

The Untold Prebiotic Potential of Carrot and Pumpkin “halwa” Pudding

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Abstract

Carrot (*Daucus carota*) and pumpkin (*Cucurbita moschata*) *halwa* are popular sweet dishes in India known for their mashed vegetable texture. The carotenoids and flavonoids present in these two vegetables have been known for their health benefits since long. Recent studies on the polysaccharides present in these vegetables has also revealed their prebiotic potential. Prebiotics are now considered important for maintaining the health of the gut microbiome, besides having other benefits for human health. The pulp of carrots and pumpkins contains several prebiotic substances, and these effects make the *halwa* puddings made out of these vegetables much more than mere desserts. By supporting the growth of beneficial bacteria residing in the gut, these prebiotic substances help in controlling harmful bacterial growth and aid the beneficial bacteria in producing useful compounds like short chain fatty acids and butyrate.

1. Introduction

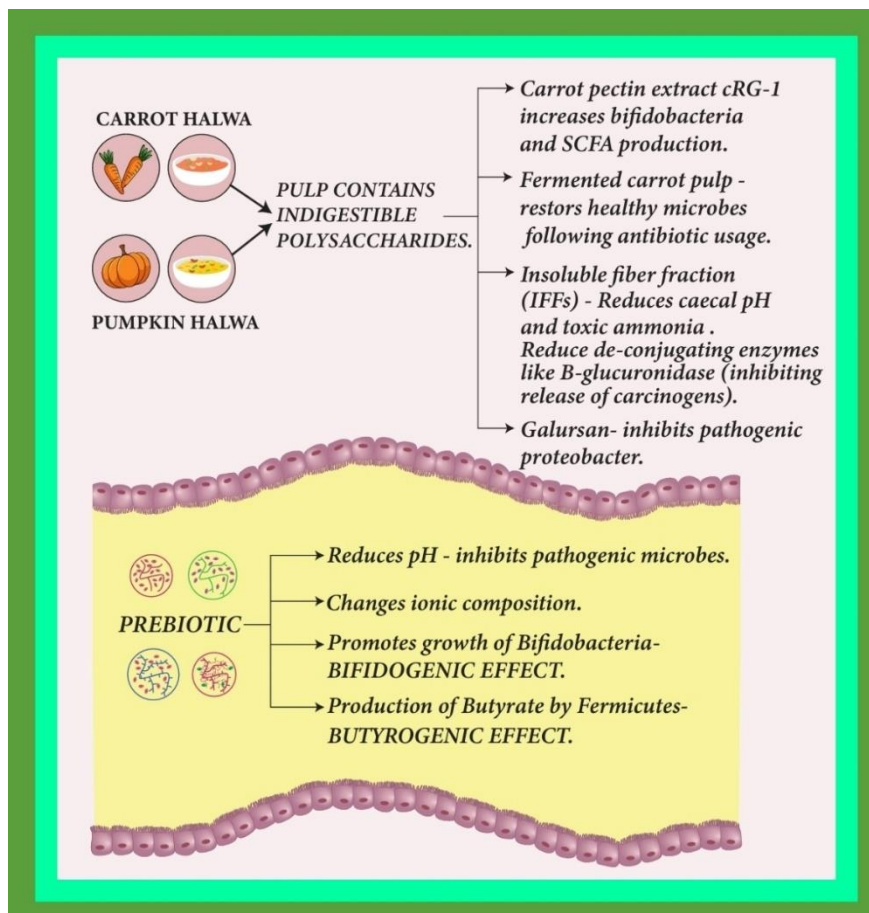
Puddings made out of vegetables and cereals have been prepared in India since millenia, and are especially served during festivals and celebrations. To prepare *halwa* the vegetables are finely grated, then sauteed in ghee, cooked in milk (optional), and sweetened with sugar. The cooking process softens the pulp and breaks down the thick cell walls, releasing the phytochemicals contained within. The pulp of carrots and pumpkins contains indigestible polysaccharides that act as prebiotics, providing various health benefits through fermentation by gut microbiota and promoting the growth of beneficial bacteria in the colon. The prebiotic substances selectively promote the growth of beneficial microbes, and also modulate the gut environment by causing changes in the pH (acidity) and ionic composition of the intra-luminal contents. These changes help in nurturing a healthier gut, promoting a robust microbiome, and preventing the overgrowth of pathogenic micro-organisms. A

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healthy gut microbiome is crucial for over-all well-being and the prebiotic properties of these vegetables can contribute to enhancing the gut health.

GRAPHICAL ABSTRACT



2. Prebiotics: Mechanism of Action and Beneficial Effects

Prebiotics were initially defined in 1995 as non-digestible dietary components that selectively stimulate the growth of specific bacteria in the colon, benefitting the host's health [1]. This definition was later refined by the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2008. To be classified as a prebiotic, a substance must meet certain criteria including resistance to degradation by stomach acid, non-metabolism by mammalian enzymes, fermentation by the gut microbiota, and selective stimulation of beneficial bacteria. These criteria ensure that prebiotics have a positive impact on the host's health by modulating the composition and activity of the gut microbiota [2].

2.1 Mechanisms by which Prebiotics Modulate Gut Microbiota

Prebiotics, as a source of energy, have a significant impact on the composition and function of gut microorganisms [3]. Diverse bacterial species have been shown to consume specific prebiotics, as evidenced by functional metagenomics studies [4]. For

example, species such as Actinobacteria, Bacteroidetes, and Firmicutes can ferment Fructo-oligosaccharides (FOS), Galacto-oligosaccharides (GOS), and Xylo-oligosaccharides (XOS). Also, certain species have the ability to degrade specific prebiotics, such as *Bifidobacterium* sp. with starch and fructans. Additionally, cross-feeding may occur where the fermentation by-products of a complex prebiotic serve as substrates for other microorganisms [5,6]. An example of this is the degradation of resistant starches by *Ruminococcus bromii* [7].

In addition to their direct impact on gut microbiota, prebiotics can modify the gut environment, by reducing the pH through the production of acidic fermentation products. This change in pH, from 6.5 to 5.5, has a significant influence on the composition and population of the gut microbiota, promoting the production of butyrate by Firmicutes, which is known as the butyrogenic effect [8,9]. Furthermore, the degradation of prebiotic polysaccharides leads to the production of Short Chain Fatty Acids (SCFAs), primarily. These SCFAs can permeate gut enterocytes (intestinal epithelial cells) and enter the bloodstream, exerting effects not only in the gastrointestinal tract but also in distant organs and systems [10].

2.2 Beneficial Effects of Prebiotics

In several studies, prebiotics have demonstrated a positive impact in enhancing health and ameliorating various disorders. They have the potential to meliorate the immune system by promoting the growth of beneficial bacteria, inhibiting harmful ones, and stimulating the expression of immune molecules [11-13]. Prebiotics such as GOS can improve skin health by enhancing water retention, ameliorating the erythema of dermatitis, and strengthening the skin barrier. They may also have a neutralizing effect on harmful compounds like phenols [14-18]. Certain prebiotics like lactulose have shown promise in treating hepatic encephalopathy, a liver condition that leads to psychiatric and neurological issues, by reducing gut ammonia levels [19,20]. Additionally, some prebiotics have been associated with increased calcium absorption, which can be beneficial for conditions like osteoporosis or low bone mass [21,22].

In gastrointestinal disorders such as Irritable Bowel Syndrome and Crohn's disease, the effect of prebiotics has been varied, with some studies reporting symptomatic improvement. Prebiotics support the growth of beneficial gut bacteria, including *Bifidobacteria* and *Faecalibacterium prausnitzii*. There is evidence suggesting that prebiotics may reduce the risk of colorectal cancer and slow its progression by promoting gut health and enhancing the function of the epithelial barrier. Furthermore, prebiotics such as FOS and GOS may help prevent Necrotizing Enterocolitis by stimulating the growth of beneficial gut microbiota [23]. Prebiotics also play a role in the gut-brain axis, influencing neural, endocrine, and immune pathways [23]. This can potentially impact mood, memory, learning, and may have benefits in managing certain psychiatric disorders. Additionally, prebiotics may have cardiovascular health benefits, by reducing inflammatory markers and improving lipid profiles. However, the evidence regarding their effects on blood triglycerides and cholesterol levels remains mixed [23].

3. Prebiotic Potential of Carrot

Carrot pomace is a high-fiber ingredient, with the latter comprising approximately 25% of the vegetable's weight. On a dry weight basis, the main fiber constituents found in carrot pomace include pectin (3.88%), hemi-cellulose (12.3%), cellulose (51.6%) and lignin (32.1%). Among these, pectin is primarily responsible for the prebiotic effects associated with carrot pomace

[24]. The prebiotic effects of carrot-derived **pectin extract cRG-I** on the gut microbiome were recently investigated using M-SHIME (Simulator of the Human Intestinal Microbial Ecosystem) technology. The results of the study demonstrated several interesting findings. Firstly, cRG-I was found to be non-digestible by host enzymes, indicating its potential to reach the colon intact, where it can interact with the gut microbiota. This characteristic is a desirable trait for prebiotics as they need to resist digestion in the upper gastrointestinal tract to reach the colon. Furthermore, the study found that cRG-I increased the levels of beneficial SCFAs in the gut. Moreover, cRG-I was shown to stimulate the growth of specific health-associated bacteria in the gut including *Bacteroides dorei*, *Faecalibacterium prausnitzii*, *Prevotella* species and *Bifidobacterium longum* [25]. These bacteria have the ability to break down cRG-I, suggesting their involvement in the fermentation of this specific prebiotic compound [25]. The significant increase in *B. longum* indicates that these bacteria consumed the side chains of cRG-I [26]. Bifidobacteria are well known beneficial bacteria in the gut that can contribute to gut health by producing SCFAs and modulating the immune system.

An animal study by Yu et al (2022) investigated the potential prebiotic effects of fermented carrot pulp in regulating and protecting the gut microbiome, specifically after antibiotic treatment. The study used mice and evaluated the effects of lactobacillus fermented carrot pulp on various aspects of health and gut microbiota. According to the study findings, mice that were treated with lactobacillus fermented carrot pulp experienced significant improvements in their health. These improvements included increased weight, an improved mental state, restored microbiota composition, and normalized bowel movements. The study concluded that fermented carrot pulp has the potential to aid in the restoration of microbiota richness and diversity, thereby regulating and protecting the gut microbiome following antibiotic therapy [27].

Ramnani et al studied the prebiotic properties of carrot-derived fiber and its effects on bifidobacteria and lactobacillus/enterococcus groups, in a double-blind, placebo-controlled trial conducted with 66 participants. The participants consumed fruit and vegetable shots containing carrot and Jerusalem artichoke inulin. The results showed that bifidobacterial levels significantly increased after consuming these shots. Additionally, there was a small increase in the Lactobacillus/Enterococcus group [28]. In another study, utilizing an in vitro fermentation model, Song et al. demonstrated that the human gut microbiota can ferment carrot juice, resulting in the production of lactate and acetate. The study revealed that carrot juice had a significant impact on the composition of the gut microbiome. Specifically, it led to an increase in the abundance of beneficial bacteria, including *Lactobacillus fermentum*, *Lactobacillus salivarius*, *Lactobacillus mucosae*, and *Bacteroides uniformis*. Furthermore, carrot juice reduced opportunistic pathogenic bacteria like *Enterococcus faecium* [29].

Chau et al investigated the effects of **insoluble fiber fractions (IFFs)** from carambola and carrot pomace on hamsters' intestinal enzymes, fecal bacterial enzymes, and biochemical parameters. The results indicated that IFFs from both carambola and carrot pomace had significant effects on various aspects of intestinal health. One notable effect was the reduction in caecal pH which refers to the acidity of the contents in the cecum, a pouch-like structure in the digestive tract [30]. Low caecal pH is beneficial because it inhibits the proliferation of harmful microbes. IFFs from carambola and carrot pomace helped to maintain a balanced and healthy gut microbiota. Additionally, the study found that IFFs led to decreased levels of caecal and fecal ammonia. Since ammonia is a toxic substance that can be produced by certain bacteria in the gut during the breakdown of proteins, lower levels of ammonia are beneficial. The IFFs also resulted in reduced fecal enzyme activities, specifically mentioning beta-

glucuronidase. *Beta-glucuronidase* is an enzyme involved in the de-conjugation of harmful compounds in the large intestine. When these compounds are de-conjugated, they can have carcinogenic effects on the colon. Therefore, lower levels of enzymes like *beta-glucuronidase* are considered beneficial, as they decrease the amount of harmful compounds and reduce the risk of carcinogenesis in the large intestine. Based on these findings, it was suggested that IFFs from carambola and Carrots could improve intestinal functions and promote overall gut health (FIG. 1). Furthermore, these IFFs have the potential to be used as functional ingredients in fiber-rich products with prebiotic properties [30].

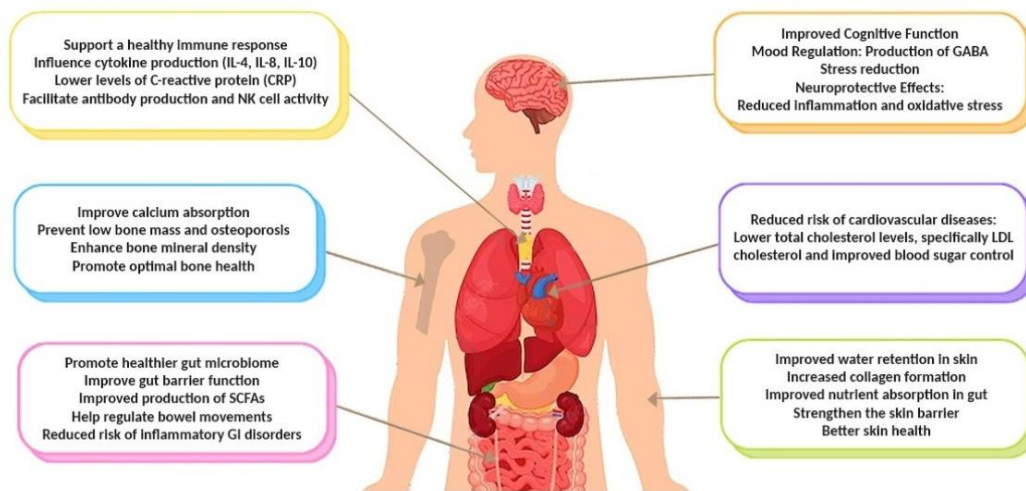


FIG. 1. Beneficial effects of carrot and pumpkin pulp prebiotics on various organ systems.

Another study by Engevik et al. investigated the prebiotic potential of **galursan HF 7K (GHF7K)**, an oligosaccharide derived from carrots, in mice as a potential alternative to antibiotics. The researchers aimed to understand how GHF7K supplementation affects the gut microbiota composition. The results showed that GHF7K supplementation did not significantly change the total number of bacterial in the gut. However, it did lead to significant alterations in the composition of both luminal (contents of the gut) and mucosa-associated (bacteria attached to the gut lining) bacterial populations at the phylum level. These changes were observed in a region-specific pattern, meaning that different parts of the gut were affected differently. The study also found that the alterations in gut microbiota correlated with changes in the ionic composition of the intestinal fluid. Specifically, GHF7K supplementation increased the concentration of chloride ions (Cl⁻) in the duodenum and jejunum, while the concentration of sodium ions (Na⁺) was increased in the caecum. Furthermore, the researchers observed an increase in number of bacteria from the Proteobacteria phylum in the ileum and colon of mice fed GHF7K. It is worth noting that an increase in Proteobacteria is also commonly associated with antibiotic use. However, the study did not report any changes in the number of beneficial bacteria genera such as *Lactobacillus* and *Bifidobacterium*, which belong to the Firmicutes phylum. The results suggest that GHF7K dietary supplementations can alter gut microbiota, potentially serving as an alternative to prophylactic antibiotic use [31]. The plausible mechanism for this effect may be that GHF7K prevents infection by either promoting the proliferation of resident Proteobacteria, thereby limiting further colonization by other pathogenic Proteobacteria, or by acting as a preferred binding substrate for Proteobacteria members. In this way, luminal GHF7K may prevent pathogenic Proteobacteria from binding to oligosaccharides on the host mucosa.

Polyphenols from carrots have been shown to possess prebiotic effects by regulating the structure of the colonic microbial community. In an in vitro model, it was observed that the abundance of beneficial microbiota increased while harmful bacteria decreased after carrot powder underwent in vitro digestion/fermentation. These findings suggest that carrot polyphenols have the potential to be used as prebiotics [32]. Another study focused on the impact of bound polyphenols on the prebiotic properties of carrot dietary fiber (CDF). Researchers removed polyphenols from CDF, resulting in dephenolized dietary fiber (CDF-DF). Despite CDF-DF and CDF having similar key features and no substantial structural differences, *Lactobacillus rhamnosus* grew more favorably with CDF. This suggests that **bound polyphenols** played an important role in enhancing the prebiotic properties of carrot-derived fiber. Interestingly, the removal of polyphenols did not significantly change the structure of the fiber [33].

4. Prebiotic Effects of Pumpkin

Pumpkin (*Cucurbita moschata*) flesh and peel are rich in fiber, with pectin and polysaccharides being the most important components. Du et al. in 2011, highlighted that pumpkin is a plentiful source of polysaccharides, which can be converted into oligosaccharides through acid hydrolysis in the stomach. *In-vitro* studies demonstrated that these oligosaccharides significantly stimulated the growth of beneficial lactobacilli. This suggests that pumpkin-derived oligosaccharides have prebiotic potential and can be utilized as beneficial ingredients [34]. Another study on a Type 2 diabetic model showed that pumpkin polysaccharide changed the gut microbiota structure by selectively enriching key species of *Bacteroidetes*, *Prevotella*, *Deltaproteobacteria*, *Oscillospira*, *Veillonellaceae*, *Phascolarctobacterium*, *Sutterella*, and *Bilophila*. Also, the pumpkin polysaccharide improved metabolic parameters such as insulin resistance and reduced serum glucose, total cholesterol, and low-density lipoprotein levels while increasing high-density lipoprotein levels [35]. In a similar study, the consumption of pumpkin polysaccharide increased the diversity of beneficial bacteria in the gut and decreased harmful bacteria such as *Clostridium*, *Thermoanaerobe*, Symbiotic bacteria, *Deinococcus*, *Vibrio haematococcus*, *Proteus gamma* and *Corio*. At the family level, pumpkin consumption significantly reduced the abundance of *Erysipelotrichaceae* and increased the *Akkermanaceae* of *Verrucobacterium* [36].

In an interesting animal study conducted by Agarkova et al. in 2019, a functional mousse (creamy dessert) made with whey protein hydrolysate (WPH) and pumpkin pectin exhibited a bifidogenic effect. The consumption of this led to a 3.7-fold increase in *Bifidobacterium* spp. in rats with antibiotic-induced dysbiosis. The findings indicate that WPH and pumpkin pectin have the potential to serve as prebiotic ingredients for the development of functional mousses that offer health benefits [37]. Moreover, a separate animal experiment studied the effect of fermented pumpkin juice on the gut microbiota of mice. The workers discovered that pumpkin juice fermented by using *Rhodobacter sphaeroides* (RPJ) reshaped the microbiome in the mice gut by increasing beneficial *Lactobacillus* and *Bifidobacterium* levels while reducing harmful Proteobacteria [38]. These studies suggest that pumpkin juice, pectin and polysaccharides, all have potential health benefits for gut microbiota.

5. Discussion

The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement from 2017 defines a prebiotic as "a substrate that is selectively utilized by host microorganisms, conferring a health benefit" [39]. The selective properties distinguish prebiotics from other substances. Prebiotics are utilized or fermented specifically by beneficial

microorganisms in the gut, such as certain species of *Lactobacilli* or *Bifidobacteria*, rather than being utilized by harmful or pathogenic bacteria. By promoting the growth of beneficial bacteria and inhibiting the growth of potentially harmful bacteria, several health benefits can result [39]. The beneficial microbes metabolize prebiotics and produce various useful substances like SCFAs (short-chain fatty acids). The selective utilization of prebiotics also results in a beneficial influence on immune parameters in gut-associated lymphoid tissues (GALT), secondary lymphoid tissue and peripheral blood [40]. Besides, prebiotics can also lower the risk of neoplasias, infections, coronary artery disease and degenerative diseases. Experimental studies have shown an improvement in glucose tolerance, a decrease in fat accumulation, inflammation, and oxidative stress [41].

Carrot pulp is a multi-nutritional food source rich in various nutrients, including vitamins, minerals, dietary fiber, and sugars. Carrots also contain an array of natural bioactive compounds, such as phenolics, carotenoids, ascorbic acid, and polyacetylenes [42]. The nonstarchy polysaccharides in raw carrots account for 2.4% of the fresh weight or 19.5% of the dry weight. Carrot fiber mainly consists of arabinose and galactose as the main sugars, with smaller amounts of glucose, rhamnose, xylose, and mannose [43]. Both carrot and pumpkin pulp contain a significant amount of pectic substances, contributing to their high water-holding capacity (WHC). The WHC of dietary fiber has been linked to the potential decrease of cholesterol absorption in the gut [44]. High WHC fibers such as those found in carrot and pumpkin pulp, possess the ability to bind to cholesterol in the digestive system, reducing its absorption into the bloodstream. This mechanism can contribute to the potential cholesterol-lowering effects associated with the consumption of high-fiber foods.

Rafiq and Sharma (2016) explored the characteristics of carrot juice as a raw material for probiotic vegetable juice by analyzing four strains of lactic acid bacteria: *L. acidophilus*, *L. Plantarium*, *L. Casei* and *Bifidum longum*. The results showed that the probiotic strains grew well in carrot juice without any nutrient supplementation [45]. Similarly pumpkin pulp has been found to have bifidogenic properties. Thus, carrots and pumpkins can act as excellent prebiotic candidates for promoting the growth and activity of probiotic bacteria in the gut, especially for individuals with lactose intolerance and those susceptible to allergic reactions from consuming dairy products. The addition of carrot and pumpkin powder can also boost the antioxidative and antimicrobial properties of various products. Kamel et al. found that incorporating carrot powder as a functional ingredient in probiotic soft cheese facilitates the growth of probiotic bacteria, making it an excellent prebiotic for enhancing the survival of probiotic bacteria in different food applications [46]. Thus, carrots and pumpkins can be added as a functional ingredient in various products to enhance their biological and nutritional content [47].

6. Conclusion

The popular Indian vegetable *halwa* puddings are not merely desserts but have great nutritional and promotive value. State-of-the-art studies have demonstrated the highly beneficial prebiotic potential of these puddings. Carrots and pumpkins are vegetables that are available almost world-wide, and puddings made out of these and similar vegetables like white gourd and beetroot, can prove to be economical functional foods. It is now known that the health benefits arising from the consumption of these vegetable puddings encompass a wide variety of systemic effects, due to the phytochemical content and pulp prebiotics. Taken on a regular basis, the *halwa* puddings can greatly enhance physical and mental well-being.

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