Chapter II

Review of Literature

Chapter-II

REVIEW OF LITERATURE

This chapter presents review of literature for the current study. This chapter mainly includes the current knowledge involving substantial findings along with theoretical and methodological contributions to the present study.

2.1 Rice

Rice is one of the major cereal crops in the world and considered as a staple food for over 3 billion people. In Asia, about 60-70% of calories of total diet is received from rice and its product. There are about 20 species of the genus Oryza and among them, most cultivated rice is *Oryza sativa*. Only a small amount of Oryza glaberina which is a perennial species is grown in Africa [13]. Rough rice is basically divided into the husk and brown rice (BR) after a threshing process. The BR, on the other hand, can be separated into starchy endosperm (about 92%), embryo (about 2%), and bran (about 6%). White rice or polished rice is obtained after removal of the bran [6]**.** The main component of rice is starch which is present in the endosperm of cereal grains and provides the necessary energy during the process of germination and seedling establishment. Starch is a semi-crystalline polymer composed mainly of linear and highly branched polymer viz. amylose and amylopectin respectively. Each polymer is composed of several D-glucose molecules and the difference lies only in the linkages between them. Amylose is a linear chain which is interlinked by α-(1→4) linkages and few branched chains [19]. Amylopectin subunits are linked by both α-(1→4) and α-(1→6), with about 95% of the glycosidic bonds being α-(1→4) linkages and roughly 5% being α-(1→6) (thesis). The highly branched nature of amylopectin is attributed to the α-(1→6) linkage. Rice is classified based on the amylose content as waxy (0-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high amylose (25-33%) rice [17]. The cooking and thermal properties of rice are the most important characteristics, and these depend mainly on the granular structure of starch and its composition.

2.1.1 Nutritional properties and processing of rice

The processing of rice after harvesting generally involves dehusking and polishing. Rice is worldwide mainly consumed in the form of milled kernels and has very less utilization as compared to other cereals such as corn, maize and wheat [17]. Moreover, some paddy lacks poor milling properties and during processing leads to broken kernels which do not get the required market value. Rice is the major source of energy in most of the Asian countries and highly desirable as stable food due to its bland taste, white colour and good digestibility. It is also used to feed infants as weaning food due to its hypo allergic nature. Moreover, according to reports, rice starch containing high amylose possess relatively lower glycemic index which is beneficial for diabetic and obese persons [17]. However, rice is deficient in vitamins and minerals such as thiamine, riboflavin, calcium, vitamin C and zinc. This may lead to deficiency diseases such as beriberi and also anemia [6]. However, brown rice (BR) which is directly obtained from the rough rice, is reported to have more nutritional components than white rice (WR) [36]. It is rich in fiber, iron, vitamins and minerals [27]. However, BR is usually less consumed due to its flavour and rough texture. Cho [6] reported that to overcome the inferior quality of BR and also to increase the nutritional property of rice, an economical method of germination can be employed.

2.1.2 Germination of paddy

Malting of cereals is one of the commercial and economic processes which was employed traditionally for brewing. a) Steeping b) Germination and c) Kilning. During steeping, the grain swiftly utilizes the oxygen dissolved in the water and fermentation begins i.e. aerobic respiration. Respiration provides the energy required for grain metabolism. This energy is derived from stored carbohydrates and the final product being ethanol and carbon dioxide. As chitting or pre-germination begins, the formation of ethanol ceases and the residual ethanol gets oxidized and thereby the oxygen uptake and carbon dioxide production occur at the equal rates [4]. The embryo is mainly responsible for the rootlet production and respiration process increases as germination proceeds. The enzyme catalyzed modifications that occur in the endosperm during germination is mainly initiated in the scutellum and aleurone layer of the grain [4]. With the onset of germination, the scutellum layer emancipates various hydrolytic enzymes under the influence of plant growth hormones such as gibberellic acid (GA_3) and causes degradation of the cell wall and starch [24]. Gibberellin is reported to be triggered by the depletion of carbohydrate reserves during respiration and this hormone is diffused to the aleurone layer through the scutellum which stimulates the release of hydrolytic enzymes. During the process of malting, the accumulation of these enzymes in the starchy endosperm and partial degradation of its structural components mainly constitute 'modification' [4].

2.1.3 Enzymatic changes during malting

Germination is a complex process during which various changes occur in the seed as it must recover physically from maturation drying and then continue with a sustained intensity of metabolism to complete all essential cellular events which enable the embryo to emerge and grow [25]. During the whole process, enzymes play a very important role since most of the energy required for metabolic activity is provided by hydrolysis of starch. Amylolytic amylase i.e. both exo-amylase and endo-amylase are the predominant enzymes produced during germination and act on starch during the process of germination. The liquefying enzymes i.e. α-amylase breaks the α-1,4 glycosidic bonds in amylose and amylopectin of starch is reported to be the principal enzymes responsible for starch degradation in seeds. However, the saccharifying enzyme i.e. β-amylase which produces glucose or maltose from the non-reducing ends provides the main source of energy to the growing embryo [6]. Apart from these enzymes, some debranching enzymes are also produced during germination which acts on the α-1, 6 linkages of starch. Dziedzoave [12] studied the enzymatic activity of malts prepared from tropical cereals and reported that the amylolytic enzyme activity was highest in rice malts whereas β-glucanase activity was highest in millet and sorghum malts. Moreover, it was observed that the optimum production of amylolytic enzymes in rice malts was between 9-13 days and the optimum time for β-glucanase development was 11 days. Moongngarm [22] compared the chemical composition of germinated rough rice and germinated brown rice and reported that the enzymes activated during germination resulted in changes in chemical components and a remarkable increase in reducing sugar was observed which is due to the starch hydrolysis. The author also reported that germination of rough rice was more suitable than brown rice at industrial level due to high germination rate and higher production yield.

2.1.4 Nutritional aspects of germinated rice

The nutritional properties of germinated rice is a topic of great interest recently since the effect of germination on physico-chemical properties of germinated rice is still under research. It is reported that various nutritional changes occur due to the action of endogenous enzymes and some new components are also observed [27]. Some of the nutrients which were observed to increase in germinated rice are lysine, vitamin E, dietary fiber, niacin, magnesium, vitamin B1, and vitamin B6. Some of the active compound which also increases are γ-aminobutyric acid, γ-oryzanol, ferulic acid, and most free amino acids [36]. Moreover, it was noted that the amount of GABA in germinated rice was almost ten times higher than in milled rice. According to reports, some nutrients were significantly higher than non-germinated rice such as fructose is 3.4 times higher, 2.75 times for reducing sugars, and 7.97 times for GABA [7]. Saman et al. [33] reported that during germination, the saccharifying enzyme which breaks down starch into reducing sugars and oligosaccharides mainly isomalto-oligosaccharides which are potentially considered prebiotic which is not found in non-germinated rice.

2.1.5 Health benefits of germinated rice

Germinated rice due to its nutritional properties possesses certain health benefits. Consumption of germinated rice is reported to prevent headache, relieving constipation, preventing cancer of colon, regulating blood sugar level and preventing heart disease. Cornejo [10] reported that germinated rice consumption can regulate heart rate, alleviation of pain and anxiety, inhibition of cancer cell proliferation due to the synthesis of bioactive compounds during germination such as γ-aminobutiric acid and antioxidants such as phenolic compounds, γ-oryzanol and vitamin E and protection for oxidative stress. It is also rich in dietary fiber which has many health benefits. The γ-oryzanol content which is present in higher quantity in germinated rice provides the antioxidative effect, prevents skin aging and also modulates cholesterol values. GABA is another bioactive compound which is also synthesized in germinated rice is a neurotransmitter which is present in abundance in the brain and spinal cord and it is known to accelerate metabolism in the brain, prevents headaches or depression and activates renal functions [27]. Prebiotic oligosaccharides produced due to hydrolysis of starch aids in improving the intestinal microflora by the selective proliferation of bifidobacteria. The isomalto-oligosaccharides (IMO) specifically helps in normalizing bowel movement, increases stool bulk, improves mineral absorption and lowers blood glucose level [33].

2.1.6 Kinetics of starch hydrolysis

Enzymatic starch hydrolysis is the major reaction that occurs during the process of germination. The hydrolysis of starch mainly occurs by the liquefying and saccharifying enzymes such as α and β amylase leading to the formation smaller molecular weight molecules such as glucose, maltose and dextrin [24]. The kinetics study of enzymatic starch hydrolysis provides optimal reaction conditions and also aids in the identification of the most effective reactor mode for carrying out the reaction. It helps in developing a mathematical model for the whole process which is an important tool for large-scale production. Presecki et al. [29] studied the kinetics of medium-temperature α-amylase hydrolyzed Huai yam powder and the effect of substrate concentration, enzyme concentration, pH and temperature were investigated. The kinetics of the hydrolysis reaction was fitted into the Michaelis-Menten equation and observed that K_m was 6.6641 mg/mL and maximum reaction rate, V_m, was 3.1588 mg/mL·min. The factors such as substrate concentration, enzyme concentration and temperature were also well established. Thus, the Michaelis- Menten equation was observed to be effective for the process due to high significance. Chandrasekar [5] worked on the kinetic properties of the amylases and proteases of dehusked unpolished rice grain ("Mottaikaruppan") which was germinated for 4 days. Both the enzymes showed variation in their kinetic properties. The amylase and protease showed zero order kinetics for 25 and 165 min respectively under optimized conditions. Moreover, the K_m values of the amylase and proteases were 4.5 (soluble starch) and 2.5g/L^{-1} (Casein) respectively. However, the author reported from this study that different fractions of the carbohydrates have to be separated and their kinetic properties need to be studied.

2.2 Vibrational spectroscopy

Vibrational spectroscopy is basically used to describe two analytical techniques- Raman and infrared (IR) spectroscopy. Both these techniques are complementary to each other and provide information on the molecular structure. This provides possibilities for structural analysis of biological material such as in food systems. In infrared spectroscopy, the sample is radiated with infrared light while in Raman spectroscopy, the sample is radiated with monochromatic visible or near infrared light from a laser. Although both the techniques are involved in detecting molecular vibrations and structures, but they do not contain the same information. The vibration in IR is only active when the dipole moment changes whereas in case of Raman spectroscopy the vibration is active when electrical polarizability changes. Therefore, Raman spectroscopy is more active for homonuclear compounds i.e. it detects bonds that connects two identical parts of a molecule such as C=C. On the other hand, IR spectroscopy is more active for heteronuclear compounds such as C=O. Another major difference is that the O–H stretching vibration is very strong in IR, but very weak in Raman because OH bonds are only weakly polarizable. Thus, water is not detected in Raman spectroscopy, but if present in the sample it dominates the IR spectrum. Schuster et al. [34] studied the hydrolysis of starch by a-amylase and amyloglucosidase and the kinetics of these reactions by FT-Raman spectroscopy. The reactions were performed one after another but in a parallel manner i.e. gelatinization, the hydrolysis of starch to dextrin by liquefaction enzyme (α-amylase), and the hydrolysis of dextrin to glucose saccharification by glucoamylase. The FT-Raman spectra showed characteristic changes specifically during the gelatinization of starch with decrease of several bands, but the increase in water signals was observed. Liquefaction was characterized by the disappearance of the band at 735 cm^{-1} and at 480 cm^{-1}. Significant changes were observed in the region of 910–935 cm^{-1} and 1127 cm^{-1}

due to the process of saccharification. Thus, there is a possibility to follow each reaction individually in a simultaneous reaction set up. These findings revealed that Raman spectroscopy can be employed potentially for monitoring important biological reactions.) Kizil [19] worked on the characterization and classification of selected irradiated starch samples by employing Fourier transform infrared (FTIR) and Fourier transforms Raman (FT-Raman) methods. The O-H (3000-3600 cm^{-1}) stretch, C-H (2800-3000 cm^{-1}) stretch, the skeletal mode vibration of the glycosidic linkage (900-950 cm^{-1}) in both Raman and infrared spectra, and the infrared band of water adsorbed in the amorphous parts of starches (1550-1750 cm-1) were employed in classification analysis of irradiated starches.

2.2.1 Chemometrics

Chemometric methods are usually classified as unsupervised and supervised based on the purpose of the data analysis, and the previous knowledge of the samples under study [21]. The unsupervised methods such as Principal component analysis (PCA), factor analysis (FA), and clusters analysis (CA) does not require any previous knowledge of the samples under study and can provide patterns, groupings, detection of outliers, etc. On the other hand, the supervised methods such as multiple linear regression (MLR), principal component regression (PCR), Partial least square regression (PLS) and linear discriminant analysis (LDA) require a set of well-characterized samples and are used for pattern recognition purposes. The supervised methods always comprise a two-stage process (a) calibration and (b) validation. In the calibration stage, each sample or object is identified as a member or not of a determined class or group, according to the previous characterization of the samples or objects (reference method). The goal is to calibrate a prediction/classification model (training set), which will be used to classify or predict the class or value of a set of new and non-characterized samples or objects. In the validation stage, the model calibrated is tested and validated.

2.3 Pasting and thermal properties of malted rice

The pasting property is one of the principal factors for determining the cooking characteristics and eating quality of the germinated rice. The modification of starch by the process of germination also brings about modification in the pasting properties. Xu et al. [37] studied the characteristics of germinated brown rice flour (GBRF) and starch (GBRS) and reported a reduction in pasting temperature from 69.40 to 66.15°C in the flour and from 65.30 to 63.55°C in the starch. Other pasting properties such as peak viscosity, breakdown and setback viscosity were also observed to be low as compared to native. The lower setback viscosity suggested that the brown rice after germination was less prone to germination. The changes in pasting properties of GBRS after germination was attributed to the action of amylases on glycosidic linkage i.e. α-1,4 glycosidic linkage leading to saccharification and easy dissolution of starch. However, the changes in GBRS was attributed to the action of protease enzymes during the process of germination which caused hydrolysis of protein and the disruption of disulfide linkages. Similar results of lowering of pasting properties after germination were reported by Chung et al. [8] and it was attributed to the starch degradation by hydrolytic enzymes. The author also reported that the proteins present in rice flour may act as an inhibitor for the breakdown of swollen granules during pasting but after hydrolysis of protein, the protein network is not formed which in turn increases the fragility of the swollen grains which reduces the viscosity. Wu et al. [36] studied the effect of germination time on physico chemical properties of brown rice flour and starch from different rice cultivars and the results showed that the changes in pasting properties of rice flour were more pronounced as compared to isolated starch. The authors in this study suggested that the pasting properties of flour could be affected by starch content amylase activity, amylose/ amylopectin ratio, protein and lipid contents and starch being the main component of rice, its hydrolysis by enzymes results in reduction of viscosity.

The characterization of cereal biopolymers by thermal analysis is important in apprehending the functionality of starch on the structural and molecular levels [1]. observed that after germination there was significant decrease in transition temperatures (T_0, T_p and T_c), ΔH_{gel} and % retrogradation both for germinated brown rice flour and germinated brown rice starch. It was also observed that the peak temperature (To) was higher in germinated brown rice flour than in germinated brown rice starch. This was due to the presence of lipids in the flour which restricts starch swelling by forming a film on its surface. Another possibility was the formation of amylose-lipid complexes upon gelatinization and therefore a higher temperature was required to melt the complexes. On the other hand, Chung et al. [8] reported that after germination no change was observed in T_o while a slight increase was observed in T_c. This was attributed to the variation e in germination conditions and environmental origin of rice samples. Moreover, a reduced enthalpy was observed in germinated brown rice as compared to native rice. This was due to the activity of the hydrolytic enzymes that disrupted the double helical structure of starch and hence the energy necessary to melt the crystalline structure of starch was very less.

2.4 Weaning formulation

In infants, breastmilk alone is preferred essential to meet the daily nutritional requirement. However, after four to six months of age, mother's milk is not sufficient to sustain normal growth and needs to be supplemented with other foods. Weaning foods should be given to the infants of this age which involves food and drinks other than breastmilk. Codex Alimentarius [9] defined baby foods are foods intended primarily for use during the normal infant's weaning period and also for the progressive adaptation of infants and children to ordinary food. They may be either in ready-to-eat form or in a dry form requiring reconstitution with water only. The characteristics needed in a weaning food are the following:

- The food should be high in energy content and fulfill the requirement of good-quality protein, vitamins, and minerals.
- The food should be in the form of a slurry or semisolid when stirred with cold or warm water or milk which shall enable the child to swallow it easily.
- The prepared food should have low dietary bulk.
- The food should be precooked and predigested or processed in such a way that it needs minimum preparation prior to feeding and is easily digested by the child.
- The food should be free from antinutritional factors and low in indigestible fiber content.
- Addition of artificial colors and flavors to weaning foods are generally not advisable, and the composition of the food must follow the guidelines and standards recommended by competent agencies, such as the Bureau of Indian Standards.

Recommended daily dietary allowances for infants

The calorie density of weaning food is very important for babies since the capacity of babies are limited and they cannot eat more than a certain amount in one feeding. Therefore, the calorie density of weaning formula should be as high as possible so that a small number of feeding is enough for the babies. The minimum energy densities of complementary foods were estimated based on the age and gastric capacity of infants, the number of meals per day, and the quantity of breastmilk consumed. Thus, the estimated energy densities for weaning formula should be 77 and 116 kcal (322 and 485 kJ) per 100 for well-nourished infants 9 to 11 months of age. However, the energy density required for adequately breastfed 6 to 8 month-old-infants is less i.e. 59 and 88 kcal, or 247 and 368 kJ. Similarly, all other nutritional requirement for infants are shown in Table 2.1 and Table 2.2.

Table 2.1: Recommended daily requirement of infants [32]

Value	0–0.5 year	0.5–1 year	Remark
Weight (kg)	6	9	
Height (cm)	60	71	
Energy (kcal/kg)	108	98	
Protein (g/kg)	2.2	1.6	
Vitamin A (μg RE)[a]	375	375	Retinol equivalents (RE). 1 RE = 1 μg retinol/6 μg β-carotene
Vitamin D (μg)[b]	7.5	10	As cholecalciferol; 10 μg cholecalciferol = 400 International Units (IU) vitamin D
Vitamin E (mg α TE)[c]	3	4	α-Tocopherol equivalents (TE). 1 μg *d*-α-tocopherol = 1 αTE
Vitamin K (μg)	5	10	
Vitamin C (mg)	30	35	
Thiamine (mg)	0.3	0.4	
Riboflavin (mg)	0.4	0.5	
Niacin (mg NE)[d]	5	6	Niacin equivalents (NE). 1 NE = 1 mg niacin/60 mg of dietary tryptophan
Vitamin B6 (mg)	0.3	0.6	
Folacin (μg)	25	35	
Vitamin B12 (μg)	0.3	0.5	
Calcium (mg)	400	600	
Phosphorus (mg)	300	500	
Magnesium (mg)	40	60	
Iron (mg)	6	10	
Zinc (mg)	5	5	
Iodine (μg)	40	50	
Selenium (μg)	10	15	

Table 2.2: Estimated amino acid requirements for infants [32]

Amino acid	Requirement (mg/100 kcal)
Histidine	26
Isoleucine	66
Leucine	132
Lysine	101
Phenylalanine	57
Methionine	24
Cysteine	23
Threonine	59
Tryptophan	16
Valine	83

2.4.1 Viscosity of gruels/ weaning formulation for babies

Increasing the gruel energy density to improve the energy intake of young children is a major concern in developing. However, these gruels are prepared mostly by starch-rich food and simply by increasing the flour concentration leads to drastic changes in gruel consistency during cooking i.e. it becomes thick and viscous which makes it difficult babies to eat-especially if the child is sick and prefers a liquid gruel. Moreover, the addition of water to lower the concentration of solid matter makes the gruel thinner which in turn reduces the calories per unit volume and it becomes difficult for the babies to consume enough volume to get the energy it needs with a small number of feedings [23]. Therefore, the concentration of ingredients in a prepared gruel is a function of the consistency or viscosity of the product that can be fed, which in turn is related to the functionality of the product ingredients, particularly starch [32]. The author also suggested a solution to reduce the dietary bulk of weaning formulation by incorporation of 5% malted barley flour since during the process of malting the starch is hydrolyzed to reducing sugars by amylolytic enzymes. The correlation between sensory methods and a viscosity range of weaning formulation as observed from literature is shown below in Table 2.3.

2.4.2 Preparation of commercial weaning formula

Processed cereal-based foods are prepared primarily from one or more milled cereals, which should constitute at least 25% of the final mixture on a dry weight basis. The cereal flour that can be used in weaning formulation according to Codex Committee are wheat, rice, barley, oats, maize, millet, sorghum, and buckwheat, and also groundnut, sesame, soybean (defatted or low-fat), and other legumes. Legumes contain anti-nutritional properties and therefore sufficient heat processing is required. Optional ingredients include protein concentrates and amino acids, fruits, nutritive sweeteners, malt, milk or milk products, fats and oils, salt (including iodized salt), and spices. Vitamins and minerals can be added in accordance with the legislation of the country in which the food is sold [32]. The nonmilk, cereal-based weaning foods are usually intended to be eaten with milk. Cereals are deficient in lysine and therefore the addition of milk which is rich in amino acids completes the nutritional value of the mixture. Canned baby foods are easily available in the market and defined by the Codex Committee as products prepared from any nutritive material commonly used as a food ingredient including cereals and milk. Strained foods are another category of baby food commercially available. It consists of fairly uniform, small particles of a size that does not require or encourage chewing before being swallowed. Junior foods ordinarily contain particles of a size to encourage chewing by infants and children. Strained foods may contain meat, fruits and vegetables such as carrots, potatoes and peas [32] (Sajilata, Singhal, & Kulkarni, 2002).

Table: 2.3 Correlation between sensory methods and viscosity range of weaning formula [23]

Number of classes	Short descriptive term or expression		Qualitative definitions	Viscosity range (Pa.s)	Viscometer type and measuring conditions
	Drinkable			<1	Haake VT500, 1 measuring system SVDin, shear rate= 83 s^{-1}, gruel temperature= 45°C
3	Spoonable		with the consistency of yogurt	1<<3	
	Thick			>3	
6	Free-flowing liquid			<1	Brookfield LVT measuring conditions not reported
	Soup like			1<<3	
	Easily spoonable			3<<6	
	Thick, batter-like			6<<10	
	Very thick, non-spoonable			10<<40	
	Dough-like			>40	

2.4.3 Preparation of weaning food using traditional technologies

Malting of cereals and legumes is a traditional process and possess nutritional benefits for weaning food preparation. In the malting process, starch is hydrolyzed to reducing sugar by hydrolytic enzymes which when added to weaning food can reduce the dietary bulk and increase the calorie intake. Griffith [16] studied the effects of blend and processing method on the nutritional quality of foods made from cereals and legumes. Weaning blends, formulated in a 60% cereal to 40% legume combination using teff, pearl millet, cowpea, and peanut, were evaluated for changes in nutritional quality due to the effects of blend and processing method. The four blends were prepared by four traditional processing methods: control (unprocessed), roasting, germination, and natural fermentation. From the results, it was observed that germinated blends showed significantly low viscosity measurements as compared to other processing methods. Germination of ingredients increased nutrient density and in vitro protein digestibility, while roasting and fermentation produced little change from the control product. Malleshi [20] prepared weaning formulations based on malted cereals and roller drying method. Malted sorghum and malted cowpea flours were blended in the proportion of 70:30 to prepare the malted weaning food (MWF). On the other hand, a precooked weaning food (RDF) was prepared by roller drying a cold-water slurry of pearled sorghum (70%) flour and toasted cowpea flour (30%). The results showed that cooked paste viscosity of MWF was considerably lower than that of RDF. The protein content of MWF was slightly higher (13.4%) as compared to RDF (13.0%) but the available lysine content of MWF protein was 3.85% and that of RDF protein was 2.95%. Moreover, it was also observed that the protein efficiency ratio for MWF (2.26) was significantly higher than that for RDF (1.87).

2.4.4 Utilization of germinated brown rice

Germinated brown rice due to higher nutritional and antioxidant properties can be used in the preparation of various functional food. Germinated grains also contain an amount of glutamic acid, alanine, and glycerine which produce a sweeter and more enhanced flavor as compared to the ordinary brown rice. Bread prepared from germinated brown rice by substituting wheat flour showed lowered specific volume of bread. The improving effect was the addition of 10 or 20% GBR than for BR. The bio-functional components i.e. GABA in BR and GBR breads, decomposed unexpectedly from the final bread. Therefore, GBR would improve the bread quality when substituted for wheat flour [35]. GABA, glutamine, and glycerin are amino acids that form certain neurotransmitters of the brain which are inhibitory and have the ability to reduce the transmittal of stress, anxiety, grief or depression related messages from the limbic system to the cortex. With a reduction in these problems will make a person feel more relaxed with a better sense of well-being [27]. Germinated rice due to the presence of simple sugars due to hydrolysis of starch has better organoleptic properties which can be utilized in the preparation of various confectionaries, health drinks and energy bars. Moreover, it can be a major source of brewing industry for preparation of beer apart from malted barley and maize. Another important use is in the preparation of weaning formulation as it can reduce the dietary bulk and improve the calorie intake of the infants.

2.5 Cytotoxicity test of food samples

Colorimetric assays of cell viability, activation and proliferation based on the use of the tetrazolium salt MTI" was very useful. The major advantages of MTT colorimetric assay include savings in the cost of reagents and equipment, low labour cost due to the elimination of sample processing steps required for liquid scintillation counting, as well as avoiding problems of safety and waste disposal associated with the use of radioisotope. However, the disadvantage of this method is that the resulting colored formazan product is insoluble, precluding direct spectrophotometric absorbance measurements without first dissolving the crystals. Although some methods have been developed to overcome these problems, but all require additional sample processing steps [31]. XTT assay is a colorimetric assay for the nonradioactive quantification of cellular proliferation, viability, and cytotoxicity. To estimate the number of viable cells 2, 3-Bis-(2-methoxy-4-nitro5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) is employed. The mitochondria of the living cells are only capable of reducing XTT, a yellow tetrazolium salt to orange coloured formazan dye, that can be measured by absorbance at 490 (or 450) nm in a microplate reader. The major advantages of XTT assay are its sensitivity and rapid analysis since it requires no solubilization step as in MTT assay [31]. The trypan blue exclusion assay is mainly employed to determine the number of viable cells that are present in the cell suspension. The assay is based on the principle that live cell with intact cell membranes excludes certain dyes such as trypan blue, eosin or propidium, whereas dead cells take up the dyes because of the loss of membrane selectivity. Thus, when a cell suspension was mixed with the dye and visually investigated under an optical microscope, it was observed that the viable cell is not stained whereas the nonviable cells are stained blue.

2.6 Invitro digestibility

Germination of cereals and legumes affect the digestibility of the weaning formulations as starch breakdown occurs during germination leading to the formation of reducing sugar. Jirapa et al, [18] studied on the nutritional quality of germinated cowpea flour (*Vigna unguiculata*) and its application in home prepared powdered weaning foods. Locally available rice, cowpea flour, banana-pumpkin slurry, and skim milk powder and sucrose in the ratio 35:35:15:15:5 were used to formulate weaning food containing not less than 15% protein. The ingredients were cooked into a slurry and oven-dried to produce flakes. The nutritional and sensory qualities of the weaning products were determined and observed that germination had little effect on the amino acid profile of cowpeas, however, in vitro protein quality and starch digestibility was improved in germinated cowpea flour. The invitro starch digestibility of weaning formula prepared from germinated cowpea flour showed higher starch digestibility than weaning food prepared from cowpea flour.

2.7 Rheology of weaning formulation

The rheological characterization of food is an essential part of product development since these properties affect processing parameters, quality of product and sensory properties [30]. The weaning period is one of the most critical phases of life since during this period infants are fed with foods such as cereals, fruits, vegetables and meat along with breast milk and derive nutrition from it. Thereby, the rheological property of the formula may affect the absorption of the nutrients, bowel movement and clearing in the digestive tract. The rheological property of food changes throughout the digestive system in which was described by [28]. The digestive system or gastrointestinal tract consists of the mouth, oesophagus, stomach, small and large intestines along with glands like the salivary glands, liver and pancreas. Digestion proceeds as it moves from the mouth through to the stomach and intestine. The salivary α-amylase and the lingual lipase enzyme initiate the digestion of carbohydrate and lipids, respectively in the mouth. The main chemical digestion takes place in the stomach where the protein is digested by pepsin. Gastric juice is produced in the stomach which possess hydrochloric acid, water, salts, pepsin, mucous and bicarbonate ions. The acidic nature of gastric juice is due to HCl (pH 1-2). It is reported that the acidic characteristic of infant's stomach is at pH 4-5 which is lower in adult stomach i.e. pH drops to <2. Dupont [11] suggested that protein digestion is similar for adults and infants, however rate of protein hydrolysis is slower in case of infants due to lower enzyme concentration. In the small intestine, food is subjected to pancreatic juice which breaks down carbohydrates (by amylase), lipids (by lipase, cholesterol esterase, phospholipase) and proteins (by trypsin, chymotrypsin). Gastric lipolysis plays an important role in fat digestion for newborns than for adults. Although salivary amylase is very low in newborn babies but is able to digest a reasonable amount of starch which is due to the presence of significant concentration of glucoamylase or amyloglucosidase in the small intestinal mucosa, with its activity over 50% that of adults. Therefore, in infants glucoamylase become an alternate enzyme for starch toleration. Prakash [28] studied the rheological properties of four different commercially available infant formulas – newborn, anti-reflux, soy and lactose-free – in an *in vitro* digestive system were investigated. The enzymatic saliva, when mixed with the formulas, showed no change in their viscosity in the mouth which may be due to the short residence time. The measurement was performed every 15 min during gastrointestinal digestion process which revealed that viscosity decreased as time progressed.

2.7.1 Rheological characteristics

Rheological parameters are used as analytical tools to provide a fundamental insight into the structural organization of food. The rheological properties of pureed foods are described by the flow models such as shear stress, shear rate, shear rate–viscosity. Weaning formulation contains a combination of both viscous and elastic behaviour which are generally recognized as viscoelastic properties. The viscoelastic property of foods is generally studied by oscillatory viscometry, which provides more details about their rheological behavior than conventional rheometric data such as flow-behavior index and consistency coefficient [2]. Ramamoorthi et al. [30] evaluated the effect of food matrix and heat treatment on the rheological properties of salmon-based baby food. The viscoelastic properties were determined by dynamic oscillatory measurements to ascertain frequency dependency of the storage and loss modulus of the system. Baby food containing salmon is rich in protein and therefore both protein content and thermal process (sterilization) may affect the viscoelastic behaviour of the baby food due to complex physico-chemical reactions. Rheological behaviours of all samples were tested over a temperature range of 25–55°C. The results showed the higher viscoelastic behaviour of samples with consistently higher storage modulus ($G^{/}$) than loss modulus ($G^{//}$) over the entire frequency range used (0.5–100 rads/s at 25°C). Thermal treatment also showed a significant effect ($p < 0.0001$) on viscoelastic behaviour of baby foods when the exponential model ($G^{/}$ or $G^{//} = A(\omega)^b$) was used. The Casson model was found to be the best for the shear rate – shear stress for all types of tested samples. Moreover, retorted samples exhibited lower yield stress than their unretorted samples. Dynamic mechanical spectroscopy and steady-shear rheological tests were carried out to evaluate viscoelastic properties of commercial sweet potato puree infant food using a controlled stress rheometer. The baby food prepared from sweet potato exhibited viscoelastic behaviour with G/ which was much greater than G// at all frequency. The baby foods showed shear thinning behaviour as the flow index was observed to be less than unity. Moreover, good fit of data by the Herschel–Bulkley model was observed.

2.7.2 Rheological models

The rheological models very often used for characterization of food products are as follows:

Power law model	$\sigma = K(\dot{\gamma})^n$	Eq. (2.1)
Casson model	${\sigma_c}^{1/2} = {\sigma_c}^{1/2} + \eta_c(\dot{\gamma})^{1/2}$	Eq. (2.2)
Harschel-Bulkley model	$\sigma = \sigma_0 + K(\dot{\gamma})^n$	Eq. (2.3)
Linear model	$\sigma = \sigma_o + \eta_c(\gamma^{\cdot})$	Eq. (2.4)
Ostwald de Waele model	$\tau = K(\dot{\gamma})^n$	Eq. (2.5)

Rheological behaviour of salmon-based baby food was studied and showed that Casson model was found to be the best for the shear rate – shear stress [30].

2.8 Sorption isotherm

Water activity (aw) is defined as the ratio of the vapour pressure of water in food to the saturation vapour pressure of water at the temperature at which the food remains in equilibrium. The relationship at equilibrium is given by Eq. 2.6.

$$a_{w}=\frac{p}{p_o}=\frac{\%RH}{100} \quad . \qquad \text{Eq. 2.6}$$

The food when kept at a particular relative humidity (RH) of atmosphere attains particular equilibrium moisture content (EMC) and the RH at that point is called as equilibrium relative humidity (ERH). The relationship of EMC-ERH for food products are temperature dependent i.e. EMC varies with temperature at a constant RH. The relation so developed is known as sorption isotherm which is useful in understanding the behaviour of food during its handling, drying, processing and storage. It is reported that aw, drying time and temperature are considered the most important factors affecting the quality of the dehydrated foods including microbial, chemical and physical aspects. Most microorganisms would not grow in foods with aw below 0.6 and to extend the shelf life of the products it is required to keep the a_w less than 0.6. Thus, sorption characteristics of dried or intermediate moisture content products should be in the range of 0 to 0.6 water activity. This would enable to predict the shelf life of packaged moisture-sensitive products by modeling moisture uptake during storage and distribution [3].

This isotherm curve can be obtained in one of two ways i.e. adsorption and desorption isotherm. On the basis of the van der Waals adsorption of gases on various solid substrates, adsorption isotherms have been classified into five general types: Type I is the Langmuir, and Type II the sigmoid-shaped adsorption isotherm; however, no special names have been given to the other three types. Types II and III are closely related to Types IV and V, except that the maximum adsorption occurs at a pressure lower than the vapour pressure of the gas [3]. Moisture sorption isotherms of most foods are nonlinear, generally sigmoidal in shape, and have been classified as Type II isotherms. Water in fresh food is suggested to be close to that of pure water i.e. unity. As the moisture content of the food lowers to about 22%, the vapour pressure level is maintained. However, the moisture level is then no longer able to sustain the vapour pressure of the food at unity, and therefore, begins to show a lowered vapour pressure, as if in solution. The changes with atmospheric humidity of this last fraction (22%) of water in dehydrated foods result in the characteristic sigmoid shape of water sorption isotherms. Type III behaviour is shown by food rich in soluble compounds such as sugars. Moisture sorption isotherm for a hypothetical food system can be divided into three main regions: Region A represents strongly bound water in food which has an enthalpy of vaporization considerably higher than that of pure water. Region-B represents firmly bound water molecules, present as multilayers above the monolayer [3]. In this region, water is available as a solvent for low-molecular-weight solutes and for some biochemical reactions. The quantity of water present in the material within this region do not freeze at the normal freezing point. In region C or above, excess water is present in macro-capillaries. This exhibits nearly all the properties of bulk water and is capable of acting as a solvent which is easily available for microbial growth. The water sorption isotherm can be classified into three categories i.e. gravimetric, manometric and hygrometric [3]. The gravimetric method involves the measurement of weight changes while manometric method involves measurement of the vapour pressure of water in the vapour space surrounding the food. Sorption isotherms by mathematical models are used based on empirical and/or theoretical criteria. Isotherm models, which can be divided into several categories; kinetic models based on an adsorbed monolayer of water (BET model), kinetic models based on a multi-layer and condensed film (e.g. GAB model), semi-empirical (e.g. Halsey model) and purely empirical models [15]. Isotherms for a variety of freeze-dried dairy powders and commercial dairy powders were predicted using a simple additive isotherm model. As errors were involved in measuring isotherms gravimetrically, the simple additive isotherm model was observed as a good method for predicting moisture sorption isotherms of dairy powders [14].

2.8.1 Isosteric heat of sorption

Two methods are mainly available for measurement of the differential heat of sorption. First is the direct calorimetric measurement of the heat evolved and the second is the application of the Clausius-Clayperon equation on the isosteric equilibrium pressures at different temperatures. However, sorption calorimetry is difficult because of the technique needed for precise measurement of the small quantities of heat evolved. For this reason, calorimetric measured heats of sorption are much less common than those calculated from the sorption isotherm. The net isosteric heat (q_{st}) is defined as the total heat of sorption in the food minus the heat of vaporization of water, at the system temperature [3]. Conventionally, q_{st} is a positive quantity when heat is evolved during adsorption, and negative when heat is absorbed during desorption. The heat of adsorption is a measure of the energy released on sorption, and the heat of desorption the energy requirement to break the intermolecular forces between the molecules of water vapour and the surface of the adsorbent. Thus, the heat of sorption is considered as indicative of the intermolecular attractive forces between the sorption sites and water vapour [3]. The isosteric heat of sorption was determined from the equilibrium adsorption data using the Clausius–Clapeyron equation. Isosteric heats of sorption were found to decrease exponentially with increasing moisture content. The enthalpy-entropy compensation theory was applied to the sorption isotherms and indicated an enthalpy-controlled sorption process.

2.9 Sensory evaluation

The sensory evaluation is one of the important parameters for food products to measure their consumer acceptability. It is observed that the weaning formulation prepared from germinated cowpea flour had higher scores for appearance, colour, aroma, flavour, texture and overall acceptability as compared to weaning formula with non-germinated cereal flour [18]. Onyeka [26] prepared weaning formulations using materials such as cereal (maize), pulses (soybean and groundnut) and tuberiferous plants (cooking banana). The grains were germinated and dried or kilned before milling and formulation. The sensory evaluation showed 90% acceptance of the weaning formula prepared from malted samples. The high scores of the malted samples could be due to the aroma and taste of roasted groundnuts. Kilning and roasting also provide characteristic aroma to the product. Moreover, malting of grains improves the organoleptic properties due to the production of simple sugars.

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