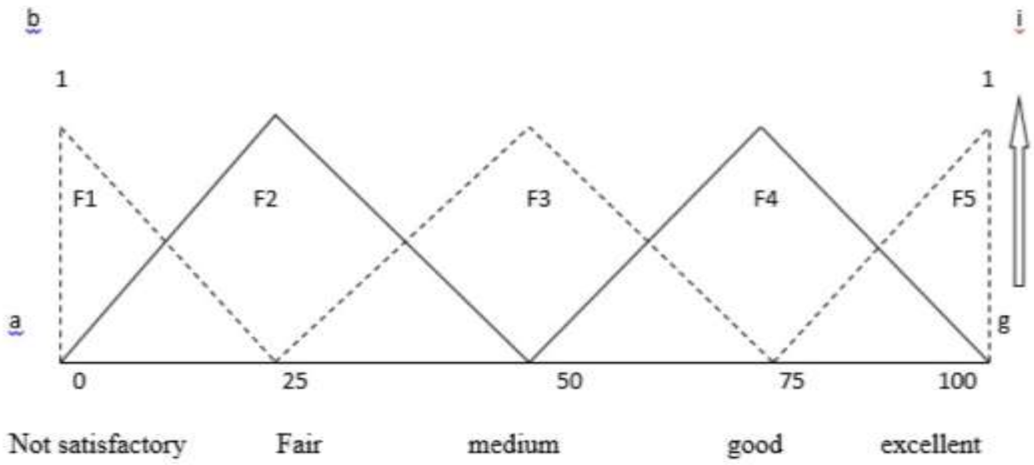


Fig.5.10 Distribution of 5-point sensory scale [2]

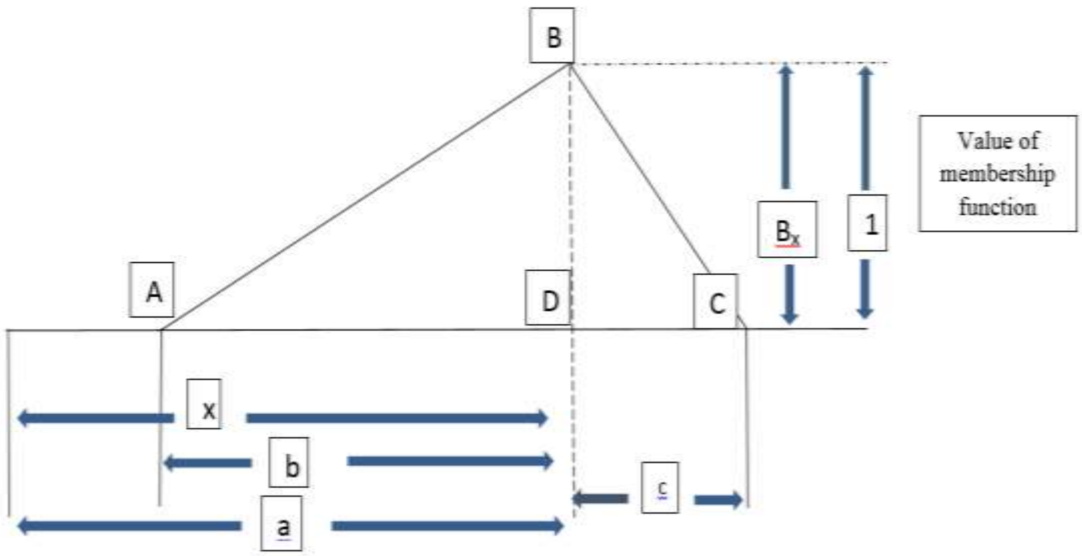


Fig.5.11 Graphical representation of triplet (a,b,c) and its membership function [2]

5.11.5 Standard fuzzy scale and ranking of products

Triangular membership function (Fig.5.10) also referred as standard fuzzy scale. The linguistic expressions of the standard fuzzy scale were set as not satisfactory/not at all necessary, fair/somewhat necessary, satisfactory/necessary, good/important and excellent/extremely important, respectively. In Fig 5.11, graphical representation of triplet (a, b, c) and was represented by a triangle ABC. The location of the centroid of the triangle ABC are depicted by the triplet (a, b, c). Triangular membership function distribution pattern of 5-point scale was presented in the Fig 5.10. and symbols were F1, F2, F3, F4 and F5. Each symbol represents sensory scales, and membership function of each of the sensory scale follows triangular distribution pattern showed in the graph where the maximum value of membership is 1, the values of which are defined by a set of 10 numbers.

$$X = \frac{a-(b-c)}{3}$$

(10)

Therefore, the ranking of control (X_C) and optimized (X_O) extrudate sample were

X_C 37.61 (11)

X_O 37.28

Value of *X* (Eq.7) ranked both the sample ranked on the basis of overall quality of sample which is as follows: X_C (control) > X_O (optimized).

5.11.6 Quality attribute ranking of extruded samples in general

Using Eq.10, quality attributes *viz.*, appearance (X_{QA}), color (X_{QC}), taste (X_{QX}), texture (X_{QT}) and mouthfeel (X_{QM}) were;

X_{QA}	67.22222
X_{QC}	51.38889
X_{QX}	45.27778
X_{QT}	57.22222
X_{QM}	50.55556

(12)

Therefore, the ranking of quality attributes was appearance >taste> color > mouthfeel >texture

Table 5.9 Membership function of 5 point linguistic scale

Scale factor	Symbols	Attribute values in fuzzy scale						
Poor/Not satisfactory	F1	1	0.5	0	0	0	0	0
Fair	F2	0.5	1	1	0.5	0	0	0
Satisfactory	F3	0	0	0.5	1	1	0.5	0
Good	F4	0	0	0	0	0.5	1	1
Excellent	F5	0	0	0	0	0	0	0.5

5.11.7 Membership function of overall sensory scores

Standard fuzzy scales values from F1, F2, F3, F4 and F5 were used to obtained value of membership function of overall scores of extrudates. Graphical presentation (Fig.11) showed that value of membership function is 1.

Bx can be expressed as,

$$B_X = \frac{X-(a-b)}{b} \text{ for } (a\text{-}b)<x<a \tag{13}$$

$$= \frac{(a+c)-X}{c} \text{ for } a<x<(a+c)$$

$$= 0 \text{ for all other values of X}$$

BX_1= (0 0.2610 0.5457 0.8304 0.8965 1 0.6408 0.3850 0.1293 0)

BX_2= (0 0.2583 0.5390 0.8197 0.9087 1 0.6537 0.3987 0.1437 0) (14)

5.11.8 Similarity analysis for products

Two samples obtained their respective membership functions (BX_1 and BX_2) from Eq. 8 BX_1 and BX_2 values were compared with the corresponding values of the membership function of standard fuzzy scale (F1-F5) from Table 5.9. Values of membership functions F1,F2,F3,F4 and F5 were obtained (Table 5.8) and also showed matrix having 10 elements. Similarity analysis is very used for distributing the overall sensory score (which has been obtained as a single triplet) out of five sensory scales of standard fuzzy scale. Similarity value (S_m) for the samples was defined as

$$S_m = \frac{F \times B^T}{Maximum\ of\ \left(F1 \times F1^T\ and\ B1\ \times B1^T\right)} \tag{15}$$

Table 5.10 Similarity values for extruded samples

Quality attributes	Similarity values for extruded samples	
	Control (C)	**Optimized (O)**
Poor/Not satisfactory	0.0373	0.0380
Fair	0.3487	0.3558
Satisfactory	0.7215	0.7278
Good	0.6665	0.6642
Very good	0.2511	0.2430
Excellent	0.0208	0.0188

In the Table 5.10, control extrudate product which has only red rice as the main ingredient showed highest similarity value 0.7215 (satisfactory) and optimized extrudate product which contain red rice and passion fruit powder showed highest similarity value 0.7278 (satisfactory). Therefore, highest similarity predicts the better acceptability by fuzzy logic tool.

5.12 Conclusion

In the present chapter, two extruded products namely C (control: red rice) and O (optimized:red rice incorporated with passion fruit powder) were carried out for sensory evaluation using fuzzy logic. Quality attribute *viz.*, appearance, colour, taste, texture and mouthfeel ranking of extruded samples in general were appearance >taste> color > mouthfeel >texture. The ranking of two products control and optimized were almost negligible X_C 37.61 > X_O 37.28. Similarity values for extruded samples showed that both samples were satisfactory.

References

[1] Bisharat, G, I., Oikonomopoulou, V, P., Panagiotou, N, M., Krokida, M, K. and Das, H. *Food Processing Operations Analysis*, Asian Books Private Limited, New Delhi,2005.

[2] Das, H. Food processing operations analysis. New Delhi: Asian Books, 2005.

[3] Jin, Z., Hsieh, F. and Huff, H, E. Effects of soy fiber, salt, sugar and screw speed on physical properties and microstructure of corn meal extrudate. *Journal of Cereal Science*, 22(2):185-194,1955.

[4] Jaya, S. and Das, H. Sensory evaluation of mango drinks using fuzzy logic, *Journal of Sensory Studies* 18:163-176, 2003.

[5] Jha, P., Das, A, J., Dash, K, K. and Deka, S,C. Sensory evaluation of black pigmented rice (*Oryza sativa cv. Poireton*) wine fortified with probiotic Lactobacillus acidophilus ATCC 4356 and Lactobacillus sakei ATCC 15521 using fuzzy. *Journal of Institute of Brewing*, 121:566-573, 2015.

[6] Lusas, E, W. and Rooney, L, W. Snack food processing. London, New York, Washington D.C.: CRC Press Boca Raton. p 639, 2001.

[7] Lazim, M, A. and Suriani, M. Sensory evaluation of the selected coffee products using fuzzy approach, *World Academy of Science, Engineering and Technology*, 50:717-720, 2009.

[8] Lincklaen, W., Westenberg, S, D., Jong, D,A., Meel, J, F, A., Quadt, E. and Duin, R, P. Fuzzy set theory applied to product classification by a sensory panel. *Journal of Sensory Studies* 4:55-72, 1989.

[9] Mezreb, K., Goullieux, A., Ralainirina, R. and Queneudec, M. Effect of sucrose on textural properties of corn and wheat extrudates. *Carbohydrate Polymers*, 64:1-8,2009.

[10] Martinez, L. Sensory evaluation based on linguistic decision analysis. *International Journal of Approximate Reasoning*, 44:148-164, 2007.

[11] Prinyawiwatkul, W., McWatters, K, H., Beuchat, L, R. and Phillips, R, D. Cowpea flour: A potential ingredient in food products. *Critical Reviews in Food Science and Nutrition*, 36:413-436, 1996.

[12] Silva, E, M, M., Ascheri, J, L, R., Carvalho, C, W, P., Takeiti, C, Y. and Berrios, J, J. Physical characteristics of extrudates from corn and dehulled carioca bean flour blend. *LWT-Food Science and Technology*, 58(2):620-626, 2014.

[13] Routray, W. and Mishra, H, N. Sensory evaluation of different drinks formulated from dahi (Indian yogurt) powder using fuzzy logic, *Journal of Food Processing and Preservation*, 36:1-10,2012.

[14] Thakur, S. and Saxena, D, C. Formulation of extruded snack food (gum based cereal-pulse blend): optimization of ingredients levels using response surface methodology. *Lebensmittel-Wissenschaf und-Technologie-Food Science and Technology*, 33:354-361, 2000.

6.1 Introduction

Extrusion cooking also known as high-temperature short time (HTST) processing technique, is gaining attention for novel food product development due to low-cost and high efficiency continuous cooking process.[28,3,12] In extruded foods base material used are cereals, starches, pulses and vegetable proteins mainly. Since these ingredients help to obtain structure, texture, bulk, mouth feel and various other characteristics desired for the final products.[20]

Moisture content in the food products can be used as the critical data which can evaluate the quality of products during food preservation. The quality of product totally depends upon the moisture content, moisture migration, or moisture uptake. Sorption or desorption state depends on vapor pressure of water present in the sample and surroundings. Equilibrium moisture content (EMC) increases with relative humidity (RH %) but decreases with increase in temperature in a particular food products. The phenomenon wherein the EMC during the adsorption and during the desorption process is different is termed as ‘‘hysterisis.’’.[22]

Water adsorption by any food products is a process where water molecules progressively and reversibly combine with the solids via chemisorption, physical adsorption, and multilayer condensation.[35] There are three region in an isotherm namely region A, B and C. In region A, water represents strongly bound water with enthalpy of vaporization considerably > pure water. The bound water (unfreezable) includes structural water (H-bonded water) and monolayer water.[17] In region B, water molecules bind less firmly. Also vaporization enthalpy is found to be slightly > pure water. Water in region C is loosely bound with the food materials.[22,23]

The moisture sorption isotherm (MSI) study is an important tool which can be used to predict the product stability, improve storing method and selection of packaging material.[4] It is also used to optimize or maximize the retention of color, flavor, texture, nutrients and biological stability.[29] Sorption isotherms of MSI information can be used to design, modelling and optimization of different processes like aeration, drying and storage respectively.[5,16] Silva et al.[30] stated that biomaterials behave as hygroscopic showed problem with moisture content and humidity and change its moisture content according to the humidity and temperature of surroundings.

6.2 Materials and methods

6.2.1 Raw materials

Optimized extruded sample was prepared by using twin extruder. Antioxidant stability during storage and MSI studies were carried in optimized sample.

6.2.2 Antioxidant stability during storage

Total phenolic content (TPC) of extruded product was evaluated during storage by using method described by Slinkard and Singleton.[34] The pre-weighed petri dishes with samples (2g) were placed in hermetically sealed glass desiccators using Na Cl (75% RH) and stored at 25˚C. The analyses of TPC was done at every 15 days interval for 120 days.[32]

6.2.3 Moisture sorption isotherm (MSI) studies

Moisture sorption isotherm (MSI) for red rice incorporated with passion fruit powder (O) extruded product was determined by Smith [32] method and later described by Plahar and Leung.[25] Products were dried at 70˚C for 24 h prior to equilibrating them. Three (25˚C,35˚C and 45˚C) different temperatures, eight saturated electrolytes solution *viz.*, lithium chloride (LiCl), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), magnesium nitrate ($Mg(NO_3)_2$), potassium iodide (KI), sodium chloride (Na Cl), potassium chloride (KCl) and potassium sulfate K_2SO_4), relative humidity (RH %) range from 11 to 97 % were used during MSI studies. Samples (2 g) were placed into a sterilized pre-weighed petri plates dishes in separate desiccators.[7] Equilibrium data was obtained after attaining three successive weight measurements which did not differ more than 0.001 g. Inside the each desiccator, 10 mL of beaker containing toluene was placed in order to prevent microbial spoilage.[8] The weight of each Petri dish containing samples was measured in 4 days interval. A graph was plotted following equilibrium moisture contents (EMC) *vs* water activity. Table 6.1 illustrated the list of salts solution with respective RH (%). The final equilibrium moisture content (EMC) was calculated by method described by Sant'Anna et al.[33] measuring the difference in the weight loss before and after drying the weight of sample at 105˚C for 3 h in a laboratory oven (Boi-Technics India). The relationship between equilibrium relative humidity (ERH) and water activity (a_w) is shown below.[31]

$$a_w = \frac{ERH}{100}$$

6.2.4. Mathematical model fitting and data analysis

Existing mathematical models were reviewed and used to predict the adsorption monolayer moisture content at different temperatures. The experimental values of EMC and ERH were obtained from experiment. Six mathematical models (Table 6.1) were fitted to experimental data (EMC versus a_w) and the constants. The models were calculated by nonlinear regression using MATLAB (Math Works, Inc., R2008a).Various researchers have been extensively using these isotherm models in food products.[9, 26] The goodness of fit and precision of selected models were on the basis of high coefficient of determination (R^2) and low root mean square error (RMSE).

Table 6.1 Equilibrium relative humidity of selected salt solution at various temperature levels[13]

RH (%) at temperature			
Saturated salt solution used	25 ˚C	35˚C	45˚C
Lithium chloride (LiCl)	11.30	11.25	11.16
Magnesium chloride ($MgCl_2$)	32.78	32.05	31.10
Potassium carbonate (K_2CO_3)	43.16	43.17	43.17
Magnesium nitrate ($Mg(NO_3)_2$)	52.89	49.91	46.93
Potassium iodide (KI)	68.86	66.96	65.26
Sodium chloride (NaCl)	75.29	74.87	74.52
Potassium chloride (KCl)	84.34	82.95	81.74
Potassium sulfate (K_2SO_4)	97.30	96.71	96.12

Table 6.2 Mathematical models used to fit the equilibrium moisture sorption isotherm of optimized product

Sl.no.	Model	Mathematical equation	Reference
1	Oswin	$M_W = A(\frac{a_w}{1-a_w})^B$	Andrade et al.[1]
2	Smith	$M_W = A + B\ln(-a_w)$	Andrade et al.[1]
3	Curie	$M_W = \exp(A + Ka_w)$	Curie [10]
4	Peleg	$M_W = Aa^c{}_w + Ba^k{}_w$	Basu et al.[4]
5	Langmuir	$\frac{1}{CM_o} = a_w(\frac{1}{M_w} - \frac{1}{M_O})$	Langmuir [18]
6	Brunauer-Emmett-Teller (BET)	$\frac{M}{M_0} = \frac{Ca_w}{(1-aw)[1+(C-1)a_w]}$	Brunauer et al.[6]

Note: $M_{w:}$ equilibrium moisture content (g water/100 g dry matter) and M_o : monolayer sorbet constant and a_W represent the water activity (decimal). A, B, K and C are respective model constants.

6.3 Results and discussion

6.3.1 Antioxidant stability during storage

The product was extruded from combination of pigmented rice and exotic fruit (passion fruit foam mat dried powder). Antioxidant stability (TPC) during storage of optimized product are presented in Fig.6.1 and values are stated in Table 6.3. From the Fig.6.1, it can be concluded that TPC (mg GAE/100 g of dry solid) showed amount of total phenolic acid changes was very less with respect to storage duration (120 days). Antioxidant and phenolic compounds degradation are affected by various parameters *viz.*, light, temperature, air oxidation phenomenons, etc. [36]

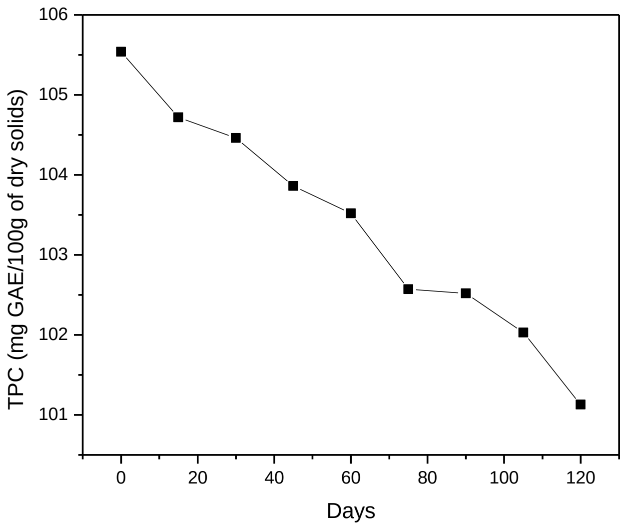


Fig. 6.1 Antioxidant stability during storage of sample

Table 6.3 Storage study of antioxidant stability of TPC

Total Days	Total phenolic content (mg GAE/100 g dry matter)
0	105.54±0.40
15	104.72±0.45
30	104.46±0.23
45	103.86±0.18
60	103.52±0.47
75	102.57±0.32
90	102.52±0.31
105	102.03±0.02
120	101.13±0.08

Note: values were calculated in triplicates (n=3) as mean ± SD

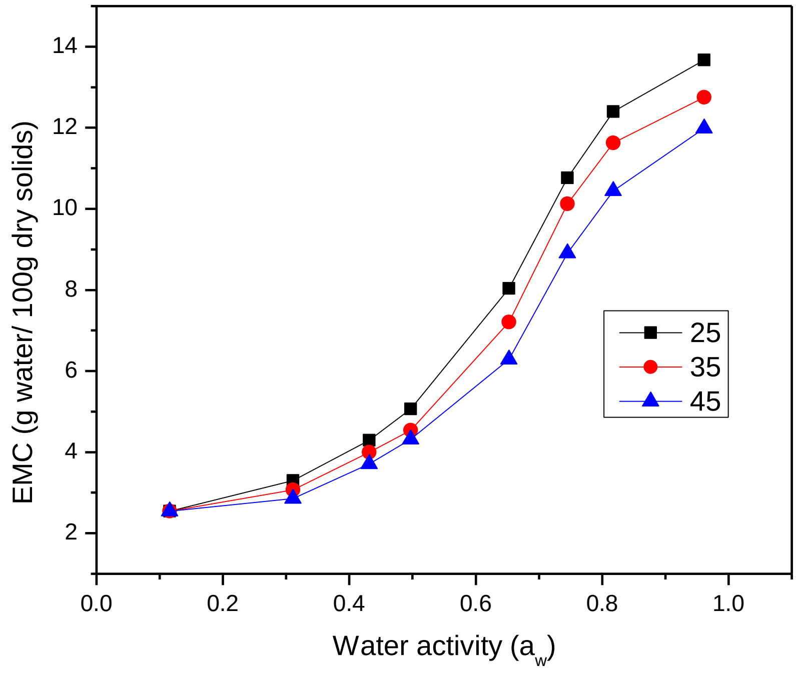


Fig 6.2 Moisture sorption isotherm (MSI) of optimized sample at three different temperatures

MSI of optimized sample at different temperatures (25C˚, 35˚C and 45˚C) are presented in Fig 6.2. The shape of the graph resembles more of Sigmoid S-shaped curves of type II types at given temperatures between water activity and EMC (dry basis) data. Many researchers have also concluded similar finding extruded products.[19, 24] Initially, slow increase in EMC was observed until 0.6 a_w and after that abrupt increase in graph was observed. Initial slow increase might be attributed to the hydrophilic nature of carbohydrates and protein in extrudate. At higher temperature, the possibilities of reduction in active sites for water binding may occur which leads to decrease EMC. Also, water molecules in the extrudates attain higher energy levels and smash away from the water binding sites, eventually led to lower EMC.[27] Demertzis et al.[11] also stated that above mentioned situation make the food material less hygroscopic at higher temperatures. In starchy food products, crystalline regions of starch (amylopectin) are reported to be unaffected to moisture diffusion process and therefore, increase in EMC may act as a plasticizers for amylose region (amorphous).[2]

6.3.2 Mathematical modeling and fitting of moisture sorption data

The sorption isotherm models, their respective constants and statistical parameters are presented in Table 6.4. The EMC values were obtained from experimental data of control and optimized extruded products at three different temperatures (25-45˚C) and water activity (a_w) ranged 0.11 to 0.97 were fitted to six MSI models. Mathematical models used were Oswin, Smith, Curie, Peleg, Langmuir and Brunauer-Emmett-Teller (BET). The model constants and statistical parameters were showed in Table 6.4 (R^2, Adj.R^2, RSME and SSE) and from the value best fit was predicted. In the optimized extruded sample, at 25˚C Langmuir model showed R^2 0.98, adj. R^2 0.97, RSME 0.62 and SSE 2.35, at 35 ˚C Peleg model showed R^2 0.99, Adj. R^2 0.99, RSME 0.33 and SSE 0.44 and finally at 45 ˚C, it was recorded that R^2 0.99, adj. R^2 0.98, RSME 0.45 and SSE 0.83. Therefore, from the data, it can be determined that at 25 ˚C, Langmuir model and at 35 and 45 ˚C, Peleg model showed better fitting during the prediction of EMC of extrudate. Various researcher reported that Peleg model showed better suitable model during the prediction of EMC of starchy powders e.g. yam, [21] potato [15] and pistachio nut [14] at different storage temperatures.

Table 6.4 Sorption isotherm models and their respective constants and statistical parameters

Storage temperature 25˚C									
Model	**Model coefficients/constants**					**Goodness of fit parameters**			
	A	**B**	**K**	**C**	**M_o**	**R2**	**Adj. R^2**	**RSME**	**SSE**
Oswin	6.43	0.24				0.8	0.77	2.04	25.19
Smith	6.36	0.23	-	-	-	0.43	0.34	3.46	72.14
Curie	1.89	0.02	-	-	-	0.39	0.29	3.59	77.32
Peleg	-109.5	123.8	1.16	1.15	-	0.98	0.96	0.79	2.5
Langmuir	-	-	-	0.73	3.01	0.98	0.97	0.62	2.35
Brunauer-Emmett-Teller (BET)	-	-	-	0.001	3.11	0.96	0.96	0.83	4.16
Storage temperature 35˚C									
Oswin	5.85	0.25	-	-	-	0.87	0.85	1.37	11.39
Smith	6.03	2.18	-	-	-	0.95	0.95	0.8	3.85
Curie	0.76	-	1.88	-	-	0.98	0.98	0.5	1.53
Peleg	0.66	12.64	1.39	-0.35	-	0.99	0.99	0.33	0.44
Langmuir	-	-	-	0.002	3.22	0.97	0.97	0.62	2.34
Brunauer-Emmett-Teller (BET)				0.003	3.19	0.98	0.98	0.47	1.34
Storage temperature 45˚C									
Oswin	5.8	0.26	-	-	-	0.91	0.9	1.08	7.07
Smith	5.51	0.33	-	-	-	0.61	0.55	2.36	33.6
Curie	0.78	-	1.86	-	-	0.39	0.29	3.59	77.32
Peleg	8.33	5.71	3.72	0.64	-	0.99	0.98	0.45	0.83
Langmuir	-	-	-	0.002	3.18	0.96	0.95	511	3.38
Brunauer-Emmett-Teller (BET)	-	-	-	0.005	3.15	0.98	0.97	0.54	1.76

Note: M_w : monolayer moisture content (g water/100 g dry matter) and M_o : monolayer sorbet constant and a_w represent the water activity (decimal). A, B, K and C are respective model constants.

6.4 Conclusion

Total polyphenols content (TPC) of extrudate showed slow degradation of antioxidant content. The sorption isotherm study of extrudate at three different temperatures (25-45˚C) and a_w level (ranged from 0.11-0.97) were carried out by standard static-gravimetric method using various salt solutions revealed Sigmoid shape resembling type II isotherm was observed which is very typical of food material. Six model fitting concluded that at 25 ˚C Langmuir model showed the best fit. And at 35 ˚C and 45 ˚C Peleg model predicted as most suitable model to practice MSI study of optimized extrudate.

Reference

[1] Andrade, P, R, D., Lemus, M,R. and Perez, C,C,E. Models of sorption isotherms for food: uses and limitations. *Vitae, Revista De La Facultad De Quimica Farmaceutica*, 18(3):325-334, 2011.

[2] Al-Muhtaseb, A,H., McMinn, W,A,M. and Magee, T,R,A. Moisture sorption isotherm characteristics of food products: a review. *Food Bio production Processing*, 80(2):118-128, 2002.

[3] Abd el-hady, E,A., Mostafa, G,A., El-samahy, S,K. and El-saies, I,A. Production of high fiber corn extrudates. *Journal of Agricultural Science, Mansoura University*, 23:1231-1245,1998.

[4] Basu, S., Shivhare, U, S. and Mujumdar, A, S. Model for sorption isotherm for food: A review. *Drying Technology*, 24(8):917-930, 2006.

[5] Bala, B, K. Physical and thermal properties of malt. *Drying Technology*, 9(4):1091-1104,1991.

[6] Brunauer, S., Emmet, P,H. and Teller, E. Adsorption of gases in multimolecular layers. *Journal of the American Chemical Society*, 60:309-319, 1938.

[7] Bisharat, G,I., Eleni, P, N., Panagiotou, N, M., Krokida, M, K. and Maroulis, Z, B. Thermal, textural, and physicochemical analysis of corn extrudates enriched with broccoli or olive paste. *International Journal of Food Properties*, 17(9):2100-2116,2014.

[8] Cassini, A, S., Marczak, L, D, F. and Norena, C,P, Z. Water adsorption isotherms of texturized soy protein. *Journal of Food Engineering*, 77:194-199,2006.

[9] Cardoso, J, M. and de Silva P, R. Hygroscopic behavior of banana (*Musa ssp*. AAA) flour in different ripening stages. *Food Bio production and Processing*, 92(1):73-79, 2014.

[10] Caurie, M. Derivation of full range moisture isotherms. In Water Activity: Influences on Food Quality (L.B. Rockland and G.F. Stewart, eds.) pp. 63-87, Academic Press, New York,1981.

[11] Demertzis, P, G., Riganakos, K, A. and Kontaminas, M, G. Water sorption isothermsfor crystalline raffinose by inverse gas chromatography. *International Journal of Food Science and Technology*, 24:629-636, 1989.

[12] Ding, Q., Ainsworth, P., Tucker, G. and Marson, H. The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice-based expanded snacks. *Journal of Food Engineering*, 66:283-289, 2005.

[13] Greenspan, L. Humidity fixed points of binary saturated aqueous solutions. Journal of Research, National Bureau of Standards (US), Series A, Physics and Chemistry, 81:89-96,1977.

[14] Hayoglu, I. and Faruk, O, G. Water sorption isotherms of pistachio nut paste. *International Journal of Food Science*, 42:224-227, 2007.

[15] Iguedjtal, T., Louka, N. and Allaf, K. Sorption isotherms of potato slices dried and texturized by controlled sudden decompression. *Journal of Food Engineering*, 85:180-190,2008.

[16] Kumar, P. and Mishra, H, N. Moisture sorption characteristics of mango-soy fortified yogurt powder. *International Journal of Dairy Technology*, 59:22-28,2006.

[17] Kinsella, J, E. and Fox, P,X. Water sorption by proteins: milk and whey proteins. CRC, *Critical Reviews in Food Science and Nutrition*, 24:91-139,1986.

[18] Langmuir, I. The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society*, 40:1361-1402, 1918.

[19] Lazou, A. and Krokida, M. Thermal characterization of corn-lentil extruded snacks. *Food Chemistry*,127: 1625-1633,2011.

[20] Launay, B. and Lisch, J, M. Twin-screw extrusion cooking of starches: Flow behavior of starch pastes, expansion and mechanical properties of extrudates. *Journal of Food Engineering*, 2: 259-80, 1983.

[21] Montes, E., Torres, R., Andrade, R., Perez, O., Marimon, J. and Meza, I. Models of desorption isotherms of yam (*Dioscorea rotundata*). *Dyna*, 72:145-152,2009.

[22] Mujumdar, A, S. and Devahastin, S. Fundamental principles of drying. In Mujumdar's Practical Guide to Industrial Drying; Devahastin, S., Ed.;Exergex Corp: Montreal,1-22,2000.

[23] Mujumdar, A,S. Ed. Handbook of Industrial Drying, 3rd Enhanced Ed; CRC Press: Boca Raton, FL, 2006.

[24] Norajit, k., Gu, B. and Ryu, G. Effects of the addition of hemp powder on the physicochemical properties and energy bar qualities of extruded rice. *Food Chemistry*, 129:1919-1925, 2011.

[25] Plahar, W, A. and Leung, H, K. Storage stability of dehydrated and Soy-fortified fermented maize meal. *Journal of Food Science*, 50:182-187,1985.

[26] Peng, G., Chen, X, Wu. and Jiang, X. Modeling of water sorption isotherm for corn starch. *Journal Food Engineering*, 80(2):562-567, 2007.

[27] Palipane, K, B. and Driscoll, R,H. Moisture sorption characteristics of in shell macadamia nuts. *Journal of Food Engineering*, 18:63-76, 1992.

[28] Ryu, G, H., Neumann, P, E. and Walker, C,E. Effects of some baking ingredients on physical and structural properties of wheat flour extrudates. *Cereal Chemistry*, 70:291-297,1993.

[29] Rizvi, S, S, H. Thermodynamic properties of foods in dehydration. In M, A, Rao, and S, S.,H. Rizvi (Eds.), Engineering properties of foods (pp. 223-309). New York: Marcel Dekker,1995.

[30] Silva, C, de L, O,C., Faria, L, J,G, de. and Costa, C, M, L. Comportamento higrosc opico de partes aereas de pimenta-de-macaco (*Piper aduncum* L.). *Revista Brasileira de Engenharia Agrícola e Ambiental*, 19(4):376-381,2015.

[31] Sahin, S. and Sumnu, S,G. Water activity and sorption properties of foods. In Physical Properties of Foods, S. Sahin and S.G. Sumnu, eds. pp. 193-226, Springer Science1Business Media, New York, 2006.

[32] Smith, P. The determination of equilibrium relative humidity or water activity in foods. A literature review, BFMIRA, Leatherhead, 29p,(Scientific and Technical Surveys,70),1971.

[33] Sant'anna, V., Englert, A, H., Correa, A,P,F., Brandelli, A., marczak, L,D,F. and Tessaro, I,C. Grape marc powder: Physicochemical and microbiological stability during storage and moisture sorption isotherm. *Food Bioprocess and Technology*, 7:2500-2506, 2014.

[34] Slinkard, K. and Singleton,V, L. Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28:49-55, 1977.

[35] Van den B, C. and Bruin, S. Water activity and its estimation in food systems: Theoretical aspects. In Water Activity: Influences on Food Quality; Rockland, L.B., Stewart, G.F., Eds.; Academic Press: NewYork, 1-61,1981.

[36] Yousif, A., Durance,T,A., Scaman, C,H. and Girard, B. Headspace volatiles and physical characteristics of vacuum microwave, air, and freeze-dried oregano (*Lippiaber landieri Schauer*). *Journal of Food Science*, 65(6):926-930, 2000.

7.1 Introduction

Diabetes mellitus is a multifarious metabolic disorder where concentration of fasting plasma glucose (FPG) is higher than 126 mg/dL, or in which blood glucose levels are above 200 mg/dL at any time of day. [2, 55] India is reported to have highest incidence of diabetics in the world with 3.8% of the rural population and 11.8% of urban population diagonised with having the disease. [52] There are two types of Diabetes mellitus namely type 1 and type 2 [27, 1] Type 2 diabetes mellitus (T2DM) accounts for approximately 90 % cases of diabetes cases and will soon declared a severe global epidemic of the twenty-first century.[26] According to King et al.[29] countries with the highest incidence of T2DM will India, China and United States. Dixit and Pokala[11] estimated that diabetes will effect over 100 million people within the Indian population by the year 2030.

Dipeptidyl peptidase-4 (DPP4) inhibitors are a novel type of oral glucose-lowering agents that regulate fasting plasma glucose, postprandial glucose, and HbA1c levels in the body. They act to halt the physiological breakdown of the incretin hormones glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), decreasing the inactivation of endogenous incretins to trigger the release of insulin in a glucose dependent manner.[12,46] Pharmacological involvement and clinically approved dipeptidyl peptidase-4 (DPP4) inhibitors are of vital importance in treating the diabetes epidemic.[15,21,22,34] Moreover, GLP-1 mimetics and GLP-1 secretagogues have proved effective in managing the disease. Glucagon-like peptide-1 (7–36)-amide is a 30-amino acid polypeptide which is secreted from gut endocrine L-cells and a potential therapeutic agent for the treatment of T2DM. GLP-1 acts as a formidable incretin to modulating postprandial blood glucose levels, stimulating glucose-dependent insulin secretion and inhibiting glucagon secretion and gastric emptying.[53,12] GLP-1 also regulates other diverse cellular processes to include increasing beta-cell mass by stimulating cell proliferation, inhibiting beta-cell apoptosis, aiding in the differentiation of primary neuronal (PC12) cells through stimulating cyclic AMP production and has suggested neuro and cardio protective effects.[32,45,51,7]

Pigmented red rice is an underutilized crop in North-eastern part of India. Over 400 plants are reported to have glucose lowering effects.[14] Lee et al.[33] reported that whole grains and cereals are recommended for diabetes to control blood glucose.This present study will investigate antidiabetic effects of *Oryza sativa* L. particularly its ability to inhibit contains DPP-4 and enhance GLP-1 secretion, to determine whether it can be incorporated into a healthy cereal product. The functional foods concept gaining much publicity amongst the health conscious population and these foods are becoming well known for their ability to prevent and manage chronic diseases such as diabetes, obesity and hypertension [44,5] Rice is an important cereal and staple food crop for half of the world's population.[42,48] Bhonsle and Sellappan[6] illustrated that some rice varieties have antidiabetic properties. This suggest that these staple food product could be demonstrated as functional food and exploited as a preventive or management therapy for diabetes.

Rice bran, a by-product of rice milling, contains many bioactive compounds such as lipophilic components including γ-oryzanol, tocotrienols, and tocopherols and phenolic compounds such as ferulic acid, sinapic acid, and protocatechuic acid.[16,47] Bioactive compounds in rice bran exhibit tremendous health benefits to combat various disease such as cardiovascular health [50] inhibition of cancer,[40] and improvements in glucose homeostasis.[31] Pigmented or colored rice bran contains both lipophilic and phenolic compounds with antioxidant effects[41,39] cancer inhibiting effects [40,10] and can inhibit α-glucosidase activity.[54] It has also been reported that rice bran may prevent the development of fructose-induced insulin resistance in rats.[23]

Extrusion cooking technology is extensively used for the development of new products in a short time period. The raw material (feed) undergoes physicochemical changes mostly starch gelatinization, protein denaturation, amylose-lipid complex formation and degradation of heat sensitive components such as vitamins, antioxidants, and pigments.[25] Using cereal and pulses as raw material various studies have reported that the extrusion cooking process results in low fat, high fibre and protein rich extrudates.[3,17,28,35] Various parameters are responsible for the outcome of extrudates to include types of extruder, feed material, material moisture content, barrel temperature, and screw speed.[30] It has been well established that diet contributes to the development of diabetes, therefore producing a nutritionally rich extrudate with anti-diabetic properties could provide a strategy for the management of disease. Various studies reported the association between the consumption of certain foods, their constituents, and the incidence of diabetes. Some studies have identified peptides and phenolic compounds which can control the level of blood glucose.[36]

In plant, various mineral elements are present and accumulate in different proportion after absorption from soil.[18] Arsenic (As) is a category 1 carcinogen by the IARC Monographs and is considered as a toxic element to humans, animals and plants. It is a consequence of different contaminants to include industrial production of pesticides, herbicides, wood preservatives and mining, and can severely compromise human health [8,38] Several species (As) are reported in rice and include arsenite (As (III)), arsenate (As (V)), dimethyl arsenic acid (DMA) and monomethyl arsonic acid (MMA).[13,43] Arsenite and arsenate are inorganic arsenic (i-As) species and are carcinogenic in nature,[37] whereas organic As species MMA and DMA are less toxic but can be cancer promoters. [24]

In this study, the potential antidiabetic effects of pigmented red rice will be investigated. Moreover, the red rice will undergo an extrusion process to determine whether this can enhance antidiabetic effects. The safety of the rice and extruded product will be assessed for their arsenic content to ensure it is within the recommended levels. Finally, rice and extrudates will undergo solvent fractionation to determine the optimal extract for the use as a therapeutic agent or incorporation as a functional food.

7.2 Material and methods

7.2.1 Materials

Red rice (*Oryza sativa* L.) locally known as *Umling ame* (UA), and white rice *Pungpo ame* (WR) were collected from Manigong Circle, Arunachal Pradesh (India). Rice bran (RB) was obtained from UA by using polisher (Satake, Japan). The removed bran rice was also collected and named as polished red rice (PRR). Extruded products: control (C) extrudate (solely red rice: UA) and optimized (O) extruded products (UA incorporated with 11.25% passion fruit powder) were prepared using a twin extruder (Model FUE-1F,Flytech Engineering, Chennai, India. Before the extrusion process, the experiments were design by using central composite design (CCD) of total 30 runs. Optimization and validation was done in previous Chapter 5(A) .

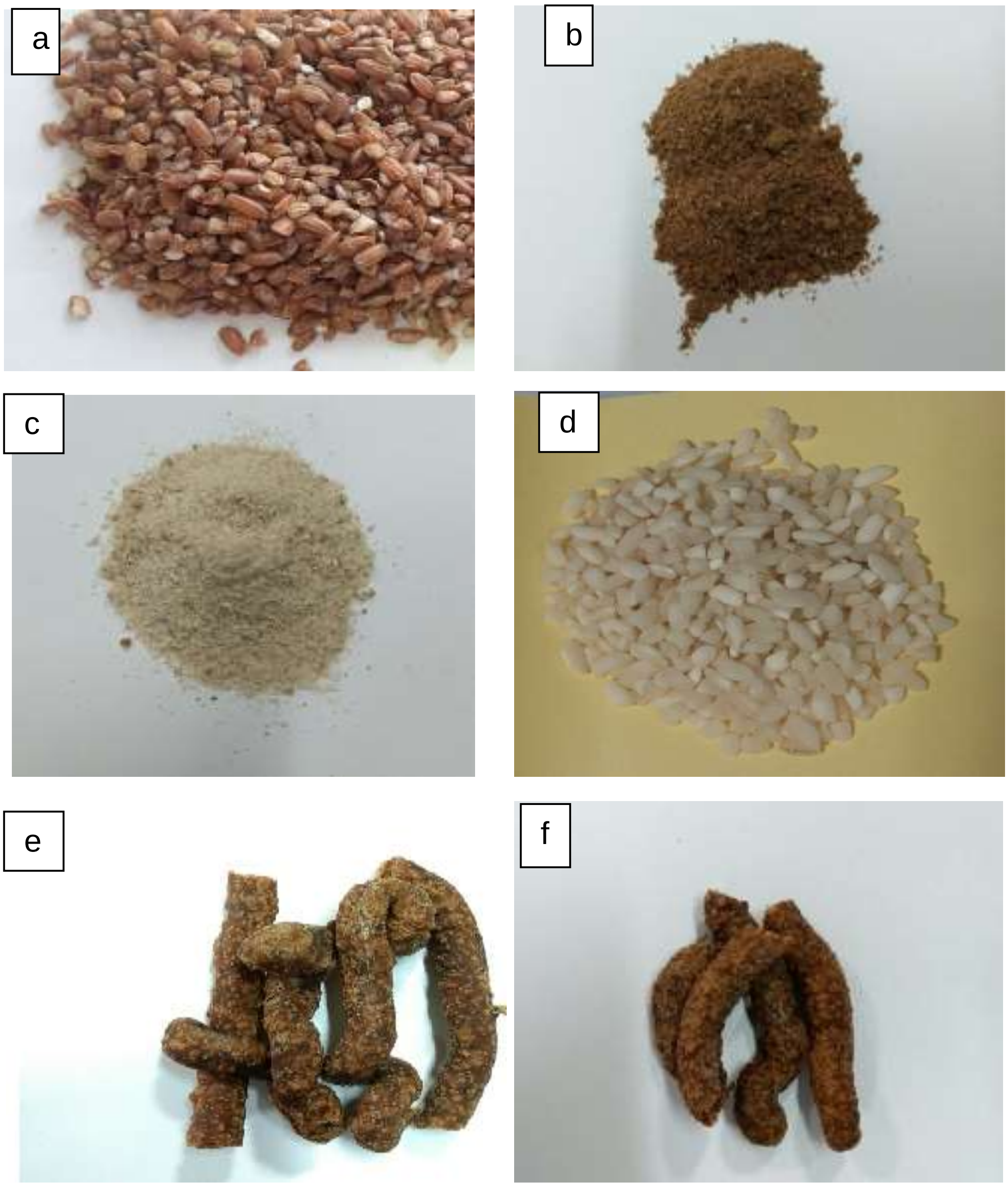


Fig 7.1. Photographs of six samples tested. (a) red rice locally known as umling ame (UA), (b) red rice bran (RB), (c) white rice locally known as pungpo ame (WR), (d) Red rice polished (PRR), (e) extruded red rice (C: control) and (f) extruded red rice incorporated with passion fruit powder (O :optimized)

7.2.2 Extraction

Bioactivity guided fractionation was carried out on rice samples (10g) by immersing the crude powder in 50 ml of solvents of different polarity *n*-hexane, 50:50 ethanol: water and water. They were kept on a roller mixer overnight at room temperature. Supernatants were obtained by filtration before allowing the solvents to evaporate on a heat block at 50˚C. Dried extracts were stored at -20 ˚C prior to use in assay.

7.2.3 Determination of DPP-4 inhibition activity

DPP-4 inhibition of rice extracts was carried out in a 96-well plate as previously described by Fujiwara and Tsuru. [20] Sample extracts were dissolved in HEPES buffer (p H 7.4) at a concentration of 2mg/ml and assessed fluorometrically using Gly-Pro-aminomethylcoumarin (1mmol/l; BaChem Ltd, Switzerland) and purified porcine DPP-4 (1U/ml: Merk Chemical, UK) Berberine (1mg/ml: Flourochem) was used as a positive control as it has already shown potent DPP-4 inhibitory activity.[2] After addition of all solution (n=3) incubate at 37˚C for 1 h with agitation. Later, 100 µL of 5mM acetic acid was added to stop the reaction. The plate was read using a fluorescent microplate reader (Tecan Saffire II, Reading,UK) at excitation and emission wavelength of 351 and 430nm,respectively.

7.2.4 STC-1 pGIP/Neo cell culture studies

STC-1 cells transfected with a plasmid (pGIP/Neo) encoding neomycin phosphotransferase were obtained from Dr. B. Wice (Washington University of St.Louis) with permission from Dr D. Hanahan (University of California, San Francisco, CA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/l D-glucose with L-glutamine, without sodium pyruvate (Gibco, Paisley, UK) and supplemented with 10% foetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 400 µg/ml geneticin (G418 disulfate salt; Sigma, UK). Cells were incubated in a 5% CO2 humidified atmosphere at 37˚C and used between passage numbers 20 - 50 when 70 – 90 % confluence had been reached. STC-1 pGIP/Neo cells were seeded in 12 well plates at a density of 2 million cells per well with 1 ml DMEM and incubated overnight at 37˚C in a 5% CO2 humidified atmosphere to allow attachment. Media was removed and cells were washed twice with HEPES buffer (pH 7.4) and pre-incubated in HEPES for 1 h. After removal of buffer, samples at 2 mg/ml were reconstituted in HEPES and added to cells in triplicate for 3 h. After the incubation period supernatant was removed, centrifuged at 1000 g for 10 min to remove cellular debris and stored at -20˚C prior to analysis.

7.2.5 Measurement of cell viability

Trypan blue was used to assess cell viability. Trypsin (1 ml) was added to each well and incubated at 37˚C for 1 min to detach cells. Following this, 1 ml of DMEM was added to neutralise trypsin and resulting solution was centrifuged at 1000 g for 5 min. The supernatant was discarded and cells were re-suspended in DMEM. The cell suspension was added to trypan blue (1:1) and cell viability measured using a Countess Automated Cell Counter (Invitrogen, Life Technologies Ltd, UK).

7.2.6 Determining GLP-1 secretion from STC-1 pGIP/Neo cells

For determining hormone secretion from STC-1 pGIP/Neo cells, GLP-1 levels were measured by means of ELISA (Millipore, UK) in accordance to manufacturer's instructions. For total GLP-1 determination, assay buffer, standards and samples were incubated in the pre-coated 96 well plate overnight at 4˚C. Plates were washed 5 times with wash buffer and detection conjugate was added to each well and incubated for a further 2 h. The plate was again washed 3 times prior to 20 min incubation with substrate. Stop solution was added to each well and the fluorescence measured on a fluorescence plate reader (Tecan Saffire II; Reading, UK) at excitation and emission wavelengths of 355 nm and 460 nm, respectively.

7.2.7 Inductively coupled plasma mass spectrometry (ICP-MS) of samples

7.2.7.1 Sample preparation for arsenic (As) speciation

Sample preparation for As speciation was described by using Signes-Pastor et al. [49] method. Samples were dried and milled for 3 min at 500 rpm with a 1 min rotation and a reverse rotation using a Retch PM100 rotary ceramic ball mill. The samples (0.1g) were weighed accurately to a weight of 0.1 g into 50 ml polypropylene centrifuge tubes to which 10 ml of 1% conc. Aristar nitric acid was added and allowed to sit overnight. Batches were prepared with a blank rice CRM (NIST 1568b Rice flour) which has the As species As_i, and dimethylarsinic acid (DMA) concentrations certified. Later, microwave digestion was done in an CEM MARS 6 instrument for 30 min at 95˚C using a 3 stage slow heating program: to 55 ˚C in 5 min held for 10 min., to 75 ˚C in 5 min., held for 10 min. to 95 ˚C in 5 min., held for 30 min. A 1 ml aliquot was transferred to a 2 ml polypropylene vial and then, 10 µl of analytical grade hydrogen peroxide was added to convert any arsenite to arsenate to facilitate subsequent chromatographic detection.

7.2.7.2 Chemical analysis

Sample were digested in 1% nitric acid and digested sample solutions were run on a Thermo Scientific IC5000 Ion Chromatography (IC) system, with an Thermo AS7,2 × 250 mm column (and a Thermo AG7, 2 ×50 mm guard column) and a gradient mobile phase (A: 20 mM Ammonium Carbonate, B 200 mM Ammonium Carbonate – Starting at 100% A, changing to 100% B, in a linear gradient over 15 min.) interfaced with a Thermo ICAP Q ICP-MS that monitored m/z+ 75, using He gas in collision cell mode. The chromatograms obtained were compared with that for authentic standards; DMA and As_i. As present under each chromatographic peak was calibrated using a DMA concentration series.

7.2.8 Statistical analysis

Statistical analysis was conducted using Graph Pad Prism version 5 software (Graph Pad Software, USA). All experiments were carried out in triplicates. Data are expressed as mean± SEM and analysis by one -way ANOVA with Tukey's multiple comparison test (*p<0.05,**p<0.01,***p<0.001).

7.3 Results and discussion

Sequential extraction was carried out on each sample. The yield obtained from the different solvents varied greatly, ranging from 0.21 – 1.91 % (Table 1). The most effective extractant was hexane, resulting in yields of ≥ 0.82 %. Extruded products produced the largest yields when compared to the raw rice materials. On extraction with hexane, C (control) and O (optimized) produced 1.44 % and 1.04 % yields, respectively. With ethanol: water as an extractant, C (control) and O (optimized) produced a yield of 1.02 % and 1.49 %, respectively, and with the use of water, C(control) and O (optimized) produced yields of 1.18 % and 1.91 %, respectively. This demonstrates that when red rice products are extruded, more compounds, or a larger amount of specific compounds, can be extracted from the material, potentially giving greater scope for the use of this product as a functional food considering the scale up processes needed to ensure the cost-benefit and feasibility of production.

Table 7.1. Percentage yield obtained from each sample when extracted using different solvents

Solvent used	Sample	Yield (%)
n-hexane	UA	0.82
	PRR	0.82
	WR	0.99
	RB	0.91
	C	1.44
	O	1.04
Ethanol:water (50:50)	UA	0.49
	PRR	1.01
	WR	0.37
	RB	1.22
	C	1.02
	O	1.49
Water	UA	0.26
	PRR	0.21
	WR	0.60
	RB	0.80
	C	1.18
	O	1.91

7.3.1 Inhibition of DPP-4 activity

Several researchers have demonstrated the ability of foods and food products to inhibit DPP-4 (refs). Here, hexane extracts of red rice and extrudates were unable to inhibit the activity of the enzyme (Fig. 2(a)). However, all ethanol:water extracts of rice samples inhibited DPP-4 activity significantly ($p < 0.001$; Fig 7. 2(b)). Specifically, RB was the most potent at inhibiting DPP-4 activity by 70.48±1.06 %, followed by UA (42.55±0.84 %), PRR (35.91±1.27 %), WR (29.14±1.23 %), O (25.49±1.86 %), then C(13.55±3.97 %). Furthermore, WR when extracted with water was able to significantly inhibit DPP-4 activity by 8.78±4.84 % when compared to a buffer control ($p < 0.001$; Fig 7. 2(c)). DPP-

4 inhibitory activity was found to be retained in the ethanol: water extracts of both extruded products C (control) and O(optimized), albeit at reduced levels. Extracts from O (optimized) containing passion fruit powder, had greater inhibitory activity than the control extrudate, suggesting that passion fruit may possess its own antidiabetic activities.

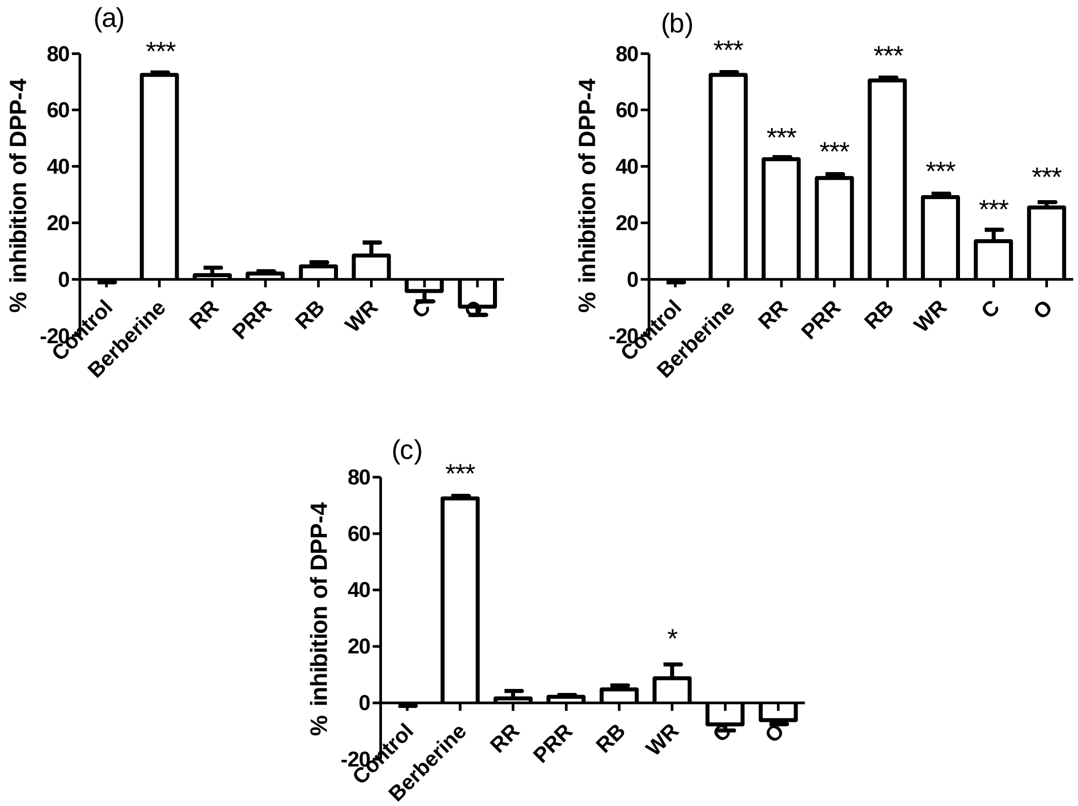


Fig 7.2. DPP-4 inhibition activity

Inhibition of DPP-4 by rice samples extracted by (a) hexane, (b) ethanol:water (50:50), and (c) water at 2 mg/ml. Bars represent mean ± SEM. Data analysed using one way ANOVA followed by Tukey's Multiple Comparisons Test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$; n=3). Berberine at 1 mg/ml was used as a positive control. RR = red rice, PRR = red rice polished, RB = red rice bran, WR = white rice, C = control extrudate and O = optimized extrudate

7.3.2 GLP-1 secretion

The ability of rice extracts to enhance GLP-1 secretion is illustrated in Fig. 7.3. *n*-Hexane extracts were able to potently stimulate GLP-1 secretion. In particular, PRR, C(control) and O(optimized) enhanced secretion of GLP-1 3.14-fold (p<0.01), 3.48-fold (p<0.001) and 6.06-fold (p<0.001), respectively when compared to buffer control (Fig. 3(a)). Ethanol: water extracts of WR and O(optimized) also significantly stimulated GLP-1 secretion 3.33-fold and 4.19-fold, respectively (p<0.001; Fig. 3(b)). None of the water extracts had any effect on incretin secretion (Fig. 7.3(c)).

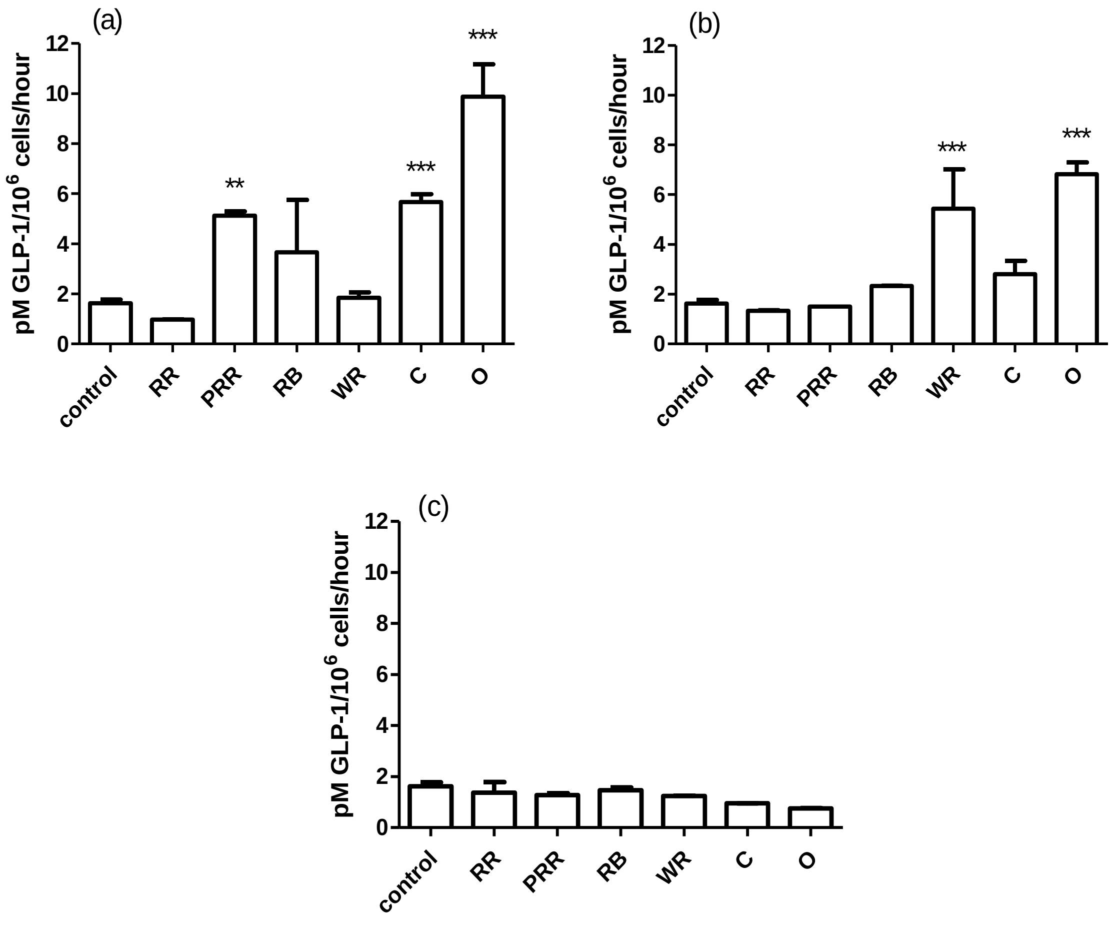


Fig.7.3 Effect of different rice sample extracts on GLP-1 secretion.

GLP-1 secretion stimulated from STC-1 pGIP/Neo cells by rice samples extracted by (a) hexane, (b) ethanol:water (50:50), and (c) water at 2 mg/ml. Bars represent mean ± SEM. Data analysed using one way ANOVA followed by Tukey's Multiple Comparisons Test

(* $p<0.05$, ** $p<0.01$, *** $p<0.001$; n=3). UA = red rice, PRR = red rice polished, RB = red rice bran, WR = white rice, C = control extrudate and O = optimized extrudate.

7.3.3. Inductively coupled plasma mass spectrometry (ICP-MS) for elemental analysis

ICP-MS was conducted in the four rice raw materials (UA, WR, RB and PRR) samples and the two extrudate products (C and O) to determine levels of arsenic. As mentioned, arsenic is highly abundant in rice and is carcinogenic in nature [13,43] therefore arsenic was measured to determine whether these rice samples were 'safe' for consumption. As shown in Table 2, DMA content was highest in UA (0.010mg/kg) > WR (0.005mg/kg) > RB, PRR, O (0.003 mg/kg) > C (0.002mg/kg). As V (i-As) content was found in significantly higher proportions and was highest in RB > C >UA > O> WR > PRR, ranging from 0.026 – 0.176 mg/kg. Australian food standard established the maximum total As content for cereals as 1 mg/kg [19], suggesting that all samples tested here are well below the recommended limits. To date, the Codex Alimentarius Commission has not formulated any formal guidelines on the safe limits of heavy metals in either white or brown rice, however Asian countries like China, whose staple food crop is rice, has recommended that the safe content for As is limited to < 0.2 mg/kg. Choi et al. [19] reported that grain milling can decreased the content of As and distribute the accumulation of As closer to the surface rather than in the inner core. These results demonstrate that extrusion processing lowers the content of DMA as it was found in lower quantities in C and O when compared to the raw rice materials. This suggests a more in depth study on the effect of extrusion on As species should be carried out as it may be a promising process which can reduce As content in rice and cereals.

Table 7.2 Inductively coupled plasma mass spectrometry (ICP-MS) analysis for arsenic species

Sample	DMA (Dimethylarsinic acid)	As V	Total (mg/Kg)
UA	0.01	0.057	0.067
WR	0.005	0.055	0.06
RB	0.003	0.176	0.179
PRR	0.003	0.026	0.029
C	0.002	0.062	0.063
O	0.003	0.057	0.06

7.4 Conclusin

In total six sample, ethanol: water extracts of rice samples inhibited DPP-4 activity significantly. Specifically, RB was the most potent at inhibiting DPP-4 activity when compared with other samples. n-Hexane extracts were able to potently stimulate GLP-1 secretion. Ethanol: water extracts of WR and OE also significantly stimulated GLP-1 secretion but none of the water extracts had any effect on incretin secretion. The ICP-MS study revealed that DMA content was highest in UA (0.010mg/kg) > WR (0.005mg/kg) > RB, PRR, O (0.003 mg/kg) > C (0.002mg/kg). As V (i-As) content was found in significantly higher proportions and was highest in RB > C > UA > O > WR > PRR, ranging from 0.026 – 0.176 mg/kg. Between two extruded products C (control) and O (optimized), the optimized extruded product containing passion fruit powder, had greater inhibitory activity than the control, suggesting that passion fruit may possess its own anti-diabetic activities. Therefore, these samples can further investigate and contributes in pharmacological research area.

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 33: S62- 90, 2010.

[2] American Diabetes Association, Standards of medical care in diabetes, *Diabetes Care* 28, S4-S36, 2005.

[3] Adamidou, S., Nengas, I., Grigorakis, K., Nikolopoulou, D. and Jauncey, K. Chemical composition and antinutritional factors of field peas (*Pisum sativum*), chickpeas (*Cicer arietinum*), and faba beans (*Vicia faba)* as affected by extrusion preconditioning and drying temperatures. *Cereal Chemistry,* 88(1):80-86, 2011.

[4] Al-masri, I. M., Mohammad, M. K. and Tahaa, M. O. Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*:1061-1066,2009.

[5] Bigliardi, B. and Galati, F. Innovation trends in the food industry: the case of functional foods. *Trends Food Science and Technology,* 31:118-29, 2013.

[6] Bhonsle, S, J. and Sellappan, K. Grain quality evaluation and organoleptic analysis of aromatic rice varieties of Goa, India. *Journal of Agricultural Science,*2(3):99-107,2010.

[7] Bose,A,K., Mocanu M, M., Carr R,D., Brand, C,L. and Yellon, D,M.Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes*, 54:146-51,2005.

[8] Bhattacharya, P., Welch, A, H., Stollenwerk, K, G., McLaughlin, M, J., Bundschuh, J. and Panaullah, G. Arsenic in the environment: Biology and chemistry. *Science of the Total Environment*, 379(2):109-120, 2007.

[9] Choi, S ,H., Kim, J, S., Lee,J,Y.,Jeon, J, S., Kim, J, W., Russo,R,E.,Gonzalez,J.,Yoo ,J ,H., Kim, K,S., Yang, J,S. and Park, K,S. Analysis of arsenic in rice grains using ICP-MS and fs LA-ICP-MS, *The Journal of Analytical Atomic Spectrometry* (JAAS),29 ,1233-1237,2014.

[10] Chen, M, H.,Choi, S, H., Kozukue, N., Kim, H, J. and Friedman, M. Growth-inhibitory effects of pigmented rice bran extracts and three red bran fractions against human cancer cells: Relationships with composition and antioxidative activities. *Journal of Agricultural and Food Chemistry,* 60:9151-9161,2012.

[11] Dixit, R. and Pokala, N. Opinion of doctors about unmet needs of rural diabetics. *International Journal of Basic and Clinical Pharmacology,* 116-120, 2016.

[12] Drucker, D,J. The biology of incretin hormones. *Cell Metabolism,* 3:153-65, 2006.

[13] D'Amato, M., Forte, G. and Caroli, S. Identification and quantification of major species of arsenic in rice. *Journal of AOAC International,* 87:238-243, 2004.

[14] Ernst, E. Plants with hypoglycemic activity in humans. *Phytomedicine,* 4:73-78,1997.

[15] Flatt, P, R., Bailey, C,J. and Green, B,D. Dipeptidyl peptidase IV (DPP IV) and related molecules in type 2 diabetes, *Frontiers in Bioscience,* 13: 3648-3660,2008.

[16] Friedman, M. Rice brans, rice bran oils, and rice hulls: composition, food, and industrial uses, and bioactives in humans, animals, and cells. *Journal of Agricultural and Food Chemistry,* 61:10626-10641,2013.

[17] Frias, J., Giacomino, S., Penas, E., Pellegrino, N., Ferreyra, V., Apro, N., Carrión, M,O. and Vidal-Valverde,C. Assessment of the nutritional quality of raw and extruded Pisum sativum L. var. laguna seeds. *LWT-Food Science and Technology,* 44(5):1303-1308,2011.

[18] Frost-Meyer, N, J. and Logomarsino, J, V. Impact of coffee components on inflammatory markers: A review. *Journal of Functional Foods,* 4(4):819-830, 2012.

[19] FSANZ. Contaminants and natural toxicants. Standard 1.4.1. Available:< http://www.foodstandards.gov.au/_srcfiles/fsc_1_4_1_Contaminants_v78.pdf >. Accessed ,2006.

[20] Fujiwara, K. and Tsuru, D. New Chromogenic and Fluorogenic Substrates for Pyrrolidonyl Peptidase. *Journal of Biochemistry*, 83(7), 1145–1149, 1978.

[21] Green, B,D. and Flatt, P,R. Incretin hormone mimetics and analogues in diabetestherapeutics, *Best Practice and Research Clinical Endocrinology and Metabolism*, 21:497-516, 2007.

[22] Green, B.D., Flatt, P.R. and Bailey, C,J. Inhibition of dipeptidylpeptidase IV activityas a therapy of type 2 diabetes, *Expert Opinion on Emerging Drugs*, 11:525-539,2006.

[23] Guo, H., Ling, W., Wang, Q., Hu, Y., Xia, M., Feng, X. and Xia, X. Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. *indica*) on hyperlipidemia and insulin resistance in fructose-fed rats. *Plant Foods for Human Nutrition*, 62:1-6,2007.

[24] Heitkemper, D, T., Vela, N, P., Stewart, K, R. and Westphal, C, S. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 16(4):299-306, 2001.

[25] Harper, J, M. Food Extruders and Their Applications; Mercier, C., Linko, P., Harper, J.M., Eds.;American Association of Cereal Chemists: St. Paul, MN, USA, pp. 1-15,1989.

[26] International Diabetes Federation. IDF Diabetes Atlas, 6th edn. Brussels, Belgium: International Diabetes Federation. Downloaded http://www.idf.org/diabetesatlas on 9 April 2017.http://www.idf. org/diabetesatlas,2013.

[27] Jain, S. and Saraf S. Type 2 diabetes mellitus-Its global prevalence and therapeutic strategies. Diabetes Diabetes and Metabolic *Syndrome*: *Clinical Research* and Reviews, 4:48-56,2010.

[28] Jisha, S., Sheriff, J, T. and Padmaja, G. Nutritional, functional and physical properties of extrudates from blends of cassava flour with cereal and legume flours. *International Journal of Food Properties,* 13(5):1002-1011,2010.

[29] King, H., Aubert, R,E. and Herman, W, H. Global Burden of Diabetes. Prevalence, Numerical Estimates and Projections. *Diabetes Care,* 21:1414-31,1998.

[30] Kumar, A., Girish, G, M., Jones, D, D. and Hanna, M, A. Modelling residence time distribution in a twin-screw extruder as a series of ideal steady-state flow reactors. *Journal of Food Engineering* 84:441-448,2008.

[31] Kim, S, M., Rico, C, W., Lee, S, C. and Kang, M, Y. Modulatory effect of rice bran and phytic acid on glucose metabolism in high fatted C57BL/6N mice. *Journal of Clinical and Biochemical Nutrition,* 47:12-17, 2010.

[32] Lee, S, H., Lee ,S., Youn, Y,S., Na, D,H., Chae, S,Y.,Byun, Y. and Lee, K,C. Synthesis, characterization, and pharmacokinetic studies of PEGylated glucagon-like peptide-1. *Bioconjugation Chemistry* 16:377-82, 2005.

[33] Lee,S., Park,H., Cho,S., Jeong,H., Hwang,H. and Lee, Y. The supplementary effects of high dietary fibre rice (SUWON 464) on blood pressure and lipid metabolism in spontaneously hypertensive rats.Poster abtract/*Cardiovascular pathology*,13:80-138,2004.

[34] Lovshin, J, A. and Drucker, D,J. Incretin-based therapies for type 2 diabetes mellitus, *Nature Reviews Endocrinology,* 5:262-269,2009.

[35] Lazou, A, E., Krokida, M, K., Karathanos, V, T. and Marinos-Kouris, D. Mechanical properties of corn-legume based extrudates. *International Journal of Food Properties,* 13(4):847-863,2010.

[36] Lacroix, I, M, E. and Li-Chan, E, C. Y. Overview of food products and dietary constituents with antidiabetic properties and their putative mechanisms of action: a natural

approach to complement pharmacotherapy in the management of diabetes. *Molecular Nutrition and Food Research*, 58:61-78,2014.

[37] Mandal, B, K. and Suzuki, K, T. Arsenic round the world: A review. *Talanta*, 58(1):201-235, 2002.

[38] Mayorga, P., Moyano, A., Anawar, H. M. and Garcia-Sanchez, A. Uptake and accumulation of arsenic in different organs of carrot irrigated with As-rich water. Clean–Soil, Air, Water, 41(6):587-592, 2013.

[39] Min, B., McClung, A, M. and Chen, M, H. Phytochemicals and antioxidant capacities in rice brans of different color. *Journal of Food Science*,76:C117-C126,2011.

[40] Nam, S, H., Choi, S, P., Kang, M,Y., Kozukue, N. and Friedman, M. Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. *Journal of Agricultural and Food Chemistry*, 53:816-822,2005.

[41] Nam, S, H., Choi, S, P., Kang, M, Y., Koh, H, J., Kozukue, N. and Friedman, M. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. *Food Chemistry*,94, 613-620,2006.

[42] Nagendra,P, M,N., Sanjay,K,R., Shravya, K, M., Vismaya, M,N. and Nanjunda, S, S. Health benefits of rice bran - A review. *Journal of Nutrition and Food Science,* 1:108,2011.

[43] Pizarro, I., Gomez, M., Palacios, M, A. and Camara, C. Evaluation of stability of arsenic species in rice. *Analytical and Bioanalytical Chemistry,* 376:102-109, 2003.

[44] Padmavathi, M. Drug delivery system in nano greens. *International Journal of Herbal Medicine*, 1(3):56-60,2013.

[45] Perry, T, Lahiri, D,K., Chen, D., Zhou, J., Shaw, K,T., Egan, J,M. and Greig, N,H. A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-me Mediated Differentiation in PC12 Cells. *Journal of Pharmacology and Experimental Therapeutics*,300(3): 958-966, 2002.

[46] Park, H., Park, C., Kim ,Y. and Rascati, K,L. Efficacy and safety of dipeptidyl peptidase-4 inhibitors in type 2 diabetes: meta-analysis. *Annals of Pharmacotherapy*, 46(3):1453-69,2012.

[47] Shao, Y. and Bao, J. Polyphenols in whole rice: Genetic diversity and health benefits. *Food Chemistry*,180:86-97,2015.

[48] Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. and Berghofer, E. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry*, 124:132-40,2011.

[49] Signes-Pastor,A,J.,Carey,M. and Meharg,A,A. Inorganic arsenic in rice-based products for infants and young children. *Food Chemistry*, 191,128–134,2016.

[50] Qureshi, A,A., Sami, S. A., Salser, W, A. and Khan, F, A. Dose dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic humans. *Atherosclerosis*, 161:199-207, 2002.

[51] Qin, Z., Sun, Z., Huang, J., Hu, Y., Wu, Z. and Mei B. Mutated recombinant human glucagon- like peptide-1 protects SH-SY5Y cells from apoptosis induced by amyloid-beta peptide (1-42). *Neuroscience Letters*,444:217-21,2008.

[52] Reddy, K,S., Shah, B., Varghese, C. and Ramadoss, A. Chronic diseases 3. Lancet,366:1746-51,2005.

[53] Wang, Q., Li ,L., Xu ,E., Wong, V., Rhodes, C. and Brubaker, P,L. Glucagon-like peptide-1 regulates proliferation and apoptosis via activation of protein kinase B in pancreatic INS-1 beta cells. *Diabetologia*, 47:478-87,2004.

[54] Yao, Y., Sang, W., Zhou, M. and Ren, G. Antioxidant and α-glucosidase inhibitory activity of colored grains in China. *Journal of Agricultural and Food Chemistry*, 58:770-774, 2010.

[55] Zendjabil, M. Biological diagnosis of diabetes mellitus, *Current Research in Translational Medicine*, 64:49-52,2016.

8.1 Conclusion

In conclusion, salient features and future scope of the present study with respect to the specific objectives, have been highlighted. Comparison of two pigmented red rice (UA: short kernel) and (LA: long kernel) and one nonpigmented (WR: short kernel) was determined. Phytochemical study of purple passion fruit was also carried using analytical instruments. Foam mat drying of purple passion fruits and characterization of the powder was done. Effect of extrusion parameters on the physicochemical, phytochemical, rheological and sensory properties of passion fruit powder incorporated red rice product were further carried out. Moisture sorption isotherm (MSI) and antioxidant activity study of optimized extruded products was evaluated. Dipeptidyl peptidase-4 (DPP-4) inhibitory activity in rice samples and its product and glucagon-like peptide-(GLP-1) secretion were finally carried out. ICP-MS for the confirmation of arsenic species in rice sample were also further investigated.

The salient findings of the thesis are summarized below:

1. **Physicochemical analysis of pigmented and nonpigmented rice and phytochemical analysis of purple passion fruit**
    - ❖ The length of UA was 5.37±0.24 mm (short type) and short and medium type grain produced higher head rice yield (HRY)
    - ❖ UA has low porosity (%) when compared with LA and WR. Low porosity had greater resistance to water-vapor escape during the drying process, which may lead to the need for higher power to drive the aeration fans
    - ❖ Texture profile analyzer (TPA) of cooked rice data revealed that the cooked UA rice had higher hardness value than the cooked LA and WR
    - ❖ Highest water uptake properties was found in UA (3.65±0.01 %) and hence, optimal cooking time (31.16±0.01 min) was also highest in UA
    - ❖ Mineral contents of rice cultivars (UA,LA and WR) reveals presence of eleven elements in three cultivars *viz.*, Al, Ca, Cu, Cr, Fe, K, Mg, Mn, Mo, Na and Zn
    - ❖ Anthocyanin content of the colored rice cultivars (UA 12.79±0.001 and LA 11.47±0.001 mg/100g) compared to white rice (1.34±0.001) further reinforced its potential for high value addition

- ❖ It is prudent to summarize that these pigmented untapped rice cultivars of Arunachal Pradesh, India have enormous potential in the field of pharmaceutical industry vis-a-vis its health benefits
- ❖ pH and ˚Brix of passion fruit pulp were 3.2± 0.15 and 16.09 ± 0.10, respectively
- ❖ FT-IR and RP- HPLC analyses of the rice cultivars deciphered the presence of seven phenolic compounds *viz.*, quinic, salicylic, quercetin, apigenin, ferulic, gallic and caffic acid which are paramount for functional foods.

2. **Foam mat drying of purple passion fruits fruit and characterization of the powder**
   - ❖ A preliminary trial was conducted to identify the effect of whipping time and methyl cellulose on foam density of passion fruit pulp
   - ❖ Foam density (FD) of the mixture was constant and minimum, up to 3 min of whipping and the highest foam density was observed at 4 min whipping
   - ❖ Increase of methyl cellulose concentration in fruit pulp, decreased FD and was proportional up to a certain value and the lowest FD (0.9615 g/cm^3) was observed at 3% methyl cellulose
   - ❖ The foam mat drying of passion fruit pulp was successfully carried out using CCD followed by response surface methodology
   - ❖ The *L**, *a**, and *b** values of the foam mat dried powder differed significantly with respect to fruit pulp
   - ❖ The DPPH scavenging activity and vitamin C content decreased in powder (60.53±0.21% and 35.19± 0.20 mg/100g) compared to the raw passion fruit pulp (70.53±0.03 %) and (60.53±0.21 mg/100g)
   - ❖ Total phenolic content (TPC) content of passion fruit powder (210.11±0.23 mg GAE/100g) increased over raw fruit pulp (206.29 ±0.10 mg GAE/100g)
   - ❖ RP-HPLC analysis revealed presence of various important bioactive compounds.
3. **Effect of extrusion cooking on the physicochemical and phytochemical properties of passion fruit powder incorporated red rice extrudates, rheology of doughs and sensory evaluation of product**

- The independent parameters were *viz.*, temperature(˚C), screw speed (RPM), feed moisture content (%) and amount of passion fruit powder (%) and dependent parameters were *viz.*, expansion ratio (%), water absorption index (%), total phenolic content (mg GAE/100g) and DPPH scavenging activity (%)
- During validation, the experiment was conducted at optimized condition and observed experimental values of ER (7.08%), WAI (2.18), TPC (130.10 mg GAE /100g) and DPPH scavenging activity (63.01%) and did not differ significantly
- DSC thermograph showed an endothermic behavior of extruded products.
- XRD analysis of samples revealed strong peaks of control sample at 2θ *viz.*, 12.72,18 and 18.82 and for optimized sample were 18.18,20 and 23.50. Weak peaks were also observed at 15, 18.82, 19.82 and 23.5. A and C-type pattern were observed
- SEM images revealed that the control sample showed a continuous structure and appeared smoother and in optimized products surface became scratched, cracked, and rougher
- RP-HPLC quantification of compounds like vitamins and anthocyanin in control (C) and optimized (O) extrudates were carried out
- The Mizrahi and Berk model showed the best fits in both red rice (R^2=0.83) and passion fruit incorporated red rice doughs (R^2=0.87) and explained the shear rheological properties
- Flow index, n value varied in control sample (0.46 to 0.47) and optimized (0.88-0.89) for Herschel-Bulky and Mizrahi and Berk model, respectively
- The oscillatory rheological properties of rice dough were affected due to the incorporation of passion fruit powder and the storage modulus (G´) was higher than loss modulus (G´´) for rice dough
- Complex viscosity (η*) of doughs were parallel to each other and decayed linearly with increase in frequency.

4. Moisture sorption isotherm (MSI) and antioxidant activity study of optimized product during storage

- No significant changes in the total phenolic content (mg GAE/100g of dry solids) during 120 days of storage of extruded product

- The MSI shape of the graph resembled more of Sigmoid S-shaped curves of type II types at given temperatures between water activity and EMC data
- Initially, slow increase in EMC was observed until 0.6 a_w. After that abrupt increase in graph was observed
- At 25 ˚C, Langmuir model described and predicted the EMC of product, 35 and 45 ˚C Peleg model predicted as most suitable model to practice MSI study of optimized extrudate.

5 Assessment of the antidiabetic potential of red rice and rice-based products

- The most effective extractant was *n*-hexane, resulting in yields of ≥ 0.82 %
- Rice bran (RB) was the most potent at inhibiting DPP-4 activity when compared with other samples
- RB was the most potent at inhibiting DPP-4 activity by 70.48±1.06 %, followed by UA (42.55±0.84 %), PRR (35.91±1.27 %), WR (29.14±1.23 %), O (25.49±1.86 %) and then C (13.55±3.97 %)
- DPP-4 inhibitory activity was found to be retained in the ethanol:water extracts of both extruded products C (Control) and O (optimized), albeit at reduced levels
- *n*-hexane extracts were able to potently stimulate GLP-1 secretion.
- In particular, PRR, C (control) and O (optimized) enhanced secretion of GLP-1 3.14-fold ($p<0.01$), 3.48-fold ($p<0.001$) and 6.06-fold ($p<0.001$), respectively
- DMA content was in the order of UA (0.010mg/kg) > WR (0.005mg/kg) > RB, PRR, O (0.003 mg/kg) > C (0.002mg/kg). As V (i-As) content (ranging from 0.026 – 0.176 mg/kg) was found significantly higher proportions and was in the order of RB > C > UA > O > WR > PRR.

Future scope of the present investigation

- Cost calculation and feasibility of developed product may be considered before coming to the market.
- Bioavailability of prepared extruded products can be carried out.
- Reduction of As V (i-As) species which is carcinogenic in nature content from rice bran can be further investigated at molecular level.

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List of Publications

1) Samyor, D., Deka, S.C. & Das, A.B. (2016). Phytochemical and antioxidant profile of pigmented and non-pigmented rice cultivars of Arunachal Pradesh, India, International Journal of Food Properties, 19, 1104–1114

2) Samyor, D., Deka, S.C. & Das, A.B. (2016). Evaluation of physical, thermal, pasting characteristics and mineral profile of pigmented and non -pigmented rice cultivars. Journal of Food Processing and Preservation, 40, 174–182

3) Samyor, D., Das, A, B. & Deka, S, C. (2017). Pigmented rice a potential source of bioactive compounds: a review. International Journal of Food Science and Technology. International Journal of Food Science and Technology, 52, 1073-1081

4) Samyor, D., Das, A.B. & Deka, S, C. (2017). In: Value Addition of Underutilized Crops of India by Extrusion Cooking Technology, Innovative Food Science and Emerging Technologies, Published by Apple Academic Press, Taylor & Francis Group (In press)

5) Samyor, D., Deka, S.C. & Das, A.B. (2017). Effect of foam mat drying on physicochemical and phytochemical properties of passion fruit powder. International Journal of Food Properties (Under Review)

6) Samyor, D., Deka, S.C. & Das, A.B. (2017). Effect of extrusion cooking on the physicochemical and phytochemical properties of passion fruit powder incorporated red rice extrudates. Food Chemistry **(**Under Review)

7) Samyor, D., Deka, S.C. & Das, A.B. (2017). Effect of passion fruit powder on rheologicalproperties of gluten free red rice dough. Journal of Texture Studies (Under Review).

Conferences /seminars /workshops

1. Samyor, D., Das, A. B. and Deka, S. C. (2013). Studies on physicochemical properties of some selected underutilized rice cultivars of Arunachal Pradesh. Presented poster at the 7th International Food Convention, IFCON 2013 w.e.f 18-21 December, 2013(venue CSIR-CFTRI, Mysore, India), Organized by AFSTI, Mysore.

2. Samyor, D., Das, A, B. and Deka, S. C. (2014). Pigmented rice- A potential source of bioactive compounds. Presented poster in National conference on Emerging Technology Trends in Agricultural Engineering. November 7-9, 2014 (ETTAE 2014). Organized by Department of Agricultural Engineering, North Eastern Regional Institute of Science and Technology, Nirjuli, Itanagar, Arunachal Pradesh-791109.

3. Samyor, D., Das, A, B and Deka, S.C. (2015) Effect of different pH and temperature on the stability of anthocyanidin content of red rice. Oral presentation in National Seminar cum Workshop on Innovative Prospects in Food Processing: Integration of Engineering and Biological Sciences 27 -28th March, 2015.

4. Samyor, D., Calderwood,D., Das,A,B., Deka, S,C. and Green,B,D.(2016). Presented e-Poster "Pigmented red rice (*Oryza sativa* L.) contains dipeptidyl peptidase-4 (DPP-4) inhibitory activity which can be incorporated into a healthy cereal product" in 1st International Conference on Food Bio-actives and Health, held on (12-15) Sept. 2016 in Norwich, UK.

5. Samyor,D., Deka,S.C. and Das,A,B.(2016).Presented a poster entitled as "Effect of passion fruit (*Passiflora edulis* Sims.) powder on dynamic oscillatory rheological properties of gluten free red rice (*Oryza sativa* L.) dough" in 2ndYoung Investigator Meeting, Cambridge, U.K on 16th September 2016.

6. Attended Global Challenge Networking Programme Event on :'Productivity'Resaerch Event (Order no. 559920946) held on Monday, 17 October 2016 from 12:30 to 15:00.

7. Attended a one day workshop on 'Research & Innovation actions Writing workshop', Friday 4 November 2016, Queen's University Belfast, United Kingdom.

8. Attended one day event on 'What Works Research'? Views from research, policy and practice, Tuesday 15 November 2016, 11.00am – 4.00pm, The Great Hall, Queen's University Belfast, UK.

9. Samyor,D., Deka,S.C. and Das ,A,B.(2017). Presented oral presentation on topic "Effect of passion fruit powder on rheological properties of gluten free dough" Trends and Innovation in Food Processing Technology: Prospects and Challenges. (TIFPT-2017). Date: 9th and 10th February, *2017,* Dept. of Food Engineering & Technology, Tezpur University, Assam

10. Samyor,D.,Deka, S,C. and Das,A,B.(2017). Presented oral presentation on topic "Quality evaluation of extrudate products by using Fuzzy logic tool" One Day UGC-SAP Seminar on Research Trends in Food Processing: Value Addition & Enterprise Development (RTiFP-2017), 27th March, 2017, Dept. of Food Engineering & Technology, Tezpur University, Assam