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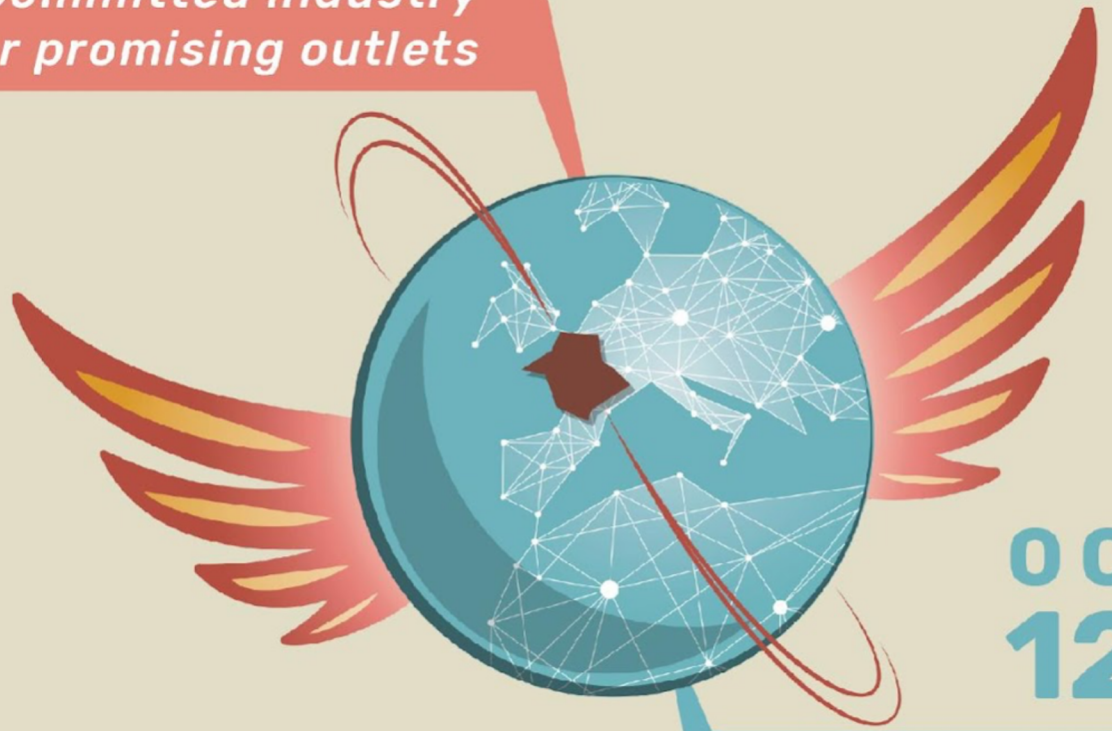

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CAN SORGHUM FLOUR REPRESENT A VALID ALTERNATIVE IN GLUTEN-FREE DIET?

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What is gluten free diet?

- Gluten free diet (GFD) is a complex diet without gluten containing food
- Consumption of a gluten-free diet requires a major lifestyle change (gluten is contained in a variety of foods that are commonly consumed in the Western diet)
- Foods containing wheat, rye, and barley should be avoided
- Rice, corn, buckwheat, soybean or tapioca flours, and potatoes are allowed

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Nutritional aspects of gluten free diet

- Patients often prefer to consume industrial gluten free products (GFPs)
- GFPs are lack of vitamins, micronutrients and fiber
- Rich in lipids
- High glycemic index
- Rice and maize usually contain high amounts of nichel that worsen gastrointestinal symptoms
- If unbalanced GFD, development of nutritional deficiency or metabolic diseases

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Clinical indications for gluten free diet

- Celiac disease (recommended)
- Non-celiac gluten sensitivity (strongly suggested)
- Irritable bowel syndrome (sometimes suggested)
- Infertility (sometimes suggested)
- Autoimmune diseases (considered)

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Celiac disease

- Celiac disease is an immune-mediated disease of the small intestine
- Triggered by the ingestion of gluten in genetically predisposed individuals
- Estimated global prevalence is 1%
- Variability of clinical manifestations (diarrhea, abdominal pain, malabsorption, dermatitis, metabolic or neuropsychiatric disorders)
- **The only treatment is a strict gluten-free diet**

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Non-celiac gluten sensitivity

- Clinical condition with gastrointestinal symptoms similar to celiac disease
- Absence of intestinal mucosal damage or serological alterations
- No evidence of malabsorption or mild forms
- Marked clinical response, with reduction of gastrointestinal symptoms, after the start of the gluten free diet
- Benefit from the diet for many years

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Other clinical conditions

- GFD is a treatment option for many patients with irritable bowel syndrome
- Some cases of infertility can improve with GFD, even in the absence of obvious celiac disease
- Some condition of autoimmunity (as thyroiditis, hepatitis or entheropathies) could benefit from GFD
- Recent studies suggest a role for GFD in autism or psychiatric disorders

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What is the role for sorghum?

- Sorghum is naturally free of gluten
- Safe and well tolerated in celiac disease or patients requiring GFD
- Modern food techniques have made sorghum based products more palatable
- Pasta, cake or baked goods are produced with satisfactory patient acceptance
- Good nutritional profile

Ciacci et al. Clin Nutr . 2007
Cayres et al., J Cereal Science, 2020

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Cereals frequently used in gluten free products compared to sorghum

	Carbohydrates (g/100g)	Protein (g/100g)	Fat (g/100g)	Minerals (mg/100g)
Rice	73.70	7.70	2.20	1.20
Maize	65	8.80	3.80	1.30
Sorghum	72.60	10.40	1.90	1.60

Moreno et al. Austin J Nutri Food Sci. 2014

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Sorghum foods from around the world

Food type	Food name	Country of origin
Part of the main meal	Couscous	India & Sahel
	Annam (Sora)	India
	Kaoliang mi fan	China
	Lehata wagen	Botswana
	Nufro	Ethiopia
	Okababa	Nigeria
Confectionary or sweet	Rawa laddu	India
	Kesari	India
Bakery products	Roti	India
	Bhakri	India
	Dosa	India
	Kisra	Sudan
	Injera	Ethiopia
	Mantou	China
Porridge	Sankati (Mudda or Kali)	India
	Kanji (Ambali)	India
	Bogobe	Botswana
	Ogi	Nigeria
	To`	West Africa
	Nasha	Sudan
	Aceda	Sudan
	Ugali	Uganda, Rwanda & Tanzania
Beverages	Rabadi	India
	Pito	Nigeria & Ghana
	Baijiu	China
	Obiolor	Nigeria & Ghana
Breakfast	Upma	India
	Idli	India
	Noodles	China
Snack foods	Popped sorghum	India

Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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But from a nutritional point of view, what do we know about sorghum?

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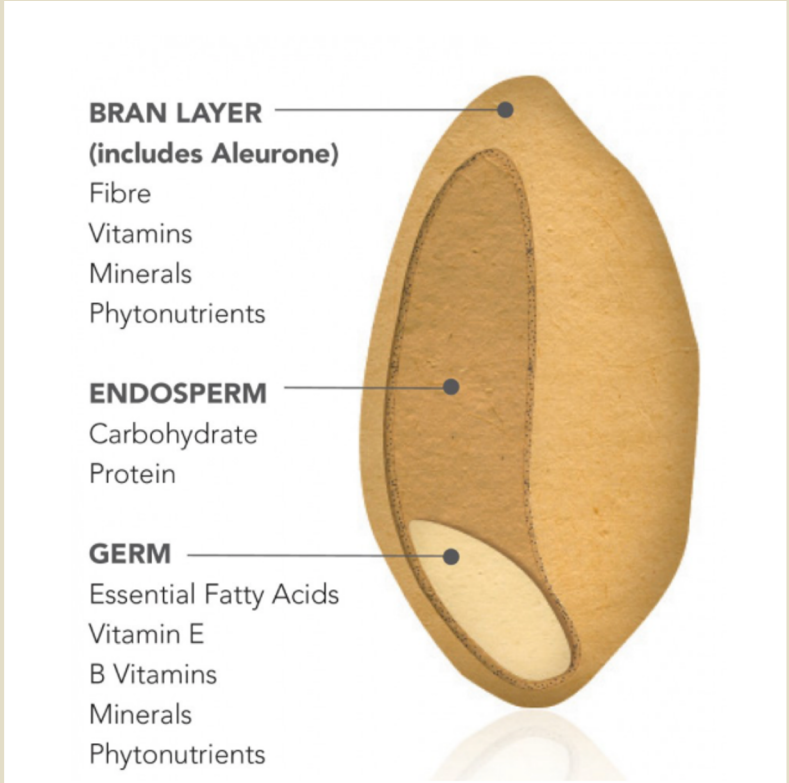
Sorghum grain composition

- Carbohydrates
- Proteins
- Lipids
- Vitamins and Minerals
- Phenolic compounds



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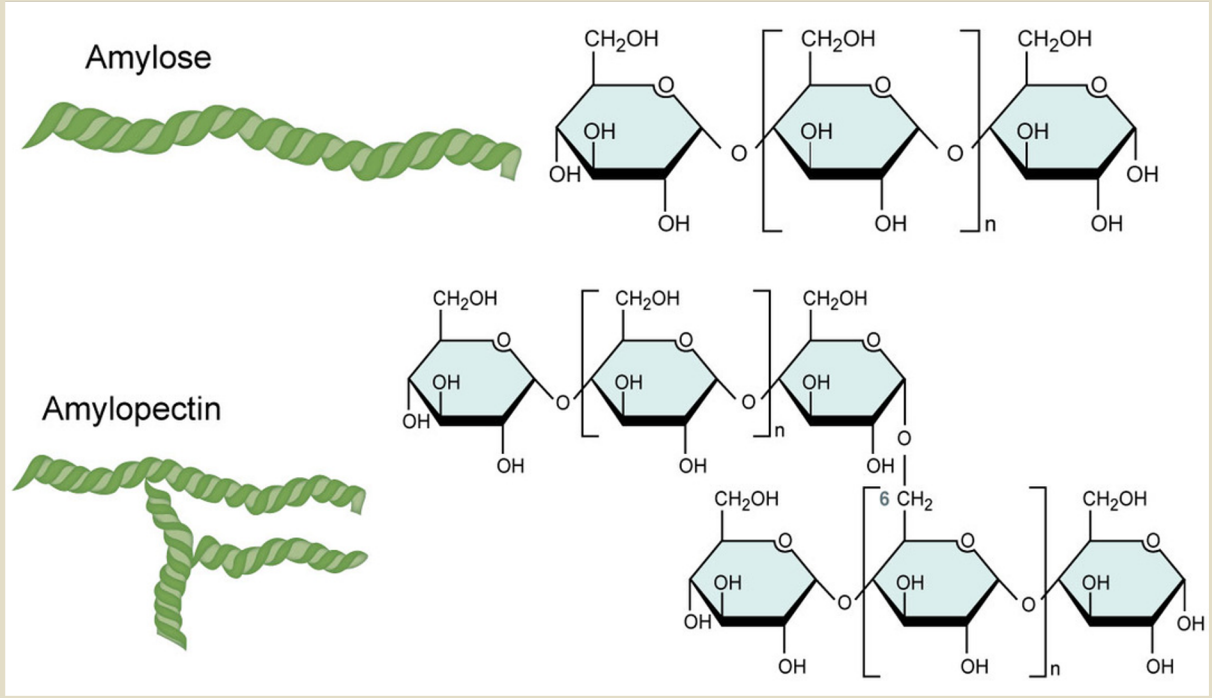
Carbohydrates

- Polysaccharides starch (amylose and amylopectin)
- Fructosan
- Raffinose
- Sucrose
- Maltose
- Free sugar 1-2%
- Non starch polysaccharides (NSPs).



Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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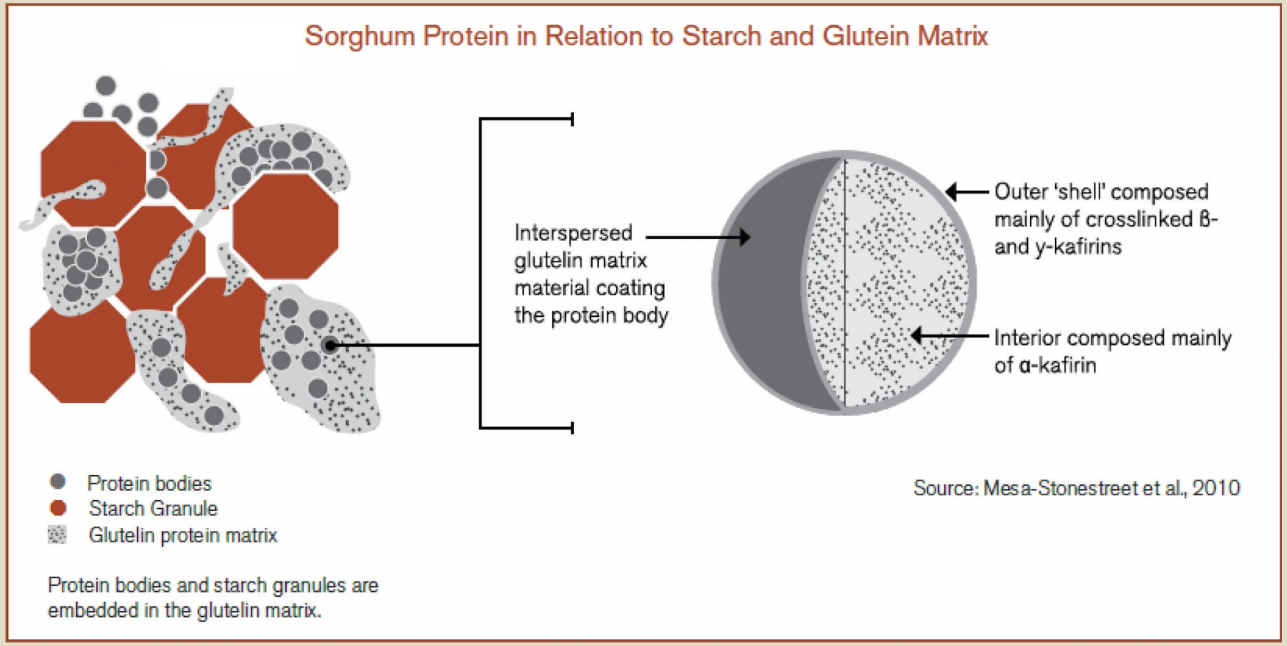
Proteins

- Albumins
- Globulins
- Glutelins
- Kafirins (high in cysteine and methionine)



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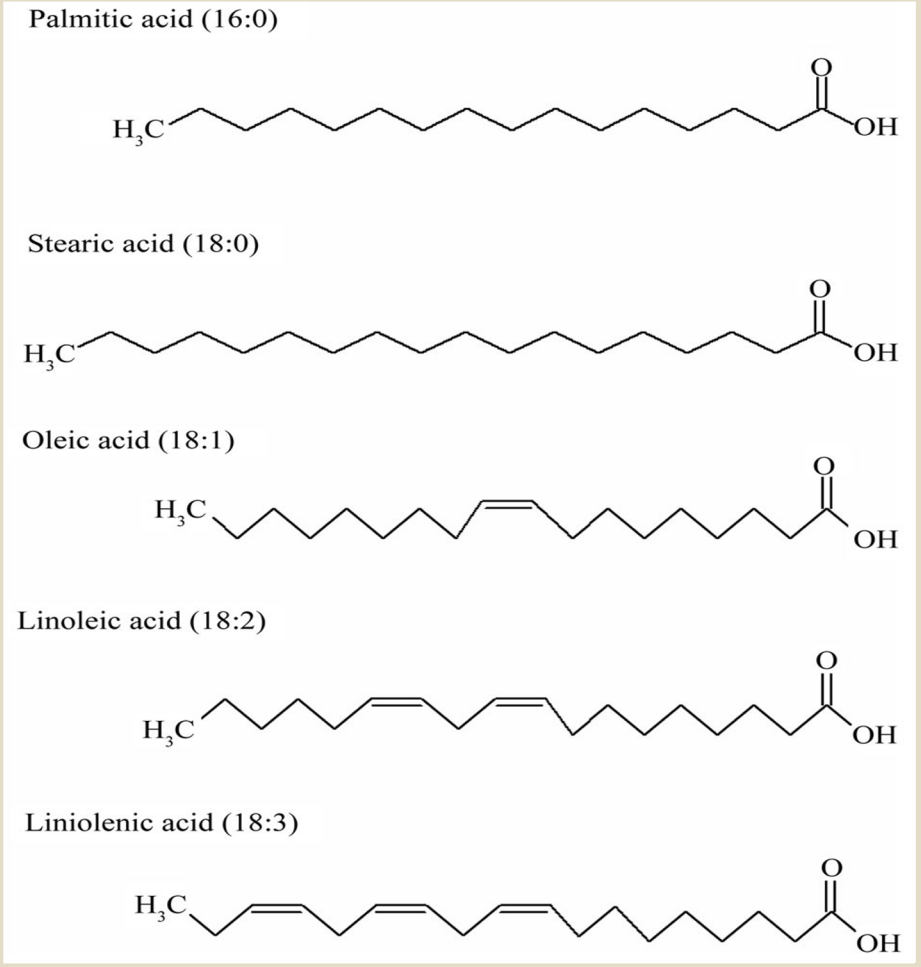
Lipids

- Sorghum grain contain 1-3% of lipids
- Linoleic 49%
- Oleic 31%
- Palmitic 14%
- Linolenic 2.7%
- Steric 2.1%



Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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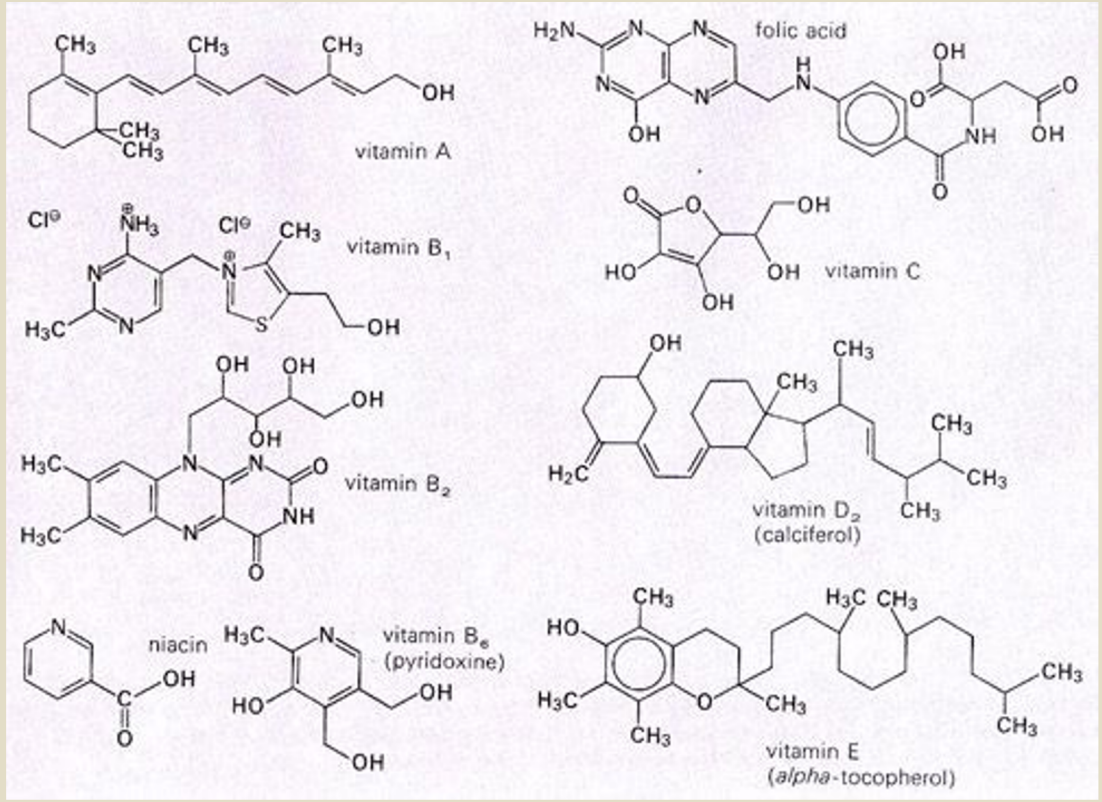
Vitamins and Minerals

- Abundance of B-carotene and tocopherols
- Lipid-soluble vitamins (Vit. A, D and K)
- Water soluble vitamins (thiamin, riboflavin and pyridoxine)
- Minerals (Magnesium, iron, zinc, copper, calcium, phosphorus)
- Phenolic compounds (flavonoids, phenolic acids, tannins)



Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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Is there any evidence on the role of sorghum in maintaining health or preventing chronic disease?

Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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Anti-diabetic activities of sorghum

Sorghum Fraction/ Bioactive Compound	Study Model	Anti-diabetic mechanism
Flavonoids	Enzyme assay	↓ α-amylase, α-glucosidase, AGEs
Sorghum extract (ethanol based)	STZ-induced diabetic rats	↑p-AMPK/AMPK
		↓ p38:p38, PEPCK, blood glucose level
Sorghum extract (methanol based)	High-fat-diet induced obese mice	↑ PPARγ and adiponectin
		↓ TNFα
Grain sorghum muffins	Healthy adult men	↓ Plasma glucose and insulin
	Enzyme assay	↑ Slowly-digestible and resistant starch
		↓ Readily-digestible starch
Flavonoids	Enzyme assay	↓ α-amylase, α-glucosidase, AGEs
Sorghum extract (ethanol based)	Enzymatic assay	↓ α-amylase, α-glucosidase
Extruded Sorghum Drink	Healthy adults	↓ Postprandial glycemia
Flavonoids	Enzyme assay	↓ α-amylase, α-glucosidase, AGEs
Sorghum extract	STZ-induced diabetic rats	↓ pAMPK and macrophage infiltration

Amarakoon et al., J Sci Food Agric, 2021

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Anti-atherosclerotic activities of sorghum

Sorghum fraction/Bioactive compound	Study model	Anti-atherosclerotic mechanism
Sorghum Lipids	Enzyme assay	↓ HMG CoA Reductase
Whole Sorghum	Mice	↑ HDL cholesterol
Sorghum Lipids	Hamster	↓ Plasma non-HDL and cholesterol absorption
Sorghum Lipids	Hamster	↑ HDL cholesterol and *Bifidobacterium*
Sorghum Phenolics	HFD fed rats STZ-induced diabetic rats	↓ Plasma cholesterol and triglycerides
Sorghum Phenolics	HUVECs	↑ HO1 and eNOS expression ↓ ICAM1, MCP1, NOX4 and CD39 expression
Sorghum Phenolics	Human blood samples from healthy males and females after 8 h fasting	↓ Platelet aggregation and PMP release

Amarakoon et al., J Sci Food Agric, 2021

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Anti-inflammatory activities of sorghum

Bioactive compounds	Study model	Anti-inflammatory mechanisms
Sorghum bran extract	TPA-induced mice	↓ Ear thickness
		↓ iNOS and COX-2
Phenols	Red blood cells	↓ AAPH-induced oxidative damage
	PMN cells	↓ ROS, cell migration
	PBMC cells	↑ IL-6, MCP-1, MIP-1α and MIP-1β
Caffeoylglycerols	LPS-induced RAW 264.7 cells	↓ iNOS and COX-2
Apigenin	LPS-induced PBMC cells	↓ COX-2
Apigeninidin		↓ PGE2
Kafirin	LPS-induced THP-1 cells	↓ IL-1β, IL-6 and TNF-α
		↓ ROS
		↓ pERK and pJNK
Phenols	HUVECs	↑ HO1 and eNOS
		↓ NOX4
		↓ MCP-1 and ICAM1
Triacylglycerol	LPS-induced RAW 264.7 cells	↓ IL-1β, IL-6, and COX-2
Unsaturated fatty acids		
Tocopherols, carotenoids		
Phenols		

Amarakoon et al., J Sci Food Agric, 2021

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Anti-cancer activities of sorghum (Apoptosis)

Bioactive compounds	Cancer pathway	Anti-cancer mechanisms	Cancer type
Hwanggeumchal sorghum extracts	Apoptosis	↑ p53 ↓ Expression and phosphorylation of STAT5b/IGF-1R ↓ HIF-1α, Bcl-2, Breast tumor kinase (Brk)	B *In vivo*
Apigeninidin		↓ Bcl-2 ↑ BAK, BAX, caspase-9, caspase-3, cleaved PARP, lamin B ↑ Release of mitochondrial cytochrome C and apoptosis-inducing factor	Leu
3-DXA extracted from red sorghum		↑ p53; ↓Bcl-2	B
3-DXA (luteolinidin and apigeninidin)		p53-independent pro-apoptotic activity	C
Anthocyanin-rich plant extracts		↓ cIAP-2, survivin, XIAP, and insulin-like growth factor binding proteins	C
Polyphenol extracts from bran		↑ ROS ↑ caspase 3, caspase 8, cleaved PARP1, cleaved caspase 3 ↓ IGF-1, IGF-2, and survivin, ↑ XIAP, ↓ SMAC	L
Sorghum extracts		↑p53, caspase 3 and 7	C
Mixed cereal grain (MCG)		↓ Colonic neoplasia ↑ p53 and mRNA of CDKN1a and caspase 3	C *In vivo*
Sorghum extract		↑ Cleaved PARP and caspase-3 ↑ pH2AX, pERK, pJNK, ATF3	C

Amarakoon et al., J Sci Food Agric, 2021

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Anti-cancer activities of sorghum (Cell cycle)

Hwanggeumchal sorghum extracts	Cell cycle	↑ p53 ↑ G1 arrest ↓ Cyclin D, cyclin E, and pRb	B
High-polyphenol extracts		↑ ROS ↑ p21, Chk2, p-Chk2	L
Anthocyanin-rich plant extracts		↑ G1 arrest	C
Mixed cereal grain (MCG)		↑ p53, CDKN1a mRNA ↓ Cyclin D1 mRNA and protein ↓ mRNA and protein of NOS2 and COX2	C *In vivo*
Sorghum extract		↑ S phase arrest, p21 ↓ CDK6	C

Amarakoon et al., J Sci Food Agric, 2021

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Anti-cancer activities of sorghum (Angiogenesis and metastatis)

Procyanidin rich extract	Angiogenesis	↓ VEGF	L *In vivo*
Hwanggeumchal sorghum extracts		↓ p-STAT5, p-STAT3, VEGF, VEGF-R2 ↓ HIF-1α	B *In vivo*
Hwanggeumchal sorghum extracts	Metastasis	↓ Metastasis from breast to lung ↓ JAK/STAT pathways	B *In vivo*
Sorghum extract		↓ Migration and invasion ↓ β-catenin, VEGF, MMP-9	C

Amarakoon et al., J Sci Food Agric, 2021

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Antioxidant activities of phenolics extracts of sorghum grains

Sorghum Source	Bioactive Extracts	Antioxidant Activity
Hongyingzi, Hongzhenzhu, Dongbei sorghum, Jiangsu sorghum, Jiliang 2 sorghum, Longza 11, black grain sorghum, white Longmi sorghum.	Caffeic acid, *p*-coumaric acid, ferulic acid, protocatechuic acid, luteolindin, apigeninidin, luteolin, apigenin, taxifolin, naringenin.	Antioxidant activities against DPPH and FRAP assays.
Tannin-containing sorghum varieties (Sumac, Hi-Tannin, Seredo, CR 35:5 × 2), non-tannin varieties (white variety, KARI-Mtama, red variety, ICSV-III), Mizzou, Tx430.	Condensed tannins, 3-DXA, phenolics.	Induced phase II detoxifying enzymes; anti-proliferative effect on esophageal, OE33, colon cancer cells.
Liberty, Mr-Buster, Cracker, IS131C, Shawaya Short Black 1.	Phenolic extracts.	Antioxidant activities against DPPH and FRAP assays; Anti-proliferative effect on Caco-2 cells.
Tx3362, Shawaya Black, Black PI Tall, Hyb 107, Hyb 115, Hyb 116, Hyb 117, Hyb 118.	Total phenolics, condensed tannins, flavan-4-ols, 3-DXA.	Antioxidant activities against DPPH and ABTS assays.

Xu et al., Foods, 2021

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Anti-proliferative effect of phenolic extracts of sorghum grains

Sorghum Source	Bioactive Extracts	Anti-Proliferative Effect
Black sorghum varieties (Macia, Sumac, PI152653, PI152687, PI193073, PI1329694, PI1559733, PI1559855, PI1568282, PI1570366, PI1570481, PI1570484, PI1570819, PI1570889, PI1570993).	Total phenolic extracts.	Anti-proliferative effect on HepG2 and Caco-2 cells: induction G1/S cell cycle arrest, activation of p53.
Red sorghum	3-DXA extracts.	Inhibitory effect on MCF7 cancer cells through up-regulating p53 and down-regulating Bcl-2 genes.
Dale, M81E	Vanillic acid, p-coumaric acid, ferulic acid, caffeic acid, apigeninidin, luteolinidin, malvidin-3-*O*-glucoside, apigenin, luteolin, trans-resveratrol, luteoferol.	Inhibitory effect on HCT116 and colon cancer stem cells through activating p53 gene.
Hwanggeumchal sorghum.	Total polyphenol extracts.	Anti-proliferative effect on MDA-MB 231 and MC7 cells: down-regulating VEGF, VEGF-R2, cyclin D, cyclin E, pRb and up-regulating p53.
TX430, Sumac.	Total phenolic extracts.	Anti-proliferative effect on HepG2 and HCT15 cells.

Xu et al., Foods, 2021

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TX430, Sumac.	Total phenolic extracts.	Anti-proliferative effect on HepG2 and HCT15 cells.

Xu et al., Foods, 2021

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Anti-diabetic and anti-atherogenic effect of phenolic extracts of sorghum grains

Sorghum Source	Bioactive Extracts	Anti-Diabetic and Anti-Atherogenic Effects
Brown sorghum varieties (SOR 01, SOR 03, SOR 08, SOR 11, SOR 17, SOR 21, SOR 24, SOR 33)	Gallic acid, chlorogenic acid, caffeic acid, ellagic acid, p-coumaric acid, quercetin, luteolin, apigenin.	Inhibitory effect on α-amylase and α-glucosidase activities.
Hwanggeumchal sorghum.	Phenolic extracts.	Reduced the serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine.
KNICS-579	Polyphenol extracts.	Reduced the concentration of triglycerides, total LDL-cholesterol and glucose.
Red sorghum	Total phenolic extracts.	Antioxidant activity against ABTS, DPPH, FRAP assays; Inhibitory effect on pancreatic lipase, α-amylase and α-glucosidase activities.

Xu et al., Foods, 2021

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Health benefits

- Absence of gluten (fundamental in gluten free-diet)

- Help in control of glycemic response (low glycemic index)
- Lower level of cholesterol absorption (policosanols)
- Rich in minerals (calcium, phosphorous) and vitamins
- Reduce chronic inflammation and oxidative stress
- Relevant in cancer and cardiovascular disease prevention
- Antimicrobial and anthelminthic activities

Khoddami, et al. Crit Rev Food Sci Nutr. 2021

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Our experience

Clinical characteristics of patients and results after 1 month of GFD sorghum based.

Patients	disease	Age	M/F	Symptoms during standard GFD	Results after 1 month of GFD sorghum based
#1	NCGS	48	M	Glucose intolerance, overweight, deficiency anemia	Improved glucose intolerance, resolved anemia
#2	NCGS	51	M	Megaloblastic anemia, chronic fatigue	Improved fatigue
#3	NCGS	31	F	Iron deficiency anemia, vitamin D deficiency	Resolved anemia and vitamin D deficiency
#4	NCGS	64	F	headache, osteoporosis, chronic fatigue	increased levels of vitamin D, improvement of pain related to osteoporosis
#5	NCGS	36	F	Headache, chronic fatigue, vitamin D deficiency	Resolved all symptoms
#6	NCGS	40	F	psoriasis, pruritus, vitamin D deficiency	Improved psoriasis
#7	CD	32	M	Glucose intolerance, hypothyroidism	Improved glucose intolerance
#8	CD	77	M	Pruritus, chronic fatigue	Resolved pruritus and fatigue
#9	CD	49	M	Overweight, chronic fatigue	Resolved fatigue, lost weight
#10	CD	15	M	Deficiency anemia, hyporexia	Resolved deficiency anemia, recovered appetite

GFD gluten-free diet; CD celiac disease; NCGS non-celiac gluten sensitivity.

Gasbarrini GB et al., Dig Liver Dis, 2021

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Future perspectives?

- Well-designed clinical trials with a large sample of individuals are needed
- Longer follow-up patients to follow for many months or years
- Evaluation of the acceptance of the diet in the long term
- Evaluation of nutritional and biometric parameters (bioimpedence, magnetic resonance of muscle)
- Evaluation of the development of chronic diseases over time

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CONCLUSIONS

- Products made with sorghum flour represent a valid alternative in GFD
- Sorghum products has a good nutritional profile
- Studies in vitro or animal models suggested several implications in maintaining health or preventing chronic disease
- Preliminary experience on humans demonstrate encouraging results in promoting well-being
- High palatability and digestibility could favor the diffusion among consumers
- The high retail price could still represent a limitation for large-scale diffusion

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