2

REVIEW OF LITERATURE

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2.6. Prebiotics and its role in management of hyperlipidemia

2.1. Prebiotics concept : An Overview

a.Definition of prebiotics

Gibson and Roberfroid (1995) first defined prebiotics as "A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'.

Prebiotics foods are capable of selectively stimulating the growth of one or more desirable or health-enhancing gut bacteria hence Gibson *et al*., 2004 defined prebiotics as "a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health".

The Food and Agriculture Organisation (2007) in its technical meeting on prebiotics classified prebiotics as " a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota".

According to International Scientific Association of Probiotics and Prebiotics (2008), 'A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.

b.Classification of prebiotics

Dietary carbohydrates must also adhere to a list of criteria in order to be classified as prebiotics(Gibson *et al*., 2004). These criteria are

1) It should not undergo hydrolysis or absorption in the upper gastrointestinal tract;

2) When it reaches the colon, it should be selectively metabolized by a limited number of beneficial bacteria;

3) It should be able to alter the colonic microflora to a healthier bacterial flora;

4) It should be capable of inducing a physiological effect that is beneficial to health

While many nutritional compounds have some degree of prebiotic activity. Roberfroid (2007) classified prebiotics into two groups inulin-type fructans and galactooligosaccharides (GOS) based on the criteria of selective fermentation and specific changes in the gastrointestinal microflora.

Fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS), lactulose and polydextrose can be classified as the most established prebiotics. Emerging prebiotics include xylooligosaccahrides (XOS),isomaltooligosaccharides (IMO) and lactitol. Industrial applications of inulin from chicory root like fructooligosaccharides (FOS), arabinoxylooligosaccharides (AXOS) and xylooligosaccharides (XOS) are also recognized especially in food industries (Stowell, 2007; Sabater-Molina *et al.,* 2009; Femia *et al.,* 2010; Xu *et al.,* 2009). Whole grains rich in resistant starch-rich have shown prebiotic characteristics and is believed to exert beneficial health effects. Mannitol, maltodextrin, raffinose, lactulose, and sorbitol are also prebiotics with proven health properties (Yeo and Liong, 2010; Vamanu and Vamanu, 2010;

Mandal *et al.*, 2009). Resistant starch-rich whole grains are considered prebiotic in nature and assumed that their consumption leads to many health benefits. These are not absorbed in small intestine of healthy individuals but later are fermented by natural microflora of the colon to produce short-chain fatty acids (SCFA) (Vaidya and Sheth, 2010). The fermentability of dietary fibers viz. oat *b*-glucan, flaxseed gum, and fenugreek gum to SCFAs suggest their potential prebiotic application in promoting human health (Lin *et al.,* 2011).

The only prebiotics for which sufficient data have been generated to allow an evaluation of their possible classification as functional food ingredients are the Inulin- type fructans which include native inulin, enzymatically hydrolyzed Inulin or oligofructose and synthetic fructooligosaccharide.

c. Characteristics of ideal prebiotics

Prebiotics should be indigestible in the stomach and the small intestine by human digestive enzymes. They should stimulate growth of selected groups of bacteria beneficial for human health, especially bacteria, such as *bifidobacteria* and have an indirect regulatory effect on the microbial equilibrium in the alimentary tract. Their metabolism should have a beneficial effect, including the production of SCFAs and organic acids, reducing the pH of intestinal contents and should be safe for human health (Gibson & Roberfroid 1995; Roberfroid, 2001)

2.2.Inulin Type Fructans

Inulin is a reserve carbohydrate found in many plants and vegetables. Inulin is classified as a fructan consisting almost entirely of linearly beta-1,2-linked fructose units with a terminal alpha1-beta2 - linked glucose molecules. Inulin belongs to the so-called non-digestible oligosaccharides (NDOs).

Inulin is stored by plants typically in the roots as a form of energy. It is the second most occurring non-structural natural polysaccharides after starch. Inulin-type fructans are natural components of several edible fruits and vegetables, and the most common dietary sources are wheat, onion, banana, garlic,leeks and has been tested in high doses on animals with no toxic effects reported (Coussement, 1999).

a. Origin and History of use of inulin

Inulin is extracted from the chicory root *(Cichorium intybus*) by means of hot water and is called native chicory inulin or standard inulin (ST-inulin).

Inulin, a carbohydrate reserve in many plants was first extracted from the root of helenum by German scientist Rose in 1804 and was later called inulin in 1918 (Madrigal and Sangronis, 2007).

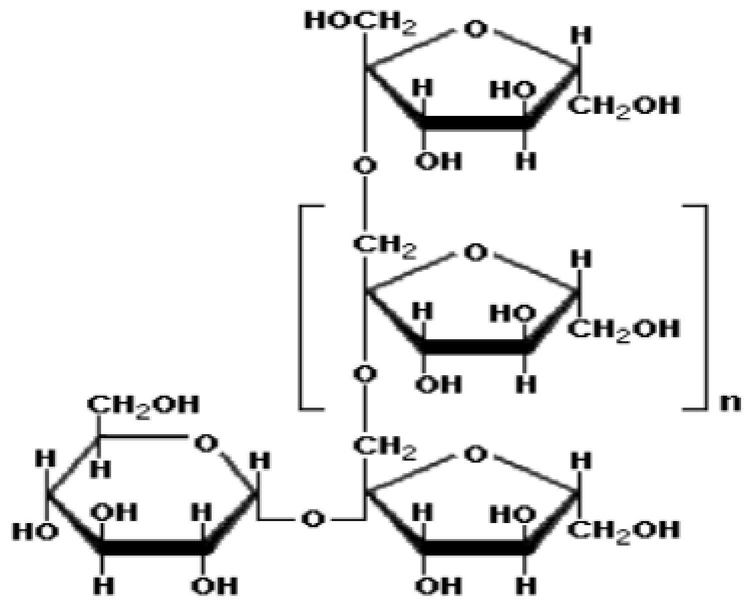
The first studies on the effects of inulin in healthy humans was during the early twentieth century (Lewis, 1912) and in 1935, Shannon and Smith studied the non-toxicology of inulin by injected intravenously with 160 g inulin. Over the past 10 years there has been a spectacular increase in the number of studies on the functional and nutritional benefits of inulin subsequently inulin changed from a subject of mere scientific interest into an industrial product with many applications.

b. Physiochemical property of Inulin-type Fructans

Chemically, inulin is a polydisperse β(2-1) fructan composed of a mixture of oligo and polysaccharides (De Leenher & Hoebregs, 1994). They are mostly formed of linear chains of fructose structured as GFn, where G= glucosyl unit, F= fructosyl unit, and n= the number of fructosyl units linked to each other (Figure 1).

This specific type of glycosidic bond gives inulin its unique structural and physiological properties. Because of the beta configuration of the bonds between fructose monomers, inulin-type fructans resist enzymatic hydrolysis by human salivary and small intestinal digestive enzymes – specific for alpha-glycosidic bonds. As a result, inulin-type fructans are indigestible and are fermented in the colon.Roberfroid, 2007)

Fructans can also be described by degree of polymerization (DP). DP refers to the number of repeat units in an oligomer or polymer chain, so DP of an individual fructan would be its number of repeating fructose units and identical to n. The degree of polymerization of the chains varies between 2 and 60 units with an average of approximately 12 in native inulin.



Chemical structure of inulin (n= number of fructosyl units)
(Zamora, 2005)

Figure 1

Inulin occurring in different plant species have varying chain lengths: wheat, onions and bananas have short-chain inulins (maximal degree of polymerization DPmax<10); dahlia tubers, garlic and Jerusalem artichoke have medium-chain inulins (DPmax<40); and globe artichoke and chicory typically contain long-chain inulin molecules (DPmax<100) (Van Loo *et al.,* 1995).

Short-chain inulin or oligofructose can be obtained by partial enzymatic hydrolysis of the native inulin, obtaining a polymerization degree between 2 and 7. Applying physical methods, long-chain inulin with an average polymerization degree varying between 22 and 25 can be obtained (Franck, 2002).

Inulin of high performance known as (HP) type of inulin has an average DP of 25 with a molecular distribution ranging from 11 to 60. The inulin molecules in chicory roots have a degree of polymerization (DP) of 3 to over 60 units and are indicated with the formula GF_n, whereas hydrolysis products are indicated with the formula F_m, with $n \geq 2$ and $m \leq 7$. Inulin molecules with a DP of 3 to 10 are called oligofructose or fructo-oligosaccharides (FOS).

c. Industrial production

The primary industrial source of pure inulin is the chicory root (*Cichorium intybus L.).* Chicory root is a biennial plant requiring soil and climatic conditions resembling those for sugar beets: deep, fertile, permeable and cool soil with pH tending towards neutral; intolerance of droughts or poor drainage.

Inulin was produced on a pilot scale in Deutsche Kulorfabrik in the early 1920s and later was extracted on an industrial scale. In 1927, it was reported that several German sugar factories extracted inulin from chicory, similar to sugar extraction from sugar beets. Like sugar from sugar beets, the extract was high in impurities that were removed by lime-carbon dioxide purification. Chilling precipitation further purified the inulin. However, the production process was deemed uneconomical due to the recovery process using precipitation by chilling (Gibson *et al.,* 1994).

During the early 1990s, several attempts were made to isolate and purify inulin and oligofructose for use as dietary supplements. Nowadays, they are used in a pure form as ingredients in many food products. Their inulin content is high (more than 70% on dry substance). The production process, which is represented in Fig. 2 involves the extraction of the naturally occurring inulin from chicory roots. Manufacturing of inulin involves typically harvesting, slicing and washing. The steps of production includes diffusion in hot water, followed by refining using technologies from the sugar and starch industries (e.g. ion exchangers), and then evaporation and spray-drying.

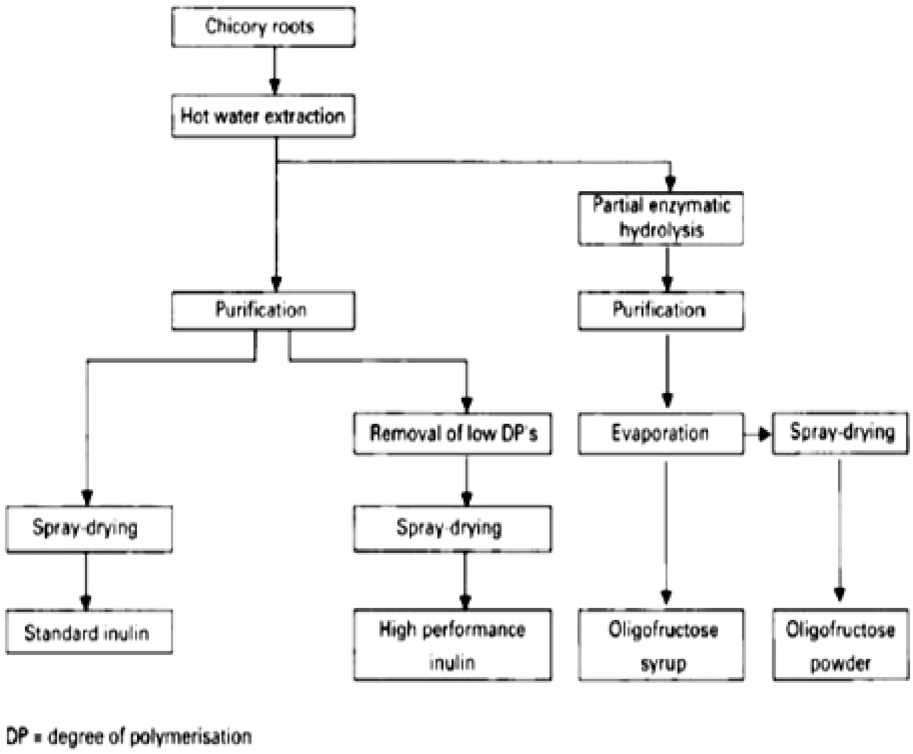


Figure 2. Steps involved in industrial production process of inulin

d. Consumption of inulin

The daily per capita intake of inulin and fructooligosaccharides in the USA varies between 1 and 4 g and in Europe between 2 and 12 g (van Loo *et al*, 1995). Most of the inulin intake from our diet (>90%) originates from wheat and onions.

An average North American diet ingests 1-4 g inulin-type prebiotic compounds daily, a low daily intake compared to a less processed diet, higher in plant foods.

In southern European countries, the consumption of inulin has been reported between 5 and 18g, due to consumption of relatively high amounts of garlic.

However studies suggests that these levels are probably too low to elevate *bifidobacteria* significantly (about one $\log^{10}$ value) in the human gut.

Fructooligosaccharides have been found to be effective in humans at doses of 4 g/day, although the initial counts of *bifidobacteria* and not just the dose of fructooligosaccharides determine the relative increase in *bifidobacteria* (Rao, 1999).

Hence, much interest exists in the approach of fortification of common foodstuffs with prebiotics (Manning & Gibson, 2004).

Inulin and oligofructose content of various foods is given in the table below

TABLE I

CONTENTS OF INULIN AND OLIGOFRUCTOSE (G/100 G)

Food item	Inulin	Oligofructose
Banana (fresh)	0.5	0.5
Garlic (fresh)	12.5	5.0
Onions (fresh)	4.3	4.3
Barley(fresh)	0.8	0.8
Asparagus (fresh)	2.5	2.5
Jerusalem artichoke (fresh)	18.0	13.5
Wheat bran (fresh)	2.5	2.5
Rye (baked)	0.7	0.7
Chicory root (fresh)	17.5	9.6
Leeks (fresh)	6.5	5.2
Wheat flour (baked)	2.4	2.4

Boeckner *et al.,* 2001 and Sensus

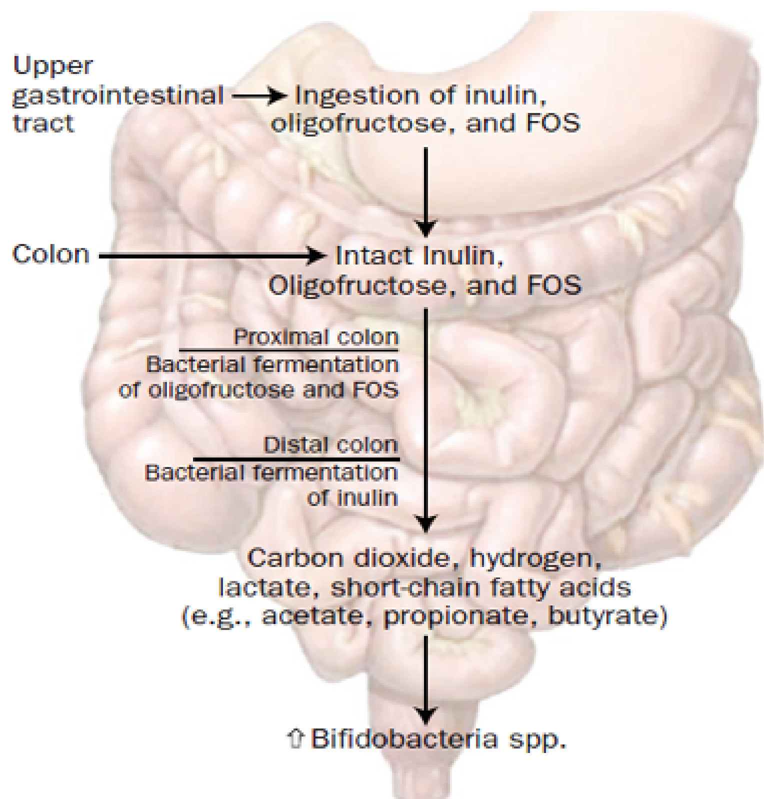
e. Metabolic Fate of Inulin-type Prebiotics

Inulin, oligofructose and FOS resist enzymatic digestion in the upper gastrointestinal tract, reaching the colon virtually intact where they undergo bacterial fermentation.

Although inulin-type prebiotics induce growth of *bifidobacteria*, they do not exert their effects in the same portion of the large bowel as degree of polymerisation influences wherein the colon fermentation occurs.

Inulin, oligofructose, and FOS are fully metabolized by the colonic microflora. The end products of fermentation are gases (such as carbon dioxide and hydrogen), lactate, and short-chain fatty acids (including acetate, propionate, and butyrate). Increased hydrogen concentrations can be observed by breath hydrogen testing.

Colonic bacterial fermentation of inulin-type prebiotics, and the by-products produced, acidify the colonic content, increase bacterial biomass (and consequently fecal mass), and modify the composition of the microflora. The primary stimulating effect of prebiotics on gut ecology is stimulation of *bifidobacteria* species growth.



Metabolic Fate of Inulin-type Prebiotics

Figure 3

The DP appears to exert some influence on the metabolic response to specific inulin-type prebiotic compounds – including potential side effects. For example, fermentation of FOS appears to occur primarily in the proximal colon (Rumessen,1990)whereas a higher proportion of the fructans in inulin appear to survive transit through the proximal colon. As a result, inulin might potentially have more positive effects on distal colonic fermentation and bacterial populations than a shorter-chain fructan-type prebiotic like FOS or oligofructose, which would be more metabolically active in the proximal colon (Figure 3).

Possibly as a result of the relative resistance to enzymatic degradation in the proximal colon, the transit time of fructans with greater DP is reportedly longer than that of short-chain fructans. Rumessen and Gudmand-Hoyer used a single-blind, crossover, randomized trial with 10 healthy adults to test the difference in intestinal transit times between oligofructose and inulin. Orocecal transit times were calculated using changes in breath hydrogen profiles. The estimated average transit time for inulin (51% of fructans with DP >12) was 75 minutes compared to 30 minutes for oligofructose (100% of fructans with DP <10). The researchers also concluded that shorter chain length (smaller DP) results in increased abdominal side effects.Regarding inulin HP, theoretically more of the fructans reach the distal colon compared to inulin. Because the biological activity of inulin-type prebiotics appears to depend partially on the molecular size, it is particularly important to consider the molecular size distribution when reviewing clinical research.

f. Safety and tolerance of inulin

In general, there are no safety concerns with the ingestion of non-digestible oligosaccharides. However, excessive intake can cause undesirable side effects such as flatulence, bloating, cramps, and liquid stools caused by gas formation and osmotic effects of short-chain fatty acids (SCFA's) and lactate formed in the colon.

Distribution of the intake over the day and ingestion with solid foods (meals), as well as adaptation of a desirable intestinal flora, may decrease such side effects although the response varies with individual (Marteau and Florie, 2001).

Side effects will be less with long-chain inulin molecules since these are fermented at a rate which is about 50% lower than that of short chain inulin molecules (Roberfroid *et al.,* 1998; Coussement 1999). Havenaar (2000) reviewed studies in humans and concluded that in adults up to 20 g/day of inulin with an average DP of 9 does not cause serious adverse side effects except mild to moderate discomfort such as flatulence in some individuals.

In the large majority of studies on the nutritional significance of inulin, intake levels were below 20 g/day. In 2 recent randomized controlled trials (RCTs; Bonnema and others 2010; Holscher *et al.,* 2014), the tolerance of moderate doses (5 to 10 g/d) of chicory oligofructose, chicory inulin, and agave inulin was confirmed.

Only mild gastrointestinal side effects (flatulence and bloating) were observed and these effects were less with native inulin than with oligofructose. If not given in excess (over 80 g/d; Clausen *et al.,*1998), inulin is completely fermented in the colon, resulting in the production of SCFA's (mainly acetic acid, propionic acid, butyric acid) and lactate.

2.3. Technological property of inulin

a. Physical properties of inulin

Niness (1999) and Franck (2002) studied the physical properties of inulin. Inulin is moderately soluble in water (10% at room temperature) producing a low-viscosity solution. At higher concentrations, a tridimensional microcrystalline gel network with a creamy structure and a fat-like mouthfeel can be formed. The sweetness of inulin is about 10% of that of sucrose. By removing the smaller inulin molecules, the sweetness is eliminated and the gel-forming capabilities are enhanced. Inulin finds extensive applications as a low-calorie fat replacer in spreads, dressings, dairy products, bakery foods and ice cream.

Inulin and oligofructose are stable to heat at pH >5. At pH values of <4, hydrolysis occurs depending on temperature and heating time (Glibowski and Bukowska, 2011).

b. Stability of inulin

The effect of environment reaction on inulin chemical stability has been analysed in a few studies (Florkowska *et al*., 2004, Kim *et al.,* 2001, Glibowski and Wasko 2008]. These studies shows the effect of pH on rheological properties of the gels formed by inulin and it was found that in the acidic environment gelation properties of inulin were deteriorated. Another study on the effect of time, temperature and pH on inulin chemical stability showed that Inulin chemical stability decreases in an acidic environment at pH≤ 4 due to the heating time and increase in temperature. In a neutral and alkaline environment inulin is chemically stable independent of pH, heating time and temperature. Inulin can have limited applications in acidicfoods (pH ≤ 4), especially heated at temperatures above 60°C. Inulin degradation does not take place in the products with pH ≥ 5 heated up to 100°C. (Glibowski and Bukowska,2011)

2.4. Applications in food products

Inulin-type prebiotics are increasingly being used for food applications. In these applications they are considered to be a functional food ingredient, added to make health claims and/or persuade the consumer the product is a healthier choice than one that does not contain inulin-type prebiotics.

Inulin is a widely used as prebiotic, sugar replacer, fat replacer and texture modifier. It is a significant ingredient used in food industry by virtue of its diversified nutritional and functional properties. Inulin, cellulose, starch, pectin, carrageenan and xanthan gum are of great attention because of their nutritional and technological properties. It has been applied in a variety of foods, either, individually, or combined with other FOSs or probiotics to develop symbiotic foods.

The inulin concentration enhances product texture; at high concentration inulin can alter the texture profile of products because of its physico-chemical significance. Inulin may also significantly affect the sensory attributes of many products. Due to its unique technological and nutritional properties inulin finds its extensive applications in food industry (Yasmeen *etal.,* 2015).

a. Fat Replacer

Inulin has a significant property to replace fat. When mixed with water or aqueous liquid it produces a particle gel network having a tiny spreadable texturer. It can be incorporated in foods for 100% fat replacement (Franck, 1993). This gel like structure is comprised of a three dimensional complex of impenetrable submicron crystalline particles of inulin in water. Longer chain lengths reduce the solubility of inulin-type fructans and result in the formation of what Niness describes as “inulin microcrystals” when mixed with water or milk, which are not discretely perceptible and have a smooth, creamy mouth feel (Roberfroid, 2007).

According to Niness (1999) standard inulin his less fat mimetic characteristics compared to high performance inulin. When inulin-type prebiotics are used to produce low-fat spreads, inulin HP is the preferred choice because of its superior fat mimetic properties and lack of sweetness.

For proper replacement of fat, not only subsequent texture and structure should be optimum but also have to understand the flavor release (de Wijk *et al.,* 2006). Inulin can perform as an excellent fat replacer in various dairy products.

b. Sugar Replacer

Inulin also exhibit as sugar replacing property. It is also combine with high sweeteners replace sugar giving a balanced sweet taste which masks the aftertaste of other sweeteners (Wiedmann and Jager, 1997).

In contrast to longer-chain, higher-molecular weight inulin-type fructans that are less soluble and fat mimetic, shorter-chain, lower-molecular weight oligofructose and FOS are preferred as sugar replacers. These shorter-chain oligomers are more soluble than sucrose, possess functional qualities similar to sugar or glucose syrup, and can have nearly 30-50 percent the sweetness of table sugar.

If the beta bond is hydrolysed between the fructose units, the oligofructose shows excellent stability during food processes even in very acidic conditions. A fructose is formed in this process that is very pronounced in low pH, less dry matter conditions and high temperature.

The oligofructose also adds in mouthfeel improvement and lowers the water activity that ensures high microbial strength. It shows the properties of humectants and also affects the freezing and boiling points.

c. Stabiliser

Inulin is used as a stabilizer in different other food items. Most of the gelling agents such as alginate, gelatine and k-carrageenans, maltodextrins and gellan gum work in synergy with inulin. It also improves the stability of emulsions and foams like ice creams, aerated desserts, sauces and table spreads. Therefore, inulin can substitute other stabilizers.

d. Texture modifier

When inulin is incorporated in low concentrations to the food, the product sensory qualities and rheological properties will not be affected due to its slight sweet or neutral taste and minimal effect on viscosity (Kalyani Nair*et al*., 2010). Because of its unique properties, inulin influence significant affects on the texture of dairy product.

Inulin influence texture modification and also improves the sensory attributes of dairy products as stated by Tungland and Meyer (2002).

Native inulin product is more viscous and more soluble than a long-chain inulin, and also functions as texture improvers in dairy products (Wada *et al.,* 2005).

DP also effect the other physico-chemical properties that includes the melting temperature (Blecker *et al.,* 2003), transition temperature of glass capability of gel formation, successive gel vigor (Meyer and Blaauwhoed, 2009), relations with different food ingredients like hydrocolloids. Such characteristics are obviously linked with technological functions of inulin especially its use as texture improver.

The fat replacement property of inulin is not only linked with the rheological modification (hardness or thickness) but also related to modifications sensory attributes like mouthfeel, smoothness and creaminess. Generally, higher concentration of inulin is needed to mimic the smoothness or creaminess in low-fat dairy products having the thickness and rheology close to those of the full fat dairy food items. This property makes it more feasible to develop and design good sensory attributes of inulin enriched low fat products. Thus, inulin application as texture improver in different dairy products is the most important area of applications.

e. Inulin in various food products

Inulin is being used in various food products to develop new functional products. Some of the food products is listed below (Gonzalez *et al.,* 2015)

Chocolate

Golob et al. (2004) used inulin to replace sucrose in formulation of chocolates. With the same purpose of replacing sucrose, different concentrations of inulin and polydextrose was used in dark chocolate at temperatures of 65°C for ten minute and 50°C for 15 minute (Aidoo *et al.,* 2013). Analysis of the rheological properties, microstructure and physical properties showed that the optimum mixture had formulation of 75.3 percent polydextrose and 24.6 percent inulin revealing that at higher concentrations of inulin, the formation of larger crystals affected the quality of the chocolate.

Kolida et al. (2007) evaluated the prebiotic effect of the daily consumption of inulin at 5 and 8 g/day on healthy subjects by supplementing with a chocolate drink for two weeks and significant improvement in concentration of bifidogenic bacteria was observed in both the doses.

Most of the reported studies of chocolate supplemented with inulin were mainly aimed at improving the foods physicochemical or sensory properties apart from its prebiotic properties.

Milk and milk beverage

Inulin is been widely used in dairy sector because of its prebiotic properties as well as the texture it imparts which is similar to fat creaminess (Meyer *et al.,* 2011).

According to a study by Yap *et al.,* 2008, administration of inulin added infant milk formula for 14 days showed positive results with reduction of *Clostridium* bacteria and an increase in the number of *bifidobacteria* after the analysis of feces.

Villegas *et al.*, (2010) compared the effects of two types of inulin (short and long chain) adding them via concentrations of between 3-8 and 0-8 percent sucrose to low-fat vanilla flavor milk beverages. On evaluation of the sensory attributes, acceptability for short-chain inulin were highest between 5 and 8 percent, and 5 percent for long-chain inulin, both with a sucrose concentration between 4 and 6.5 percent.

In the case of milk and milk beverages, the addition of inulin is mainly focused onconferring prebiotic potential and on evaluating sensory properties through both in vivo and microbiological assays.

Yoghurt

Inulin is used to enhance the probiotic activity of many food products such as yogurt. In a study reported by Mazloomi *et al*., 2011 on incorporation of inulin in a probiotic low-fat yoghurt at concentrations of 1 and 2 percent resulted in increase in viability of the bacteria during storage without any effect on the sensory characteristics and physicochemical of the yogurt

In most cases, the study of yogurt fortified with inulin is directed at the effect on the product's rheological and textural properties and, when the product is symbiotic that is primarily on the influence it has on the survival of probiotics. The physical, microbiological and sensory tests carried out in many supporting studies indicated a good rate of survival for probiotics after the addition of inulin.

Ice-cream

Low fat ice-cream and a probiotic ice-cream with inulin showed more favorable texture characteristics, in terms of firmness and melting, while the survival rate of the bacteria was better with oligofructose added ice cream(Akalin and Erisir,2008).

In another study by Pandiyan *et al.,* 2012 on addition of 3 percent inulin in the preparation of synbiotic ice cream showed that the survival of *L. acidophilus* increased with the application of inulin and the sensory properties were not affected even after 7 and 15 days storage at both 18 to 23°C.

El-Nagar *et al.* (2002), found positive relationships between sensory properties, changes in texture and the rheological characteristics obtained by adding inulin at 5, 7 and

9 percent to a frozen yogurt, concluding that blend properties improved as the inulin percentage increased.

Hence, from these studies it can be observed that the addition of inulin ice-cream generally improves texture conditions by increasing firmness and reducing melting time, increases the survival of probiotics during the development of synbiotic ice-cream and substitutes fat without altering the product's sensory attributes.

Cheese

Cottage cheese has also been supplemented with prebiotics and probiotics in order to obtain a synbiotic product.

Araújo *et al.,* (2010) developed a cottage cheese by mixing 8 percent inulin with a probiotic strain *Lactobacillus delbrueckii* which resulted in a synbiotic product of acceptable sensory characteristics.

According to Buriti *et al.,* (2007), inulin content of 2.5/portion 50g is sufficient to consider it as a prebiotic taking into account the probiotic's gastrointestinal resistance, the products can be considered a food with functional potential.

The application of inulin in cheese is primarily to get a synbiotic effect on the product. The final product is evaluated for inulin content, resistance in the gastrointestinal tract and physicochemical and sensory properties. However, it is necessary to complement this evidence with in vivo assays in order to evaluate indicators confirming consumer benefits.

Hennelly *et al*., (2006) developed a product with inulin incorporated into the imitation cheese matrix at a level of 3.44 g/100 g cheese and it was able to directly replace 63 percent of the total fat in the formulation without any significant effect on the melting characteristics. However at the higher level of inulin incorporation cheese hardness may increase at temperatures above 55 ^{o}C.

Bread and biscuits

Inulin is used as a fat and sugar replacer in bread and biscuits development.

Fat mimetic property of inulin was studied by Rößle *et al.* (2011) where the effect of inulin and oligofructose as fat replacers on quality attributes of quick breads (scones) were evaluated and the results indicates that a product similar to the control was obtained while achieving a reduction in fat and sugar with a mixture of margarine (3.53 percent), oligofructose (10 percent) powdered sugar (0.55 percent) and inulin (5.92 percent).

In a study by Zahn *et al.,* 2010, inulin at concentrations of 50, 75 and 100 percent were used as a substitute for fat in the preparation of muffins and it was found that the product's sensory properties was negatively affected to a significant degree by higher inulin concentrations. The effect on the texture and sensory properties of the product showed that with increase in the percentage of inulin, the moisture and crumb density also increased and it was concluded that a concentration of up to 50 percent inulin can obtain a product similar to the control.

Hempel *et al.* (2007) used inulin syrup made from Jerusalem artichoke tubers in its commercial form in wafer cracker. The syrup was ultrafiltrated in order to modify the content of free sugar and minerals and both were lyophilized. Wafers with different types of flour such as wheat, spelled wheat, rice and mixtures were used and the results showed that ultrafiltration syrup prevented the formation of compounds that affect the color of the wafers. This study helped to understand that in order to maintain the sensory quality and physical characteristics of the baked products, it is necessary to maintain a balance between the inulin addition and the flour replacement.

Inulin is used as a fat substitute in most bakery products and biscuits. Almost all cases report that hardness increases with the percentage increases of inulin. However no trials of theseproduct's prebiotic potential were reported.

Pasta

The effect of the addition of inulin to a gluten-free cornmeal paste over on the sensory characteristics of dry spaghetti and the rheological properties of pasta dough was evaluated in Mastromatteo *et al*. (2012) study. Inulin incorporated at 5 and 7.5 percent samples demonstrated high strength and high values of elongation and shear viscosity. The sensory quality of the dry spaghetti was generally good however the quality of the cooked spaghetti decreased inversely with the concentration of inulin.

Pasta enriched with 11 percent inulin administered to healthy volunteers over a period of between two and five weeks reported significantly positive effects both on the peptides of the intestine and on the factors involved in the modulation of the gastric emptying rate (Russo *et al.,* 2011)

Inulin is sometimes applied not only for enhance texture, but also to provide a potential prebiotic for the pasta and there are trials that provide evidence.

f. Inulin fortified food products

The practice of adding inulin to other foods is expanding, with new application emerging every day. Other examples of fortified products with inulin are as follows.

Milk dessert

Formulations of Inulin mixtures of 3-9 g/100 g in combination with different degrees of polymerization with sucrose and lemon flavoring was developed in a study by Arcia *et al.,* 2011 to obtain a low-fat milk dessert with prebiotic properties. The findings showed no significant differences in the acceptability between the regular product and that supplemented with 5.5 g/100 g of inulin.

Amaranth bar

Dias and Gomes (2010) developed an amaranth product in the form of bars at University of Sao Paulo in Brazil to which a mixture of inulin and oligofructose (17 g/100 g) was added. On evaluation it showed good results and was found to be acceptable both in sensory attributes and the calorific value. The product had the added advantage of being low calorie with high fibre content.

Meat balls

Inulin gel was used to replace fat in meat balls. Meat balls with inulin added as a fat substitute differed in their basic chemical composition in comparison to that of the control. The replacement of 25% and 50% pork back fat by inulin gel resulted in a reduction of fat content by approx. 21% and 43%, and energy value by 13% and 34%, respectively. Overall accepatability of 10% added inulin gel were slightly higher in terms of juiciness, aroma and taste compared to the other variations. The introduction of inulin to meatballs inhibited the adverse effect of storage time on their sensory attributes(Flaczyk *et al*, 2009).

Burger

Inullin has been used in meat products as an functional additive. Cegielka and Tambor (2012) added inulin in four formulations at concentrations of 1, 2 and

3 percent in chicken burgers and analyzed their physico-chemical and sensory properties. The results showed that the thermal processing yield and the shear force was not affected by the addition of inulin and all formulations with 1 percent inulin concentration was found to be highly acceptable.

Gluten free cookie

Maghaydah *et al* (2013) developed inulin enriched gluten-free cookies. Inulin was added at four levels (3.0%, 3.5%, 4.0% and 4.5%) to the gluten-free cookie control formula (corn flour, corn starch, rice flour and lupine flour) Findings showed that the total dietary fibre content increased with the inulin level but the spread factor decreased with increasing inulin level. The sensory attributes were not compromised with the addition of dietary fibre (inulin) and incorporation of 4.0% inulin had highest acceptance.

Commercial uses of prebiotics

A commercial example of a prebiotic is Actilight, produced by the French company Vivis from beets, and added to products such as cookies and soups. The Japanese company, Beghin Meiji Industries, produces milk enriched with a soluble fraction of dietary fiber, and Bauer (Germany) produces the fermented product probiotic plus oligofructose, which contains two probiotic bacterial strains and the prebiotic Raftilose.

The best-known prebiotic preparation produced using inulin from chicory is Raftilose. Another popular prebiotic, containing oligofructose, is a line of Raftiline preparations. Both above-mentioned preparations have been deemed as fit for human consumption as additives to dairy products.

Other known commercial preparations containing FOS include NutraFlora, Actilight, Neosugar and Meioligo. However, it needs to be remembered that the daily consumption of prebiotics should be limited to several grams. Excessive consumption of these preparations has a laxative effect and causes strong bloating.

2.5. Nutritional applications of prebiotics

a. Inulin as a dietary fibre

Inulin is a storage carbohydrate in plants, having fructose moieties joined by -(2-1)-d-frutosyl linkages and is resistant to digestion in the human small intestine due to the - configuration of anomeric C-2 but it can be fermented in large intestine (Apolinario *et al.,* 2014). Almost 90% of the inulin passes to the colon and digested by bacteria present there (Cherbut, 2002). It is thus a significant component of the dietary fiber complex and is labeled as dietary fiber on food items.

FDA in 2002 have classified inulin as dietary fibre. Inulin is a carbohydrate which is resistant to digestion by digestive enzymes and absorption in the small intestine. They improve the bowel movements by increasing the faecal water content and biomass and due to such property which are characteristic of dietary fibre, inulin was regarded as a fibre by Roberfroid (1993).

Inulin has features that are unusual from other fibres due to their peculiar fermentation significances. Therefore, inulin as a dietary fibre can be used as a considerable approach to achieve balanced diet.

b. Calorific value of inulin

The low calorific value (1.5 kcal/g or 6.3 kJ/g) of inulin is due to its non-digestibility in contrast to its constituent monosaccharide moieties. On the evidence of In-vitro and In-vivo results, the energy rate of inulin and oligofructose was reported to be 1.5 kcal/g (Roberfroid, 1999). Further scientific observations have also proved less energy account of inulin, where, the energy value ranging from 1 and 1.5 kcal/g is being used for food tagging (Flamm *et al.,* 2001).

2.6. Health benefits of prebiotic

The colonic microflora has a profound influence on health (Steer *et al.,* 2000). Prebiotics constitute a well-known class of functional foods. The use of prebiotic as functional food ingredients to manipulate the composition of colonic microflora in order to improve health is of currently a great concern (Aryana andMcGrew, 2007; Coppa, Zampini, Galeazzi, & Gabrielli, 2006; Losada & Olleros, 2002; Manning & Gibson, 2004).

Prebiotic exhibit an array of health benefits which include lowering of lipid fractions, stimulate absorption of several minerals to improve mineralization of bone by increasing the availability of Ca, Mg, Zn and Iron etc. Effects of prebiotics depends on dose, the time of administration, content of calcium in the diet and age.

Bacterial metabolites are stipulated to be the main effectors of most observed effects. The most important metabolites are the short-chain fatty acids (SCFA) acetate, propionate and butyrate (Cummings JH. *et al*., 2001). Prebiotic consumption can double the pool of SCFA in the gastrointestinal tract.

a. Hypolipidemic effect

Prebiotics play an important role in the management of cardiovascular diseases. Regular consumption of wholegrain is implicated in reduced risk of the vascular occurrences (Harris and Kris-Etherton, 2010).

Dietary intake of durum wheat has been suggested to increase ferulic acid concentration in blood plasma and is propound to be a puissant factor for the health benefits promulgated for the role of dietary fiber in management of cardiovascular diseases (Napolitano *et al.,* 2009).

The consumption of inulin-type fructans has been associated with improved lipid profile and, consequently, reduction of cardiovascular risk (Ooi *et al*., 2010).

Supplementation of both glucomannan and inulin-type fructans in both normal and moderately hyperlipidimic subjects decreases TAG and cholesterol level (Gallaher *et al.,* 2002).

Consumption of prebiotic enriched soy food resulted in increase in high-density lipoprotein and reduction in low density lipoprotein cholesterol and in hyperlipidemic adult (Wong JMW *et al*., 2010). Gut peptides is responsible for cascade of events devoted to control food intake, body weight, and glucose metabolism.

Causey *et al.,*(2000) observed a significant decrease of blood triglyceride levels after 3 week of ingesting 20g inulin daily and a trend to cholesterol reduction They also found that serum LDL-cholesterol decreased and serum HDL-cholesterol increased following the administration of inulin compared to the control group in hypercholesterolemic men.

b. Satietogenic effect

The "satietogenic" effect of prebiotics results from the excessive production of gut peptides (GLP-1, glucagon-like peptide-1 and PYY) and a decrease in ghrelin peptide (Delzenne *et al.,* 2005).

SCFAs promote secretion of GLP-1, one of the major incretin hormones primarily synthesized by entero-endocrine L-cells. This hormone inhibits glucagon secretion, decreases hepatic gluconeogenesis, improves insulin sensitivity, and enhances central satiety, resulting in weight loss (Ahren *et al.,* 2004).

The effect of prebiotics on satiety level was studied in ten healthy human subjects where results showed decreased satiety levels after prebiotic supplementation with resultant increase in postprandial plasma gut peptide concentration (Cani *et al.,* 2009) .Fermentable dietary fibers as short-chain FOS can be supplemented in foods to induce satiety and thus prevent obesity (Hess *et al.,* 2011).

Prebiotics may prove to be a useful tool for controlling food intake, increase satiety and thus lowering obesity risk.

Increased production of satiogenic intestinal peptide

The reduction of body fat contributes to the improvement of the lipid profile, since there is a reduction in the amount of free fatty acids for the hepatic synthesis. It has been suggested that the consumption of inulin-type fructans may increase the production of satiogenic intestinal peptides and, consequently, reduce body fat.

c. Anticarcinogenic effect

Inulin-type fructans have been shown to inhibit the formation of aberrant crypt foci (ACF), a biomarker of colon cancer, and reduce tumour incidence.

More pronounced effects were achieved by synbiotics (mixtures of probiotics and prebiotics) and long-chain inulin-type fructans compared to short-chain derivates especially in the more distal parts of the colon (Van Loo, 2004; Pool-Zobel, 2005).

Butyrate has been evidenced to suppress expression of transcription factor NF-κB in HT-29 cell lines, whereas acetate is known to increase the peripheral blood antibody production and NK activity in cancer patients (Macfarlane *et al.* 2008).

At least two mechanisms have been proposed to explain the effect of prebiotics on the development of cancer:

The first is the production of protective metabolites. Butyrate is a common fermentation end product and is known to stimulate apoptosis in colonic cancer cell lines, and it is also the preferred fuel for healthy colonocytes (Prasad, 1980).

According to Manning & Gibson, 2004, shift of colonic metabolism away from protein and lipid metabolism towards more benign end products (saccharolysis)

Prebiotics such as barley when germinated may help in prevention of colitis related colon cancer (Koh and Kim, 2011). Prebiotics possess protective effect against colon carcinogenesis due to its fermentation by intestinal microflora producing short chain fatty acids.

Synbiotic approach in prevention of colon cancer have proved to have a synergistic effect in improving colon carginogenesis.

d. Immunomodulatory effect

Inulin promote a positive modulation of the immune system (Delgado *et al*., 2011). The proposed mechanisms by Schley and Field (2002) underlying the immunomodulating effects are direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine; production of SCFA from fermentation; and modulation of mucin production the mechanisms for immunomodulation and the ultimate impact on health. Consumption of fermentable fibres help modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation (Swanson *et al.,* 2002; Gibson *et al*, 2004).

A study on a prebiotic mixture of short-chain GOS, long-chain FOS and pectin-derived acidic oligosaccharides resembling the composition of oligosaccharides in human milk, promote T Helper 1 (Th1) and regulatory T cell (Treg)-dependent immune responses and induce down regulation of IgE-mediated allergic responses. Additionally, the prebiotic administration does not interfere with the desired vaccine-specific serum antibody responses in healthy term infants (Stam *et al,.* 2011).

e. Gastrointestinal Health

Inulin-type prebiotics to infants to determine whether prebiotics might help prevent occurrences of acute diarrhea. The findings do not suggest a clinical role for reducing the incidence or severity of diarrhea in infants.

Fermentation of carbohydrates stimulates colonic motility. Addition of long chain inulin with DP more than 25 in the diet showed significant increase in stool frequency in subjects with constipation (Hond *et al* 2000).

f. Enhance mineral absorption

Extensive experimental studies in animals suggest that prebiotics such as inulin-type fructans can increase the absorption of minerals such as calcium, magnesium, iron, and zinc (Greer *et al*., 2006). The role of prebiotics in mineral absorption and thus total bone mineral mass accumulation is important.

Significant increase in whole-body bone mineral content (BMC) and whole-body bone mineral density was observed on supplementation of Inulin type fructans in male rats (Roberfroid *et al*.,2002).

In postmenopausal women fructooligosaccharides increased magnesium absorption and status after five weeks (Tahiri *et al*., 2001).

In healthy young men, the consumption of 40g inulin/day for 28 days resulted in a significant increase in calcium absorption (Coudray *et al*, 1997).

Griffin *et al*., 2002 showed in girls, who were around the menarche, that a mixture of oligofructose and long-chain inulin at a dose of 8 g/d, but not of oligofructose alone, increased true calcium absorption.

Abrams *et al.,* (2005) reported an increase not only in calcium absorption, but also in the whole-body mineral content and density when a combination of short- and long-chain inulin-type fructans were consumed by pubertal adolescents for a period of one year. It was found that all prebiotics does not promote mineral absorption to the same extent and the effects were more pronounced when long-chain fructans were present. It has been suggested that polymorphisms of the specific vitamin D receptor (Fok1) gene directly affect bone mineralization during pubertal growth through an effect on calcium absorption. The Fok1 effect on whole body bone mineral density (BMD) was only significant for those with high calcium intake.

The most convincing data in humans for improvement in mineral absorption is seen in adolescents and postmenopausal women.

Supplementation of 10gm/day of a 1:1 mixture of oligofructose (average DP of 4) and long-chain inulin fructans (average DP of 25) in postmenopausal women showed a significant increase of 8.4% in calcium and 9.5% in magnesium absorption. This further emphasizes that the benefit is best achieved with a combination of both short - and longchain fructans.

Several mechanisms have been proposed to explain the effect on mineral absorption and metabolism.

The first is that the intake of prebiotics acidifies the intestinal contents, which aids the solubilisation of minerals (Coudray *et al.*, 2003).

Another mechanism is given by Scholz-Ahrens *et al*, 2001 where bacterial fermentation products, predominantlylactate and butyrate enlarge the absorptionsurface of minerals by promoting proliferation of enterocytes.

Othermechanisms that have been proposed include suppression of bone resorption rate relative to bone formation rate (Zafar *et al.*, 2004), release of bone-modulating factors such as phytoestrogens (Ohta *et al*., 2002) and improvement in gut health and gut-associated immune defence (Scholz-Ahrens *et al*, 2007).

2.7. Prebiotics and its role in management of hyperlipidemia

Prebiotics are based on the concept that modulation of the gut microbiota maintain and/or promote health using non-digestible, fermentable carbohydrates. Evidences have proposed Inulin type fructans as modulators of microbial ecology and physiology in animals and humans(Delzenne *et al*., 2011) and gut microbiota play a critical role in the development of inflammation and metabolic disorders such as obesity (Cotillard *et al.,* 2013; Le Chatelier *et al.,* 2013) diabetes mellitus(Karlsson *et al.,* 2013; Qin *et al.,* 2012) and stroke (Wang *et al.,*2011).

Dewulf *et al.,* 2013 conducted a trial that assessed gut microbiota composition in the meta analysis. The findings suggested that prebiotic treatment (inulin/oligofructose 50/50 mix) led to an increase in *bifidobacterium* and *faecalibacterium prausnitzii,* which are negatively correlated with the serum lipopolysaccharide (pro-inflammatory component) levels(Creely *et al.,* 2007

Liu *et al*., (2017) in the meta analysis of the effect of fructans on blood lipid profile and glucose level concluded that dietary inulin type fructans supplementation may be beneficial for LDL-c reduction across all study populations whereas HDL-c improvement and glucose control were observation only in small T2DM subgroup.

Wu*et al.,* 2015 examined the effects of inulin-type fructans on 3 major atherosclerosis associated blood lipid profiles that is HDL-C, TG, and LDL-C and the results of the meta-analysis showed that the effect on HDL-C, TG, and LDL-C were 0.05 mmol/L, -0.08 mmol/L and -0.16 mmol/L, respectively. TG level decreased dramatically by 0. 17 mmol/L in the dyslipidemia group and in the diabetes group, a noticeable increment of 0. 15 mmol/L was found for HDL-C and a reduction of 0. 30 mmol/L was found for TG. Thus it was concluded that daily intake of proper amount of inulin-type fructans can increase serum HDLC level and decrease TG and LDL-C levels, which is especially beneficial for dyslipidemia and diabetic patients

In a study by Dehghan *et al.,* 2014, significant reduction in the plasma TC and increase in HDL-c was observed during the 6–8 weeks of inulin intervention compared with the control group

The beneficial effects on triglycerides and cholesterol LDL by administering inulin have been detected, without effects with FOS. Most of this effect may be due to increased loss of bile salts in the feces, which can range between 20 and 80%, producing secondarily a decrease in total body cholesterol (D. A. de Luis *et al*.,2011).

Russo *et al.,* 2008 reported that inulin type fractions supplementation reduced triglyceride level and increased HDL –c level. According to Higgins*et al.,* 2003 and Genta *et al.,* 2009, a significant reduction in the LDL-c was observed when compared with the controls. It was also reported that the consumption of inulin alone improved the lipid profile and lowered the TC and the LDL-c but showed no effect on the other blood lipids on obesity and insulin resistance in humans.

The effect of fructan (long chain inulin) supplementation on hepatic lipogenesis and cholesterogenesis in normal subjects in a double-blind, placebo-controlled crossover study was studied and results confirm the experimental data obtained in animals that the hepatic de novo lipogenesis was reduced by feeding fructans at a moderate dose (10g inulin per day for 3 weeks) (Letexier *et al*., 2003).However, there is no significant modification of cholesterol synthesis.

In a meta-analysis by Brighenti *et al,* 2007 with 290 subjects, intake of inulin type fructans was associated with a significant decrease in serum triacylglycerol by 0.17 mmol/L or 7.5%, regardless of health conditions.

However in a recent meta-analysis, reduction in the plasma TC, LDL-c and TG concentration, with increased HDL-c in the diabetes trials was observed where 513 adult participants with a BMI>25 kg/m^2 were supplemented with dietary prebiotics (Beserra *et al.,* 2015) The findings indicated that the consumption of inulin alone improved the lipid profile and FOS did not have an effect on the lipid profile. This observation can be attributed to the fact that the two fructans differ in their chain length, inulin with high degree of polymerization (DP) which is between 10 and 60 while FOS has a DP <10 which is a partially hydrolysed product of long-chained inulin(Roberfroid *et al.,* 2010).

As a result of the higher DP than that of FOS, inulin exert the prebiotic effect that is promoting growth and/or activity of probiotics and benefits to the host until it reaches the end of the colon(Liu TW *et al.,* 2016). However, mechanism explaining the effect with experiments in-vitro and in-vivo are required .

Several mechanisms have been put forward to explain a hypolipidemic effect of inulin type fructans:

1. Decreased hepatic lipogenesis

Delzenne NM. *et al.,* 2007 hypothesized that the consumption of inulin-type fructans reduces the *de novo* synthesis of fatty acids in the liver. Delzenne and Kok, 2001 observed a 40% decrease in the amount of mRNA of fatty acid synthase (FAS). These results demonstrated that inulin-type fructans are able to alter the gene expression of enzymes involved in hepatic TG synthesis, contributing to the improvement of the lipid profile. It was reported that the consumption of inulin-type fructans is not capable of increasing hepatic lipid catabolism, since it does not alter the activity of the enzyme carnitine palmitoyl transferase. This finds support that consumption of inulin-type fructans is associated with reduced serum TG by increased extrahepatic lipid catabolism (Daubioul *et al*., 2000 and Delzenne *et al*., 2002).

2. Alteration of blood glucose and insulinemia

Nondigestible carbohydrates reduce peak levels of blood glucose after a meal and consequently the induction of lipogenic enzymes via an increased gene transcription (Roberfroid, 2000).

Serum glucose and insulin also exert great influence on lipid profile, with high levels of these usually associated with a worse lipid profile. It is known that glucose is able to stimulate gene transcription of the enzymes involved in lipogenesis, and the presence of insulin potentiates this effect thereby exerting great influence on the lipid profile.

3. Increased production of SCFA

The SCFA (propionate, butyrate, and acetate) are the major end products of bacterial fermentation of carbohydrates that are not digested in the upper gastrointestinal tract(St-Onge *et.al.,* 2000) such as inulin-type fructan. The ratio of acetate to propionate reaching the liver is a putative intermediate marker predicting the potential lipid lowering properties of prebiotics

Another pathway through which inulin exert its potential health benefits is through the production of short chain fatty acid in the colon. FOS or inulin was degraded by gut microbiota into short-chain fatty acid, such as acetate, propionate and butyrate.Delzenne and Williams, 2002; Letexier *et al*., 2003; Wong *et al*., 2006).

Butyrate inhibits the synthesis of cholesterol in the liver through the down-regulation of the liver lipogenic pathway and provides a source of energy for human colon epithelial cells (Rossi *et al.,* 2005)

According to Arora *et al*., 2011, propionate is able to inhibit the synthesis of cholesterol and triglycerides at a concentration of 10-20 mmol/L are necessary.

The SCFA propionate is capable of lowering serum cholesterol levels because it inhibits the activity of 3-hydroxy-methyl-3-glutaryl-CoA (HMG-CoA) reductase enzyme; redistributes plasma cholesterol to the liver; increases the synthesis and secretion of bile acids, since it stimulates the activity of 7α-hydrolase enzyme by increasing the concentration of mitochondrial succinyl-CoA and inhibits expression of the genes involved in intestinal cholesterol biosynthesis

Increased fecal excretion of bile salts and cholesterol

Precipitation and excretion of bile acids to the intestine, which requires the liver to utilize cholesterol for further bile acid synthesis is associated with reduction of serum cholesterol is reduced (Pedersen *et al.,* 1997); Delzenne *et al.,* 2002 provided mechanisms postulating the efficacy of inulin in reducing serum cholesterol. Inulin decreases the pH of the cecum and binds soluble bile acid to bacteria or insoluble compounds resulting in increase in fecal bile acid excretion which increases the utilization of cholesterol to synthesis bile acid in the liver and thus decreases the liver cholesterol concentration.

According to Guo *et al*., 2011, fermentation of inulin-type fructans in the intestinal mucosa leads to the production of organic acids, reducing the pH in the intestinal lumen. Thus, the bile acids become less soluble and may be eliminated with the feces, which reduces their intestinal absorption and as a consequence of the fermentation process, the production of butyrate increases the thickness of the intestinal wall, which hinders the absorption of cholesterol molecules (Ooi *et al.,* 2010).