International Journal of Medical Science and Pharmaceutical Research

ISSN(e): 3050-5720
ISSN(p): 3050-5712

Vol. 3 (5): 218-225, May 2026

Home Page: https://ijmspr.org/

Impact of Food Processing on Health: A Comparative Study

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KEYWORDS:

Food Processing; Inflammation; Oxidative Stress; Diabetes Mellitus, Type 2; Ultra-Processed Foods.

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DOI: 10.55677/IJMSPR/2026-3050-I506

Published: May 16, 2026

ABSTRACT

Food processing plays a pivotal role in determining nutritional integrity and its subsequent impact on human health. The present study was designed to comparatively evaluate the health implications of foods across varying levels of processing—natural, processed, and ultra-processed—using selected samples of milk, coffee, and tomato classified according to the NOVA system. Aqueous extracts of each sample were prepared and investigated for anti-inflammatory potential through hydrogen peroxide quantification and for anti-diabetic activity using the α-amylase inhibition assay. The findings revealed a progressive elevation in hydrogen peroxide concentration and activity from natural to ultra-processed foods, reflecting an increased oxidative and inflammatory burden associated with higher degrees of processing. In contrast, α-amylase inhibitory activity was most pronounced in natural food samples, moderate in processed variants, and markedly reduced in ultra-processed products across concentrations ranging from 10–50 µg/ml. The overall inhibitory hierarchy was observed as: Natural > Processed > Ultra-processed.

Collectively, the results underscore that extensive industrial processing may amplify inflammatory potential while attenuating bio-functional efficacy, thereby contributing to metabolic dysregulation. These findings reinforce the importance of prioritising natural and minimally processed foods in dietary patterns to mitigate the risk of chronic metabolic disorders, including type 2 diabetes.

Cite the Article: *Kalva, K.S., Reddy, S.S., Sriya, S., Jyotshna, B. (2026). Impact of Food Processing on Health: A Comparative Study. International Journal of Medical Science and Pharmaceutical Research, 3(5), 218-225. https://doi.org/10.55677/IJMSPR/2026-3050-I506*

INTRODUCTION

Food is any edible substance consumed to provide nutritional support, supplying carbohydrates, proteins, fats, vitamins, and minerals (Prathusha G et al., 2025) required for energy, growth, tissue repair, and overall health (Dr. Yogesh Kumar Kosariya et al,.2025). Based on origin, foods are classified as plant-based, animal-based, and microorganism-based. Plant foods such as grains, fruits, vegetables, legumes, and tubers provide fibre, vitamins, minerals, and bioactive compounds like polyphenols and flavonoids with antioxidant and anti-inflammatory properties (Zhu Q, et al,.2025). Animal-based foods, namely, meat, eggs, fish, and milk, are rich in high-quality protein and essential micronutrients (Sheffield S et al,.2024). Microorganism-based foods such as spirulina and fermented products contribute proteins, essential fatty acids, and beneficial metabolites (Alfadhly NKZ, et al,.2022). However, the growing consumption of ultra-processed foods has become an important public health concern, as it is strongly linked with chronic inflammation and escalating incidence of type 2 diabetes.

A widely accepted system for classifying foods by processing level is the NOVA classification, developed by Brazilian researchers. It categorises foods into:

Group 1: Unprocessed or Minimally Processed Foods – Natural foods altered only by basic processes, namely, cleaning, drying, freezing, or pasteurization (e.g., fruits, vegetables, whole grains, milk, eggs (Monteiro CA et al,.2018).

Group 2: Processed Foods – Foods made by adding salt, sugar, or oil to natural foods to enhance shelf life or taste (e.g., cheese, canned fish, salted nuts (De Araujo et al,.2022).

Group 3: Ultra-Processed Foods (UPFs) – Industrial formulations containing additives such as emulsifiers, artificial flavors, and stabilizers, with little intact whole food (e.g., sugary drinks, instant noodles, packaged snacks, processed meats (Elizabeth L et al,.1955).

Food processing aims to improve safety, extend shelf life, enhance convenience, ensure quality control, and increase availability. Methods include pasteurization, sterilization, canning, freezing, drying, fermentation, salting, sugaring, and modified atmosphere packaging. Processing can have both positive and negative nutritional effects. It may inactivate pathogens, improve digestibility, and enable fortification. However, excessive processing can reduce fiber, antioxidants, and heat-sensitive vitamins (namely, vitamin C as well as B-complex vitamins) (Van Boekel et al,.2010), alter glycemic index, and introduce additives, excess sugars, sodium, and unhealthy fats.

Studies have shown that ultra-processed foods are typically energy-dense but nutrient-poor. High intake is associated with obesity, cardiovascular disease, metabolic disorders, certain cancers, and mental health concerns. One key mechanism linking UPFs to disease is chronic inflammation. Diets rich in added sugars, refined carbohydrates, trans fats, and highly processed ingredients promote oxidative stress and activate inflammatory pathways such as NF-κB. C-reactive protein (CRP), TNF-α, and interleukins are among the inflammatory markers that rise as a result (Martins GMDS et al,.2022).

There is strong epidemiological evidence linking high UPF consumption with type 2 diabetes. Ingredients such as high-fructose corn syrup and refined starches cause rapid blood glucose spikes, repeated insulin release, and eventual insulin resistance. Trans and saturated fats (Odegaard AO, et al,.2006) impair insulin signaling, while certain emulsifiers and artificial sweeteners may disrupt gut microbiota (Ruiz-Ojeda FJ,et al,.2019), increasing intestinal permeability and systemic inflammation. Over time, these metabolic disturbances increase the risk of prediabetes and diabetes.

In contrast, diets rich in minimally processed plant foods support healthy gut microbiota, reduce inflammation, improve glycemic control, and lower chronic disease risk. Whole foods provide fiber, antioxidants, and phytochemicals that protect against metabolic dysfunction.

Although many studies have shown the negative health effects of ultra-processed foods, there is still a need to better understand how food processing affects inflammation and metabolic disorders. In addition, awareness about the long-term health effects of processed diets is still limited. Therefore, this study attempts to explore the correlation between food processing, inflammation, and the development of type 2 diabetes. To achieve this, a literature-based approach is adopted to review and analyze existing scientific evidence on the health impacts of ultra-processed foods.

MATERIALS AND METHODOLOGY:

MATERIALS

1.1. Selection and Classification of Samples

Samples were procured locally and categorised based on the NOVA classification system.

* Procurement: Buffalo milk and curd were obtained from a dairy farm, while vegetables and commercial products were sourced from local supermarkets.

1.2. Chemical Reagents

The study utilised analytical-grade reagents for enzymatic and oxidative assays.

In vitro Anti-Inflammatory Assay Materials:

Oleic Acid	Used in the preparation of the substrate solution
10% NaOH	For the dissolution of fatty acids,
Tween-60	An emulsifier is used to stabilise the substrate mixture.
Boric Acid (0.2 mol/L)	Used to adjust the total volume and pH (9.0)
Tris buffer	Consisting of 0.015 mol/L $CaCl_2$ and 13% sucrose at pH 8.2.

In vitro Anti Diabetic Assay Materials:

α-Amylase Enzyme	Primary molecular target for treating DM
1% starch solution	Polysaccharide substrate
DNSA Reagent	Used to terminate the reaction and measure reducing sugars
Phosphate Buffer	To maintain the required physiological pH for enzyme activity,
Acarbose	A Complex oligosaccharide used as the standard reference\alpha-amylase inhibitor.

METHODOLOGY

In vitro Anti-Inflammatory Assay

Determination of Hydrogen Peroxide Content:

0.4mL of oleic acid was measured and mixed thoroughly with 0.5mL of 10% NaOH to confirm complete dissolution. Distilled water has been added gradually until a clear and colourless solution was obtained. Then, 20mL of this solution has been taken and 0.1mL of Tween-60 (emulsifier) was added. 0.2mol/L boric acid (pH 9.0) was used to get the final volume down to 100 mL. The produced solution was diluted forty times before usage, resulting in a final concentration of 2.57 mmol/L of linoleic acid.

Tris buffer containing 0.015mol/L CaCl2 and 13% sucrose (pH 8.2) was combined with 0.5 mL of the sample solution. For 10min, mixture has been agitated at 40°C. The crude extract has been extracted via centrifuging it for five minutes at 4000 rpm. The extract was stored under refrigeration for further analysis.

0.2mL of prepared extract has been mixed with 2.8mL of substrate solution. The optical density (OD) was measured and recorded at 234 nm at 15-second intervals. The procedure was performed for all selected samples (DSHS Peiris, et al,. 2025).

Anti-diabetic Activity Assay

α-Amylase Inhibitory Activity

A solution of α-amylase (0.5mg/mL) was made. The sample was combined with the enzyme at several doses (100–500 µg/mL). The mixture was mixed with 100µL of 0.2mM phosphate buffer (pH 6.9) and a 1% starch solution. For 5min, reaction has been incubated at 37°C. Two millilitres of 3,5-dinitrosalicylic acid (DNSA) reagent were added to halt the reaction. After that, the reaction mixture was heated for 15 minutes at 100°C in a boiling water bath. Ten millilitres of distilled water were added to the mixture once it had cooled. A spectrophotometer was used to measure the absorbance at 540 nm in order to calculate the enzyme activity. The procedure was repeated for all selected samples (Kadali SLDV et al,.2017).

Statistical Analysis

Every experiment was carried out three times. Differences between the natural, processing, and ultra-processed groups have been considered significant at $p<0.05$. The outcomes are shown as Mean ± Standard Deviation (SD).

3. RESULTS

1. Selection of samples

Milk, Coffee, and tomato were selected, which are available in all three forms.

Table 1: Selected samples available in 3 different forms

SAMPLE	NATURAL FOOD	PROCESSED FOOD	ULTRA-PROCESSED FOOD
Milk 	Buffalo milk 	Curd 	Ice cream 
Coffee 	Coffee beans 	Nescafé coffee powder 	Id liquid 
Tomato 	Tomato 	Tomato puree 	Ketchup 

2. Preparation of food extracts

5g of sample has been mixed with 50ml of distilled water and centrifuged to obtain a clear extract.

3. Analysis of Anti-inflammatory Activity

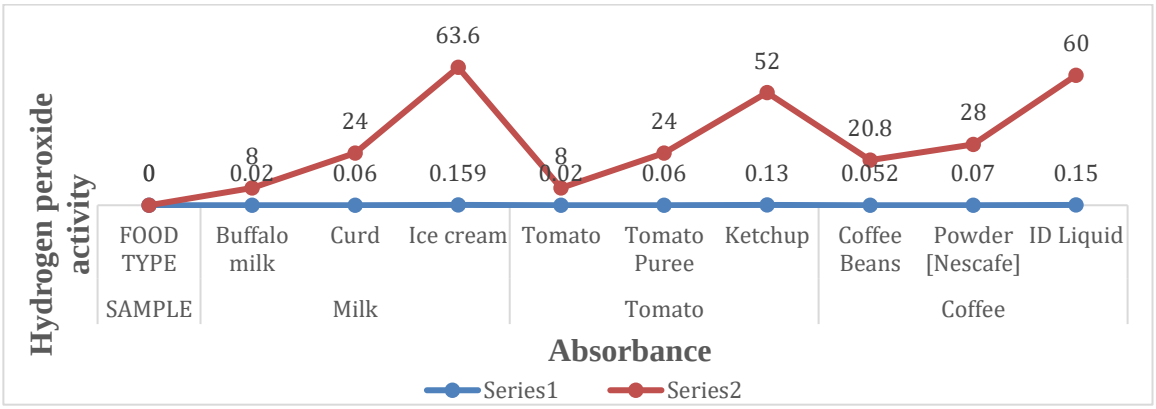

Figure 1: Determination of Hydrogen Peroxide Content of various food samples

Hydrogen peroxide levels were measured spectrophotometrically to assess oxidative potential in various food samples. In dairy products, buffalo milk showed the lowest activity (8), curd had moderate activity (24), and ice cream showed the highest (63.6). Similarly, in coffee samples, coffee beans exhibited the lowest activity (20.8), instant coffee showed moderate activity (28), and liquid coffee recorded the highest (60). Among tomato products, fresh tomato had the lowest activity (8), tomato puree showed moderate levels (24), and ketchup had the highest (52). Overall, processed foods showed higher oxidative activity, while natural foods showed lower, with moderately processed items falling in between.

4. Analysis of Anti-diabetic Activity

Table 2: α-amylase %inhibition of Milk samples

SAMPLE	FOOD TYPE	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
MILK	**Natural food (Buffalo milk)**	80.68%*	79.54%*	71.59%***	56.81%***	47.72%***
	Processed food (Curd)	78.40%	69.31%**	55.68%	50%	35.22%
	Ultra-processed food (Ice-cream)	72.72%	60.22%	45.45%	38.63%	22.72%

Note: * indicates statistically significant, ** indicates very statistically significant, ***indicates extremely statistically significant.

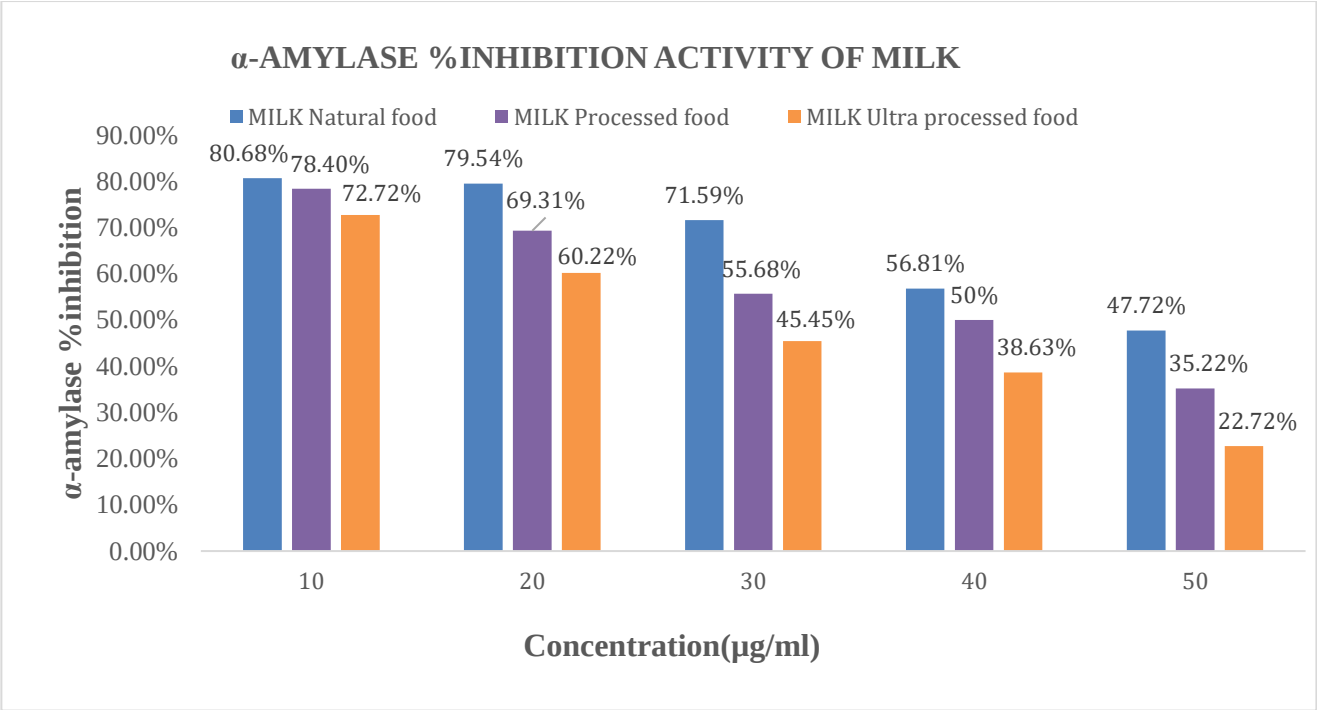

Figure 2: α-amylase %inhibition activity of Milk samples.

Milk extracts (10–50 µg/mL) exhibited concentration-dependent α-amylase inhibition, with buffalo milk showing the highest activity, followed by curd and ice cream.

Table 3: α-amylase absorbance of Coffee samples

SAMPLE	FOOD TYPE	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
COFFEE	Natural food (Coffee beans)	64.77%***	54.54%***	47.72%***	38.63%***	31.81%***
	Processed food (Nescafe powder)	51.13%**	42.04%*	34.09%	23.86%	15.9%
	Ultra-processed food (ID liquid)	43.18%	32.95%	18.18%	10.22%	3.40%

Note: * indicates statistically significant, ** indicates very statistically significant, *** extremely significant

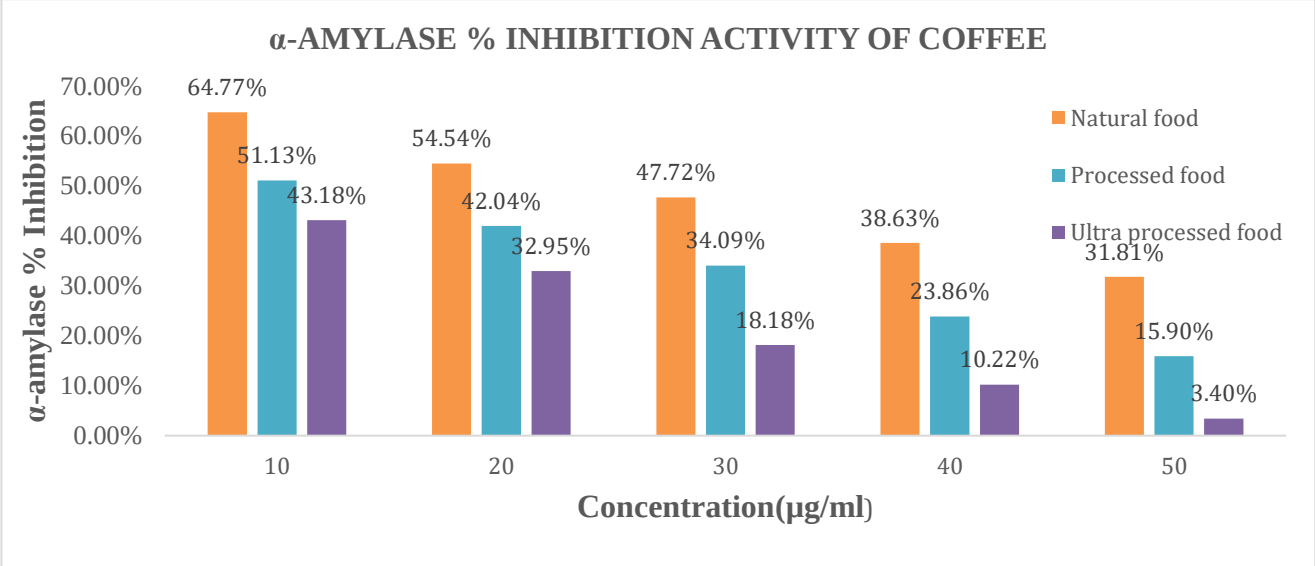

Figure 3: α-amylase % inhibitory activity of Coffee samples.

Coffee extracts exhibited concentration-dependent α-amylase inhibition (10–50 µg/mL), with coffee beans showing the highest activity, followed by instant coffee powder and liquid coffee.

Table 4: α-amylase %inhibitory activity of Tomato samples.

SAMPLE	FOOD TYPE	10µg/ml	20µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
TOMATO	Natural Food (Tomato)	88.63%***	86.63%	81.81%*	76.13%***	73.86%**
	Processed Food (Tomato puree)	71.59%*	61.36%*	60.22%	59.09%	56.81%
	Ultra-processed food (Kissan Ketchup)	32.95%	60.22%	21.59%	14.77%	9.09%

Note: * indicates statistically significant, ** indicates very statistically significant, ***indicates extremely statistically significant.

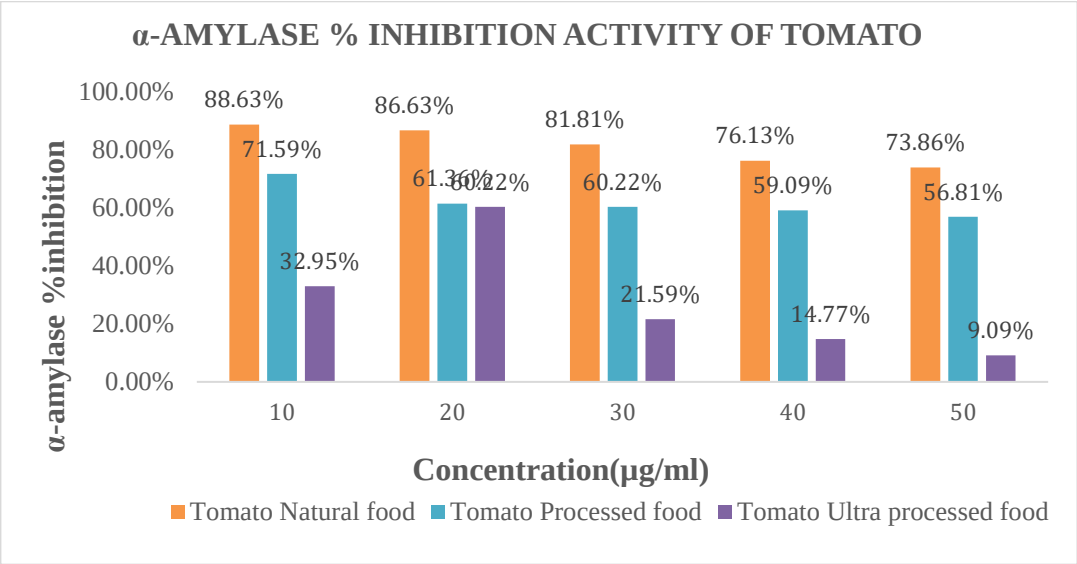

Figure 4: α-amylase %inhibitory activity of Tomato samples.

Tomato extracts demonstrated concentration-dependent α-amylase inhibitory activity across the tested range (10–50 µg/mL). Fresh tomato exhibited the highest inhibitory potential, followed by tomato puree, while ketchup showed the lowest activity. Overall, α-amylase inhibition declined progressively with increasing degree of processing.

ACARBOSE (α-amylase inhibitor):

Table 5: α-amylase %inhibitory activity of Acarbose

CONCENTRATION (µg/ml)	PERCENTAGE INHIBITION
10	81.81%
20	84.09%
30	86.36%
40	90.90%
50	97.72%

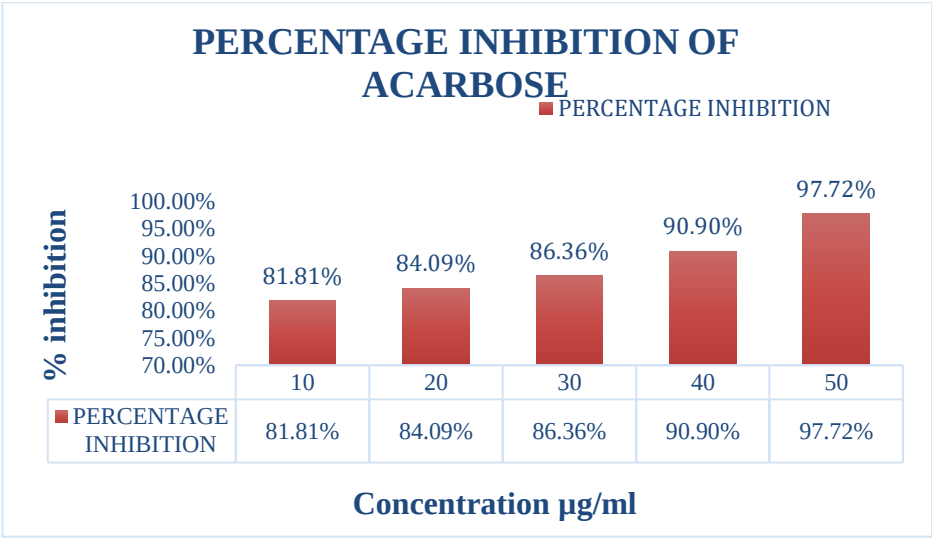

Figure 5: α-amylase inhibitory activity of Acarbose

Acarbose was employed as the standard drug for evaluating α-amylase inhibitory activity, against which the inhibitory potential of milk, coffee, and tomato samples was comparatively assessed across concentrations ranging from 10–50 µg/mL. At 10

µg/mL, acarbose exhibited 81.81% inhibition, which was comparable to natural tomato (88.63%) and milk (80.68%), but markedly higher than coffee samples. At 20 µg/mL, acarbose (84.09%) maintained strong inhibition, closely aligning with tomato (86.63%) while surpassing milk and coffee extracts. At 30 µg/mL, acarbose (86.36%) continued to demonstrate superior activity, exceeding milk, coffee, and processed tomato samples. At 40 µg/mL, the inhibitory effect of acarbose (90.90%) remained pronounced, distinctly higher than all tested food samples. Finally, at 50 µg/mL, acarbose achieved maximal inhibition (97.72%), significantly outperforming milk, coffee, and tomato extracts, all of which exhibited comparatively reduced activity. Overall, acarbose consistently demonstrated a concentration-dependent and superior inhibitory efficacy, serving as a robust benchmark, while natural samples showed moderate activity that declined with increasing concentration, particularly in processed and ultra-processed forms. This pattern may be attributed to the fact that even natural foods, when consumed in excessive concentrations, can contributeto metabolic imbalances such as diabetes, thereby reducing their inhibitory effectiveness.

4. DISCUSSION

"Reactive oxygen species (ROS)," a group of chemically reactive molecules produced during oxidative metabolism, includes hydrogen peroxide. The formation of ROS may occur as a by-product of normal cellular metabolic processes—such as mitochondrial autoxidation of components within the respiratory chain—or it may be deliberately produced by specific enzymes whose primary function is the generation of ROS (Wittmann C , et al,. 2012). When the production of reactive oxygen species (ROS) exceeds the body's antioxidant capacity, a condition known as oxidative stress occurs, causing damage to lipids, proteins, and DNA. An imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defenses leads to an increased oxidative burden, commonly observed in chronic diseases The accumulation of this damage is closely associated with the development of various diseases, including, diabetes mellitus, and inflammatory diseases. (Shaymaa Galeel Shamran, et al .,2025)

A surge of hydrogen peroxide recruits neutrophils, the immune system's first responders, to the wound site to eliminate microorganisms, remove damaged tissue, and initiate inflammation. In foods and beverages, hydrogen peroxide mainly originates from the autoxidation of food components (Kaur N Kumar, et al, 2021). To control foodborne pathogens, physical and chemical methods such as heat, radiation, and chemical sanitizers are commonly used; however, they can sometimes reduce food quality. (Dhay Ali Abed, et al,. 2026)

Experimental results showed that when foods are processed, they tend to produce more hydrogen peroxide, revealing that ultra-processed foods produce more hydrogen peroxide when compared to processed and natural foods. Consumption of ultra-processed foods with enhanced hydrogen peroxide production, which contributes to oxidative stress and increases susceptibility to inflammatory diseases.

A calcium-dependent metalloenzyme called α-amylase breaks down polysaccharides into simpler sugars like glucose and maltose that help in digestion (Navjot Kaur et al 2021). Mammalian α-amylase is primarily synthesized and secreted by the salivary glands and pancreatic acinar cells (Kimie Date, et al,. 2019). Furthermore, the enzyme raises blood glucose levels and promotes postprandial hyperglycemia. One well-known therapeutic target for the treatment and maintenance of postprandial blood glucose increases is α-amylase.

Acarbose is a complex oligosaccharide that inhibits a number of the intestinal enzymes that break down complex carbohydrates. By inhibiting the activity of these enzymes, acarbose reduces the absorption of dietary carbohydrates and the subsequent postprandial increase in blood glucose and insulin levels.

At lower concentrations (10–20 µg/mL), the natural food sample (buffalo milk) exhibited statistically significant α-amylase inhibitory activity comparable to acarbose, while at higher concentrations (30–50 µg/mL) the difference became highly significant. In contrast, the processed sample (curd) showed significant inhibition only at lower concentrations and no notable difference at higher levels, suggesting partial loss of bioactivity due to processing. Notably, the ultra-processed sample (ice cream) displayed no significant inhibitory effect across all tested concentrations, indicating a marked decline in α-amylase inhibitory potential with increasing levels of processing.

The processed sample (Nescafé coffee powder) exhibited significant α-amylase inhibition only at lower concentrations (10–20 µg/mL) and showed no notable difference from acarbose at higher levels, indicating reduced inhibitory potency due to processing. In contrast, the natural sample (coffee beans) demonstrated significant activity at lower concentrations, with markedly enhanced significance at higher concentrations (30–50 µg/mL). Meanwhile, the ultra-processed sample (ID liquid coffee) showed no significant inhibition across all concentrations, highlighting a substantial loss of bio-functional efficacy with ultra-processing.

Similarly, the ultra-processed sample (Kissan ketchup) displayed no significant α-amylase inhibitory activity across the tested range, indicating a pronounced decline in bioactivity. The processed sample (tomato puree) showed inhibition only at lower concentrations (10-20 µg/mL), with diminished effectiveness at higher levels (30-50µg/mL), suggesting partial attenuation due to processing. In contrast, the natural sample (fresh tomato) exhibited significant inhibitory activity at lower concentrations, with highly significant effects observed at higher concentrations.

Experimental results showed that aqueous extracts of all the ultra-processed food samples were less prone to inhibition of α-amylase in comparison with natural foods, whereas processed foods had moderate inhibition of α-amylase, standing second on the list, natural being the first. The α- amylase inhibition order is as follows:

Natural > Processed > Ultra-processed food.

The above study highlights the fact that consumption of ultra-processed foods is at a higher risk of causing Type 2 diabetes compared to natural foods at the same concentration.

5. CONCLUSION

From the study conducted, we can conclude that Ultra-processed foods (UPFs) elicit markedly elevated inflammatory responses and exert a more profound disruption of glucose homeostasis compared with natural and minimally processed foods. The high concentrations of added sugars, refined fats, sodium, and synthetic additives commonly present in UPFs contribute to persistent low-grade inflammation and the development of insulin resistance. Regular consumption of UPFs has been consistently associated with an improved risk of type 2 diabetes and other inflammation-mediated metabolic disorders. In contrast, natural and minimally processed foods demonstrate lower inflammatory potential and confer protective effects on metabolic health.

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