

# Chapter 4

## Functional Cereals for Gluten Intolerance



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### 4.1 Introduction

Modern food diets primarily rely upon the extensive usage of the meal or refined flour of bread wheat. Gluten intake induces inflammatory response in some consumer's and damage villus of the small intestine and leads to the flattening of jejunal mucosa (Shan et al., 2002). In adults, diarrhea or constipation, aphthous ulcers, sore tongue and mouth, dyspepsia, abdominal pain, fatigue, infertility, bloating (weight-loss), neuropsychiatric symptoms, bone pain (osteoporosis), weakness (myopathy, neuropathy) are the primary symptoms, which appear after the intake of gluten. Whereas, infants may show diarrhea, abdominal distension, failure to thrive, anorexia, vomiting, psychomotor impairment, etc., upon the consumption of gluten products. Therefore, such ailments appeared after the consumption of gluten is known as gluten-intolerance (CD) or coeliac disease (CD). The presence of a specific category of gliadins and glutenins in wheat and prolamins (alcohol soluble proteins) from rye and barley are responsible for such immunogenic reactions (Farrell & Kelly, 2002; Fasano & Catassi, 2001; Murray, 1999; Vader et al., 2002). Analysis of the immune epitope database (IEDB) revealed the presence of 190 T-cell stimulatory epitopes for celiac disease in wheat. Among these, 94, 74, and 12 epitopes, respectively, linked with CD are encoded by  $\alpha$ -,  $\gamma$ -, and  $\omega$ -gliadins of wheat. Whereas, 8 and 2 epitopes, respectively, encoded by the low molecular weight and high molecular weight genes in wheat for CD are reported (Comino et al., 2013).

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The first approach to combat CD is to exclude wheat flour from the diet and rely upon the composite flours from pseudocereals, pulses, etc., in routine diets. The second approach is the enzymatic treatment of wheat flour or gluten to hydrolyse all/immunogenic proteins or the genetic engineering and plant breeding approaches to silence the immunogenic proteins from wheat and other cereals. A large population in India consumes vegetarian diets, which are based upon the usage of wheat, and rice, the alternatives of wheat with similar viscoelastic properties are limited and also fail to mimic the texture and sensory attributes of wheat products. Therefore, the evaluation of the physico-chemical properties and development of new formulations based on gluten-free composite flour for making high-quality gluten-free products are gaining high popularity among researchers globally. The functional properties, nutritional values, dietary fiber content, and glycemic index of these products are also different from the products of flour from bread wheat. Starches from maize, rice, tubers, and plantains, in combination with dairy products, gums and hydrocolloids, etc., are widely used to produce quality gluten-free products. However, the poor-quality crumb texture and volume of gluten-free bread are major problems (Gujral et al., 2003; Viridi & Singh, 2020). Quinoa, amaranthus and buckwheat are pseudocereals, and are rich source of iron and fibre. Quinoa contains high levels of riboflavin and buckwheat flour is rich in niacin (US Department of Agriculture, 2000), therefore, pseudocereals may serve as suitable diet for CD patients. The functional properties of starch and proteins derived from sorghum and pseudocereals, and possible modifications to improve the functionality of gluten-free products are discussed.

## 4.2 Sorghum

### 4.2.1 Starch Characteristics of Sorghum

*Sorghum bicolor* (L.) Moench is commonly known as great millet, miloa, jowar/jowari and durra. Sorghum is a grass species which belongs to the family Poaceae, a highly thermo tolerant crop, considered as model crop for studying drought and heat stress. Sorghum is the fifth most important crop after wheat, rice, maize and barley, which is grown in arid and semi-arid agro climatic zones of the world (<https://www.fao.org/in-action/inpho/crop-compendium/cereals-grains/en/>). Khan et al. (2013) reported starch content between 55.6% and 70.0% for the dry grain weight of sorghum cultivars. Sorghum with amylose content of ~25% is considered as normal ( $WxWxWx$ ), whereas, waxy sorghum ( $wxwxwx$ ) is deficient in amylose and composed of 100% amylopectin, while hetero-waxy ( $WxWxwx$  or  $Wxwxwx$ ) type of sorghum contains intermediate amount (~15%) of amylose content (Sang et al., 2008). Amylose content of 18.2–28.8% for Indian wheat was reported by Singh et al. (2010a), which is close to sorghum starches. Morphological studies revealed the presence of irregular-polyhedral and spherical granules of starches in

sorghum (Sandhu et al., 2021). The presence of pores on sorghum starches was also observed (Benmoussa et al., 2006; Huber & Bemiller, 2000; Singh et al., 2010b). Round, doughnut-shaped and polygonal morphology also observed for sorghum starches (Benmoussa et al., 2006). The presence of radial, tube-like channels in the sorghum starch granules were also reported by Huber and BeMiller (2000). The presence of pores, channels may facilitate water access to the granule interior. Amylose content between 11.2% and 28.5% for starches from Indian sorghum was observed against 23.7, 14.0 and 0%, respectively for normal, heterowaxy and waxy sorghum starches (Sang et al., 2008) (Table 4.1). Therefore, normal and heterowaxy sorghum starches consist of high levels of amylose content. The variable solubility behaviour of starches from different sorghum cultivars was also reported. Swelling index ranged between 19.0% and 5.0%, while swelling power (SP) ranged between 6.2 and 15.3 g/g for Indian sorghum starches (Singh et al., 2010a). The solubility of 5% and SP of 8.79 g/g for Nigerian sorghum starches was also reported by Olayinka et al. (2008). The solubility between 17.4% and 22.5%, and SP between 13.8 and 15.2 g/g, for US sorghum starches was also observed (Subrahmanyam & Hosene, 1995). The swelling power of starches from various Indian wheat varieties was ranged between 13.1 g/g and 24.9 g/g (Singh et al., 2010a). These findings thus demonstrated a wide variation in the SP and solubility of sorghum starches from different varieties, which may be affected by the genetic composition of sorghum cultivars, and by the environmental factors. SP and solubility indicate the strength of interaction between the starch chains found between the crystalline and amorphous domains (Singh et al., 2010a; Punia et al., 2020; Punia Bangar et al., 2021a, b). Starches with higher crystallinity and amylose content have poor SP than starches with higher amylopectin. Higher SP for starches with a greater proportion of short chain amylopectin was observed against starches with a large proportion of long chain amylopectin molecules (Singh et al., 2010a). The formation of a stronger crystalline network in starch granules may be attributed to a higher proportion of the long chains of amylopectin. Therefore, higher SP indicates a weaker interaction among the amorphous and crystalline regions through starch chains. The transition temperatures  $T_o$ ,  $T_p$ , and  $T_c$  between 66.1°C and 73.12°C, 70.1°C and 77.79°C, and 75.0°C to 81.24°C, respectively, while the enthalpy of gelatinization ( $\Delta H_{gel}$ ) between 9.26 and 13.5 J/g for Indian sorghum starches was reported by Singh et al. (2010a) (Table 4.1). Seven US sorghum starches showed  $\Delta H_{gel}$  between 2.84 and 3.39 J/g, while  $\Delta H_{gel}$  of 7.45 J/g for ten Zimbabwean sorghum starches (Beta et al., 2001) and  $\Delta H_{gel}$  of 13.7 J/g for Nigerian sorghum starch by Gaffa et al. (2004) was reported. Similarly,  $T_o$ ,  $T_p$ , and  $T_c$  of 67.9 °C, 70.7 °C, and 75.7 °C, respectively for normal sorghum starch had been observed (Sang et al., 2008). An average  $T_p$  of 67.4 °C for ten Zimbabwean sorghum starches (Beta et al., 2001), and  $T_c$  of 90 °C for the Nigerian sorghum starches was also reported (Gaffa et al., 2004). Starches from Indian wheat were exhibited  $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H_{gel}$  of 55.6–57.3 °C, 60.6–62.1 °C, 65.3–67.5 °C, and 8.0–10.2 J/g, respectively (Singh et al., 2010a) (Table 4.1). Difference between  $T_c$  and  $T_o$  is known as the difference in gelatinization ranges, (R). The crystalline domain of a starch granule is composed of small crystallites,

**Table 4.1** Thermal Properties (onset, peak and end of gelatinization) of flour from different cereals and pseudo cereals

Source	Amylose (%)	T <sub>o</sub> (°C)	T <sub>p</sub> (°C)	T <sub>c</sub> (°C)	ΔH (J/g)	Reference
Indian wheat	18.2–28.8	55.6–57.3	60.6–62.1	65.3–67.5	8.0–10.2	Singh et al., 2010a
Indian sorghum starch	11.2–28.5	66.1–73.12	70.1–77.79	75.0–81.24	9.26–13.5	Singh et al., 2010b
Sorghum starch (normal)	23.7	67.9	70.7	75.7	–	Sang et al., 2008
Sorghum starch (waxy)	0	67.7	73.0	82.1	14.7	Sang et al., 2008
Sorghum starch (hetero waxy)	14.0	69.6	72.8	78.6	13.7	Sang et al., 2008
Amaranths ( <i>A. hypochondriacus</i> )	5.8 <sup>Δ</sup>	63.20–70.01	68.88–72.88	74.47–76.95	8.50–13.94	Singh et al., 2014; <sup>Δ</sup> Gamel et al., 2005
Amaranths ( <i>A. caudatus</i> )	4.4 <sup>ε</sup>	60.46–63.28	65.05–67.05	70.93–74.40	11.55–14.38	<sup>ε</sup> Okuno and Sakaguchi, 1981 Gamel et al., 2005
Buckwheat <sup>a</sup>	15.95 <sup>×</sup>	59 <sup>×</sup>	66 <sup>×</sup>	72 <sup>×</sup>	–	<sup>×</sup> Hager et al., 2012
Buckwheat (1:2 moisture content)	16–18	59.5 to 64.1	63.7–68.4	81.7–85.8	14.5–15	Yoshimoto et al., 2004
Common buckwheat (1:2)	25.6–28.6, 34.5–34.5 g	58.6–60.2	61.5–64.3	70–73	14–15.3	Lu & Baik, 2015
Common buckwheat (1:4 moisture content)		61.2	66.1	75.2	9.0	Li et al., 2014
<i>Chenopodium Quinoa</i>	4.62	52	58	64	–	Hager et al., 2012
<i>Chenopodium Quinoa</i>		53.9	60.6	66.0	10.3	Srichuwong et al., 2017
<sup>a</sup> <i>Chenopodium Quinoa</i>	8.4	57.4	66.0	72.7	8.4	
Pearl millet (1:2 moisture content)	21–25	66.2–67.2	69.7–71.4	86.3–91	14.3–14.7	Gaffa et al., 2004
Foxtail millets	16.9–17.5	55	57.5	62	–	Wankhede et al., 1979
Finger millet	38.6	62.5	69	74	–	Malleshi et al., 1986
Maize	17.5–22.1	64.0–68.9	68.9–72.1	73.2–76.8	8.1–11.2	Sandhu & Singh, 2005

<sup>a</sup>Flour; T<sub>o</sub>: Onset temperature; T<sub>p</sub>: Peak temperature; T<sub>c</sub>: End temperature; ΔH: Enthalpy of gelatinization

and the marginal differences in the crystal strength were attributed to variation in R (Banks & Greenwood, 1975).

The enthalpy of retrogradation (ΔH<sub>ret</sub>) of gelatinized starches indicates tendency to retrograde or recrystallize upon cooling of starch paste after gelatinization. While the ratio of ΔH<sub>gel</sub> and ΔH<sub>ret</sub> is defined as percentage retrogradation (%R). Therefore,

a higher value of  $\Delta H_{\text{ret}}$  indicates the lower tendency of starches to retrograde and vice-versa.  $\Delta H_{\text{ret}}$  is an indication of the unravelling and melting of double helices formed during storage and influenced by the amylopectin unit chain length distribution (Shi & Seib, 1992). The unravelling and melting of the double-helical regions during the gelatinization of starches are affected by the chain length distribution of amylopectin (Shi & Seib, 1992). Therefore, the breakdown of starch granules during heating, i.e., gelatinization upon heating, and reannealing of starch granules upon cooling relies on the structural arrangements of starch chains within the crystalline and amorphous domains of non-gelatinized starch granules (Perera & Hoover, 1999).  $T_o$ ,  $T_p$ , and  $T_c$  between 46.2 and 52.6 °C, 54.18 and 58.61 °C and 61.4 to 65.9 °C, respectively for retrograded Indian sorghum starch pastes stored in a refrigerator were observed.  $\Delta H_{\text{ret}}$  between 1.11 J/g and 4.31 J/g for retrograded Indian sorghum starch pastes were reported (Singh et al., 2010a). These findings thus demonstrated lower transition temperatures and  $\Delta H_{\text{ret}}$  of stored starch pastes than the transition temperatures of gelatinization and  $\Delta H_{\text{gel}}$  fresh starch dispersions. This implies that some of the Indian sorghum cultivars have starches with higher enthalpy of retrogradation ( $\Delta H_{\text{ret}}$ ) thereby lower syneresis values and therefore, can be used for making gluten-free products with long storage-shelf life. These findings thus revealed that sorghum starch has higher gelatinization temperature (68–78 °C), than the gelatinization temperature of starches from maize (62–72 °C) and barley (51–60 °C) along with a higher degree of retrogradation (Collar, 2017; Hosene, 1994). The low number of short chain amylopectin in sorghum may attribute to higher gelatinization temperature and higher degree of retardation than remained cereals (Ai, 2013). Studies have shown that higher gelatinization temperature may have adverse effect on the quality and sensory of baked products (Taylor & Dewar, 2001). However, the texture and sensory of gluten-free chapatti, pan cake, and other food products may not be affected by high gelatinization temperature of sorghum starches.

The pasting properties of starches are crucial for the final texture, sensory, and consumer acceptability. Therefore, the pasting temperature, the breakdown-, and final viscosity of the sorghum starches were evaluated by a rapid visco-analyzer. The peak viscosity (PV) and hot paste viscosity (HPV) ranged from 2541 to 4698 cP, and 919 to 2629 cP, respectively for Indian sorghum starches. The breakdown (BDV) and final viscosity (FV) varied from 911 to 2645 cP and 2314 to 4743 cP, respectively, while the setback viscosity from 1067 cP to 2114 cP for Indian sorghum starches was observed (Singh et al., 2010a). Zimbabwean sorghum cultivars exhibited average PV, HPV, CPV, BD, and SB of 3984 cP, 1392 cP, 2928 cP, 2592 cP, and 1536 cP, respectively (Beta et al., 2001). Whereas, the PV, BD, and SB of 2004 cP, 144 cP, and 1476 cP, respectively for Nigerian sorghum starch was reported by Gaffa et al. (2004). The pasting temperature ranged from 75.2 °C to 80.9 °C for Indian sorghum cultivars and 69 to 70.3 °C for Zimbabwean sorghum varieties (Beta et al., 2001). On the contrary, starches from Nigerian sorghum cultivars were exhibited the PT of 82.6 °C (Gaffa et al., 2004). PT between 82.3 and 89.6 °C for starches from various Indian wheat varieties was observed. Starches with higher PT and higher amylose content demonstrated lower peak, trough, breakdown, setback,

and final viscosity (Singh et al., 2010a). The amylose and amylopectin content, agroclimatic conditions, and genetic composition of sorghum influence the pasting properties greatly. Ratnavathi and P. J. (2014) and Khoddami et al. (2021) concluded that sorghum flour with higher hot peak paste viscosity, setback viscosity, water uptake, and low gelatinization temperature are highly suitable for flat breads such as chapatti, whereas sorghum flour with high gelatinization temperature and low peak paste viscosity may be highly suitable for the preparation of stiff porridge such as Indian Sankhati and African tô. The starch digestibility of 33–48% for sorghum starches against 53–58% for corn starches by Sikabbubba (1989) was evaluated. The digestibility of starches from floury and corneous sorghum grains was also different; with starch from the former type of grain revealed higher digestibility than the later one. The lower size particles of the floury grain of sorghum may be digested rapidly by starch solubilizing enzymes *in vitro* and may be attributed to a higher digestibility. A lower starch digestibility of normal sorghum than the waxy was also reported by Hibberd et al. (1982). Since most of the corn starch is utilized by industries for the manufacturing of breakfast cereals, snacks, etc., the availability of corn starch in India is limited. Majority of gluten-free products rely upon corn, rice and potato starches; therefore, sorghum starch may be a good alternative of corn starches. The functionality of sorghum starches is also equivalent to corn starches. Starch from sorghum can be produced by wet milling technology, which is available for corn starch production. These findings thus imply that starches from normal/corneous sorghum starches have a better alternative to corn starches.

#### 4.2.2 *Composition and Functionality of Sorghum Proteins*

The protein content in sorghum ranged from 80 to 84% of the total grain nitrogen, whereas the germ and pericarp of sorghum contained protein content between 9.4 to 16% and 3.0 to 6.5%, respectively (Serna-Saldivar & Rooney, 1995; Taylor & Schüssler, 1986). Majority of seed storage proteins stored in protein bodies are made from the surrounding layers of lipids. Higher content of  $\alpha$ -kafirins, and minor stock of  $\beta$ -, and  $\gamma$ -kafirins, inside the protein bodies (0.3–1.5  $\mu\text{m}$ ) of the sorghum endosperm was observed, whereas, higher proportion of  $\beta$ -, and  $\gamma$ -kafirins in the peripheral region of protein bodies was found. Higher cysteine content in  $\beta$ -, and  $\gamma$ -kafirins attributed to crosslinking with each other, which led to the formation of a shell around  $\alpha$ -kafirins inside the protein bodies. The minor proportion of glutelin, globulins and albumins in the protein matrix of the sorghum endosperm were also reported. The glutelin, globulin and albumin content of 33.4%, 7.0% and 5.6%, respectively was reported in the matrix of sorghum endosperm by Virupaksha and Sastry (1968). The  $\alpha$ -kafirins showed two subunits namely  $\alpha 1$ -, and  $\alpha 2$  kafirin with the molecular weight of 23,000 Dalton (Da) and 25,000 Da, respectively. The expression of 19 kafirin encoding genes in sorghum was reported which may encode different subunits of  $\alpha$ -kafirins proteins (Xu & Messing, 2008). A gene encoding a methionine rich kafirin, known as  $\delta$ -kafirins, with molecular weight of 16,000 kDa

was also reported from sorghum, which accounts for only 1% of total grain proteins. In 92.9 g/kg (N x 5.81) crude protein, the average kafirin content of 48.2 g/kg for 33 Australian sorghum lines/cultivars was observed (Selle et al., 2020), which was 51.9% of total grain nitrogen, therefore, kafirin represents a major proportion of the protein content in sorghum. A substantially higher leucine content of 62.7% in the Australian sorghum kafirin was also observed (Selle et al., 2020). Studies have shown that very high leucine content-based diets may not be suitable for the good performance of the broiler chickens (Selle et al., 2020). Amino acid composition analysis revealed lower lysine and threonine content in the grains of sorghum, as observed for maize (Table 4.2). Since kafirins are homolog of zein proteins of maize, lower lysine and threonine content in sorghum may also be associated with the higher abundance of these endospermic proteins of sorghum. The prolamins also have some degree of viscoelastic properties which rely on the purification methods and proportion of the individual subunits in the purified fraction of each prolamins. The purified zein and kafirin proteins with higher proportion of  $\alpha$ -prolamins showed best viscoelastic properties and the presence of cysteine residues in some of the prolamins subunits showed deleterious effect on the viscoelastic properties of prolamins (Oom et al., 2008; Schober et al., 2011). Therefore, the viscoelastic properties of maize and sorghum proteins can be modified by different processing methods, and could be an alternative source of wheat gluten to enhance the quality of bread upto some extent.

Wet cooking of sorghum resulted in the di-sulfide crosslinking of kafirins, which led to their poor solubility and digestibility. Thus, the digestibility of sorghum decreases upon wet cooking. A higher increase in the proportion of antiparallel  $\beta$ -sheets and decrease in the  $\alpha$ -helices may have attributed to the poor solubility of sorghum kafirins (Duodu et al., 2001). Popping and dry-roasting of sorghum grains also did not affect the digestibility of sorghum significantly; however, the addition of reducing agents during cooking enhanced the digestibility of cooked flour (Correia et al., 2010; Hamaker et al., 1987; Parker et al., 1999). These findings thus revealed that sorghum proteins can be used to enhance the viscoelastic properties gluten-free composite dough systems and starches obtained from sorghum can be a very good source of slow digestible starches.

### 4.3 Roles of Pseudocereals in Health and Nutrition

Pseudocereals, which include amaranth, buckwheat, chenopods and millets etc. provide better nutrition than most major crops and are multipurpose crops. Since these are gluten-free and have superior nutritional attributes, however, the utilization of pseudocereals in making processed food require detailed analysis of structural and functional properties of starch and proteins. The pasting and thermal profile of starches, functional properties of foam of flour, starch and proteins from different pseudocereals appeared to be differential and are discussed here in brief to better understand the utilization of flour, starch and proteins in the designing of different types of gluten-free products.



**Table 4.2** Amino acid composition of important cereals, pseudocereals and pulses

Source	Jowar	Maize	Rice, raw, milled	Wheat flour, refined	Wheat, whole	Amaranth seed, black	Amaranth seed, pale brown	Quinoa	Tartary buckwheat (B-121)	Common buckwheat (Tomotake et al. (2006))	Bengal gram, whole	Cowpea, white	Lentil, whole, brown	Peas, dry	Rajmah, red (Kidney bean)
Histidine	2.07 ± 0.20	2.70 ± 0.21	2.45 ± 0.30	1.95 ± 0.23	2.65 ± 0.31	1.86	1.98 ± 0.50	2.98	294.45 ± 37.90	2.52	2.51 ± 0.18	3.25	2.07 ± 0.14	2.34 ± 0.09	2.70 ± 0.30
Isoleucine	3.45 ± 0.63	3.67 ± 0.22	4.29 ± 0.23	3.19 ± 0.27	3.83 ± 0.20	2.82	2.85 ± 0.04	3.75	75.95 ± 7.24	3.12	4.34 ± 0.23	4.4	4.17 ± 0.05	3.87 ± 0.46	4.23 ± 0.38
Leucine	12.03 ± 1.51	12.24 ± 0.57	8.09 ± 0.40	6.22 ± 0.46	6.81 ± 0.33	4.83	4.94 ± 0.17	6.08	988.87 ± 28.17	5.94	7.40 ± 0.31	7.96	7.36 ± 0.34	7.02 ± 0.38	7.78 ± 0.71
Lysine	2.31 ± 0.40	2.64 ± 0.18	3.70 ± 0.39	2.05 ± 0.18	3.13 ± 0.26	5.45	5.50 ± 0.35	5.55	315.72 ± 45.76	5.68	6.59 ± 0.25	7.14	6.78 ± 0.51	7.12 ± 0.51	6.71 ± 0.81
Methionine	1.52 ± 0.50	2.10 ± 0.17	2.60 ± 0.34	1.64 ± 0.20	1.75 ± 0.21	1.86	1.95 ± 0.12	2.24	403.42 ± 14.96	2.3	1.16 ± 0.16	1.53	0.84 ± 0.03	0.68 ± 0.19	0.88 ± 0.40
Cystine	1.06 ± 0.30	1.55 ± 0.14	1.84 ± 0.18	2.03 ± 0.27	2.35 ± 0.23	1.6	1.51 ± 0.15	1.85	55.09 ± 2.64		1.27 ± 0.09	0.6	1.18 ± 0.04	0.82 ± 0.15	0.70 ± 0.18
Phenylalanine	5.10 ± 0.50	5.14 ± 0.29	5.36 ± 0.43	4.29 ± 0.28	4.75 ± 0.38	3.98	4.75 ± 0.41	4.35		4.3	6.26 ± 0.70	5.63	4.61 ± 0.68	4.76 ± 0.23	5.90 ± 0.56
Threonine	2.96 ± 0.17	3.23 ± 0.29	3.28 ± 0.27	2.34 ± 0.08	3.01 ± 0.17	3.02	2.99 ± 0.21	3.01	770.66 ± 35.84	3.5	3.55 ± 0.31	4.1	3.35 ± 0.05	3.65 ± 0.15	4.18 ± 0.65
Tryptophan	1.03 ± 0.21	0.57 ± 0.12	1.27 ± 0.14	1.04 ± 0.16	1.40 ± 0.10	1.5	1.69 ± 0.10	1.25		2	0.95 ± 0.07	0.92	0.76 ± 0.04	0.86 ± 0.19	1.05 ± 0.27
Valine	4.51 ± 0.71	5.41 ± 0.71	6.06 ± 0.02	4.01 ± 0.44	5.11 ± 0.05	4.34	4.30 ± 0.27	4.55	243.89 ± 22.90	4.26	4.58 ± 0.51	5.31	4.85 ± 0.06	4.67 ± 0.66	5.07 ± 0.71
Alanine	9.19 ± 1.12	7.73 ± 0.46	5.51 ± 0.40	2.98 ± 0.37	3.64 ± 0.21	4.26	3.83 ± 0.64	4.35	214.64 ± 16.74	3.89	4.67 ± 0.56	5.06	5.75 ± 0.61	4.51 ± 0.44	4.57 ± 0.53
Arginine	3.96 ± 0.43	4.20 ± 0.24	7.72 ± 0.55	3.49 ± 0.28	5.13 ± 0.33	7.77	7.21 ± 0.91	7.85	1475.60 ± 45.76	11.16	8.59 ± 0.58	7.44	7.44 ± 2.06	8.09 ± 0.30	6.10 ± 0.85
Aspartic acid	7.09 ± 0.86	6.55 ± 0.59	8.73 ± 0.80	4.63 ± 0.39	5.44 ± 0.33	12.57	12.70 ± 2.25	8.4	89.26 ± 4.74	9.54	11.78 ± 1.60	11.01	12.48 ± 1.70	11.34 ± 0.72	10.50 ± 0.85
Glutamic acid	21.54 ± 2.81	19.39 ± 0.70	18.92 ± 1.76	31.57 ± 1.80	27.06 ± 1.76	16.12	17.39 ± 1.68	13.75	1372.94 ± 45.80	19.38	17.27 ± 1.08	18.2	17.25 ± 1.68	17.52 ± 0.81	16.01 ± 2.17
Glycine	3.08 ± 0.25	3.27 ± 0.15	4.18 ± 0.16	3.21 ± 0.20	4.19 ± 0.23	8.5	8.28 ± 0.35	4.8	467.6 ± 26.35	5.66	3.95 ± 0.16	4.09	4.78 ± 0.08	4.19 ± 0.16	3.78 ± 0.49
Proline	6.99 ± 0.92	7.88 ± 0.71	4.31 ± 0.78	9.23 ± 0.64	10.25 ± 1.49	3.76	3.83 ± 0.45	5.67	329.1 ± 20.76	7.93	3.74 ± 0.19	4.05	5.01 ± 1.49	3.73 ± 0.19	3.40 ± 0.58
Serine	4.02 ± 0.43	4.58 ± 0.44	4.95 ± 0.21	4.77 ± 0.39	4.80 ± 0.14	7.79	7.27 ± 0.46	4.56	426.16 ± 6.64	4.61	5.10 ± 0.65	4.8	5.51 ± 0.21	4.83 ± 0.38	5.74 ± 0.90
Tyrosine	3.61 ± 0.25	3.71 ± 0.18	4.36 ± 0.41	2.62 ± 0.15	3.12 ± 0.31	2.85	3.10 ± 0.34	1.98	346.33 ± 14.38	3.03	2.88 ± 0.15	3.25	2.40 ± 0.77	3.25 ± 0.19	3.12 ± 0.37

Jowar: *Sorghum vulgare*; Maize, dry: *Zea mays*; Rice, raw, milled: *Oryza sativa*; Wheat flour, refined: *Triticum aestivum*; Wheat, whole: *Triticum aestivum*; Amaranth seed, black: *Amaranthus cruentus*; Amaranth seed, pale brown: *Amaranthus cruentus*; Quinoa: *Chenopodium quinoa*; Tartary buckwheat (B-121): Common buckwheat: *F. esculentum* (Tomotake et al. (2006)); Bengal gram, whole: *Cicer arietinum*; Cowpea, white: *Vigna catjang*; Lentil, whole, brown: *Lens culinaris*; Peas, dry: *Pisum sativum*; Rajmah red/Kidney bean: *Phaseolus vulgaris*

Source: Longvah, T., Ananthan, R., Bhaskarachary, K., & Venkaiah, K. (2017). Indian food composition tables, National Institute of nutrition, Indian Council of Medical Research Department of Health Research, Ministry of Health and Family Welfare, government of India, Jamat Osmania (PO), Hyderabad – 500,007, Telangana, India

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### 4.3.1 *Amaranth*

#### 4.3.1.1 Starch Characteristics of Amaranth

Amaranth, buckwheat chenopods and millets are considered as main pseudocereals. The nutritional quality of amaranth is superior from other pseudocereals. Amaranth grains consist of 73.7–77.0% carbohydrates, 12.5–15.5% proteins, 7.1–8.0% lipids, 3.0–3.5% mineral content, and 19.5–49.3% dietary fiber contents (Pedersen et al., 1990). Major proportion of carbohydrates in amaranth is starch and accounts for 62–65%. Amaranth starch composed of amylose and amylopectin. Where amylose is a linear polymer of glucose, amylopectin is highly branched in nature, made from a linear chain of (1 → 4)-linked  $\alpha$ -D-glucose and short chains of (1 → 6)- $\alpha$ -D-glucose-linked branches. Amylopectin content for amaranth ranges between 90 and 98% of the total starch, with 1700 amylopectin/molecules and exhibits smooth polymodal chain length distribution. The degree of polymerization (DP) for amaranth amylopectin also ranges between 11–12 (Singh et al., 2014; Wilhelm et al., 2002). The size of amaranth starch granules ranges between 0.5 and 2.5  $\mu\text{m}$ , are polygonal in shape and show unimodal size distribution. Though amaranth starch granules are similar to rice, granules of starches from other cereals are larger in size. The pasting ( $T_0$ ) and gelatinization temperature ( $T_p$ ) for amaranth starches ranges from 69 °C to 72 °C and 60 °C to 77 °C, respectively (Zhu, 2017). However, the amylose content, genotype, crystallinity and the presence and/or the absence of amylose-lipid complexes may affect the pasting behaviour of amaranth starches. Amaranth starches contain a minor proportion of starch bound lipids of 0.16–0.28% which also affects their pasting and functionality (Hoover et al., 1998). The small size granules of amaranth starches with low amylose content attributed to a lower breakdown viscosity and a more stable paste upon gelatinization. The small size, granular structure, low amylose content and high tendency to loosen crystallinity results in fast digestibility thereby considered as high glycaemic food. The rapidly digestible starch (RDS) of 30.7% and predicted glycemic-index of 87.2 for the raw seeds of amaranth had been reported earlier. Food with higher content of rapidly digestible starch is considered as high glycaemic food (Capriles et al., 2008). Glycemic index represents the levels of carbohydrate in food in response to postprandial glucose levels after the consumption of food (Jenkins, 2007). Therefore, amaranth may be adversely affecting the postprandial glucose of consumers suffering from diabetic and cardiovascular disease. However, amaranth proteins are having very high nutritional profile mimicking to the nutritional profile of milk. Amaranth grains are rich in minerals like phosphorous, iron, potassium, zinc, calcium, and vitamins such as vitamin B-complexes, vitamin E along with polyphenols such as flavonoids, caffeic acid, p-hydroxybenzoic acid, and ferulic acid. *Amaranth hypochondriacus* from Mexican Highlands showed high content of rutin (4.0–10.2 mg/g flour) and nicotiflorin (7.2–4.8 mg/g flour) (Barba de la Rosa et al., 2009).

#### 4.3.1.2 Composition and Functionality of Amaranthus Proteins

Pulse proteins are deficient in sulfur rich amino acids i.e., cysteine and methionine, while cereal proteins are poor in lysine and tryptophan amino acids (Table 4.2). Conversely, amaranth proteins are rich in essential amino acids such as cysteine and methionine amino acids (Table 4.2). Grains, endosperm, and germ contain protein content of 11% to 17%, 35% and 65%, respectively, whereas average protein content of 15% and 85%, respectively for germ and the endosperm of other cereals has been observed, hence, amaranth is a suitable alternative source of wheat gluten (Singh et al., 2019). As per the Osbornes' (Osborne, 1924) solvent-specific solubility-based classification of seed storage proteins, amaranth grain revealed the accumulation of 51% albumins, 16% globulins, 24% glutelins, as a major fraction, whereas, 1.4% to 2.0% alcohol-soluble prolamines, as minor protein fraction of grain proteins had been reported (Gorinstein et al., 1991; Martínez et al., 1997). Cereals such as maize and wheat, on the contrary, contain alcohol-soluble prolamines as the major storage proteins (Gorinstein et al., 1991). Conversely, a major proportion of grain storage proteins in leguminous crops are salt-soluble globulins. A higher proportion of lysine and valine amino acid residues in the albumin and globulin fraction of the amaranth grain proteins were observed. A higher proportion of leucine and histidine amino acid residue in the glutenin subunits proteins of amaranth were also noticed. The nutritional quality indicators, such as protein digestibility, lysine availability, protein efficiency ratio, etc., are also fairly good for amaranth proteins thus imply that amaranth grain are good source of cereal and pulse proteins (Paredes-Lopez, 2020). Amaranth whole-meal flour showed average protein digestibility of 74.2%, which is significantly improved after thermal processing of grain, like popping, roasting etc., (Bejosano & Corke, 1998). The presence of anti-nutritional substances may have affected the protein digestibility of amaranth whole-meal flour. Whole meal of thermally processed grains of amaranth demonstrated a superior protein digestibility, which may be attributed to the thermal inactivation of anti-nutritional substances. The protein digestibility corrected amino acid score of 0.40 and 0.57, respectively, for wheat and oats was observed against 0.64 for amaranth (Bejosano & Corke, 1998). Therefore, the protein digestibility corrected amino acid score of amaranth whole meal flour was also superior. Apart from these, the functionality of proteins is also important for the development of new product formulations. Gluten-free muffins prepared from amaranth protein isolates demonstrated superior texture (volume, height, springiness, cohesiveness) and sensory attributes (crust, color, appearance and overall acceptance) when compared with muffins made from gluten fortified batter (Shevkani & Singh, 2014).

The presence of angiotensin-converting enzymes inhibitor peptides was reported for the peptides derived from 11S globulin sub-fraction of amaranth. Amaranth glutelin showed antihypertensive and anticarcinogenic activities, which attributed by the presence of the lunasin-like polypeptide in glutelin (Barrio & Añón, 2010; Sabbione et al., 2016). The antioxidant activity of amaranth proteins also enhanced after the gastrointestinal digestion also affects the fatty acid metabolism in liver thus confirmed the hypotriglyceridaemic effect in rat (Escudero et al., 2006) (24). The

colonic epithelial cells show a decrease in the expression of *CCL20* gene at transcript levels in the presence of the amaranth peptide (SSEDIKE), therefore, amaranth peptides also possess anti-inflammatory properties. Bioactive peptides with AWEEREQGSR, YLAGKPQQEH, IYIEQGNGITGM, and TEVWDSNEQ amino acid (aa) residues from 11S globulin protein of *Amaranthus mantegazzianus* revealed antioxidant activity. Furthermore, cationic peptide with HVIKPPSRA and KFNRPETT aa residues and a neutral peptide with aa sequence of GDRFQDQHQ demonstrated in vivo inhibition of  $\text{Cu}^{2+}/\text{H}_2\text{O}_2$ -induced oxidation of low-density lipoproteins (García Fillería & Tironi, 2017; Orsini Delgado et al., 2016). Gluten-free muffins prepared from *Amaranthus* protein isolates showed texture and sensory properties like the muffins prepared from protein isolates from kidney bean and field pea. Similarly, the edible/biodegradable film-forming properties of amaranth proteins were also comparable to pulse proteins.

### 4.3.2 Buckwheat

*Fagopyrum esculentum* (common buckwheat) and *Fagopyrum tataricum* (tartary buckwheat/bitter buckwheat) are widely cultivated in America, Europe, and Asia. On the contrary, *Fagopyrum dibotrys* or *Fagopyrum acutatum* and *Fagopyrum cymosum*, known as golden or tall buckwheat, are a less cultivated buckwheat with potential significance in Asia (Liu et al., 2006). The consumption of buckwheat increased tremendously because of its disease healing and prevention attributes (Ahmed et al., 2014; Cai et al., 2004; Guo et al., 2013; Li & Howard Zhang, 2001). On dry basis, the grain contains ~70% starch, ~12% protein, ~10% dietary fibres, ~3% lipids, 2.5% ash content (Zhu, 2016; Food Data Central, 2020). Hence, starch is a major proportion of buckwheat grains. A wide variation in amylose content in the starch of different buckwheat accession was reported. Minor content of important minerals (Mg, K), vitamins (B, C, E), flavonoids (rutin, quercetin), D-chiro-inositol, fagopyritols, and polyunsaturated essential fatty acids (linoleic acid) in the grains of buckwheat were also observed (Li & Howard Zhang, 2001; Wijngaard & Arendt, 2006). The presence of these biomolecules led to various health benefits like anti-hypertension, hypocholesterolemic activity, fat storage suppression in body, antioxidant and free radical scavenging, anti-inflammatory, etc., (Ahmed et al., 2014; Li & Howard Zhang, 2001; Wijngaard & Arendt, 2006). However, the higher abundance of tannins, protease inhibitors, and phytic acid affects the starch digestibility.

#### 4.3.2.1 Composition and Functionality of Buckwheat Starches

Amylose content ranges between 23 and 29.1% for starches from 30 genotypes of common buckwheat (Ikeda et al., 1997). The amylose content between 3.8% and 16% for waxy or mutant buckwheat was also observed (Gregori & Kreft, 2012). The

morphological analysis shows that starch granules had smooth surface and less spherical shape, A type polymorph, with polygonal structure. The starch granules showed average granule size of  $\sim 6\text{--}7\ \mu\text{m}$  and ranged between  $\sim 2$  to  $15\ \mu\text{m}$  (Qian et al., 1998; Zheng et al., 1997). Therefore, the buckwheat starch granules are smaller in size as compared to other cereals (Gregori & Kreft, 2012; Jane et al., 1994; Liu et al., 2015a, b; Vallons & Arendt, 2009). Degree of polymerization (DP) of 94,900 with a minimum and maximum range between 38,000 and 134,000, for starches from buckwheat was observed. Therefore, common buckwheat starch had much higher DP than amaranth, wheat, quinoa, and proso millet than waxy maize (Praznik et al., 1999). DP with two peak maxima ranged between 1020 and 1380 for buckwheat starches. Amylose in common buckwheat starches showed the distribution of chain length from 3.1 to 4.3, with average chain length between 280 and 380 glucosyl residues. Therefore, the common buckwheat amylose resembled with the starches from wheat and barley. According to crystal types, the starches are grouped into A, B, and C types. A type buckwheat starch granules consist of a higher proportion of DP 6–12 chain-length amylopectin molecules compared to amylopectin in B type starch granules, whereas, a lower proportion of medium (DP 16–24) and long chain (DP 25–60) amylopectin molecules in A type starch granule of buckwheat was observed (Punia et al., 2021; Sanderson et al., 2006). Buckwheat starches revealed the higher chain length (CL) (23–24) glycosyl-residues than cereals. Higher amounts of extra-long chains in buckwheat amylopectin could be due to long CL and lower short-to-long chain ratio of amylopectin chains (Hanashiro et al., 2005; Yoshimoto et al., 2004). The gelatinization properties of the buckwheat starches using differential scanning calorimetry (DSC) were accomplished, which revealed that cultivars type, moisture conditions and scanning rate ( $^{\circ}\text{C}/\text{min}$ ) differentially affect the  $T_o$ ,  $T_p$  and  $T_c$  of buckwheat starches.  $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H$  ranged from  $59.5$  to  $64.1\ ^{\circ}\text{C}$ ,  $63.7$  to  $68.4\ ^{\circ}\text{C}$ ,  $81.7$  to  $85.8\ ^{\circ}\text{C}$ , and  $14.5$  to  $15\ \text{J/g}$ , respectively for starches from different common buckwheat accessions (Yoshimoto et al., 2004) (Table 4.1). It ranged from  $58.6$  to  $60.2\ ^{\circ}\text{C}$ ,  $61.5\text{--}64.3\ ^{\circ}\text{C}$ ,  $70\text{--}73\ ^{\circ}\text{C}$ , and  $14\text{--}15.3\ \text{J/g}$  for common buckwheat starches (Lu & Baik, 2015). On the contrary, at higher moisture (1:4) and thermal scanning rates ( $10\ ^{\circ}\text{C}/\text{min}$ ),  $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H$  of  $61.2\ ^{\circ}\text{C}$ ,  $66.1\ ^{\circ}\text{C}$ ,  $75.2\ ^{\circ}\text{C}$ , and  $9.0\ \text{J/g}$ , respectively for common buckwheat accession was observed (Li et al., 2014). Similar observations of different DSC parameters for tartary buckwheat were also noticed (Zhu, 2016 and reference therein). The apparent amylose content in summer and autumn harvested buckwheat grains of similar accession was similar, thus implying that the amylose content in buckwheat was not affected by agronomic practices (Hurusawa & Miyashita, 1965). The gelatinization profile of buckwheat starches was not affected by the amylose content, however, the chain length distribution of amylopectin revealed strong correlation to  $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H$  of buckwheat starches. Lower  $T_p$  for starched having a higher proportion of amylopectin with DP 7–11 was noticed, whereas buckwheat starches with a higher proportion of amylopectin of DP 12–17 showed a higher  $\Delta H$  (Noda et al., 1998). A wider gelatinization temperature attributed structural heterogeneity in buckwheat starches than maize and wheat starches. A lower  $T_o$  and  $T_p$ , and higher  $\Delta H$  for buckwheat than rice and maize starches was also reported by Zheng et al.

(1997). On the contrary, higher  $T_o$ ,  $T_p$ , and  $T_c$  for common and tartary buckwheat starches than potato starches but lower from maize starches were also reported by Gao et al. (2016). However, lower  $\Delta H$  for buckwheat starches was also observed Gao et al. (2016). On the other hand, lower  $T_p$  and  $T_c$  of wheat starches than both type of buckwheat starches was also noticed (Li et al., 1997; Qian et al., 1998). Variation in the amylopectin content, DP and morphology of starch granules etc., may be attributed to differential gelatinization behaviour of starches of the buckwheat. Buckwheat starches showed higher peak viscosity than wheat starches (Acquistucci & Fornal, 1997; Li et al., 1997; Praznik et al., 1999), however, the pasting viscosity of buckwheat starches was lower from the potato and higher from the maize starches (Gao et al., 2016). Gao et al. (2016) revealed higher breakdown viscosity for different buckwheat starches, as compared to starches from potato and maize; higher setback viscosity from maize starches while lower from potato starch. The presence of extra-long chains of amylopectin in rice had related to the lower breakdown viscosity (Han & Hamaker, 2001).

Buckwheat starches exhibited lower syneresis as against wheat and maize (Qian et al., 1998). Syneresis was positively correlated to the amylase and resistant starch content of cooked groats of buckwheat (Lu & Baik, 2015). The gelatinization profile of retrograded starches was also evaluated, and  $T_o$ ,  $T_p$ ,  $T_c$ , and  $\Delta H$  ranged from 39.3 to 41.5 °C, 49.2 to 51.2 °C, 59.2 to 60.9 °C, and 4.6 to 5.6 J/g, respectively for gelatinized and retrograded buckwheat starches (Lu & Baik, 2015). Lower storage induced water syneresis for buckwheat starches upon storage 3–10 days at 4 °C, and better stability to syneresis after freeze-thawing of gelatinized buckwheat starches at -12 and 25 °C was also reported (Qian et al., 1998). Therefore, buckwheat starches with lower syneresis may be useful to make processed food with long storage-shelf life. However, the application of buckwheat starch in food industry is limited, which may be because of high cost of production and the availability of raw material. Buckwheat starch supplemented cake demonstrated poor baking performance and lower sensory attributes (Lorenz & Dilsaver, 1982). Higher accumulation of long chain amylopectin molecules, wide variation in gelatinization profiles, and small granule size may have contributed to poor baking performance of buckwheat starches. However, the small granule size of starches may be used to replace fats in water-in-oil emulsions (Singer, 1994). Where the octenyl succinic anhydride modified buckwheat starches showed enhanced hydrophobic properties thus can be used as emulsifiers to stabilize pickering emulsions (Timgren et al., 2011). These findings thus revealed that buckwheat starch can be used to prepare the gluten-free products after improvement in the gelatinization profiles through plant breeding or genetic engineering approaches (Zhu, 2016).

#### 4.3.2.2 Structural and Functional Characteristics of Buckwheat Proteins

Buckwheat grain contains ~12% of total proteins, which are primarily albumins, globulins, prolamins and glutelins, as depicted in other cereals. Gálová et al. (2019) also revealed that the grains of common buckwheat exhibited 45% of albumin and

globulins, 15% glutelins, and 3% prolamins. On the contrary, the grains of rye and oats consist of 33% and 26% albumins and globulins thus imply that buckwheat has higher proportion of albumin and globulins. Rye grains have 39% of prolamins and 18% of glutelins content was observed against 15% of prolamins and 45% of glutelins for oats. Thus, the proportion of prolamins and glutelins was lower in buckwheat than rye and oats (Gálová et al., 2019). Large diversity in the amino acid composition of buckwheat grains was also observed (Syta et al., 2016). Common and tartary buckwheat grains are found deficient in leucine, cysteine as compared to other cereals (Bhinder et al., 2020; Motta et al., 2019; Zhang et al., 2017) (Table 4.2). Also, imbalance in the amino acid composition of buckwheat was remarkable; however, a high biological value with the amino acid score of 100 for buckwheat proteins was distinguishable (Syta et al., 2016; Syta et al., 2018). SDS-PAGE analysis revealed the presence of 30 to 50 kilo Dalton (kDa), 24 kDa, 19 kDa, 16 kDa and 10 kDa polypeptides (PP) in common buckwheat (Alonso-Miravalles & O'Mahony, 2018). The identity of 50 kDa PP may have appeared to be 13S legumin like and 8S vicilin-like globulin-type PPs, whereas the small molecular weight PPs of 10 to 15 kDa could be albumins (Alonso-Miravalles & O'Mahony, 2018). Though SDS-PAGE analysis not revealed significant differences between PPs of common and tartary buckwheat (Zaika et al., 2019), 2D-PAGE analysis revealed significant variations in protein bands between both types of buckwheat accessions (Capraro et al., 2018). The *in vitro* digestibility of buckwheat proteins was affected by polyphenols (Chen et al., 2019). Buckwheat PPs of 31 and 45 kDa exhibited resistance to proteolytic cleavage, when subjected to simulated gastric and duodenal digestion for 120 min. Whereas, under similar experimental conditions, a 50 kDa PP of buckwheat remained undigested till 180 min exposure to simulated digestion (Gálová et al., 2019).

Attempts have been made to enhance the digestibility of buckwheat proteins. The digestibility of buckwheat protein isolates was enhanced after ultrasound treatment (Jin et al., 2021), and the digestibility of buckwheat proteins improved up to 1% after microwave treatment at 2450 MHz at 850 watts for 30 min; 4% after high pressure treatment at 600 MPa pressure for 30 min at 60 °C temperature and 7% after boiling treatments (Deng et al., 2015). However, the protein digestibility of Tartary buckwheat flour was not affected after hydrothermal treatments (Chen et al., 2019). The effect of extrusion cooking on buckwheat protein digestibility remains unexplored. The hypolipidemic effect of Tartary buckwheat proteins by *in vivo* and *in vitro* was validated (Zhang et al., 2017; Zhou et al., 2018), which may appear due to the presence of quercetin in buckwheat flour and the conversion of cholesterol in bile acids (Zhang et al., 2017). Mora et al. (2019) demonstrated blood pressure lowering property and antihypertensive potential of buckwheat peptides by inhibiting the activity of angiotensin-converting enzyme. Peptides derived from the digestion of buckwheat proteins by trypsin and alcalase (gastrointestinal enzymes) demonstrated the inhibitory activity against dipeptidyl peptidase IV enzyme which thus confirmed the antidiabetic properties. Superior dipeptidyl peptidase inhibitory activity was also demonstrated by peptides derived from the hydrolysis of buckwheat proteins than barley and oat peptides. Dipeptidyl peptidase IV is a



homodimeric serine peptidase that control the secretion of insulin and glycemic control in human (Wang et al., 2015). The intravascular thrombosis and cardiovascular diseases (CVD) in humans is caused by the aggregation of platelets, therefore, the inhibition of platelet aggregation can help to overcome CVD in humans. Buckwheat protein hydrolysates inhibited the platelet aggregation in a dose-dependent manner and showed superior platelets aggregation inhibitory activity than barley protein hydrolysates (Yu et al., 2016a). The interaction of peptides with amino acid residues composition of ALPVDVLANAYR, ALPIDVLANAYR, EFLAGNNKR, GEEFDAFTPK, GEEFGAFTPK, LQAFEPLR, QLAQIPR, QKEFLAGNNK, and TNPNSMVSHIAGK to cyclooxygenase-1 (COX1) through computation modelling was also predicted (Yu et al., 2016b). These findings thus revealed the role of buckwheat protein hydrolysates in the prevention of CVDs in human. Increase in growth of *Bifidobacterium species*, *Enterococcus* and *Lactobacillus*, *Enterococcus* and *Lactobacillus* and decrease in the *E. coil* cell load in the gut of mice after feeding Tartary buckwheat protein rich diet was observed (Zhou et al., 2018). The presence of polyphenols and resistant carbohydrates in buckwheat proteins may attribute to the enhance the growth of gut microbiota in the gut of mice. Allergic responses such as asthma, allergic rhinitis, atopic dermatitis, anaphylaxis, urticarial and enterocolitis were also reported after the consuming buckwheat products. (Miyazaki et al., 2019; Nagai, 2017; Satoh et al., 2020; Satou et al., 2019). Vicilin-like proteins of 55 and 19 kDa, trypsin inhibitory protein of 9 kDa and 16 kDa, 13S protein of 22 kDa, 13S globulin of 22 kDa and proteins with molecular weight of 61, 48 and 45 kDa as major immune-responsive proteins in human were identified (Cho et al., 2014; Satoh et al., 2020; Zheng et al., 2018). Among these proteins, 13S globulin as major allergic protein was recognised (Sano et al., 2014). These findings thus revealed that though buckwheat has good health improving characteristics, the allergic reaction needs to be investigated in detail in populations of diverse origins and ethnicities. Processing of buckwheat flour at ultra-high pressure and hydrolysis with alkaline protease reduced the allergic response of buckwheat proteins upto 100% levels (Lee et al., 2017). Plant breeding approaches can also be undertaken to silent or stop the accumulation of 13S globulin in modern buckwheat cultivars. Therefore, the application of buckwheat proteins in the development of gluten-free products require more detailed scientific investigations.

### 4.3.3 Quinoa

Quinoa (*Chenopodium quinoa*, Willd.;  $(2n = 4x = 36; x = 9)$ ), spinach and beets are the members of the Chenopodiaceae family and 250 species of *Chenopodium* genus are found world-wide. People of Andes i.e., Peru and Bolivia, domesticated *Chenopodium* thousands of years ago because of a rich source of proteins with a balanced composition of essential amino acids (Filho et al., 2017; Jancurová et al., 2009). Presently quinoa is largely cultivated in Argentina, Bolivia, Chile, Colombia,



Ecuador and Peru (FAO, 2012). *Chenopodium quinoa* designated as “pseudo-cereal” or a oleaginous “pseudo-seeds” because of the unique panicle-type inflorescence, protein rich grain composition, high sulfur and lysine content (Filho et al., 2017; Vega-Gálvez et al., 2010; Repo-Carrasco-Valencia and Serna, 2003). *Chenopodium* can grow in diverse climatic conditions, its presence can be observed from sea level to 4000 m above sea level, temperature from  $-4^{\circ}\text{C}$  to  $38^{\circ}\text{C}$  and humidity between 40% to 88% (Bojanic, 2011). Quinoa is highly tolerant to drought and salt conditions (Jacobsen, 2003). Therefore, different accessions of quinoa have great diversity in physico-chemical, morphological and nutritional quality of quinoa, which may be attributed to its diverse geographic distribution and agro-climatic conditions. The grain of quinoa may be of white, black, red, yellow, etc., in colour (Bhargava et al., 2006; Ruiz et al., 2014; Vega-Gálvez et al., 2010).

#### 4.3.3.1 Morphology, Structure, and Chemical Properties of Quinoa Starch

Quinoa seeds contain 53.5–69.2% of starch on dry matter basis and is a prime component of the grains. Starch granules are small in size with diameter of 1-3  $\mu\text{m}$  and localized in the perisperm of quinoa seeds (Lorenz, 1990; Ruales & Nair, 1994). Some studies revealed size of granule between 0.4–2.0  $\mu\text{m}$  (Li & Zhu, 2017a, b; Lindeboom et al., 2004). The presence of starch bound proteins causes aggregation in QS granules, and these aggregates can be disaggregated into single granules by proteolytic cleavage of starch-bound proteins (Atwell et al., 1983; Ruales & Nair, 1994). The size of 10–30  $\mu\text{m}$  for spherical or oblong shaped aggregates was observed against 14,000–20,000 for single granule size of QS granules (Ando et al., 2002; Lorenz, 1990; Ruales & Nair, 1994; Srichuwong et al., 2017). Polygonal, angular, and irregular shapes for QS starches was observed (Lindeboom et al., 2004; Li & Zhu, 2017). The degree of crystallinity for QS ranged from 21.5 to 43.0%, therefore, QS starches exhibited a lower degree of crystallinity than starches of normal maize, garden orache and amaranth while it was higher from kañiwa, barley, adzuki bean and barley starches (Qian & Kuhn, 1999; Steffolani et al., 2013; Tang et al., 2002; Wright et al., 2002). Amylose content between 4–10.9% for QS from size exclusion chromatography was observed, whereas, debranched QS exhibited 3.5% to 27.0% amylose content. Hence, the presence of amylopectin in starch may interfere with the QS estimation (Li & Zhu, 2017). The degree of polymerization (DP) of amylopectin plays an important role to determine the functionality of starch. Fluorophore-assisted capillary electrophoresis (FACE) and high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are widely used to analyse the DP of amylopectin. QS contained higher proportion of amylopectin with short chains with DP of <12 and a lower proportion of amylopectin with DP of <13–35 as compared to waxy maize starches (Li & Zhu, 2017; Srichuwong et al., 2017; Inouchi et al., 1999). QS revealed a higher ratio between short and long chains of amylopectin (Bertoft et al., 2008; Li & Zhu, 2017). The average chain length of 16–17 glucosyl residues for amylopectinin different QS by

HPAEC-PAD was analysed, whereas SEC revealed the average chain length distribution between 18–21 glycosyl residues (Li & Zhu, 2017; Tang et al., 2002; Watanabe et al., 2007; Watanabe, 2008). The gelatinization profile of QS starches by DSC was also evaluated.  $T_p$  and  $\Delta H$  ranged between 56.2 and 65.0 °C, 10.8 and 14.4 J/g, respectively for starches from different quinoa starches as reported by Li et al. (2016).  $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H$  of 53.9 °C, 60.6 °C, 66.0 °C, and 10.3 J/g, respectively for quinoa starches was noted by Srichuwong et al. (2017) (Table 4.1). The gelatinization profile ( $T_o$ ,  $T_p$ ,  $T_c$  and  $\Delta H$ ) of white sorghum, red sorghum, millet, corn, wheat, and amaranth starches was higher than quinoa starches (Srichuwong et al., 2017). These findings thus revealed that QS exhibited  $\Delta H$  similar to sorghum, garden orach, and kañiwa, whereas, maize, adzuki bean, and amaranth starches hold higher  $\Delta H$  than QS starches. QS starches demonstrated a higher  $\Delta H$  as compared to wheat and barley starches (Inouchi et al., 1999; Qian & Kuhn, 1999; Steffolani et al., 2013; Tang et al., 2002; Wright et al., 2002). Variations in amylose and amylopectin content among starches from different botanical sources may be linked to wide differences in  $\Delta H$ , which is linked to the fine structure of amylopectin (Li & Zhu, 2017a). The swelling power of starches is also correlated to amylose content in starches. The peak viscosity of 2860 cp for quinoa starches was observed, whereas, PV ranged between 2240 and 2400 cp for sorghum starches, 2000 cp for amaranth starches, between 1850 and 1891 cp for corn and millet starches, and 1319 cp for wheat starches was reported by Srichuwong et al. (2017). Pasting temperature of 65.3 °C and 63.5 °C for sweet and bitter quinoa was also observed (Wright et al., 2002). While, the peak-, hold-, breakdown-, final and setback viscosities ranged from 367.3 and 402.5 cp, 357.9 and 369.4, 9.4 and 33.1, 495.9 and 495.1, 138.0 and 125.8 cP, respectively, for sweet and bitter starches (Wright et al., 2002). These findings thus demonstrated that peak viscosity (PV) of quinoa starch was higher than that of most other starches. The swelling power and pasting properties of starches are influenced by the interaction of proteins, lipids and non-starch polysaccharides during gelatinization. High swelling power attributed to lower pasting temperature of amaranth starches. Lower amylose content (1.2%) allows starch granule to swell more which led to rupture of starch granules at lower temperature this leads to lower pasting temperatures. The presence of lipids does not affect the pasting properties of amaranth starches due to lower amylose content in amaranth starch. On the contrary, amylose content ranged between 22.9–25.8% for wheat, millet, corn and sorghum starches, which led to a stronger amylose-lipid interaction, associated with higher pasting temperature, lower peak- and breakdown viscosities. QS starches consist of average 8.2% amylose content, and small granule size, which, therefore, resulted in the intermediate or moderate amylose-lipid interactions during gelatinization. Weak starch-lipid interactions, moderate levels of amylose content in QS may have resulted in higher peak- and lower breakdown viscosities. Starches with higher peak viscosity and lower setback viscosities could be used to prepare the food withhold/maintain the consistency of gel but not to be solidified upon cooling, thus it can be said that quinoa starches can be used to prepare rice puddings, instant creamy deserts, and also used as fat replacer for mayonnaise or water-in-oil emulsions.

#### 4.3.3.2 Composition and Functionality of Quinoa Protein

Protein content ranged between 12% and 23% which is higher from cereal grains, while the grain content in quinoa was lesser from pulses (Abugoch James, 2009). Intriguingly, majority of the protein content localized in the embryo of quinoa. Protein content between 15.6 and 18.7% for six quinoa varieties of Southern Europe was reported by Rodríguez Gómez et al. (2021). Quinoa proteins are primarily composed of 37% of globulin and 35% of albumins, however these are deficient in prolamins or least detected. Lower proportion of prolamins of 0.7% to 7.0% for quinoa seed was reported by Abugoch James (2009). The composition of amino acid for quinoa was also evaluated. Quinoa proteins are rich in lysine (5.1–6.4%) and methionine (0.4–1%) amino acids (Prakash and Pal, 1998) (Table 4.2). Superior content of essential amino acids such as histidine and lysine for quinoa was also observed. Histidine of 28.8 mg/g, and lysine content of 54.2 mg/g for quinoa was reported. These findings thus revealed a high nutritional potential of quinoa along with a source of high-quality proteins. Protein efficiency ratio (PER) and digestibility of quinoa proteins is comparable to the casein protein of milk, while the PER of washed quinoa than raw quinoa was also superior. The presence of saponins may have affected the PER and digestibility of quinoa proteins (Gross et al., 1989; Ruales & Nair, 1993). The digestibility and functionality of quinoa proteins can be enhanced by heat, hydrothermal, microwave and baking treatments. The role of 7S and 11S globulins was crucial in protein aggregation during heating at different pH and temperature regimes (Van de Vondel et al., 2021). However, heat-induced gelling behaviour of quinoa proteins need more detailed investigations. Quinoa seed storage 11S globulin is composed of hexameric protein comprise of six pairs of acidic and basic subunits, which are connected to each other by disulfide bridges (Brinegar & Goundan, 1993). Similarity analysis of 11S globulin at amino acid levels revealed its high homology with glycinin, therefore, quinoa 11S globulin protein is as designated as chenopodin (Barrett, 2006). Chenopodin is a major seed storage protein of quinoa which is 37% of the total seed storage proteins. The acidic and basic subunits of chenopodin (11S globulin) exhibited molecular weight of 30–40 kDa and 20–25 kDa, respectively, which linked together via disulphide bonds (Abugoch James, 2009; Brinegar & Goundan, 1993). Higher content of asparagine, aspartic acid, arginine, serine, leucine, glycine, glutamine-glutamic acid for chenopodin was also observed (Brinegar & Goundan, 1993). Therefore, the amino acid composition of chenopodin matched the leucine, isoleucine, and phenylalanine, and tyrosine amino acid composition of the standards of FAO protein reference (FAO, 1973). A protein with 8–9 kDa with 35% proportion of the total grain protein in quinoa was also identified by Osborne (1924) method. The 8–9 kDa protein was 2S-type protein and belongs to albumins. The 2S-type albumin was rich in arginine, cysteine, and histidine (Brinegar et al., 1996). These findings thus revealed that the 11S and 2S-type protein in *Chenopodium* are the major seed storage proteins and reservoirs of essential amino acids. The quinoa protein isolates exhibited water holding capacity of 2.8–4.5 mL of water/g of sample, while soy protein isolates exhibited the WHC of 4.3 mL of water/g of sample. Thus, the WHC of quinoa

protein isolates was comparable soy protein isolates and can be used to fortify gluten-free products.

## 4.4 Millets

Abiotic and biotic challenges, global warming and abrupt climatic conditions, shrinkage of arable land by urbanization, rising price and increasing demand of cereals globally, are ongoing challenges for cereal production. However, millets are one of the widely consumed grains in arid and semi-arid regions of Asia (India and China) and Africa (Dhull et al., 2021; Yousaf et al., 2021). Millets can withstand up to 64 °C temperature and 350–400 mm annual rainfall (Chivenge et al., 2015). Millets being a C4 crop have highly efficient photosynthesis system and require only 6–8 weeks for seed maturation which may attribute to high yield and thermo tolerance (Hariprasanna et al., 2014). Also, millets are considered as “poor man food” because of lower price and readily availability for population lives in semi-arid and arid zones (Amadou et al., 2013). Millets are considered as first ancient grains domesticated for human use. Millets are round shape small-seeded grains of the Poaceae family, which are of seven types of namely foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), barnyard millet (*Echinochloa crus galli*) and little millet (*Panicum sumatrense*) (Guenard, 2021; Punia Bangar et al., 2021a, b, c, d; Siroha et al., 2021). The total millet production of 31,019,370 tonnes in 2018 was estimated which secured sixth position among other cereals. The largest proportion of millet was produced by India during 2018 followed by Niger, Sudan, and other countries (FAOSTAT, 2020).

### 4.4.1 Composition and Functionality of Millet Starches

Millet starch revealed amylose content between 6 and 38.6%, lipid between 0.16 and 2.9%, protein 0.2 and 4.3% and ash content of 0.02 to 1.4% (Zhu, 2014 and references therein). The higher accumulation of glycine, glutamine, and aspartic acid in the granules of the foxtail millet starches was also reported by Wankhede et al. (1979). The presence of neutral lipids (linolenic acid), phospholipids lipids (palmitic acid), and glycolipids in the pearl millet starch granules in free and bound form were reported by Hoover, 1995. Polygonal morphology depicted the majority of millet starches (Zhu, 2014 and references therein). The DP affects the pasting properties of starches and DP ranged from 1060 to 1250 and 9000 to 9100, respectively for amylose and amylopectin from pearl millet starches. Whereas, the molecular weight of pearl millet amylose and amylopectin ranged from 105 to 106 and 107, respectively (Madhusudhan & Tharanathan, 1996; Wankhede et al., 1979). The

chain length (CL) distribution of glucosyl moieties influences the molecular weight of amylose and amylopectin of a starch granule. The CL between 260 and 270 glucosyl residues with four chains per amylose molecules was observed. Where amylopectin from pearl millet exhibited CL of 18 and 21 glucosyl residues, the external CL ranged between 12 and 14. Along with that, the internal chain length ranged between 4.8 and 6.3 glucosyl residues was also observed (Annor et al., 2014; Gaffa et al., 2004; Madhusudhan & Tharanathan, 1996). It is proposed that the internal chains of amylopectin constitute the amorphous region of a starch granule, whereas, the crystalline region of a starch granule is formed by the external chains of amylopectin (Pérez & Bertoft, 2010). The swelling power and solubility of millet starches between the temperatures range of ~50–90 °C was observed. Therefore, the swelling power and solubility of millet starches is lower than potato starches. Higher amylose content may also be linked with low swelling power and solubility of millet starches at lower temperature. Higher leaching of amylose in rice also linked to higher gruel solid loss which was associated with leaching of amylose during the gelatinization of rice starches followed by gel formation. Since millet starches are composed of higher amylose content, it is imperative that the pasting and rheological properties of these starches should also be analysed. PV between 345 and 425 RVU, BDV between 183 and 237 RVU, SBV between 142 and 188 RVU, and PT between 72.0 and 78.5 °C for different genotypes foxtail millet had been reported (Liu et al., 2011). The thermal behaviour of millet starches by using DSC was also analysed and very high diversity in thermal properties of various accessions of millet was noticed. Pearl millet starches revealed the  $T_o$ ,  $T_p$ ,  $T_c$ , and  $\Delta H$  between 66.2 and 67.2 °C, 69.7–71.4 °C, 86.3–91.0 °C, and 14.3–14.7 J/g, respectively (Gaffa et al., 2004) (Table 4.1).  $T_p$  and  $\Delta H$  of between 62.4 to 75 °C and 8.2–13.5 J/g, respectively, for the different accessions of foxtail millets were observed. On the contrary, proso millet starches exhibited  $T_p$  between 65.8 and 80.2 °C, while  $\Delta H$  was ranged between 6.4–11.4 J/g for proso millet starches (Fujita & Fujiyama, 1993). Similar findings for foxtail millet were also observed by Wankhede et al. (1979) (Table 4.1). The starches from pearl millet exhibited good freeze thaw stability than corn and wheat starches (Hoover, 1995), while pearl millet starch revealed poor freeze thaw stability as compared to maize starch (Yañez et al., 1991). The freeze thaw stability is largely influenced by amylose content and proportion of shorter unit chains of amylopectin in starches, and may be attributed to variation in freeze thaw stability among different millet genotypes (Srichuwong et al., 2012). Therefore, detailed investigation of different genotype of millets to improve the functionality of millet starches is required. The glycaemic index of starches is determined by their susceptibility against starch hydrolysing enzymes. Roopa and Premavalli (2008) observed total starch (TS) between 39.7 and 50.1%, RDS between 8.3 and 11.1%, SDS between 26.5 to 35.3%, RS between 0.8 and 1.0% for the flour of finger millet. Whereas, Annor et al. (2013) noticed a total starch (TS) of 83%, RDS of 11.5%, SDS of 31.3%, and RS of 40.1% for Kodo millet flour. On the contrary, Kodo millet starches revealed TS, RDS, SDS, and RS of 94.2%, 21.8%, 33.2% and 37.5%, respectively (Annor et al., 2013). These findings thus revealed that

millet starches exhibited relatively fair content of SDS and RS, which is very useful for persons suffering from many chronic life-style related ailments.

#### **4.4.2 Composition and Functionality of Millet Proteins in Millets**

The protein content varied among different millets cultivars. Protein content of 14.5% (8.6–19.4%), 11.7% (6.0–14.0%), 13.4% (6.4–15.9%), and 8.0% (6.9–10.9%), respectively for pearl, foxtail, proso, and finger millet, was observed. These findings thus revealed that among different millets, the pearl millets contain the highest protein content of 14.5% whereas, finger millet exhibited lower protein content 8.0% (Taylor & Taylor, 2017). The large protein-rich germ and smaller endosperm may be attributed to high protein content in pearl millet (Serna-Saldivar & Rooney, 1995). The analysis of proteins revealed the presence of albumins and globulins, prolamins, and glutelins in the grain of different millets. Albumin and globulins of different millets exhibited higher abundance of Glutamine/glutamate, asparagine/aspartic acid, arginine, alanine, and leucine, while higher abundance of Glutamine/glutamate, alanine, leucine, proline, and valine in the prolamins subfractions of different millets was observed. On the contrary, the glutelin fraction of different millets revealed the higher accumulation of glutamine/glutamate, arginine, leucine, asparagine/aspartic acid, proline, alanine, methionine, and phenylalanine amino acids. These findings thus demonstrated that the millets are poor in lysine content (21–37 mg/g protein), as observed for other cereals such as wheat etc. (Table 4.2). However, different millets exhibited higher leucine content (122–135 mg/g protein) which may attribute to the higher accumulation of prolamins in these cultivars (Taylor & Taylor, 2017).

### **4.5 Conclusions**

The utilization of gluten-free cereals like sorghum and rice, pseudocereals such as amaranth, quinoa, millets, and buckwheat are gaining popularity. However, significant variation in the composition and functional properties of starches and proteins in pseudocereals vary significantly. Starches from a few Indian sorghum cultivars revealed higher enthalpy of retrogradation and lower synereses values thus can be utilized as superior alternatives of corn starches. Sorghum starches, therefore, can be used for making gluten-free products with long storage-shelf life. On the contrary, proteins in grain sorghum are poor in lysine and threonine amino acid content. Sorghum kafirin proteins showed a higher proportion of  $\alpha$ -prolamins with viscoelastic properties which can be used as an alternative to gluten to enhance the viscoelastic properties of gluten-free dough, thus leading to enhance the texture



characteristics of gluten-free food products. The poor solubility of sorghum kafirin after wet cooking needs to be addressed in the future. The small size, granular structure, low amylose content and high tendency to loosen crystallinity, and fast digestibility of amaranth starches attributed to consider it as a high glycaemic food. Therefore, amaranth starches cannot be incorporated into gluten-free products. On the contrary, a higher proportion of lysine and valine amino acid residues in the albumin and globulin fraction of the amaranth grain proteins, while a higher proportion of leucine and histidine amino acids in glutenins of amaranth proteins were observed. Also, the protein digestibility and lysine content for amaranth proteins are also good, therefore, amaranth could serve as a superior alternative to the nutrition-rich source of proteins. Common buckwheat amylose resembled the starches from wheat and barley but have lower synereses values. Therefore, buckwheat starches can be used to improve the shelf-life of gluten-free products. Common and Tartary buckwheat grains are deficient in leucine, cysteine amino acids with imbalance amino acid composition, inferior protein digestibility, and allergic response. Therefore, the nutrition and functional characteristics of buckwheat protein are inferior to cereals. Quinoa starches consist of an average of 8.2% amylose content, and small granule size, which, therefore, have a higher peak- and lower breakdown viscosities. Therefore, quinoa starches can be used for making semi-solid foods like pudding and deserts and also replace fat from mayonnaise or water-in-oil emulsions. Quinoa proteins are rich in lysine (5.1–6.4%), methionine (0.4–1%), and histidine amino acids. Also, quinoa proteins revealed high protein efficiency ratio and superior digestibility, which is comparable to the casein protein of milk, therefore quinoa protein isolates can serve as superior alternatives to wheat gluten and rice-bran proteins, which have poor solubility and foaming properties. Though millet proteins are deficient in lysine content (21–37 mg/g protein), pearl millet starches are rich in amylose, hold higher SDS value, and superior freeze-thaw stability than corn and wheat starches, therefore, millet flour could serve as the base of gluten-free flour. Therefore, improvement in the functional properties of starches and proteins of pseudocereals by using processing technology, genetic engineering, and plant breeding approaches in the future will be in the prime focus.

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