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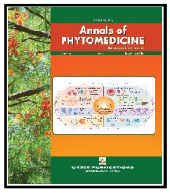

Review Article : Open Access

Cassava (*Manihot esculenta* Crantz) resistant starch: Structural and pharmacological perspectives

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Abstract

Cassava (*Manihot esculenta* Crantz) resistant starch (RS) has emerged as an important functional dietary component due to its beneficial effects on glycemic regulation, gut health, and metabolic wellness. This review highlights the structural mechanisms, phytochemical interactions, and pharmacological significance of resistant starch formation in Cassava. Structural characteristics including amylose content, crystallinity, hydrogen bonding, and chain organization strongly influence gelatinization, retrogradation, and enzymatic resistance of starch granules. Thermal processing and cooling enhance the formation of resistant starch fractions, particularly RS3, while amylose-lipid and amylose-phenolic interactions promote RS5 formation. Cassava also contains bioactive phytochemicals such as flavonoids, phenolics, carotenoids, and phytosterols that contribute antioxidant, anti-inflammatory, hypoglycemic, and prebiotic activities. Physiologically, resistant starch escapes small intestinal digestion and undergoes colonic fermentation, producing short-chain fatty acids, especially butyrate, which improve gut microbiota composition, insulin sensitivity, and metabolic homeostasis. The review further discusses the influence of genotype selection and processing technologies on resistant starch enhancement and digestibility. Overall, Cassava resistant starch represents a promising phytomedicinal and nutraceutical ingredient for the development of low-glycemic functional foods targeting diabetes, obesity, and other metabolic disorders.

1. Introduction

Resistant starch (RS) is a key dietary carbohydrate fraction that resists small intestine digestion, profoundly influencing glycemic management and metabolic health (Fuentes-Zaragoza *et al.,* 2010; Englyst *et al.,* 1992). With over 500 million people worldwide affected by type 2 diabetes and refined carbohydrates exacerbating postprandial hyperglycemia, RS serves as a low glycemic substrate fermented in the colon to mitigate these risks (Zhao *et al.,* 2024; Birt *et al.,* 2013). RS evades hydrolysis by mucosal maltase glucoamylase and pancreatic α-amylase, lowering the glycemic index (GI) of starchy foods. RS incorporation via retrogradation or native granules shifts meals to low GI categories (<55), unlike rapidly digestible starch (RDS) that drives high GI spikes (>70) (Vital *et al.,* 2018). Clinical trials demonstrate 30-40 g/day RS reduces fasting glucose by 0.5-1 mmol/l in type 2 diabetes patients (Lockyer and Nugent, 2017), with digestibility varying by type: RS2 (native granules) holds integrity to 60-70°C, RS3 (retrograded) features B type crystallinity for steric enzyme hindrance, and RS4/RS5 rely on chemical or lipid barriers (Englyst *et al.,* 1992; Chang *et al.,* 2023a, 2023b). Colonic fermentations by microbiota like *Ruminococcus bromii* and *Bifidobacterium* yields short chain fatty acids (SCFAs), predominantly butyrate (up to 70% of the pool), enhancing insulin sensitivity *via* GLP-1/GLP-2, bolstering gut barrier function, and curbing inflammation through HDAC inhibition. RS confers benefits like 10-15% LDL cholesterol reduction, heightened satiety, and lowered colon cancer risk *via* butyrate apoptosis in neoplastic cells; meta-analyses affirm stronger effects in metabolic syndrome versus healthy individuals (Eerlingen *et al.,* 1995; Ma *et al.,* 2020). Yet, *in vitro* models overestimate ileal RS recovery by 5-10% relative to ileostomy data, while microbiota heterogeneity and suboptimal doses (<10 g/day) limit efficacy, necessitating tailored approaches (Ashwar *et al.,* 2017). Tuber crops such as Cassava (300 Mt/year yield), potato, and sweet potato dominant in global starch supply and India's diets offer untapped RS *via* high amylose genotypes and processing (*e.g.,* cooling boosts RS3 5-10-fold) (Wang *et al.,* 2024). Unlike prior reviews on RS modification in roots/tubers or general retrogradation kinetics, this review uniquely integrates kinetic

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modelling (E_a = 150-200 kJ/mol gelatinization) with thermodynamic transitions and genetic regulation in tuber starch (Raigond *et al.,* 2015), addressing gaps in scalable RS enrichment for low GI foods. While existing works describe RS types, health effects, or isolated processing (*e.g.,* octenyl succinylation in Cassava/potato), they lack systemic synthesis of structure-kinetics-property links for tubers amid rising diabetes burdens. This provides a mechanistic roadmap for optimizing RS in tuber dependent diets, filling voids in precision modulation and industrial applications. Despite substantial advances in starch science, a key challenge remains in integrating these physicochemical insights with nutritional outcomes. Most existing studies address either structural transformations or health implications in isolation, limiting the ability to design starch-based foods with targeted functional properties. There is a need for a comprehensive framework that links starch structure, processing conditions, digestion kinetics, and physiological effects.

Therefore, the present review aims to provide an integrated perspective on resistant starch formation in tuber crops by combining structural, kinetic, and thermodynamic approaches with nutritional relevance. Particular emphasis is placed on understanding how processing conditions and genotype characteristics influence resistant starch content and how these changes translate into improved glycemic control, gut health, and overall metabolic benefits. This approach seeks to bridge the gap between starch chemistry and functional nutrition, enabling the development of next-generation food products with enhanced health-promoting properties. In addition to resistant starch, underutilized tuber crops contain diverse phytochemicals including phenolic acids, flavonoids, anthocyanins, carotenoids, and saponins that contribute antioxidant, anti-inflammatory, and metabolic regulatory properties. The synergistic interaction between resistant starch and these bioactive compounds may enhance the functional and pharmacological value of tuber-based foods.

2. Classification of resistant starch and its mechanism of development in tuber crops

Resistant starch (RS) has been defined as the fraction of starch and the product of starch degradation that remains undigested in small intestine of the healthy individual and that is fermented in the colon by beneficial gut microbiota such as *Bifidobacterium, Ruminococcus bromii, Lachnospiraceae* (Fuentes-Zaragoza *et al.,* 2010; Zhao *et al.,* 2024). These bacteria (primary degraders depolymerize the residual granular architecture, providing acetate and other intermediates that fuel cross feeding to butyrate producers (*e.g., Eubacterium rectale*) and produce short chain fatty acids such as butyrate (Vital *et al.,* 2018). The concept was first proposed by Englyst *et al.* (1992), a classification of dietary starch into three categories such as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) on the basis of enzymatic hydrolysis with pancreatin and amyloglucosidase (Englyst *et al.,* 1992). RS further classified as RS1, RS2, RS3, RS4, and RS5 (Zhao *et al.,* 2024).

2.1 RS 1: Physically inaccessible starch

RS 1 occurs naturally in the food sources such as whole grains, legumes, and seeds (Lockyer and Nugent, 2017). It is physically entrapped in the cell wall, limiting the digestive enzymes like α-amylase to access food material primarily due to microstructural barriers. Thus, it depends on the processing behaviour, milling, chewing, and prolonged cooking progressively disrupt cell wall integrity and reduce the RS1 fraction (Raigond *et al.,* 2015).

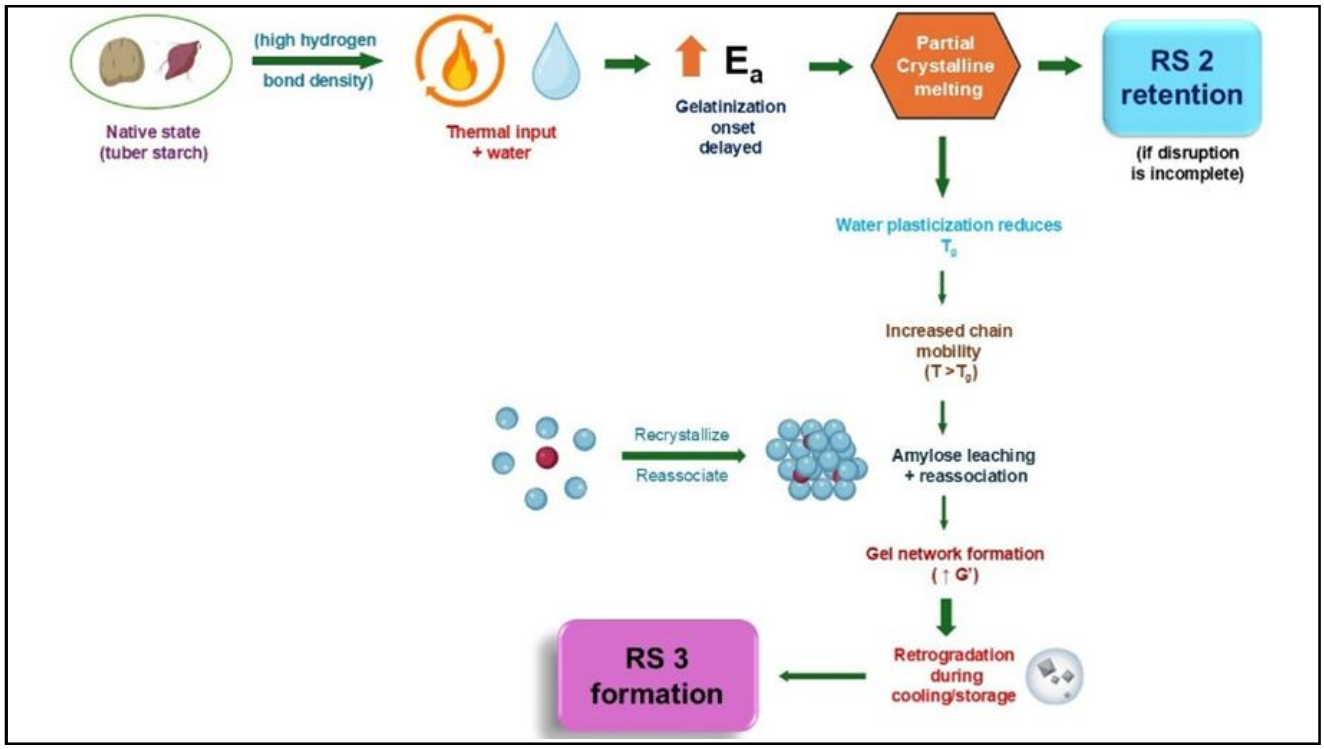

Figure 1: Mechanistic pathway of RS2 retention and RS3 formation during gelatinization-retrogradation of tuber starch (This figure is prepared by us by using the PowerPoint presentation).

2.2 RS 2: Resistant granules

Type 2 Resistant Starch is the ungelatinized starch granules in native form and indigestibility is due to compact, semi-crystalline architecture of starch granules (Chang *et al.,* 2023b). It is due to long amylose chains and densely branched amylopectin co-assemble into growth rings as amorphous and crystalline lamellae alternatively (Snelson *et al.,* 2019). High amylose content and longer amylopectin chains increase chain entanglement and double-helix packing, elevating gelatinization temperature and reinforcing crystallite stability, while limiting mobile amorphous domains accessible to α-amylase (Tian *et al.,* 2023; Ma *et al.,* 2018).

2.3 RS 3: Retrograded starch

Type 3 RS is formed when the gelatinized starch is cooked and cooled (Eerlingen *et al.,* 1995; Feng *et al.,* 2024). Mechanism involved is when gelatinized α-1,4/α-1,6 glucan chains realign into densely packed double- and single-helical domains and upon cooling, amylose and outer amylopectin chains nucleate and grow B-crystallites, driven by extensive intra and intermolecular hydrogen bonding, yielding high relative crystallinity, retrogradation enthalpy and short-range order that restrict amylase accessibility and water mobility (Villas-Boas *et al.,* 2020; Ding *et al.,* 2025). It can be developed through physical modification such as high hydrostatic pressure, ultrasound, extrusion, autoclaving, microwave cooking, and heat-moisture treatment. This facilitates the generation of small molecules and increase crystallinity during retrogradation, influencing the reduction in digestibility (Ma *et al.,* 2020). The mechanism of formation of RS3 starch is described in Figure 1.

2.4 RS 4: Chemically modified starch

Chemically modified resistant starch type 4 (RS4) developed from covalent derivatization of hydroxyl groups on amylose and amylopectin (*e.g.,* cross linking with sodium trimetaphosphate/ sodium tripolyphosphate, sodium phytate, or citric acid; esterification with octenyl succinic anhydride or acyl anhydrides), which introduces bulky substituents and inter or intra chain crosslinks that hinder α amylase access and limit water penetration (Ashwar *et al.,* 2017; Liu *et al.,* 2025). Phosphate or citrate bridges reinforce the granular matrix, elevate gelatinization temperatures, decrease swelling power and solubility, and partially disrupt long range crystalline order while often maintaining the native A or B type polymorph, producing a densely packed, low mobility network with enhanced enzymatic resistance (Ding *et al.,* 2020; Cavallo *et al.,* 2024). Distinct RS4 architectures (*e.g.,* OSA starch, starch citrates, phytate cross linked, OSAPS-lipid composites) differentially shape gut microbiota, selectively enriching SCFA producing genera and modulating *Bifidobacterium, Lactobacillus, Dorea, Coprococcus*, and other taxa, thereby linking precise chemical topology of the modified glucan network to prebiotic functionality and glycemic attenuation (Wang *et al.,* 2024; Saavedra-Cordova *et al.,* 2025).

2.5 RS 5: Amylose lipid complexes

Type 5 RS is produced by gelatinization with the fatty acids and phenolic compounds like lauric acid, stearic acid, gallic acid and quercetin. Fatty acids such as lauric acid and stearic acid interact with amylose chains to form V-type crystalline structures that reduce enzyme accessibility. In addition, phenolic compounds such as gallic acid and quercetin can interact with amylose through extensive hydrogen bonding between their hydroxyl groups and the hydroxyl residues of amylose chains and hydrophobic interactions, promoting molecular ordering and stabilization of V-type crystalline complexes. These interactions strengthen the compact starch network, restrict α-amylase access, and enhance resistance to enzymatic digestion. Consequently, amylose-lipid-phenolic complexes contribute to increased RS5 formation and improved hypoglycemic functionality (Ma *et al*., 2018; Fitriati *et al*., 2025).

Following the classification of resistant starch (RS) types (RS1-RS5), their structural underpinnings must be bridged with digestion kinetics to elucidate functional resilience in tuber starch, where discrepancies in ileal digestibility (*e.g.,* Cassava RS2 vs potato RS3) necessitate integrated analysis of granule architecture, gelatinization thermodynamics, and retrogradation pathways for predictive low GI kinetic models.

3. Structural kinetics of RS formation in tubers

RS3 formation in tuber crops arises from structural reorganization during time and temperature dependent thermal processing, governed by dynamic interplay between molecular mobility and thermodynamic stability. This process begins with gelatinization, where thermal transitions with water disassemble the native granular structure via first order kinetics, yielding amorphous solubilized starch. Subsequently, cooling induces retrogradation, restructuring the amorphous matrix into ordered, digestion resistant B type crystalline domains. Figure 2 illustrates the interconnected dynamics of gelatinization, degradation, and retrogradation driving RS formation.

3.1 Gelatinization kinetics of resistant starch formation in tuber crops

Gelatinization kinetics governs the transformation of digestible starch into resistant starch (RS) through progressive structural and thermodynamic changes in tuber crops such as Cassava, potato, and sweet potato. Native tuber starch granules possess high thermal stability due to extensive intra- and intermolecular hydrogen bonding, particularly within amylopectin-rich crystalline regions. During heating in the presence of water, these bonds gradually rupture, promoting granule swelling and amylose leaching. Compared with cereal starch, tuber starch exhibit slower gelatinization kinetics and greater thermal resistance because of their stronger hydrogen-bonded architecture (Renzetti *et al*., 2021). The gelatinization process proceeds through sequential stages involving hydration, double-helix dissociation, granule swelling, and phase transition. Initially, water penetrates amorphous regions and hydrates phosphate esters, initiating molecular mobility (Han *et al*., 2019). This is followed by disruption of amylose-amylopectin double helices, a process requiring substantial activation energy in RS-rich tubers due to their compact molecular organization. Consequently, these exhibit broader gelatinization temperature ranges and lower enthalpy (–H) values (Chien-Chun Huang, 2009). Subsequent swelling and structural disintegration alter rheological behavior, producing weaker and more heterogeneous gel networks with reduced storage modulus (G2) and complex viscosity compared with normal starch (Chipón *et al*., 2022). Glass transition temperature (T_g) further regulates starch chain mobility during cooling and storage. Declining T_g enhances amylose reassociation and amylopectin recrystallization, facilitating the formation of retrograded resistant starch (RS3), which is associated with improved thermal stability and reduced enzymatic digestibility (Renzetti *et al*., 2025). Together, these kinetic and structural transformations determine the functional and nutritional properties of resistant starch in tuber-based foods.

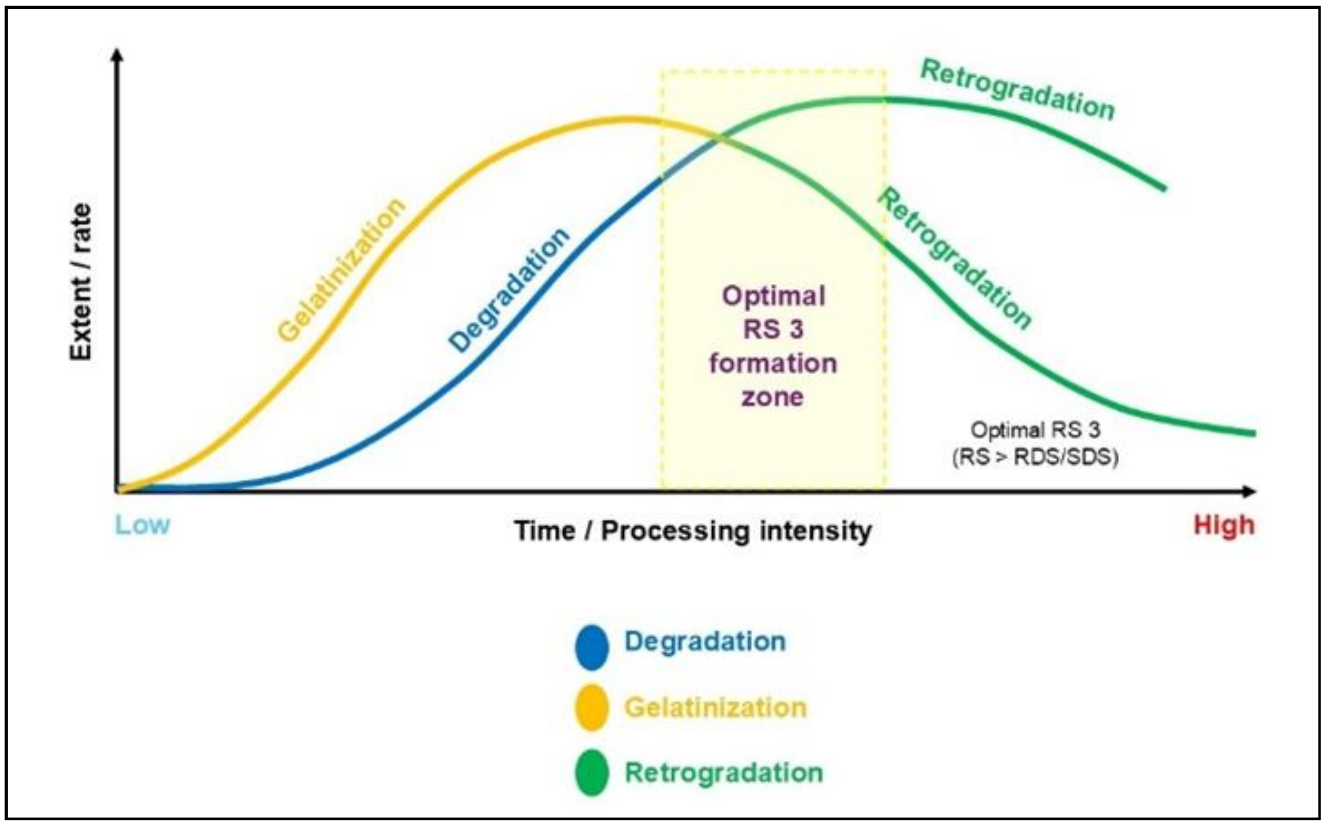

Figure 2: Kinetic competition model of resistant starch formation in tuber starch (This figure is prepared by us by using the PowerPoint presentation).

3.2 Thermodynamic framework for tuber RS gelatinization

Gelatinization kinetics play a central role in resistant starch (RS) formation in tuber crops through two major mechanisms: preservation of ungelatinized granules contributing to RS2, and retrogradation-induced recrystallization leading to RS3 formation during cooling and storage (Renzetti *et al*., 2025). This temperature-dependent process is governed by Arrhenius kinetics:

$$k = Ae^{\{-Ea/RT\}} \qquad \text{(Equation 1)}$$

where, k is the gelatinization rate constant, A is the pre-exponential factor, E is activation energy, R is the gas constant, and T is absolute temperature. RS-rich tuber starch such as potato and Cassava possesses higher activation energy due to dense hydrogen bonding and compact amylose-amylopectin architecture, resulting in slower gelatinization rates and broader temperature transitions (T_o-T_p-T_c) that favor RS retention and its temperature range described in Table 1 (Li, 2021; Renzetti *et al*., 2025). Potato starch, for example, exhibits high activation energy during gelatinization at 60-70°C because of strong amylose-associated glycosidic interactions (Schirmer *et al*., 2015). Partial gelatinization disrupts these hydrogen bonds and exposes hydroxyl groups, enhancing starch-water interactions and subsequent molecular rearrangement (Xu *et al*., 2021).

Under non-isothermal conditions, gelatinization behavior is described through endothermic heat flow and enthalpy changes:

$$EHF(T) = \Delta H'(T) = (d\Delta H(T))/dT \qquad \text{(Equation 2)}$$

with rate constant during non-isothermal conditions expressed according to Arrhenius equation (Li and Hu, 2021),

$$k = k_0 e^{\{-Ea/RT\}} \qquad \text{(Equation 3)}$$

An increase in the rate constant reflects enhanced molecular mobility and amylose reassociation, accelerating RS formation under optimal thermal conditions. Variations in kinetic behavior among tuber crops are strongly influenced by amylose content, crystalline structure, and granule morphology.

Thermodynamically, RS formation is governed by Gibbs free energy:

$$\Delta G = \Delta H - T\Delta S \qquad \text{(Equation 4)}$$

where, –G represents the Gibbs free energy change, –H = enthalpy change associated with bond formation or disruption, –S = changes in molecular order, and T = absolute temperature. Negative –G values during cooling and storage indicate spontaneous recrystallization, where enthalpy-driven molecular ordering exceeds entropy loss. This promotes formation of thermally stable retrograded RS3, thereby enhancing the functional and nutritional quality of tuber-derived starch. (Li and Hu, 2021). The mathematical models for describing gelatinization are summarized in Table 2.

Table 1: Gelatinization temperature ranges across tuber crops (native starch forms)

Tuber crops	T_o (°C)	T_p (°C)	T_c (°C)	References
Potato (*Solanum tuberosum* L.)	53	59	66	Mojo-Quisani *et al*., 2024
Anchote (*Coccinia abyssinica*)	66	70	74	Abera *et al*., 2019
Cassava (*Manihot esculenta* Crantz)	60	66	72	Nwokocha *et al*., 2009
Cocoyam (*Colocasia esculenta*)	73	76	80	Nwokocha *et al*., 2009
Sweet potato (*Ipomoea batatas* (L.) Lam. var. *batatas*)	65	75	85	Wang *et al*., 2024
Taro (*Colocasia esculenta* (L.) Schott)	75	80	93	Jane *et al*., 1992; Hui *et al*., 2024
Arrow root (*Maranta arundinacea* L.)	76	80	86	Charles *et al*., 2016
Oca (*Oxalis tuberosa*)	55	59	65	Zhu and Cui 2020
Lotus root (*Nelumbo nucifera* Gaertn.)	62	69	75	Wang *et al*., 2024

3.3 Degradation kinetics of resistant starch formation

Following gelatinization, starch granules transition from ordered crystalline structures to amorphous and hydrated states, increasing susceptibility to enzymatic hydrolysis. In this phase, α-amylase adsorbs onto starch granule surfaces and cleaves α-1,4 glycosidic linkages, leading to progressive shortening of glucan chains (Tako *et al*., 2014). The degradation process is governed by a degradation rate constant (k_d), which depends on enzyme accessibility and substrate availability. Variations in k_d influence chain-length distribution, thereby regulating the balance between starch digestibility and resistant starch (RS) formation. Enzymatic degradation kinetics are closely associated with glass transition temperature (T_g) and retrogradation behavior, both of which determine the resistance of starch to enzymatic breakdown (Tian *et al*., 2023). Under substrate-excess conditions, the hydrolysis reaction follows Michaelis-Menten kinetics adapted through the Langmuir adsorption model:

$$v_0 = \{k_{cat} E_0^{mass} S_0\} / \{K_{1/2} + {}^{mass} S_0\} \quad \text{(Equation 5)}$$

where, v_ϵ is the initial hydrolysis velocity, k_{cat} is the catalytic constant, E_ϵ is enzyme concentration, ${}^{mass}S_\epsilon$ is substrate mass load, and $K_{1/2}$ represents half-saturation mass. Conversion to molar affinity constants is expressed as:

$$K_M = K_{½}{}^{kin}\Gamma_{max} \quad \text{(Equation 6)}$$

Enzyme adsorption onto starch surfaces at 37°C follows the Langmuir binding isotherm:

$$\Gamma = \{{}^{ads}\Gamma_{max} E_{free}\} / \{K_d + E_{free}\} \quad \text{(Equation 7)}$$

where, Γ denotes bound enzyme concentration, K_d is the dissociation constant, E_{free} is free enzyme concentration, and ${}^{ads}\Gamma_{max}$ represents saturation coverage. Lower enzyme binding affinity and restricted substrate accessibility in retrograded starch contribute to enhanced RS stability and reduced digestibility (Tian *et al.,* 2023).

3.4 Enzymatic kinetics during degradation of starch granules

Enzymatic degradation of starch granules is commonly described using the Michaelis-Menten kinetic model, which evaluates hydrolysis efficiency through the Michaelis constant (K_m) and maximum reaction velocity (V_m). These parameters are obtained from the Lineweaver-Burk relationship:

$$1/V = \{Km/Vm \,.\, 1/S\} + 1/Vm \quad \text{(Equation 8)}$$

where, V represents the reaction rate and *S* denotes substrate concentration. In potato starch, conventional enzymolysis exhibits lower catalytic efficiency, whereas sonoenzymolysis significantly enhances hydrolysis kinetics by increasing V_m and reducing K_m (Wang *et al*., 2017). Ultrasound treatment promotes cavitation-induced mass transfer and mechanical disruption of starch granules, improving enzyme accessibility and substrate interaction. This enhanced hydrolysis generates short linear glucan chains that readily undergo reassociation and retrogradation during cooling, thereby promoting resistant starch type 3 (RS3) formation. Consequently, ultrasound-assisted enzymatic treatment represents an effective strategy for improving resistant starch yield and modifying the functional properties of tuber starch.

3.5 Role of glass transition temperature in RS formation

In tuber crops such as potato, Cassava, and sweet potato, glass transition temperature (T_g) regulates resistant starch type 3 (RS3) formation by controlling molecular mobility during retrogradation in the post-gelatinization amorphous state (Tananuwong *et al*., 2004). After gelatinization, starch remains in a metastable state where amylose and amylopectin chains undergo reassociation and recrystallization. The retrogradation rate depends on storage temperature relative to T_g (Mizuno *et al*., 1998). Below T_g, the starch matrix remains rigid and restricts chain diffusion, thereby inhibiting nucleation and crystallization necessary for RS3 formation (Charoenrein *et al*., 2013). Above T_g but below melting temperature (T_m), the matrix becomes rubbery, enhancing molecular alignment and crystallization and this was described in the Figure 3. Retrogradation is maximized around 4°C, where chain mobility optimally promotes amylose reassociation before thermal disorder increases (Haralampu 2000; Zhao *et al*., 2017). Thus, Tg links polymer dynamics with crystallization kinetics during RS3 formation. These structural transitions are commonly analyzed using DSC, RVA, rheometry, solid-state NMR, polarized microscopy, small-angle X-ray scattering, and X-ray diffraction techniques (Li 2022).

3.6 Kinetics of retrogradation facilitating RS3 formation

Retrogradation following gelatinization occurs in two stages: nucleation, involving amylose chain alignment, and crystal growth, characterized by double-helix formation and B-type crystallization (Arýk Kibar *et al*., 2011). These structural changes are evaluated through small-amplitude oscillatory shear (SAOS) rheometry using

elastic modulus (G2), which reflects intermolecular bonding strength, and viscous modulus (G3), associated with molecular friction and mobility. Differential scanning calorimetry (DSC) further determines onset (T_o), peak (T_p), conclusion (T_c) temperatures, and retrogradation enthalpy ($-H_r$) (Farhat *et al*., 2018; Wang *et al*., 2015; Fang *et al*., 2020 a,b). Retrogradation kinetics are commonly described using Consecutive Reaction (CR) and Avrami models, which indicate first-order kinetic behavior through exponential crystallization patterns. Higher rate constants correspond to rapid reassociation, nucleation, and crystallization during cooling (Li, 2022; Li, 2024; da Rosa Zavareze *et al*., 2012). Consequently, high-amylose potato and Cassava varieties exhibit faster and greater RS3 formation than waxy starch (Figure 4). However, Cassava starch generally produces lower RS yields than potato starch due to its comparatively lower amylose content, highlighting the importance of crop-specific optimization strategies. The comparative yield of crop-specific resistant starch content is listed in Table 3 along with the modification methods.

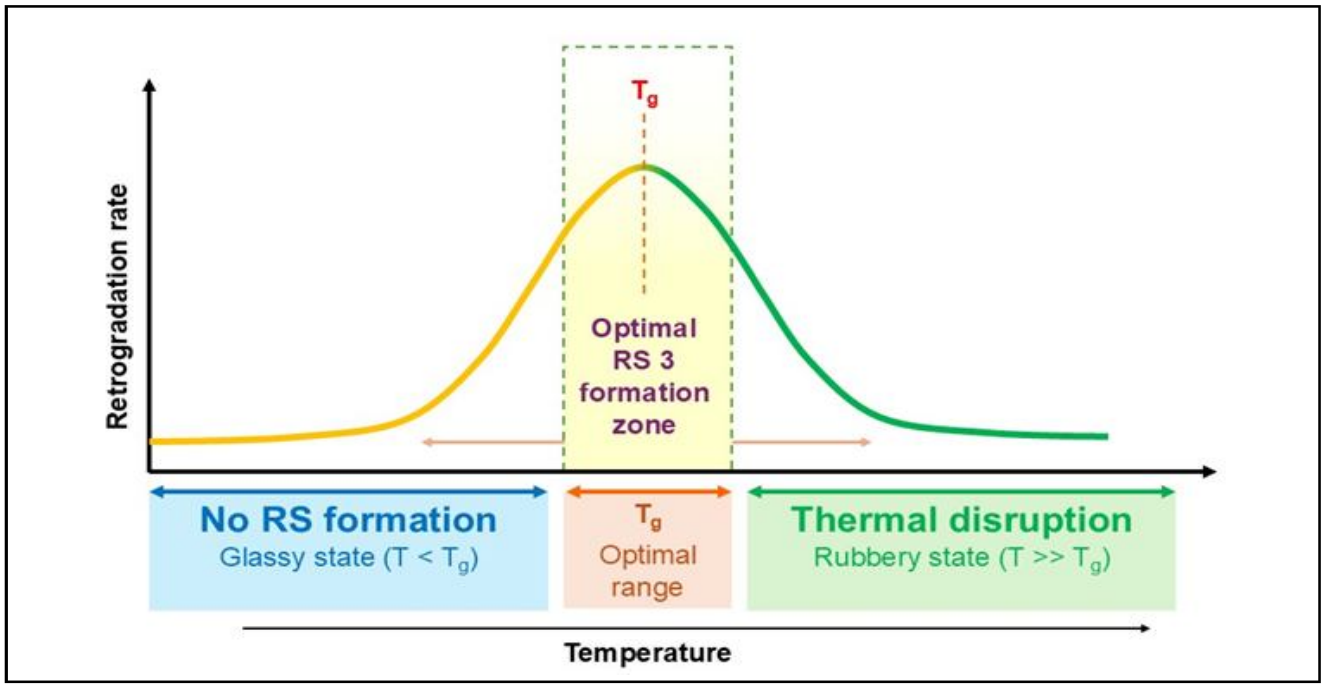

Figure 3: T_g controlled retrogradation for resistant starch formation (This figure is prepared by us by using the PowerPoint presentation).

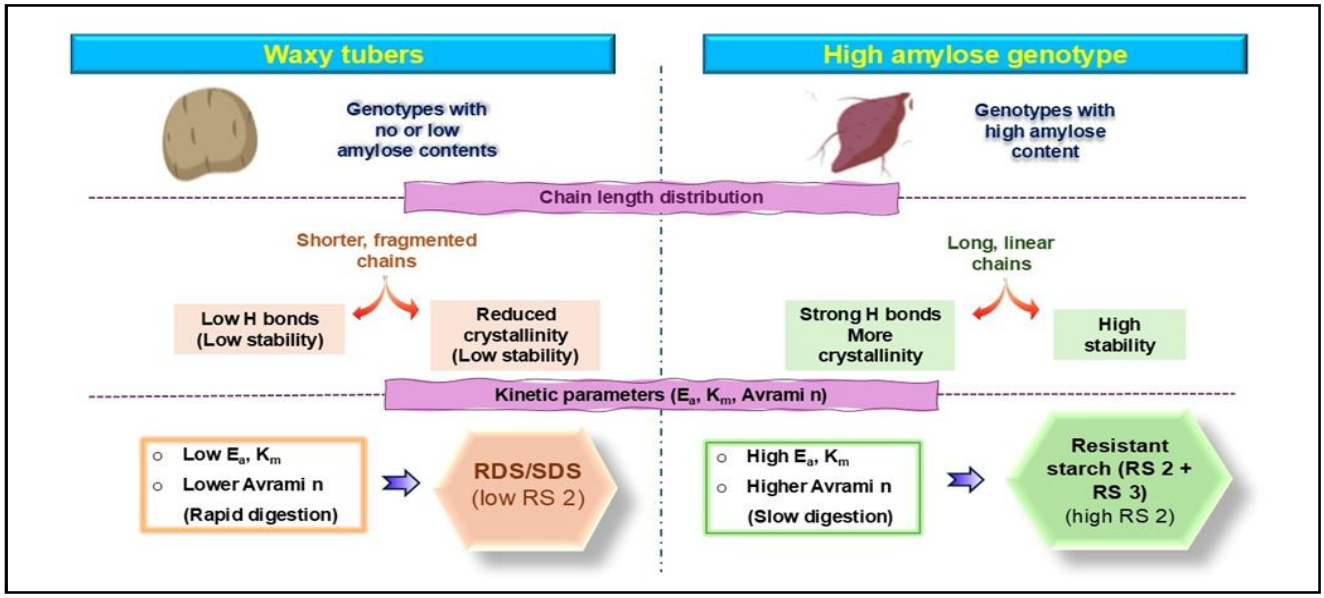

Figure 4: Genotype driven kinetics of resistant starch formation in tuber crop (This figure is prepared by us by using the PowerPoint presentation).

Table 2: Mathematical models used for fitting starch gelatinization thermograms (Li, 2022a; 2022b)

Kinetics model	Equation	Parameters	Suitable conditions
First order kinetics	$\Delta H(t) = (\Delta H\infty) \times (1 - e^{-kt})$	$\Delta H(t)$ is the gelatinization enthalpy at time t;$\Delta H\infty$ is the maximum gelatinization enthalpy;k is the rate constant.	Isothermal gelatinization with an excess of water
n^{th} order kinetics	$\Delta H(t) = \Delta H\infty - [\Delta H\infty^{(1-n)} - (1 - n)kt]^{1/(1-n)}$	$\Delta H(t)$ is the gelatinization enthalpy at time t;$\Delta H\infty$ is the maximum gelatinization enthalpy;k is the rate constant;n is the reaction order.	Non-isothermal gelatinization with an excess of water
Asymmetric multiphase gelatinization kinetics	$\Delta H(t) = \Sigma\Delta Hi(t)$, $i = 1,2,\ldots\ldots,\infty$.	$\Delta H(t)$ is the gelatinization enthalpy at time t;$\Delta Hi(t)$ is the gelatinization enthalpy of i^{th} component at time t, which can be either described by a first order or n^{th} order kinetics equation.	Non-isothermal gelatinization with a limited moisture content

Table 3: Characteristics of resistant starch formation in specific tuber crops

Tuber crop	RS type	RS yield (%)	References
Potato (*Solanum tuberosum* L.)	RS2	56.43%	Thuy *et al*., 2022
Potato (*Solanum tuberosum* L.)	RS4 (Octenyl succinylation)	29.1%	Remya *et al*., 2017
Potato (*Solanum tuberosum* L.)	RS4 (Phosphorylation with mixture of sodium trime taphosphate (STMP) and sodium tripolyphosphate (STPP)	36-66%	Woo and Seib 2002
Cassava (*Manihot esculenta* Crantz)	RS4 (Octenyl succinylation)	27.9%	Remya *et al*., 2017
Cassava (*Manihot esculenta* Crantz)	RS5	25-30%	Faridah *et al*., 2021
Arrow root (*Maranta arundinacea* L.)	RS3 (Autoclaving + cooling)	5.02%	Halim *et al*., 2023
Sweet potato (*Ipomoea batatas* (L.) Lam. var. *batatas*)	RS3 (Heat moisture treatment)	22.7%	Shin *et al*., 2004
Yam (*Dioscorea alata* L.)	RS2	13-21%	Kouadio *et al*., 2013

4. Structural changes during RS formation

Retrogradation to form resistant starch type 3 (RS3) in tubers induces profound structural reorganization, elevating amylose content, ordered double helices, relative crystallinity, and compact granule architecture, all enhancing RS yield and enzyme resistance (Sangwongchai *et al.,* 2024). High amylose potato varieties exemplify this as cooking yields 13% RS (dry matter) than 4% in normal cultivars, rising to 20% post cold storage; dietary fiber concurrently increases from 5-7% to 10-14%. These shifts stem from genetic modifications in high amylose tubers, boosting cellulose while reducing pectin in cell walls (Zhao *et al.,* 2018). Amylopullulanase hydrolysis reveals B type crystallinity (21.32% increased), reduced porosity (33.18%), and tight amylose amylopectin alignment; higher retrogradation enthalpy ($-H_r$) and lower peak temperatures further confirm altered granule perfection for RS functionality (Das and Banerjee, 2022; Shin *et al.,* 2024).

5. Changes in functional properties during RS formation

Physical treatments such as autoclaving, heat-moisture treatment, extrusion, ultrasonication, and microwave processing, along with chemical modifications including esterification, acetylation, cross-linking, and amylose-lipid complexation, significantly alter tuber starch functionality and promote resistant starch (RS) formation.

i. **Water absorption capacity**: Enzymatic and chemical modifications enhance water absorption by cleaving glycosidic bonds and generating shorter, highly hydrophilic chains. In contrast, retrograded RS exhibits reduced water absorption due to tightly packed amylopectin double helices (Abioye *et al*., 2017).

ii. **Solubility and swelling power**: Retrogradation decreases water accessibility, thereby reducing solubility and swelling power in Cassava starch by limiting granule expansion and hydration (Abioye *et al*., 2017).

iii. **Viscosity**: RS formation generally lowers viscosity because hydrolysis-induced chain shortening reduces paste-forming ability (Reddy *et al*., 2013; Reddy *et al*., 2015; Bajaj *et al*., 2022).

iv. **Thermal stability**: Retrogradation-induced recrystallization enhances the thermal stability of RS-rich starch (Reddy *et al*., 2015).

v. **Retrogradation and T_g**: Progressive retrogradation increases glass transition temperature (T_g), resulting in a more rigid matrix with restricted molecular mobility and improved long-term RS stability (Abioye *et al*., 2017; Ma *et al*., 2018).

Overall, high amylose content promotes RS formation through molecular reassociation and crystallization, generating compact structures that restrict enzymatic digestion despite increased surface area in smaller starch granules.

6. Phytochemical constituents of Cassava and resistant starch complexes

Recent studies demonstrate that Cassava resistant starch (RS) functions not merely as a low-digestible carbohydrate fraction, but as a bioactive matrix whose functionality is substantially enhanced through interactions with endogenous and exogenous phytochemicals. Fatty acids and phenolic compounds including lauric acid, stearic acid, gallic acid, and quercetin significantly enhanced resistant starch type V (RS5) formation following heat-moisture treatment, increasing RS content from 1.98% in native starch to 50.09% and 47.75% in lauric acid-quercetin and stearic acid-quercetin complexes, respectively (Fitriati *et al*., 2025). Mechanistically, these compounds promote V-type amylose inclusion complex formation, resulting in compact crystalline structures with reduced enzymatic susceptibility, thereby decreasing rapidly digestible starch while increasing slow digestible and resistant starch fractions. This highlights the potential of phytochemical-assisted starch restructuring as a strategy for developing low-glycemic functional foods. Cassava also contains diverse bioactive metabolites including flavonoids (rutin, kaempferol, apigenin), hydroxycoumarins (scopoletin, esculetin), phytoalexins, phytosterols, and fatty acids that collectively contribute antioxidant, anti-inflammatory, hypoglycemic, antibacterial, and hypocholesterolemic activities (Scaria *et al*., 2024). In particular, retrograded RS3 Cassava starch demonstrated cholesterol-lowering effects *in vivo*, suggesting that fermentation-derived short-chain fatty acids may regulate lipid metabolism and improve gut health. Similarly, RS3-tapioca enriched with phenolics such as tannins exhibited inhibitory activity against α-amylase and α-glucosidase, alongside suppression of TNF-α and prostaglandin-mediated inflammatory pathways (Mohidin *et al*., 2023). These findings indicate that the therapeutic effects of Cassava RS are strongly influenced by associated phytochemicals rather than resistant starch alone. Furthermore, heat-moisture-treated Cassava starch containing 42.53 g/100 g RS exhibited prebiotic functionality by supporting lactic acid bacterial growth and enhancing yoghurt stability (Mwizerwa *et al*., 2017). Such effects are primarily linked to colonic fermentation of RS into short-chain fatty acids, particularly butyrate, which exerts anti-inflammatory and colon-protective functions. However, the physiological efficacy of Cassava RS is highly dependent on processing-induced structural modifications including gelatinization behaviour, amylopectin branch-chain distribution, and amylose-lipid complexation (Chisenga *et al.,* 2019).

Despite promising pharmacological and techno-functional attributes, most available evidence remains limited to *in vitro* analyses and animal-based investigations, with insufficient human clinical validation. Moreover, the coexistence of beneficial phytochemicals and anti-nutritional cyanogenic glucosides underscores the need for optimized processing approaches that maximize RS functionality while ensuring safety. Therefore, future research should focus on elucidating phytochemical-starch molecular interactions, gut microbiome modulation, and clinical efficacy to establish Cassava resistant starch as a scientifically validated phytomedicinal functional ingredient.

7. Antioxidant and anti-inflammatory significance

Cassava leaves are increasingly recognized as a valuable source of bioactive phytochemicals with significant antioxidant and anti-inflammatory potential. They contain flavonoids, phenolic compounds, carotenoids, terpenoids, tannins, vitamins, and micronutrients, including rutin, kaempferol, kaempferol-3-O-rutinoside, β-carotene, and vitamin C, which collectively contribute to their therapeutic efficacy (Cai *et al*., 2025; Meilawaty *et al*., 2019). Mechanistically, flavonoids such as kaempferol suppress inflammation through inhibition of the NF-κB signaling pathway, while Cassava leaf extracts also inhibit COX-2 expression and protect neutrophil membranes from lipopolysaccharide-induced damage, indicating strong immunomodulatory and antioxidant functions (Meilawaty *et al*., 2019). *In vivo* studies further confirmed anti-inflammatory activity through inhibition of carrageenan- and histamine-induced oedema (Okechukwu *et al*., 2013). Cassava leaf extracts also exhibit concentration-dependent free radical scavenging and cytokine inhibition, suggesting protective effects against oxidative stress-associated metabolic disorders (Imane Boukhers *et al*., 2022). Network pharmacology analyses demonstrated that Cassava leaf phytochemicals target multiple pathways associated with inflammation and tissue repair, indicating a multitarget therapeutic mechanism (Cai *et al*., 2025). Furthermore, incorporation of Cassava leaves into flour significantly enhanced antioxidant and anti-inflammatory properties while reducing peak glycemic response, highlighting their potential in functional food development and metabolic disease management (Imane Boukhers *et al*., 2024). Although, Cassava leaves demonstrate promising pharmacological potential, current evidence is predominantly limited to *in vitro* and preliminary animal studies. Further clinical validation, phytochemical standardization, and bioavailability studies are necessary to establish their therapeutic efficacy and safety as phytomedicinal ingredients.

8. Pharmacological role in glycemic regulation and metabolic Health

Resistant starch (RS) represents a physiologically important fraction of dietary starch that escapes enzymatic digestion in the small intestine and undergoes fermentation in the colon. This reduced digestibility significantly alters glucose release kinetics, resulting in lower postprandial glucose and insulin responses compared with rapidly digestible starch (Birt *et al*., 2013). Structurally modified resistant starch, particularly RS2 and RS3, exhibit enhanced crystallinity and reduced enzyme accessibility, thereby slowing starch hydrolysis and lowering glycemic index (GI). For instance, resistant starch-modified taro starch demonstrated a reduced GI of 51.9 compared with 60.6 in native starch (Simsek and El, 2012). Similarly, tuber crops such as elephant foot yam, yam bean, and taro exhibited

comparatively low GI values when co-consumed with rice, effectively moderating meal glycemic load and counteracting the rapid digestibility associated with refined carbohydrate-rich diets (Kumar *et al*., 2024). The delayed digestion of RS suppresses rapid glucose absorption and attenuates insulin spikes, thereby improving insulin sensitivity and contributing to dietary management of type 2 diabetes and metabolic syndrome (Birt *et al*., 2013). In addition, resistant starch promotes satiety through delayed gastric emptying and fermentation-mediated release of satiety hormones including glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). The reduced caloric availability of RS further supports body weight regulation and obesity prevention (Lockyer and Nugent, 2017). However, despite substantial evidence supporting glycemic benefits, the physiological response to resistant starch remains influenced by RS type, processing conditions, food matrix interactions, and individual gut microbiota variability, highlighting the need for more standardized clinical investigations.

9. Gut microbiota modulation and prebiotic functionality

A major functional attribute of resistant starch is its prebiotic potential, arising from selective fermentation by colonic microbiota. Since RS escapes upper gastrointestinal digestion, it serves as a substrate for beneficial microbial populations including *Bifidobacterium*, *Ruminococcus bromii*, and members of *Lachnospiraceae* (Fuentes-Zaragoza *et al*., 2010; Zhao *et al*., 2024). Fermentation of RS produces short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, which exert profound effects on gastrointestinal and systemic health. Among these metabolites, butyrate is particularly important as the primary energy source for colonocytes and a key regulator of intestinal barrier integrity. Butyrate enhances mucin expression, strengthens epithelial tight junctions, modulates immune signaling, and suppresses intestinal inflammation, thereby contributing to improved gut homeostasis (Lockyer and Nugent, 2017). SCFA production also influences systemic metabolic regulation through modulation of inflammatory pathways, glucose metabolism, and appetite signaling. Consequently, resistant starch consumption has been associated with improved gastrointestinal health, enhanced microbial diversity, and reduced chronic low-grade inflammation (Figure 5). Although, the prebiotic functionality of RS is well established, fermentation efficiency and SCFA production vary considerably depending on starch structure, botanical source, degree of retrogradation, and host microbial composition. Therefore, future research should focus on personalized nutritional responses and microbiome-specific interactions to optimize resistant starch-based therapeutic applications.

10. Therapeutic potential in chronic disease management

Beyond glycemic regulation and gut health, resistant starch demonstrates considerable therapeutic potential in the prevention and management of chronic metabolic and inflammatory disorders. Many of these effects are mediated through SCFA production, particularly butyrate, which exhibits anti-inflammatory, anti-proliferative, and immunomodulatory activities. Resistant starch has been implicated in reducing risk factors associated with obesity, hyperlipidemia, ulcerative colitis, diverticulitis, and colon cancer through modulation of gut microbiota composition, improvement of lipid metabolism, and maintenance of colonic epithelial integrity (Birt *et al*., 2013; Das *et al*., 2024). Furthermore, emerging evidence suggests a possible role of resistant starch in neuroprotective pathways and gut-brain axis regulation, including potential implications in neurodegenerative disorders such as Parkinson's disease (Becker *et al*., 2022). The anti-carcinogenic potential of RS is primarily associated with butyrate-mediated regulation of apoptosis, inhibition of inflammatory mediators, and suppression of carcinogenic processes within the colon. Simultaneously, improved satiety, reduced glycemic fluctuations, and enhanced insulin sensitivity collectively contribute to broader metabolic benefits. Nevertheless, despite promising therapeutic outcomes, current evidence remains heterogeneous due to variations in RS classification, dosage, processing methods, and study design. Many reported benefits are derived from experimental and short-term interventions, while long-term human clinical validation remains limited. Thus, further mechanistic and translational studies are required to establish resistant starch as a clinically validated nutraceutical component for chronic disease prevention and metabolic health management.

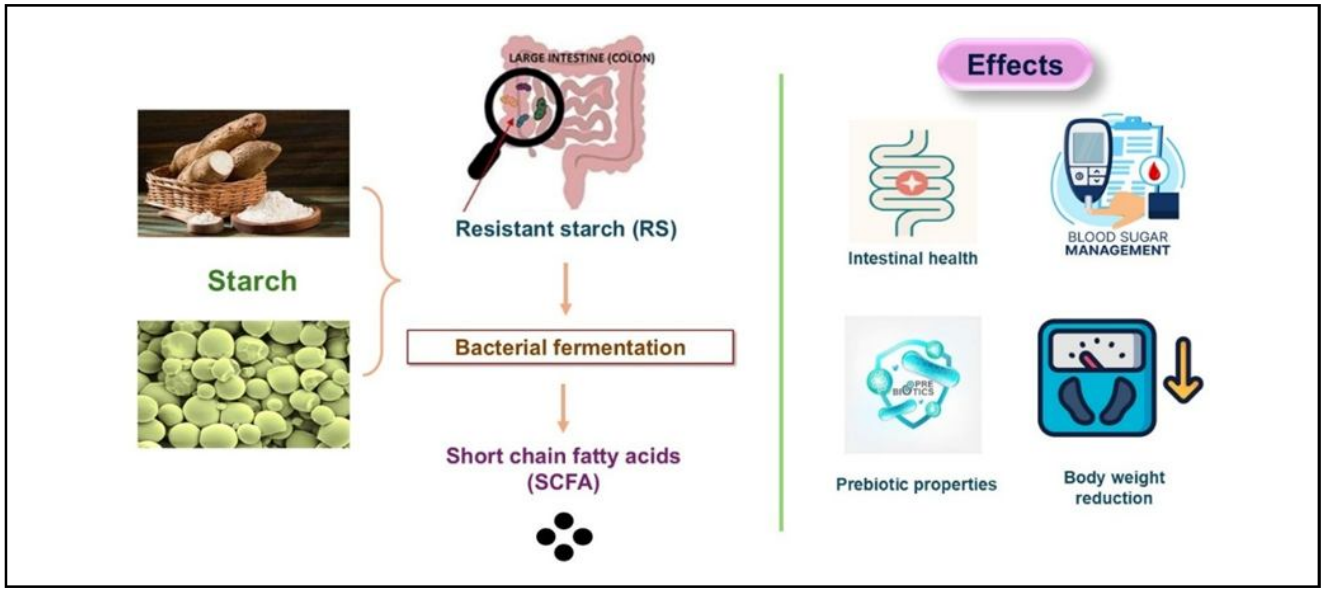

Figure 5: Influence of resistant starch in human health (This figure is prepared by us by using the Power Point presentation).

11. Research gaps and future directions

Despite growing interest in Cassava resistant starch (RS), several knowledge gaps remain that limit its effective utilization in functional foods and health applications.

i. Future research should integrate starch structural characterization with nutritional, physiological, and metabolic evaluations to better understand the relationships among starch structure, digestibility, gut fermentation, and health outcomes.

ii. Standardized processing protocols, including optimization of thermal treatment, retrogradation conditions, moisture levels, and storage parameters, are needed to maximize RS yield and ensure reproducibility across studies.

iii. Further investigation of Cassava genotypes, amylose architecture, granule organization, and starch biosynthesis pathways will support the development of varieties with enhanced RS content and functionality.

iv. Advanced analytical tools such as solid-state NMR, synchrotron X-ray diffraction, and thermal analysis should be employed to elucidate structural changes associated with RS formation.

v. Emerging technologies, including ultrasound, extrusion, microwave-assisted processing, high-pressure treatment, and pulsed electric fields, warrant systematic evaluation for their effects on RS formation, nutritional quality, and industrial scalability.

vi. Greater attention should also be given to interactions between RS, endogenous phytochemicals, and gut microbiota, particularly their roles in short-chain fatty acid production, immune regulation, and metabolic health.

vii. Since most current evidence is derived from *in vitro* studies, well-designed *in vivo* experiments, clinical trials, and long-term dietary interventions are essential to validate the health benefits of Cassava RS.

Addressing these gaps will facilitate the development of RS-enriched Cassava products as affordable functional foods and nutraceuticals for managing diabetes, obesity, gastrointestinal disorders, and other non-communicable diseases.

12. Conclusion

Resistant starch has emerged as an important functional dietary component with considerable potential in improving metabolic health, glycemic regulation, and gastrointestinal function. This review demonstrates that resistant starch formation in Cassava is strongly influenced by the interaction between starch structure, processing conditions, and molecular reorganization during gelatinization and retrogradation. Structural characteristics such as amylose content, chain length distribution, crystallinity, and hydrogen bonding play a decisive role in determining starch digestibility and resistant starch yield. Processing interventions, particularly controlled thermal treatment and cooling, promote the formation of resistant starch fractions, especially RS3, thereby reducing enzymatic susceptibility and slowing glucose release. The physiological significance of resistant starch extends beyond reduced digestibility. Through colonic fermentation, resistant starch enhances the production of short-chain fatty acids, particularly butyrate, which contributes to improved gut microbiota composition, intestinal health, insulin sensitivity, and metabolic homeostasis. These functional properties highlight the growing importance of Cassava resistant starch as a promising nutraceutical ingredient for dietary management of type 2 diabetes, obesity, and other metabolic disorders. Furthermore, the review emphasizes that strategic selection of Cassava genotypes combined with optimized processing approaches can substantially enhance resistant starch content and functional quality. The integration of structural, nutritional, and physiological perspectives provides a comprehensive framework for developing Cassava-based functional foods with potential therapeutic relevance. Future research should focus on validating these health-promoting effects through *in vivo* and clinical studies, while also exploring the application of resistant starch-enriched Cassava products in functional nutrition and phytomedicine-based dietary interventions. Such advancements will strengthen the role of Cassava resistant starch as a sustainable functional ingredient for improving human health and metabolic wellness. Therefore, underutilized tuber crops should be recognized not only as alternative resistant starch sources but also as multifunctional reservoirs of bioactive phytochemicals with significant nutraceutical and pharmacological potential.

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Abbreviations

E_a – Activation energy

EHF – Endothermic heat flow

GI – Glycemic index

GLP – Glucagon like peptide

HDAC – histone deacetylase

LDL – Low density lipoprotein

OSA – Octenyl succinic anhydride

RDS – Rapidly digestible starch

RS – Resistant starch

SCFA – Short chain fatty acids

SDS – Slowly digestible starch

T_c – Final temperature

T_o – Onset temperature

T_p - Peak temperature

Author contributions

T. Varshaa collected the literature and prepared the initial draft of the manuscript. **M. Velmurugan** and **T. Krishnakumar** revised the manuscript, refined the content, and contributed to shaping the overall structure. **C. Indu Rani** and **P. Arutchenthil** edited the

manuscript for intellectual content and clarity. **K. Thirukumaran** and **P. Veeramani** provided critical feedback to the manuscript. All authors reviewed the manuscript and approved the final version for submission.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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