Actazin™ A Novel Whole Kiwifruit-Based Ingredient for Promoting Regularity and Relief of Mild Constipation Naturally

Correspondence: Joseph L. Evans, PhD
P and N Development Ventures, Saint Louis, MO 63108 USA
Email: jevans@pndvhq.com
Executive Summary

Globally, many people suffer from a variety of digestive disorders such as bloating, constipation, diarrhea, gas, heartburn, acid reflux, irritable bowel syndrome (IBS), and many others. Gut and digestive health is central to overall systemic health, and is impacted by a wide array of genetic, environmental, dietary, hormonal, and microbial influences. To address the incidence of these growing conditions, digestive health-positioned products (foods/drinks, retail sales) reached approximately $55 billion globally and $2.5 billion (estimated) in the US in 2016, dominated by fiber, prebiotics, probiotics, and enzymes. These older, mature ingredient options suffer from a variety of limitations including lack and/or consistency of efficacy in most individuals, poor tolerability, and paucity of clinical substantiation for relevant digestive health claims.

Actazin™ is a wholefood-based nutritional ingredient option, crafted from the nutrient-dense New Zealand green kiwifruit; Actazin™ was recently introduced to the marketplace to address the limitations of the existing digestive health ingredient options. Actazin™ consists of a variety of bioactive components including fiber and polyphenolics, contributing to its prebiotic activity, along with the kiwifruit-unique protein-degrading enzyme, actinidin. Actazin™ use is supported, in part, by the traditional use of whole kiwifruit for routine maintenance of digestive health and for the symptomatic relief of constipation. Evaluation of Actazin™ in healthy individuals supports its use to increase the frequency of bowel movements. Competitive analyses of Actazin™ compared to existing digestive health ingredients reveals a highly favorable profile that merits it consideration by product development professionals for inclusion in new or existing digestive health products.

To address the incidence of these growing conditions, digestive health-positioned products (foods/drinks, retail sales) reached approximately $55 billion globally and $2.5 billion (estimated) in the US in 2016, dominated by fiber, prebiotics, probiotics, and enzymes. These older, mature ingredient options suffer from a variety of limitations including lack and/or consistency of efficacy in most individuals, poor tolerability, and paucity of clinical substantiation for relevant digestive health claims.
Introduction

Among the plethora of prescient conclusions attributed to Hippocrates, the undisputed father of scientific, evidence-based medicine, one famously credited to him is that “…All disease begins in the gut…” Not bad, as unifying hypotheses go, especially in the days before the widespread availability of the internet and 23andMe™ genetic profiling. Although we now know with certainty that all disease does not begin in the gut, let’s not quibble about minor details and pay proper homage to Hippocrates. A large number of conditions and diseases really do have their origin in the digestive system, and if left untreated, result in a sub-standard quality of life and can lead to more serious illnesses and even death (Figure 1).

Globally, many people suffer from a variety of digestive disorders such as...

- Irritable Bowel Syndrome (IBS)
- Diarrhea
- Constipation
- Acid Reflux
- Heartburn
- Gas
- and many others...

Death by Diseases of the Digestive System (per 100,000 inhabitants)

Figure 1. Death by diseases of the digestive system according to country

Gut and digestive health is central to overall systemic health, and is impacted by a wide array of genetic, environmental, dietary, hormonal, and microbial influences. Digestive health is defined here as the optimal digestion, absorption, and assimilation of food. The digestive system is absolutely required for our bodies to convert the dubious assortment of foods and drinks we consume into the requisite amino acids, carbohydrates, fats, and vitamins vital for our cells to survive, fulfill their individual and specialized functions, and replicate. The type, frequency, and quantity of food and drink we consume on a daily basis largely determines our health status, by influencing our body weight and composition, energy level, mood, hormone production, and providing the nutritional milieu that determines the composition our gut microbiome (bacteria, fungi, viruses). As we now know, the composition of our gut microbiome influences virtually every aspect of our physiology, and plays a critical role in regulating our health and risk for developing disease [1].

In light of the widespread extent and prevalence of gastrointestinal issues faced by so many consumers, it is not surprising that this is a major product category for functional food, nutraceutical, and dietary supplement product development professionals. In 2016, digestive health-positioned products (foods/drinks) reached approximately $55 billion (global retail sales), and accounted for the third largest product category in the $594 billion worldwide Health and Wellness market (global retail sales), only trailing products in the general well-being (eg multi-vitamins, etc) and weight-management categories [2]. In the US in 2016, digestive health-positioned products reached an estimated $2.5 billion in retail sales, ranking 4th behind sports/energy/weight loss ($12.6 billion), general well-being ($5.4 billion), immune/cold/influenza ($2.7 billion), and equivalent to heart health product retail sales ($2.5 billion) [3].

Probiotics and fiber have dominated the scene and driven growth in the digestive health category while, simultaneously, suffering from significant class-specific limitations: existing probiotics are simply not sufficiently effective for most individuals seeking gastrointestinal relief, while the high fiber amount required for relief and other health benefits are simply too aggressive for many individuals to endure. In addition, the higher fiber doses are for acute symptomatic relief, and are generally not tolerated or sustained for long-term use. Now, from the untainted and picturesque countryside of New Zealand, there rises up a new, wholefood-based nutritional ingredient option, crafted from green kiwifruit. This ingredient is Actazin™, and is the topic of this white paper.

“A large number of conditions and diseases really do have their origin in the digestive system, and if left untreated, result in a sub-standard quality of life...”
Prevalence and Significance
Gastrointestinal and liver diseases impose a substantial worldwide burden, and are responsible for approximately 8 million deaths per year worldwide. Diarrheal disease is the second leading cause of death among children <5 years old, with the majority of these deaths occurring in Africa. Gastrointestinal-related cancers are responsible for nearly 40% of all cancer-related deaths, with a toll of nearly 3 million deaths per year estimated in 2008.

Other gastrointestinal conditions, including constipation, are common worldwide in adults and children, which can severely impact the individual’s feeling of general wellbeing and overall quality of life [4,5]. While these are comparatively less serious conditions (vs the ones mentioned above), they are of significant importance for those who are afflicted. It is estimated that up to 20% of the world’s population suffer from this condition with women and individuals aged over 65 years being most frequently affected [4,5].

Factors such as gender, aging, socioeconomic status and educational level can affect the prevalence of constipation worldwide. The symptoms of constipation include incomplete evacuation after excretion, bloating pressure in the rectum, abdominal bloating and pain, and obstructive evacuation during defecation.

Limitations of Current Nutritional Options
The intervention options for constipation remain difficult and challenging, and many individuals are dissatisfied with current medications and non-prescription options [6,7]. Current interventions for constipation include lifestyle and dietary modifications (primarily fiber and probiotics), as well as pharmacological interventions (ie stool softeners, osmotic laxatives, and stimulant laxatives). The worldwide growth in the incidence of gastrointestinal disorders has created an immediate need to identify alternative safe and effective remedies.

A food-based approach to prevent constipation should be considered as an effective long-term solution, especially in comparison to the acute use of aggressive laxative products [8]. It is generally regarded that adequate daily intakes of fiber-rich fruits and vegetables along with adequate hydration reduce the incidence of constipation [7,8]. Food ingredients such as psyllium and wheat bran (ie major sources of fiber) are the most studied for preventing constipation. In addition, probiotics are another approach commonly used to ameliorate digestive discomfort [9]. However, few individuals are able to achieve the recommended daily intake of fruits / vegetables and / or fiber, and those that do report a significant difficulty in tolerating such an excessive recommended amount. On the other hand, probiotics are so diverse that the specific bacterium(a) responsible for delivering gastrointestinal relief for most individuals has yet to be identified. In light of the severity, magnitude, and significance of the problem, additional options for the relief and / or prevention of gastrointestinal dysfunction are clearly warranted. The unique benefits for digestive health offered by a new kiwifruit-derived ingredient are poised to address this growing and serious problem.
Kiwiﬁruit, described in early 12th century China and introduced to Europe in the 18th and 19th centuries via the efforts of various merchants and explorers (eg Scottish botanist Robert Fortune), was brought to New Zealand from China in the early 20th century, by the school teacher Isabel Fraser [10]. The kiwiﬁruit (genus *Actinidia*) is now an iconic symbol of New Zealand, and has acquired status internationally of being a “frutto della salute”. Although there are many species and cultivars of kiwiﬁruit, by far, the most important commercial species accounting for virtually all international trade, are green kiwiﬁruit (*Actinidia deliciosa* cv. ‘Hayward’) and gold kiwiﬁruit (*Actinidia chinensis* cv. ‘Hort16A’) [10;11]. Aside from their color difference, green kiwiﬁruit possess a bold, sweet and sour taste, while gold kiwiﬁruit have a milder, sweeter taste.

The potential and substantiated health beneﬁts of both major types of kiwiﬁruit have been the subject of an increasing number of peer-reviewed studies since 1977 [12-14]. Among its many reported health beneﬁts, green kiwiﬁruit are widely used as a gentle, safe, and effective nutritional intervention for gastrointestinal discomfort, especially constipation [15], while consumption of gold kiwiﬁruit has been linked to improved immunity [16;17]. Zespri®, a major supplier of green and gold kiwiﬁruit headquartered in New Zealand, ﬁled (in May 2014) the ﬁrst self-substantiated health claim for green kiwiﬁruit in New Zealand, under the Food Standards Australia-New Zealand Standard for Nutrition, Health, and Related Claims. The claim reads “Zespri® green kiwiﬁruit can contribute to normal bowel function”.

The claim referenced above is based on 10 years of data from clinical trials, and supporting evidence from other research, a summary of which follows. In a cross-over trial, thirty-eight healthy, elderly (> 60 years) consumed their regular diet, with or without 1 kiwiﬁruit daily per 30 kg body weight (about 2.5 kiwiﬁruit for a 165 lb (75 kg) person) for 3 weeks, followed by a 3 week cross-over period [18]. Based on self-reporting via a daily diary, the inclusion of kiwiﬁruit led to bulkier and softer stools, as well as more frequent stool production and greater ease of defecation.

In another study, researchers from the University of Hong Kong evaluated the effect of a 4-week treatment of 2 kiwiﬁruit daily in 33 constipated Chinese patients and 20 healthy volunteers [19]. The responder rate was 55% in the constipated group [19]. The mean complete spontaneous bowel movement increased signiﬁcantly after treatment (P = 0.013). There was also improvement in the scores for constipation (P = 0.02) and self-reported satisfaction of bowel habit (P = 0.001), as well as a decrease in days of laxative used (P = 0.003). There was also improvement in transit time (P = 0.003) and rectal sensation (P < 0.05). In contrast, no change in the bowel symptoms or anorectic physiology was seen in the healthy subjects.

Another study examined the impact of a 4-week kiwiﬁruit intervention on bowel function in patients with irritable bowel syndrome (IBS) characterized by constipation [20]. Forty-one patients and 16 healthy adults consumed 2 kiwiﬁruit daily, while 13 control IBS patients consumed 2 placebo capsules daily. After 4-weeks, weekly defecation frequency signiﬁcantly increased in the IBS group of participants who consumed kiwiﬁruit (P < 0.05), while colon transit time signiﬁcantly decreased (P < 0.026). These ﬁndings suggest that consumption of kiwiﬁruit shortens colon transit time, increases elimination frequency, and improves bowel function in adults diagnosed with IBS with constipation. The potential effects of kiwiﬁruit in individuals with other subtypes of IBS (eg diarrhea, mixed diarrhea / constipation) will require further investigation. No published data that identiﬁes a minimally efficacious daily quantity for whole kiwiﬁruit for increasing bowel movement frequency are available, but the diversity of beneﬁcial constituents contained in a single kiwiﬁruit support its consumption on a regular basis. A summary of the published clinical studies along with a preliminary report of a recently completed study evaluating the effects of green kiwiﬁruit...
on bowel movement frequency are provided in Table 1. Collectively, the studies support the beneficial effects of daily consumption (4 weeks) of 2 green kiwifruit on the frequency of complete spontaneous bowel movements and related indices of gastrointestinal function.

Kiwifruit Fiber Composition and Hydration Properties

The dietary fiber content of green (Hayward) and gold (Gold3, SunGold) kiwifruit is 3.0 g/100 g1 and 1.4 g/100 g2, respectively, and comprises approximately 1/3 soluble and 2/3 insoluble fiber [21]. The soluble fiber consists of mostly pectic polysaccharides, and the insoluble is mostly cellulose and hemicelluloses along with a small amount of pectin. The pectin molecules, in their role as structural components of the kiwifruit cell wall, bestow the superior hydration benefits of kiwifruit fiber.

Interestingly, kiwifruit fiber has unique hydration properties. It has the capacity to swell or ‘gel’ (ie, the volume fiber has in water after passively settling), reaching over three times its volume in the original fruit [22]. Compared to rehydrated dietary fiber preparations, the swelling capacity of freeze-dried kiwifruit fiber is twelve times higher than wheat bran, more than six times higher than apple fiber, and one and a half times higher than psyllium [22]. Kiwifruit fiber also has high water retention capacity- the amount of water that is bound to insoluble fiber and is not separated from fiber by centrifugation [23]. The water retention capacity of kiwifruit fiber is 12-13 g water/g insoluble fiber, which is about twice that of apple fiber and four times that of wheat bran.

The hydration properties of the fiber affect the dynamics of macronutrient absorption, reducing mixing in the bowel and diffusion [24,25]. Kiwifruit fiber may also add to fecal bulking [22]; however, it is also completely fermentable by the gut microflora and, therefore, may play a significant role in the modulation of the composition of the microflora [22].

By comparison, the hydration properties of inulin and fructose oligosaccharides (FOS) are less robust due to their chemical structures and properties. Inulin is a naturally occurring, non-structural carbohydrate (fructose) polymer characteristic of most plants. It is believed to be used as an energy storage molecule. Due to its typical chain length (ie degree of polymerization) of ~ 60 fructose units, it is very soluble in aqueous solution, and thus is not a very effective hydrator. FOS is even more water soluble than inulin, and thus is resistant to hydration. Thus, the greater molecular size (degree of polymerization), different subunit composition, and function as a structural component of pectin and related molecules largely accounts for the excellent hydration properties of kiwifruit fiber.

The nutritional composition of each species has been well characterized [26]. Kiwifruit, typically weighing about 100 g

---

per med-large fruit, is a nutrient-dense food. Based on 100 g edible flesh, green kiwifruit is low in calories (~60 kcal), yet an exceptionally good source of vitamin C (~90 mg), an excellent source of potassium (~310 mg), along with folate (~25 µg) and vitamin K (~40 µg), and a supplementary source of dietary fiber (2-3 g) [11]. Not only is green kiwifruit high in potassium, but it contains some magnesium (~17 mg) and very little sodium (<5 mg) — a combination of minerals that favors normal blood pressure levels.

Kiwifruit also contains a variety of bioactive phytochemicals such as polyphenolics and carotenoids (eg lutein, β-carotene) that contribute to the overall health benefits of kiwifruit [27]. New Zealand is exposed to increased UV radiation as compared to other countries around the world of similar latitude. As a compensatory response, plants respond to the elevated levels of UV-B light by increasing their synthesis of secondary metabolites such as polyphenolic compounds and carotenoids. Polyphenols are natural compounds produced by microorganisms and plants, and are typically involved in the defense against ultraviolet radiation or from pathogens or predators. Besides functioning as direct antioxidants, epidemiological studies and associated meta-analyses conclude that long term consumption of diets rich in plant polyphenols offer protection against development of certain cancers, cardiovascular diseases, type 2 diabetes, osteoporosis, and neurodegenerative diseases [28]. The combination of kiwifruit fiber, non-digestible carbohydrates, vitamins, and polyphenolics also influence the gut microbiome profile, by suppressing the growth of pathogenic bacteria while increasing the growth of lactic acid bacteria [29].

Another unique component of green kiwifruit is the cysteine protease enzyme, actinidin, which comprises about 40% of the soluble protein [30]. Actinidin catalyzes the hydrolysis of peptide bonds in proteins present in meat, fish, eggs, etc, and kiwifruit marinade has been used commercially as an effective meat tenderizer. In vitro work has indicated that actinidin enhances protein digestion in the stomach and small intestine [31;32]; subsequent studies have established that actinidin promotes protein digestion in vivo [33;34].

Kiwifruit is suitable for all age groups. However, for a very small number of individuals, kiwifruit may trigger an allergic response. Virtually all food groups can cause an allergic reaction in some sensitized individuals. Globally, the incidence of food allergy is reported to be increasing. However, the prevalence of kiwifruit allergy in the general population has been estimated to be less than 2% [35]. People with allergies to latex, birch pollen, and dust mites are more likely to be allergic to kiwifruit. Care should be taken when first introducing kiwifruit or new varieties of kiwifruit to children, particularly those with other known food allergies. People with a known kiwifruit allergy should avoid all kiwifruit.
Table 1. Summary of Clinical Trials Conducted on Whole Green Kiwifruit (*Actinidia deliciosa* cv. 'Hayward') on Frequency of Bowel Movements

<table>
<thead>
<tr>
<th>STUDY</th>
<th>STUDY SIZE</th>
<th>STUDY DESIGN</th>
<th>DOSE</th>
<th>PLACEBO</th>
<th>PARAMETERS ASSESSED</th>
<th>OBSERVED BENEFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rush et al (2002);[18]</td>
<td>42 (elderly, ≥60)</td>
<td>Cross-over: 3-week treatment period</td>
<td>100 g per 30 kg</td>
<td>None - control period of not eating kiwifruit</td>
<td>Number of stools, consistency, volume and ease of passing</td>
<td>Increased frequency (P = 0.012) and volume (P = 0.002); improved ease of defecation (P &lt; 0.0001).</td>
</tr>
<tr>
<td>Chan et al (2007);[19]</td>
<td>33 constipated; 20 healthy</td>
<td>Single center, case control study: 2-week baseline, 4-week treatment period</td>
<td>2 per day (1 in morning, 1 in evening)</td>
<td>None - compared to healthy and baseline</td>
<td>Straining, Bristol stool score, use of laxatives and satisfaction with bowel habits</td>
<td>Constipated cohort - Complete spontaneous bowel motion (CSBM) significantly increased (P = 0.013), 54.8% showed an increase of ≥1/week. Healthy cohort - CSBM change not significant (P = 0.31)</td>
</tr>
<tr>
<td>Chang et al (2010);[20]</td>
<td>60 IBS-C; 16 healthy</td>
<td>1-week baseline, 4-week treatment (kiwifruit or placebo), 1-week washout</td>
<td>2 per day</td>
<td>2 caps containing 0.75 g glucose daily</td>
<td>Frequency of defecation, volume, consistency, color</td>
<td>Significant increase in frequency overtime for the IBS/C treatment group (P = 0.033). Significant increase in frequency in the treatment IBS/C group compared to the placebo group after 1 week (P &lt; 0.05), but not after 2 – 4 weeks</td>
</tr>
<tr>
<td>Gearry et al (2016)[3]</td>
<td>19 IBS-C; 19 constipated; 20 healthy</td>
<td>Crossover: 2-week lead-in; 4-week treatment (kiwifruit or psyllium), 4-week washout; 4-week treatment (kiwifruit or psyllium)</td>
<td>2 per day</td>
<td>None; psyllium used as reference intervention</td>
<td>Frequency of defecation, ease, stool form, GI symptom rating scale, IBS-QOL</td>
<td>Significant increase in stool frequency in IBS-C (P &lt;0.05) and constipated + IBS-C (P&lt;0.01, combined data) vs lead-in; significant improvements (P&lt;0.05 or better) in most secondary outcomes in each group</td>
</tr>
</tbody>
</table>

Overview and Uniqueness

Actazin™ is a food-quality ingredient derived entirely from New Zealand green kiwifruit (Actinidia deliciosa cv. ‘Hayward’), with pre-clinical and clinical data supporting its use as a digestive health ingredient. The process to create Actazin™ begins with premium green kiwifruit sourced exclusively from Zespri-approved growers and harvested from the pristine orchards of New Zealand – removing the skin and seeds - then cold-drying the bright-green nutritious flesh into unadulterated, free-flowing powder, for use in functional foods, nutraceuticals, and dietary supplements. The process to create Actazin™ is gentle and chemical / solvent-free. This results in a final product that is, essentially, lyophilized green kiwifruit powder, chemically unchanged from the pure, non-GMO, additive-free Actinidia deliciosa cv. ‘Hayward,’ from which it is derived. Actazin™ is Kosher- and Halal-certified, and is available as an organic ingredient.

Actazin™ contains a totally unique combination of bioactive nutrients, that effectively and gently support the digestive and laxation processes. These constituents include 1) Soluble and insoluble fiber (recognized universally as health-promoting ingredients), 2) Polyphenolic compounds, that can act either as direct antioxidants, or indirect antioxidants (via upregulation of the transcription factor Nrf2), 3) Prebiotic substrates including fiber, carbohydrates, and polyphenolics (as microbiome modulators: ie promoting growth of beneficial bacteria and /or inhibiting the growth of pathogenic bacteria), and 4) Kiwifruit-unique proteolytic enzyme actinidin, which enhances protein digestion.

Although a significant amount of research has been conducted into the effects of kiwifruit on digestion and constipation, the exact mode(s) / mechanism(s) of action of kiwifruit has not yet been identified. A large body of research on individual constituents and classes of compounds does exist, and this research supports the idea that the individual kiwifruit constituents contribute individually and in combination to the overall health effects, offering multi-tiered digestive (and other) health benefits.
Primary Bioactive Components

**ACTINIDIN**

Some varieties of kiwifruit such as green kiwifruit contain a highly active proteolytic enzyme, actinidin, whereas others, such as gold kiwifruit, have only trace levels of this enzyme [30;36]. Actinidin belongs to the family of cysteine proteases and contains a free sulfhydryl group essential for its enzymatic activity. Some of the other thiol/sulfhydryl proteases include fruit bromelain, chymopapain, and papain. The important features of actinidin include a wide pH range for catalytic activity along with stability at high concentrations at moderate temperatures. The enzyme is susceptible to oxidation, a feature in common with other plant thiol proteases [30;31].

It has long been known that raw kiwifruit prevents the solidification of gelatin jellies, and kiwifruit has been studied as a means of tenderizing meat [37]. Anecdotal claims that the consumption of kiwifruit also assists in gastric digestion have existed for many years. Using an *in vitro* gastric digestion model, the effect of an actinidin-containing extract of kiwifruit was tested on a wide range of food proteins [31]. Under these simulated digestion conditions, the actinidin-containing extract enhanced the digestion of several proteins, particularly some caseins (milk proteins) over and above that found with pepsin alone. Further, for some protein sources, the extract resulted in different types of peptides than those derived with pepsin alone [31].

In a simulated gastric model, actinidin was found to denature at pH 2 [38]. However, the presence of food dilutes HCl to raise pH. Using an *in vitro* digestive model, another study reported that increasing pH from 1.5 to 2.5 significantly reduced pepsin–mediated degradation of kiwifruit enzymes [39]. Thus, it is likely that not all actinidin is deactivated in the stomach. A summary of the most important actinidin studies is provided in Table 2.
Table 2. Summary of Actinidin Publications

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>SUMMARY</th>
</tr>
</thead>
</table>
| Montoya et al (2014)[33] | • In this study, the effect of dietary actinidin on stomach emptying and gastric digestion of 6 dietary protein sources was determined in growing rats.  
• Dietary protein sources were beef muscle (meat), gelatin, gluten, soy protein isolate, whey protein isolate, and zein.  
• Diets were fed with green kiwifruit (Hayward) or Gold (HORT 16A) – the latter has almost no actinidin.  
• Dietary actinidin increased gastric protein digestion and/or accelerated stomach emptying for several dietary protein sources, including beef muscle, gluten and soy protein.  
• Stomach emptying may be influenced by gastric protein digestion, and dietary actinidin can be used to modulate stomach emptying and protein digestion in the stomach of some dietary protein sources but not others. |
| Montoya et al (2013)[34] | • The study investigated the effect of dietary actinidin on the rate of digestion of beef muscle (meat) proteins in the stomach, and on the rate of stomach emptying in growing pigs.  
• The rate of stomach emptying was faster when actinidin was present in the diet: the half-time of emptying was 137 vs 172 min for the diets with and without actinidin, respectively.  
• The presence of dietary actinidin increased the digestion of beef muscle protein and, more specifically, those proteins with a high molecular weight.  
• In conclusion, dietary actinidin fed in the form of fresh green kiwifruit increased the rate of gastric emptying and the digestion of several beef muscle proteins. |
| Kaur and Boland (2013)[32] | • Green kiwifruit consumption has long been thought to assist in the digestion of food proteins due to the presence of actinidin.  
• This chapter reviews the recent findings of both laboratory and animal studies on the effect of green kiwifruit on the digestion of a range of common food proteins and protein-rich foods including milk, meat, fish, eggs, legumes and cereal proteins.  
• Clear evidence is provided that both green kiwifruit, and the enzyme actinidin itself, can provide enhanced upper-tract digestion (particularly in the stomach) of a variety of food proteins, which lends support to a role for dietary kiwifruit as a digestive aid.  
• The effect of kiwifruit on digestion varied according to the type of protein, with strong enhancement of digestion of casein (the major milk protein), yoghurt and cottage cheese protein, beef, chicken and fish muscle proteins (meat), and wheat gluten. Effects on zein (corn protein), soy protein, whey protein (the minor protein fraction from milk) and collagen (connective tissue protein) were minimal.  
• A study using pigs showed conclusively that actinidin is responsible for the enhanced gastric break down of food proteins. |
| Boland (2013)[30] | • Actinidin is the predominant enzyme in kiwifruit, and can play a role in aiding the digestive process.  
• Protein is a minor but significant component of kiwifruit. |
| Chalabi et al (2014)[40] | • The proteolytic activity of actinidin was compared to papain on several different fibrous and globular proteins under neutral, acidic and basic conditions.  
• Actinidin was found to have no or limited proteolytic effect on globular proteins such as immunoglobulins from sheep, rabbit, chicken and fish, or on bovine serum albumin, lipid transfer protein and whey proteins (α-lactalbumin and β-lactoglobulin) compared to papain.  
• In contrast to globular proteins, actinidin could hydrolyze collagen and fibrinogen perfectly at neutral and mild basic pH. Moreover, this enzyme could digest pure α-casein and major subunits of micellar casein especially in acidic pH.  
• These results are consistent with many others published, eg Kaur and Boland [32], suggesting that actinidin has little effect on intact globular proteins, such as unheated whey proteins, but is very effective against unstructured proteins, such as the caseins, and fibrous proteins such as collagen, and fibrinogen.  
• The effect of heating of globular proteins on their resistance to actinidin appears not to have been explored. |
Kiwifruit Polyphenols

Whole kiwifruit is rich in phenolic compounds [27,43]. There is some evidence that polyphenolics could affect regularity by influencing colonic micro flora. An in vitro study found that many polyphenols affect the ability of both pathogenic and beneficial gut bacteria to adhere to cultured epithelial cell walls. Many polyphenols were found to inhibit pathogenic bacteria at doses likely to be present in the gastrointestinal tract, but to differing degrees. Quercetin was one of the most active with the lowest minimum inhibitory concentrations for all the four bacteria tested [44].

Some have speculated that chlorogenic acid could possess mild laxative action. Although not fully understood, the mild laxative action of prunes is thought to be largely due to their high fiber and sorbitol content. Kiwifruit is low in the non-absorbable sugar alcohol, sorbitol. Prunes are also high in phenolic compounds, and early studies on the laxative action of prunes speculated that caffeic acid and chlorogenic acid were the active principles. In vitro experiments showed that chlorogenic acid altered the sodium electrochemical gradient in intestinal tissue, impairing the efficiency of glucose uptake. In vivo, this could cause more glucose to pass into the bowel and enhance microbial fermentation [45].

Although these experimental findings hint that some polyphenols could subtly influence regularity by affecting bacterial adhesion or enhancing bacterial fermentation, a potential role for polyphenols in promoting regularity awaits further investigation.

POLYPHENOLICS

General
Polyphenolic compounds are natural compounds commonly found in fruits, vegetables, and plant-based beverages including tea and coffee. They are secondary metabolites thought to be produced in defense against ultraviolet radiation and pathogens. The major chemical classes include phenolic acids, flavonoids, stilbenes, and lignans [28]. Initially attracting interest due to their in vitro antioxidant properties, the multiple health benefits of polyphenols are now well established from a multitude of epidemiological and intervention studies. Although the ability of polyphenolics to neutralize free radicals and other reactive molecules likely contributes to their health benefits, their ability to modulate gene expression, the endogenous antioxidant system, and cellular signaling pathways is also very important. Furthermore, it is now recognized that dietary polyphenols are extensively metabolized in vivo and that the chemical, biophysical and biological properties of their metabolites are, in most cases, quite different from the ones of the parent molecules [41]. The modulation of these pathways involves complex cell signaling either occurring at the cell surface or the phenol compounds reach the nucleus directly, and either way result in up and down regulation of gene expression [42].
**Actazin™ Polyphenols**

Polyphenolic compounds present in Actazin™ have been qualified and quantified by New Zealand Plant & Food Research (Palmerston North, New Zealand), and are presented in **Figure 2**. The predominant polyphenolic compounds identified belong to the hydroxycinnamic acid derived or related compounds. In addition, procyanidin B2, epicatechin and quercetin glycosides were identified, consistent with previous literature discussing phenolic compositions of kiwifruit and kiwifruit juice [27,43,46]. A summary of the recent research pertaining to the polyphenolic compounds present in Actazin™ is provided in **Table 3**.

**Figure 2. Representative polyphenolic profile of Actazin™**
### Table 3. Selected Research on Polyphenolic Compounds Identified in Actazin™

<table>
<thead>
<tr>
<th>POLYPHENOLIC CLASS</th>
<th>POLYPHENOLS IDENTIFIED IN ACTAZIN™</th>
<th>BRIEF SUMMARY</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxycinnamic Acids</td>
<td>E-caffeoyl-3-glucoside; E-caffeoyl-4-glucoside; Neochlorogenic acid</td>
<td>Review of the anticancer properties of hydroxycinnamic acids. HAs have antioxidant activity – they are reported to scavenge free radicals and proxidant metals. The potential anticancer mechanisms of HAs involve effects on cellular differentiation, proliferation and apoptosis; effects on proteins and enzymes at a molecular level; and effects on immune function and chemical metabolism.</td>
<td>Rocha et al (2012)[41]</td>
</tr>
<tr>
<td>Flavonoids (flavan-3-ols)</td>
<td>Procyanidin B2</td>
<td>The present results show that PB2 protects against oxidative injury in colonic cells and up-regulate the expression of GSTP1 via a mechanism that involves ERK and p38 MAPK activation and Nrf2 translocation. These results provide a molecular basis for the potential contribution of PB2 in the prevention of oxidative stress-related intestinal injury and gut pathologies.</td>
<td>Rodríguez-Ramiro et al (2012)[47]</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>Review of the role of epicatechin in cell signaling and potential for protection from cardiac and vascular diseases and cancer. Epicatechin can inhibit NF-κB at different levels in the activation pathways, including: • inhibition of NADPH-oxidase by directly binding to the enzyme, regulating calcium influx, or inhibiting ligand binding; • direct scavenging of free radicals; • interaction with the DNA-binding site of the NF-κB proteins and inhibiting gene transcription. • Like PB2 above, epicatechin can modulate the [ERK and Nrf2 pathways.</td>
<td>Fraga and Oteiza (2011)[48]</td>
<td></td>
</tr>
<tr>
<td>Quercetin glycosides</td>
<td>Quercetin rhamnoside</td>
<td>Anti-inflammatory properties of quercetin and isorhamnetin were accompanied by an increase in heme oxygenase 1 protein levels, a downstream target of the transcription factor Nrf2, known to antagonize chronic inflammation. Furthermore, proinflammatory microRNA-155 was down-regulated by quercetin and isorhamnetin but not by Q3G. Finally, anti-inflammatory properties of quercetin were confirmed \textit{in vivo} in mice fed quercetin-enriched diets (0.1 mg quercetin/g diet) over 6 weeks.</td>
<td>Boesch-Saadatmandi et al (2011)[49]</td>
</tr>
</tbody>
</table>
PREBIOTIC ACTIVITY

A number of studies have been conducted on fresh and freeze-dried green kiwifruit and its effect on modulating gut microbiota (a summary of research is provided in Table 4). The results of these studies support the microbiome-modulating effects of the components in kiwifruit, and are consistent with the results obtained using Actazin™.

Two independent in vitro models assessing the prebiotic activity of Actazin™ have been developed and performed at New Zealand Plant & Food Research. Specifically, the prebiotic and anti-pathogenic effects of Actazin™ on common single-strain bacterial growth (beneficial vs pathogenic) were assessed in a high-throughput, bacterial growth assay [50]. Additionally, the effects of Actazin™, alone and in combination with a commercial probiotic (Lactobacillus species), were evaluated on short-chain fatty acid (SCFA) production, following fermentation in a mixed human fecal / microbial culture model, simulating human distal colon conditions.

Single-Strain Bacterial Growth Assay

Two types of common probiotic bacteria (Bifidobacteria and Lactobacillus species) along with three species of potentially pathogenic bacteria (Escherichia coli, Staphylococcus aureus, Salmonella enterica) were incubated overnight with increasing amounts of Actazin™. Actazin™ was previously subjected to simulated gastric digestion [51], and then dissolved to a known concentration in either water or dimethyl sulfoxide (DMSO). Results showed that Actazin™ (both water and DMSO material), in a concentration-dependent manner, supported the growth of the two probiotic strains but did not support the growth of the pathogenic bacteria, compared to the control (no Actazin™ present; Figure 3).

Based on the known quantity of Actazin™ ingredient used as the starting material and the amount of Actazin™ yielded after the simulated digestion, it was possible to calculate the amount of Actazin™ required to support an approximate 90% increase (vs control) in probiotic bacteria, in this experimental model. The resulting calculation suggests that, under these experimental conditions, approximately 25 mg of Actazin™ per one billion (1 x 10⁹) colony forming units (CFUs) is required to achieve an approximate 90% increase in probiotic growth. While not necessarily enabling a generalization of the prebiotic, growth promoting activity of Actazin™ in combination with all probiotic strains, this model does provide direct evidence of the selective, prebiotic activity of Actazin™ with these two strains of Bifidobacterium and Lactobacillus.
Han et al (2011)[52] • Thirty-two pigs were fed the control diet or one of the three test diets containing either cellulose, freeze-dried green kiwifruit or kiwifruit fiber as the sole fiber source for 14-day study.
• Inclusion of freeze-dried kiwifruit and kiwifruit fiber into diets altered the bacterial community, indicating the presence of two distinct clusters.
• Quantification of different bacterial groups by qPCR demonstrated that pigs fed the freeze-dried kiwifruit or kiwifruit fiber diets had a significantly higher number (P < 0.05) of total bacteria and Bacteroides group and a lower number of Enterobacteria and Escherichia coli group, as well as a greater ratio of Lactobacillus to Enterobacteria when compared to pigs fed the control or cellulose diets.
• Conclusions: Green kiwifruit modulated the colonic microbiota, leading to an improved intestinal environment in growing pigs.

Parkar et al (2012)[53] • As compared to the control (inulin), green kiwifruit increased Bifidobacterium spp.
• This was accompanied with increases in microbial glycosidases, especially those with substrate specificities relating to the breakdown of kiwifruit oligosaccharides, and with increased generation of short chain fatty acids.
• The microbial metabolic activity was sustained for up to 48 h, which we attribute to the complexity of the carbohydrate substrate provided by whole kiwifruit.
• Kiwifruit fermenta supernatant was also separately shown to affect the in vitro proliferation of Bifidobacterium longum, and its adhesion to Caco-2 intestinal epithelial cells.
• Collectively, these data suggest that whole kiwifruit may modulate human gut microbial composition and metabolism to produce metabolites conducive to increased bifidobacteria-host association.

Parkar et al (2010)[54] • The role of kiwifruit pectin as a functional food ingredient capable of beneficial gut health effects in vitro was investigated.
• Six different pectins were obtained from the kiwifruit cultivar Actinidia deliciosa ‘Hayward’
• The potential gut health benefit of kiwifruit pectin was tested with respect to its influence on bacterial adhesion to intestinal epithelial cells (Caco-2) in vitro.
• The most effective kiwifruit pectin (monoK pectin), obtained by re-solubilization with monopotassium phosphate, was compared with three commercial functional polysaccharides - citrus pectin, guar gum and inulin.
• The monoK pectin was superior to inulin, a standard prebiotic in enhancing the adhesion of Lactobacillus rhamnosus and decreasing the adhesion of Salmonella typhimurium to Caco-2 cells. The adhesion of Bifidobacterium bifidum was significantly enhanced only by inulin and citrus pectin, while guar gum had no effect on adhesion of any of these bacteria.

Rosendale (2012)[55] • Microbial ecology and metabolism analysis for 3 individuals following 48 hours of in vitro fecal fermentation using green kiwifruit as the substrate.
• In the absence of host carbohydrate, kiwifruit carbohydrates were differentially utilized to drive microbial diversity, with similar by-product production.
• The starting ecology of each individual influenced quantitative and qualitative microbial changes; but not necessarily the metabolic by-product production.

Table 4. Summary of Selected Literature Evaluating Kiwifruit as a Prebiotic Substrate

<table>
<thead>
<tr>
<th>REFERENCE</th>
<th>SUMMARY</th>
</tr>
</thead>
</table>
| Han et al (2011)[52]       | • Thirty-two pigs were fed the control diet or one of the three test diets containing either cellulose, freeze-dried green kiwifruit or kiwifruit fiber as the sole fiber source for 14-day study.  
• Inclusion of freeze-dried kiwifruit and kiwifruit fiber into diets altered the bacterial community, indicating the presence of two distinct clusters.  
• Quantification of different bacterial groups by qPCR demonstrated that pigs fed the freeze-dried kiwifruit or kiwifruit fiber diets had a significantly higher number (P < 0.05) of total bacteria and Bacteroides group and a lower number of Enterobacteria and Escherichia coli group, as well as a greater ratio of Lactobacillus to Enterobacteria when compared to pigs fed the control or cellulose diets.  
• Conclusions: Green kiwifruit modulated the colonic microbiota, leading to an improved intestinal environment in growing pigs. |
| Parkar et al (2012)[53]    | • As compared to the control (inulin), green kiwifruit increased Bifidobacterium spp.  
• This was accompanied with increases in microbial glycosidases, especially those with substrate specificities relating to the breakdown of kiwifruit oligosaccharides, and with increased generation of short chain fatty acids.  
• The microbial metabolic activity was sustained for up to 48 h, which we attribute to the complexity of the carbohydrate substrate provided by whole kiwifruit.  
• Kiwifruit fermenta supernatant was also separately shown to affect the in vitro proliferation of Bifidobacterium longum, and its adhesion to Caco-2 intestinal epithelial cells.  
• Collectively, these data suggest that whole kiwifruit may modulate human gut microbial composition and metabolism to produce metabolites conducive to increased bifidobacteria-host association. |
| Parkar et al (2010)[54]    | • The role of kiwifruit pectin as a functional food ingredient capable of beneficial gut health effects in vitro was investigated.  
• Six different pectins were obtained from the kiwifruit cultivar Actinidia deliciosa ‘Hayward’  
• The potential gut health benefit of kiwifruit pectin was tested with respect to its influence on bacterial adhesion to intestinal epithelial cells (Caco-2) in vitro.  
• The most effective kiwifruit pectin (monoK pectin), obtained by re-solubilization with monopotassium phosphate, was compared with three commercial functional polysaccharides - citrus pectin, guar gum and inulin.  
• The monoK pectin was superior to inulin, a standard prebiotic in enhancing the adhesion of Lactobacillus rhamnosus and decreasing the adhesion of Salmonella typhimurium to Caco-2 cells. The adhesion of Bifidobacterium bifidum was significantly enhanced only by inulin and citrus pectin, while guar gum had no effect on adhesion of any of these bacteria. |
| Rosendale (2012)[55]       | • Microbial ecology and metabolism analysis for 3 individuals following 48 hours of in vitro fecal fermentation using green kiwifruit as the substrate.  
• In the absence of host carbohydrate, kiwifruit carbohydrates were differentially utilized to drive microbial diversity, with similar by-product production.  
• The starting ecology of each individual influenced quantitative and qualitative microbial changes; but not necessarily the metabolic by-product production. |
Figure 3. Actazin™ supports the growth of two common probiotics in a bacterial growth assay.

Actazin™ Supports Growth in Vitro of Commonly Used Single Strain Probiotics

- E.coli Nissle (Water Extract)
- Staphylococcus aureus (Water Extract)
- E.coli Nissle (DMSO Extract)
- Salmonella enterica (Water Extract)
- Staphylococcas aureus (DMSO Extract)
- Salmonella enterica (DMSO Extract)

Figure 3. Actazin™ supports the growth of two common probiotics in a bacterial growth assay
Mixed Human Fecal / Microbial Culture Model
Actazin™ was previously subjected to simulated gastric digestion [51], dissolved to a known concentration in water, and added to fermentation medium containing a sample from a healthy adult human donor (n=3 individual donors). The medium was allowed to ferment, under anaerobic microbial growth conditions, for up to 48 hours simulating in situ colonic fermentation. Additional incubation conditions included Actazin™ in combination with a commercial probiotic (Lactobacillus species), Lactobacillus species alone (no added Actazin™), and control (no added Actazin™).

RESULTS
Results showed that Actazin™ alone supported higher short-chain fatty acid (SCFA) levels over ~12-24 hours (vs control) (Figure 4). The Actazin™ plus Lactobacillus probiotic combination yielded an intermediate level of SCFA, while the Lactobacillus probiotic (alone) had the lowest level of SCFA (vs the other 3 incubation conditions).

Figure 4. Actazin™ supports the increase in short-chain fatty acids in a mixed human fecal / microbial culture model
Butyrate is an essential metabolite in the human colon, as it is the preferred energy source for the colon epithelial cells, contributes to the maintenance of the gut barrier functions, and has immunomodulatory and anti-inflammatory properties [56–58].

**RESULTS**

Results showed that Actazin™ alone and Actazin™ plus *Lactobacillus* probiotic combination supported a higher butyrate level over ~12–24 hours (vs control; purple symbols) (Figure 5). The *Lactobacillus* probiotic (alone) had the lowest level of butyrate (vs the other 3 incubation conditions).

*FIBER*

Actazin™ contains 14–20% dietary fiber. Kiwifruit fiber has unique hydration properties, as described above, which affects nutrient absorption and may add to fecal bulk. The fiber is also largely fermented, potentially leading to changes in the composition of the microflora and the production of short-chain fatty acids, both of which can impact gut motility as well as having other health benefits.

![Butyrate] Over Time

*Figure 5. Actazin™ supports the increase in butyrate in a mixed human fecal / microbial culture model*
Clinical Evidence to Support Actazin™

A randomized, double-blind, placebo-controlled, crossover study was conducted utilizing two doses of Actazin™ administered to healthy and functionally constipated individuals [59]. This study demonstrated that in healthy individuals (n=19), at a dose of 2,400 mg daily, Actazin™ administration (4 weeks) produced a statistically significant (P < 0.05) increase in the number of daily (weekly) bowel movements when compared to washout as baseline (P = 0.014; 10.2% increase). A lower dose of 600 mg was also investigated, and showed an equivalent numerical increase in bowel movements (P = 0.060; 7.4% increase). There was no significant change in the Bristol Stool Chart scores suggesting that while the number of stools increased, their form was not adversely affected (i.e., did not induce looser stool form). There was a higher self-reported score for flatulence at the dose of 2,400 mg daily in the healthy cohort; however, this was not accompanied by an increase in bloating and/or abdominal pain, so it could be considered indicative of active fermentation of Actazin™ components in the gut. A summary of the study along with the results are presented in Tables 5 and 6.

Post-hoc analysis was carried out on a sub-group of defined responders (n=14/19; 74% responded), defined as those individuals showing an increase of at least 1 bowel movement per week over the preceding washout period on at least one of the non-placebo interventions.

Table 5. Summary of Actazin™ Clinical Trial

<table>
<thead>
<tr>
<th>SUBJECTS</th>
<th>STUDY DESIGN</th>
<th>DOSE</th>
<th>PLACEBO</th>
<th>OUTCOME MEASURES</th>
<th>OBSERVED BENEFIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 Healthy (36.4 yr average, 23.3 BMI, 3/19 Male) 9 C3 Functionally constipated (44.2 yr, 24.8 BMI, 1/9 Male)</td>
<td>Randomized, double-blind, placebo-controlled crossover</td>
<td>600 mg &amp; 2,400 mg daily</td>
<td>Isomalt (4 x 600 mg caps)</td>
<td>Primary: significant increase in stool frequency; Secondary, Clinical: improvement in stool form, GR-IBS and IBS specific QOL scores; Secondary, Scientific: changes in faecal microbial populations, increase in short-chain fatty acids</td>
<td>2,400 mg dose of Actazin™ significantly increased stool frequency compared to baseline. 600 mg dose of Actazin™ near significance (P = 0.061) increased stool frequency compared to baseline Significant increase in stool frequency for sub group of responders.</td>
</tr>
</tbody>
</table>

Data from Ansell et al (2015)[59].
Table 6. Number of Bowel Movements and Percent Change from Washout in the Healthy Cohort and Responder Subgroup

<table>
<thead>
<tr>
<th>HEALTHY GROUP (N = 19)</th>
<th>DAILY (WEEKLY) BOWEL MOVEMENTS</th>
<th>WEEKLY INCREASE PER WEEK</th>
<th>% INCREASE OVER WASHOUT</th>
<th>P, ANOVA</th>
<th>P VS WASHOUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washout, Overall Mean</td>
<td>1.08 (7.56)</td>
<td>0</td>
<td>0</td>
<td>0.002</td>
<td>_</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.12 (7.84)</td>
<td>0.28</td>
<td>3.7</td>
<td>_</td>
<td>0.377</td>
</tr>
<tr>
<td>Actazin™ 600 mg</td>
<td>1.16 (8.12)</td>
<td>0.56</td>
<td>7.4</td>
<td>_</td>
<td>0.060</td>
</tr>
<tr>
<td>Actazin™ 2,400 mg</td>
<td>1.19 (8.33)</td>
<td>0.77</td>
<td>10.2</td>
<td>_</td>
<td>0.014</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RESPONDERS SUB-GROUP* (N = 14; 74%)</th>
<th>DAILY (WEEKLY) BOWEL MOVEMENTS</th>
<th>WEEKLY INCREASE PER WEEK</th>
<th>% INCREASE OVER WASHOUT</th>
<th>P, ANOVA</th>
<th>P VS WASHOUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washout, Overall Mean</td>
<td>1.18 (8.26)</td>
<td>0</td>
<td>0.0</td>
<td>&lt; 0.001</td>
<td>_</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.27 (8.89)</td>
<td>0.63</td>
<td>7.6</td>
<td>_</td>
<td>0.117</td>
</tr>
<tr>
<td>Actazin™ 600 mg</td>
<td>1.35 (9.45)</td>
<td>1.19</td>
<td>14.4</td>
<td>_</td>
<td>0.005</td>
</tr>
<tr>
<td>Actazin™ 2,400 mg</td>
<td>1.39 (9.73)</td>
<td>1.47</td>
<td>17.8</td>
<td>_</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

4. Participants were classified as responder (showed an increase by at least 1 bowel movement per week over the preceding washout period on at least one of the non-placebo interventions) or non-responder. Data from Ansell et al (2015) [59].
It is important to note that an increase of ≥ 1 bowel movement per week in a symptomatic population is considered a clinically meaningful magnitude of effect by the United States Food and Drug Administration [60], and would significantly improve the symptoms of sufferers of mild or occasional constipation. Actazin™ (2,400 mg/day) produced this magnitude of effect in the healthy cohort, while Actazin™ (600 and 2,400 mg/day) produced this magnitude of effect in a sub-group of responders in the healthy cohort. Due to poor recruitment of individuals into the functionally constipated cohort (primarily due to potential subjects refusing to abstain from eating kiwifruit, an absolute exclusion criterion), there was insufficient statistical power to perform an appropriate analysis.

No published data is available that identifies a minimally efficacious daily quantity for whole green kiwifruit for increasing bowel movement frequency. However, 2,400 mg of Actazin™ is approximately the equivalent of 25% of a whole green kiwifruit (by weight). Twenty-four hundred mg of Actazin™ produced a clinically meaningful increase in bowel movements in healthy adults, and 600 mg of Actazin™ produced an equivalent clinically meaningful increase (and just missed reaching statistical significance; P < 0.06). Thus, a daily dose of 2,400 mg of Actazin™ is functionally equivalent, in terms of laxation benefit, to 2 whole green kiwifruit, keeping in mind that 1 whole fresh kiwifruit contains a substantial amount of water.
**Safety of Kiwifruit and Actazin™**

The Natural Standard Monograph (2010) reported kiwifruit to be likely safe when consumed in amounts naturally found in foods. A number of clinical studies have been conducted on whole green kiwifruit (Table 1), and all have reported green kiwifruit (2 kiwifruit per day) to be safe and well tolerated. Consumption of Actazin™ at the two levels of intake (600 mg and 2,400 mg) for 28 days was well tolerated with no serious adverse events reported. Twenty-four hundred mg of Actazin™ is approximately the equivalent of 25% a whole kiwifruit, so this amount falls well within this definition, and is considered as safe.

**Who Would Benefit from Actazin™?**

The following individuals are likely to benefit from regular supplementation with Actazin™: those individuals prone to mild-moderate constipation, including the elderly, those on certain prescription medications, and those individuals who suffer from mild-moderate stress; healthy men and women who desire to maintain the regularity and quality of their bowel daily movement(s) and maintain a healthy intestinal microbiome. Children who are constipated might also benefit from Actazin™.

Thus, a daily dose of 2,400 mg of Actazin™ is functionally equivalent, in terms of laxation benefit, to 2 whole green kiwifruit, keeping in mind that 1 whole fresh kiwifruit contains a substantial amount of water.

---

**Recommended Use / Advantages of Actazin™**

<table>
<thead>
<tr>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>To promote bowel regularity</td>
</tr>
<tr>
<td>To gently facilitate bowel regularity</td>
</tr>
<tr>
<td>To gently increase the number of daily/weekly bowel movements</td>
</tr>
<tr>
<td>To provide a natural source of fiber, polyphenols, and proteolytic, protein-digestive enzyme</td>
</tr>
<tr>
<td>To support healthy gut microbiome modulation by providing prebiotic material to indigenous bacteria and in combination with commercial probiotic(s)</td>
</tr>
<tr>
<td>To facilitate protein digestion in the gut, via the proteolytic enzyme, actinidin</td>
</tr>
<tr>
<td>To offer a natural, clean-label ingredient</td>
</tr>
<tr>
<td>To offer an ingredient that simulates whole fruit</td>
</tr>
<tr>
<td>To offer an ingredient that is effective with no major side effects</td>
</tr>
<tr>
<td>To offer an ingredient that is safe for children</td>
</tr>
<tr>
<td>To offer an ingredient that possesses excellent organoleptic properties (ie appearance, taste, aroma, texture/feeling in the mouth)</td>
</tr>
</tbody>
</table>
 Formats and Applications

Actazin™ is a compatible ingredient for inclusion with the following:

- Digestive health formulations
- Detoxification formulations
- Formulation with probiotics
- Green food blends (capsules, tablets, powders etc.)
- Fruit and veggie formulations
- Whole food vitamin and mineral complexes
- Enzyme formulations
- Super-fruit products
- Fiber formulations

 Dosage Recommendation

 Digestive Discomfort

To facilitate laxation and increase stool frequency, Actazin™ can be taken up to 2,400 mg per day until discomfort passes.

 Digestive Health Maintenance

For ongoing digestive health maintenance, Actazin™ can be taken at 600 mg per day to provide nutrients on a consistent basis to help regularity, and support a healthy gut environment.

 Digestive Health Companion Formulation

Actazin™ in combination with one or more probiotic(s) to promote the selective growth of beneficial bacteria:

- A minimum of 25 mg Actazin™ per billion colony forming units of probiotic; or
- A minimum of 250 mg Actazin™ in formulation with >10 billion colony forming units of probiotic5

 Nutrient Composition (Energy, vitamins, minerals)

The nutrient composition of Actazin™ is provide in Table 7.

5. Actazin™ has not been evaluated at ratios lower than 25 mg per 1 billion colony forming units (CFUs)
### Table 7. Representative Macronutrient Content of Actazin™

**TYPICAL NUTRITION INFORMATION – SUBJECT TO NATURAL AND SEASONAL VARIATIONS**

**SERVING SIZE: 600 MG (1 TO 4 SERVINGS DAILY)**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Average Quantity per Serving</th>
<th>Average Quantity per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>8.6</td>
<td>1435</td>
</tr>
<tr>
<td>Protein, g</td>
<td>0.020</td>
<td>3.4</td>
</tr>
<tr>
<td>Fat, total, g</td>
<td>0.018</td>
<td>3.0</td>
</tr>
<tr>
<td>Saturated, g</td>
<td>0.0039</td>
<td>0.66</td>
</tr>
<tr>
<td>Unsaturated, g</td>
<td>0.014</td>
<td>2.4</td>
</tr>
<tr>
<td>Monounsaturated, g</td>
<td>0.0032</td>
<td>0.53</td>
</tr>
<tr>
<td>Polyunsaturated, g</td>
<td>0.011</td>
<td>1.8</td>
</tr>
<tr>
<td>Trans fat, g</td>
<td>Not detected</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>0.40</td>
<td>67</td>
</tr>
<tr>
<td>Sugars, total, g</td>
<td>0.28</td>
<td>46</td>
</tr>
<tr>
<td>Glucose, g</td>
<td>0.13</td>
<td>21</td>
</tr>
<tr>
<td>Fructose, g</td>
<td>0.15</td>
<td>25</td>
</tr>
<tr>
<td>Lactose, g</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Maltose, g</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sucrose, g</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>0.084</td>
<td>14</td>
</tr>
<tr>
<td>Insoluble fiber, g</td>
<td>0.068</td>
<td>11</td>
</tr>
<tr>
<td>Soluble fiber, g</td>
<td>0.014</td>
<td>2.3</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>0.080</td>
<td>13</td>
</tr>
<tr>
<td><strong>Total phenolics, mg gallic acid equivalents</strong></td>
<td><strong>4.8</strong></td>
<td><strong>800</strong></td>
</tr>
</tbody>
</table>

6. A dose of 3,400 mg of Actazin™ has been shown in a clinical trial to facilitate increased bowel movements in healthy individuals [59]. A 600 mg dose is recommended to maintain bowel regularity.

7. Based on calculation from average quantity per 100 g.
Compared to other options, Actazin™ is the superior, overall choice based on, arguably, the most important criteria to evaluate / compare individual ingredients. As a whole kiwifruit-based ingredient, it is novel compared to the existing, more mature gut and digestive health category options, with a greater diversity of collective relevant claims along with a clear message of differentiation (eg activity due to multiple active components vs a single mode of action exhibited by other ingredients).

Advantages vs Competitive Ingredients

When considered, in total, with the clinical results obtained with whole kiwifruit, the clinical substantiation of Actazin™ supports its use in a) healthy b) elderly, c) functionally constipated, and d) IBS-C individuals.
The versatility of Actazin™ is superior, and is supported by its compatibility with multiple delivery formats; this is critical for acceptance by product development professionals and consumers. A major strength of Actazin™ is its potential for clean-label and sustainability benefits. Finally, two of the most important criteria, effective dose and cost per dose clearly support consideration of Actazin™ over competitive ingredients. Effective doses for fibers are known to be very high (~38 g/day men; ~25 g/day women), while effective doses for probiotics for the clinical improvement of digestive health symptoms are not clearly defined, and will likely vary for individual probiotic strains, and whether use is for digestive health maintenance or relief of symptoms.

Table 8. Competitive Analyses of Actazin™ vs Other Ingredients in Market Space

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>ACTAZIN™</th>
<th>WHOLE FRUIT</th>
<th>PREBIOTICS</th>
<th>PROBIOTICS</th>
<th>FIBER</th>
<th>ENZYMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novelty</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ability to differentiate from competitors</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Diversity of marketing claims</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Scientific substantiation of claims</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Compatibility with multiple delivery formats</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Convenience of use by consumers</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Clean label potential</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Sustainability potential</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Effective dose (higher score = lower dose)</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cost per effective dose (higher score = lower cost)</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total Score</strong></td>
<td><strong>43</strong></td>
<td><strong>31</strong></td>
<td><strong>28</strong></td>
<td><strong>31</strong></td>
<td><strong>28</strong></td>
<td><strong>23</strong></td>
</tr>
</tbody>
</table>

All criteria scored on a scale from 1 (minimum, least) through 5 (maximum, most). Safety, regulatory, and competitive landscape were not listed as criteria for scoring, since these criteria are viewed as equivalent for all ingredients.


actazin™ natural kiwifruit powder

For more information, please contact Chris Johnson

Mobile: +64 21 724 008 | Email: chris.johnson@anagenix.com