QUALITY OF YOGHURT ENRICHED BY INULIN AND ITS INFLUENCE ON HUMAN METABOLIC SYNDROME

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Abstract. The aim of this study was to determine the impact of biologically active inulin-type prebiotic, introduced into the yogurt, on the quality characteristics of the product. The influence of inulin-enriched yogurt on health characteristics of patients with metabolic syndrome was determined.

Enrichment of yogurt with inulin improved the sensoric properties (flavor and consistency) of the product. Individuals with metabolic syndrome showed significant decrease of high density lipoprotein cholesterol ($p<0.05$), low density lipoprotein cholesterol ($p<0.05$) and malondialdehyde ($p<0.01$) after a 28-days period of consumption of yogurt with inulin. Consumption of inulin-enriched yogurt had a tendency towards lower total cholesterol and had no effect on the triglyceride concentration. No significant effect on glucose concentration was observed. Reduction of both systolic and diastolic blood pressure was found after a 28-days period of consumption of yogurt with inulin. According to these findings, consumption of yogurt enriched with inulin might have a beneficial effect on patients with metabolic syndrome and improve their health.

Keywords: inulin, yoghurt, quality, metabolic syndrome.

INULINU PAPILDYTO JOGURTO KOKYBĖ, JO ĮTAKA SVARBIAUSIEMS ŽMONIŲ, SERGANČIŲ METABOLINIU SINDROMU, SVEIKATOS RODIKLIAMS

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Santrauka. Straipsnyje išanalizuota į jogurtą pridėto prebiotiko inulino įtaka produkto kokybiniams rodikliams (rūgštingumui, klampumui, sensorinėms ir sineretinėms savybėms) bei jų pokyčiams laikymo metu.

Nustatyta, kad inulinas pagerino jogurto sensorines bei sineretines savybes, padidino klampumą, kartu pagerėjo produkto konsistencija. Taip pat įvertintas produkto, papildyto inulinu, poveikis sergančiųjų metaboliniu sindromu sveikatai. Pavartojus produktą su inulinu 28 dienas, nustatyta teigiama jo įtaka sergančjųjų metaboliniu sindromu kraujo biocheminiams rodikliams: reikšmingai sumažėjo mažo tankio lipoproteinų ir didelio tankio lipoproteinų cholesterolio koncentracija (pastaroji atitiko normą). Tas nulėmė ir bendro cholesterolio koncentracijos mažėjimo tendenciją. Be to, reikšmingai sumažėjo malondialdehido koncentracija ir neženkliai sumažėjo oksiduotų mažo tankio lipoproteinų kiekis kraujyje. Tyrimo metu kraujo triacilglicerolių ir gliukozės koncentracija nepakito. Asmenims, 28 dienas vartojusiems jogurtą su inulinu, sumažėjo ir arterinis kraujospūdis. Tyrimo duomenimis, jogurtas, papildytas inulinu, turėjo teigiamos įtakos sergančjųjų metaboliniu sindromu sveikatai.

Raktažodžiai: inulinas, jogurtas, kokybė, metabolinis sindromas.

Introduction. Functional foods are rising food markets around the world and are expected to belong to the emerging trends in the food industry in the new millennium (Schaarsma, Korstanje, 2004; Luchina, 2003). Many laboratories are studying the mechanism of action of individual bioactive food ingredients and foods containing them (Halliwell, 2004). Joint efforts of Lithuanian scientists and producers are aimed at scientific research and production of foods conforming to the functional foods category such as yogurt and sausages with probiotic cultures and different types of prebiotics (Liutkevičius et al., 2011). Inulin is one of the most

popular prebiotics in food research field.

Inulin is one of inulin-type prebiotic substances with high fructose moieties number per molecule (Robertfroid, 2006; Kelly, 2008). Although inulin is abundant in such root vegetables as onion, Jarusalem artichokes, and leeks, it is also widely available commercially as a beneficial food supplement with prebiotic effect. In 1995, Gibson and Roberfroid coined the definition of prebiotics as "non digestible substances that provide a beneficial physiological effect stimulating the favorable growth or activity of a limited number of indigenous bacteria" (Gibson, Roberfroid, 1995).

Due to β bond inulin and other inulin type fructans are indigestible by human enzymes, therefore participating in a bacterial fermentation process in the colon. Inulin is degraded to lactic and short chain fatty acids thus acidifying the local environment and promoting growth of favorable Bifidobacteria. The beneficial effect of inulin and other inulin-type fructans is now well established in various studies. Inulin-type fructans decrease bowel transit time, absorb bile salts, increase fecal bulk mass as well as IgA levels, improve stool consistency and provide other systemic effects (Moro et al, 2003; Alliet et al., 2007). A number of studies demonstrate that inulin-type fructans modulate the secretion of gastrointestinal peptides involved in appetite regulation as well as in lipid metabolism. Moreover, a large number of animal studies and preliminary human data show that inulin-type fructans reduce the risk of colon carcinogenesis and improve the management of inflammatory bowel diseases (Russo et al., 2010).

The metabolic syndrome (MS) is characterised by the presence of several cardiovascular risk factors, including dyslipidemia, hypertension, insulin resistance and abdominal obesity (Gregor et al., 2006). To our knowledge effect of inulin supplementation has never been tested on subject with diagnosed metabolic syndrome, although limited and conflicting data about the inulin effect on subjects with isolated criteria of metabolic syndrome, e.g. only obese patients, or in patients with increased serum LDL cholesterol concentration, are available (Letechier et al., 2003).

Inulin has been shown to reduce postprandial glycemia and insulinemia. Because normal digestion does not break inulin into monosacharides, inulin does not elevate blood glucose level or stimulate insulin secretion. Therefore, several studies have found a positive effect of inulin on the management of diabetes, although there are substantial numbers of other studies that have failed to demonstrate such effect (Letechier et al., 2003; Jackson, 1999).

Inulin effects on lipids has been investigated in several studies. Inulin has been suggested to have beneficial effects on lipids, especially ability to lower triglyceride levels in blood. Even though convincing lipid lowering effect of inulin and other inulin-type fructans have been demonstrated in animals, human studies have shown more conflicting results (Beylot, 2005). Inulin type prebiotics have been most commonly reported to have no significant effect on lipid levels in studies examining patients with normal lipid levels (Balcazaramunoz et al., 2003; Russo et al., 2008). Testing individuals with elevated lipids has shown mixed results, with the majority of the studies reporting no benefit from supplementation. Several studies reported that inulin administration reduces serum triglycerides and, in some cases, cholesterol in healthy hyperlipidemic volunteers or causes a significant reduction in total cholesterol, LDL-cholesterol, VLDL-cholesterol and triglyceride concentrations in obese individuals with hyperlipidemia (Balcazaramunoz et al., 2003).

The reactive radicals can attack biomolecules, resulting in their oxidative degradation to lipid peroxides, conjugated dienes and malondialdehide. These oxidation products have damaging effects on cells and may be essential in triggering various diseases like stroke, diabetes, cancer and other chronic diseases or cause various complications (Youssef et al., 2002). Oxidized LDL in the blood is associated with oxidative stress, subclinical atherosclerosis development and may be a useful marker for identifying patients with cardiovascular risk. Studies demonstrating the effect of inulin on oxidation indices, e.g. oxidized LDL or malondialdehide, are lacking (Stoyanova et al., 2011).

The aim of the study was:

- To determine the impact of biologically active inulin-type prebiotic on the quality of yogurt produced, and its changes during the storage,
- To determine the influence of yogurt enriched by inulin-type prebiotic on the data on health characteristics such as concentration of plasma lipids, glucose, oxidation indices and anthropometric measurements of individuals with metabolic syndrome.

Materials/Methods

The DVS YC-180 starter (Chr. Hansen company, Denmark), containing yoghurt cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus),* probiotic cultures *(Lactobacillus acidophilus* La-5 and *Bifidobacterium* Bb-12) and RAFTILINE® ST (Orafti, Belgium), containing more than 90 % of prebiotic inulin were used for investigation.

A control yoghurt sample was produced from homogenized milk according the following technological scheme: milk heating and homogenization (60 °C, 170 bar) → pasteurization (90–95 °C/ 5 min) → cooling till temperature of souring (41±2 °C) → souring (41±2 °C/ 5–6 h, till pH 4.5–4.6 was reached) → mixing → fast cooling till 20 °C → cooling (4–6 °C).

Yoghurt with prebiotic RAFTILINE® was produced by the same technological scheme. RAFTILINE® was dissolved in a small quantity of milk and mixed with the main part of milk before its homogenization and pasteurization.

The samples of milk for yogurt were soured by introducing 0.025% of yogurt starter DVS YC-180, 0.02% *L. acidophilus* La-5, and 0.01 % *Bifidobacterium* Bb-12. The samples of yogurt produced were investigated in the fresh state and after 14 days of storage at 4–6 °C.

In the order to estimate the quality of enriched yogurt, active acidity of the yogurt was determined by pH-meter

766 (Calimatic, Germany). Rheological properties were measured by Hoeppler rheoviscometer (Germany) and rotative viscometer RHEOTEST-2, and sensoric properties were determined according to LST ISO 8589.

The samples of fresh industrially produced yogurt containing inulin-type prebiotic were supplied to the Vilnius University Hospital Santariškių Clinics for medical investigation.

Twenty five individuals have been randomly selected from list of patients with metabolic syndrome of Vilnius University Hospital Santariškių Clinics. The response rate was 71%.

The inclusion criteria were as follows:

- Males and females aged between 18 and 75.
- Patients suffering from metabolic syndrome according to International Diabetes Federation criteria.
- BMI: < 40 kg/m^2.
- Systolic blood pressure: ≤ 160 mmHg, and diastolic blood pressure: ≤ 100 mmHg.
- Glycosylated hemoglobin: <7.5%.
- Alanine aminotransferase (ALT) activity in blood not exceeding threefold increase: < 120 U/L.
- Creatinine concentration in blood: <133 μmol/L for males and <124 μmol/L for females.
- Hemoglobin concentration in blood: ≥ 120 g/L for males and ≥ 100 g/L for females.
- Patients did not take any drugs to diminish body weight over a period of the past 3 months.

The exclusion criteria were as follows:

- Chronic diarrhoea over the past 1 month.
- Increasing or decreasing body weight by more than 10% over a period of the past 3 months.
- Mental illness.
- Heavy drinkers (consumed amount of pure alcohol more than 60–80 g/day and 20–40 g/day for men and women respectively).
- Pregnancy or breastfeeding.
- Patients with stage III or stage IV heart failure.
- Patients suffering acute coronary syndrome over the past 6 month period.
- Patients suffering from acute diseases or exacerbation of chronic diseases.
- Intolerance to dairy products.

Study design

Patients were between 45–75 years of age (average age 60 years) recruited for the time of the study. The patients modified their diet by 125 ml of yogurt consumed daily over a period of 28 days. At the same time patients were encouraged not to change their dietary habits. The nutrient composition of yogurt is shown in Table 1. The patents were invited to arrive at the hospital between 7:30 a.m. and 9:00 a.m. after having fasted for 12 hours. The morning dose of the prescribed drugs was taken. Blood pressure was measured twice by a mercury manometer, pulse rate was measured once after resting supine for 5 minutes. The blood sample was taken on the first visit before the dose of yogurt has been consumed and on the second visit after the last dose of yogurt has been consumed (on the 29th day).

The study was approved by the Lithuanian Bioethics Committee (Order No. 41).

Table 1. **Nutrient composition of the yogurt, used in the study expressed per 100 mL** (data in parenthesis represent the content per 125 mL of yogurt)

Nutrient composition	Amount per 100 (125) mL of yogurt
Energy (kcal)*	35.4 (44.2)
Protein (g)	3.99 (4.98)
Carbohydrates** (g)	4.7 (5.8)
Total fat (g)	0.07 (0.09)
Inulin (g)	4 (5)

* Energy from inulin not included; ** Carbohydrates do not include any amount of inulin

Biochemical analyses

Cholesterol and triglyceride concentrations in serum were analyzed by enzymatic colorimetric methods (ARCHITECT ci8200, ABBOTT, USA). Low density lipoprotein cholesterol concentration was calculated using *Friedewald* formula. High density lipoprotein cholesterol was analyzed by the accelerator selective detergent method (ARCHITECT ci8200, ABBOTT, USA).

Plasma glucose concentration was analyzed by hexokinase enzymatic method (ARCHITECT ci8200, ABBOTT, USA). Glycosylated hemoglobin in blood was analyzed by immunoturbidimetric microparticle agglutination inhibition method (ARCHITECT ci8200, ABBOTT, USA). The serum insulin was measured by chemiluminescent microparticle immunoassay (ARCHITECT ci8200, ABBOTT, USA).

Serum malondialdehide concentration was assessed spectrophotometricaly using tiobarbituric acid reactants (Halvoet et al, 1998). Oxidized low density lipoproteins (LDL) in plasma were detected by *ELISA* (Mercodia, Sweden) based on the direct sandwich technique in which monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule (Halvoet et al, 2001).

Statistical methods

Statistical analysis was performed using SPSS (Statistics Base 19.0). Dependent *t-test* for paired samples was carried out to examine the differences between the baseline and 28-day follow-up results. The significance level of α=0.05 was used for all the analyses.

Results

Quality of yogurt, enriched with inulin

The influence of RAFTILINE® ST containing biologically active inuline-type prebiotic on the main quality characteristics (physico-chemical, structural-mechanical and sensory) of fresh probiotic yoghurt, containing 2.5% fat as well as yogurt after storage was investigated. The content of inulin in enriched yoghurt was 0.2%.

The influence of inulin on acidity and syneretic properties of yogurt is shown in Table 2.

Table 2. **Influence of inulin on acidity and syneretic properties of yogurt**

Indices	Yogurt			
	Fresh		After storage 14 days at 4±2 °C	
	Control	Containing 0.2% of inulin	Control	Containing 0.2% of inulin
Acidity: titratable, °T active, pH	 90 4.58	 90 4.59	 96 4.48	 95 4.54
The content of separated whey, established by centrifugation: g %	 26.00±0.26 52.0	 25.51±0.13 51.02	 24.15±0.13 48.30	 22.95±0.27 45.90

Table 3. **Influence of inulin on rheological and sensory properties of yogurt**

Indices	Yogurt	
	Control	Containing 0.2% of inulin
Viscosity, mPa.s: η_p η_s η_{at} KMS_η At_η, % N_η, %	 658.2 181.5 217.3 3.6 33.0 72.4	 760.1 189.5 246.3 4.0 32.4 75.1
Sensory properties: Taste Flavour Consistency Colour Common estimation, points	 8.5±0.35 9.3±0.45 8,5±0,50 5±0.0 31.3	 9.2±0.27 9,5±0.35 9.3±0.27 5±0.0 33.0

According to the data represented in Table 2, enrichment of yogurt with inulin does not influence the titratable and active acidity of the product. Syneresis of the fresh product containing inulin was lower compared with the yogurt without additives. During the storage, the negligible increase of acidity and decrease of whey separation was established in all samples of yoghurt.

The influence of the enrichment of yogurt with 0.2% of inulin on the rheological and sensoric data of the product is shown in Table 3.

It was determined that indices which characterize viscosity of inulin enriched yogurt are higher than of traditional yogurt. Besides that the enrichment of yogurt with inulin improved its taste, flavour and consistency.

The results of lipids, carbohydrates, MDA, oxidized LDL and other measurements of the group of individuals with metabolic syndrome before and after diet supplementation by inulin enriched yogurt are presented in Table 4.

Table 4. **Serum levels of lipids, carbohydrates, MDA, electrolytes and other characteristics of the group of individuals with metabolic syndrome before and after diet supplementation by inulin enriched yogurt** (n=25)

Indices	Before inulin enriched yogurt consumption Mean±SD	After inulin enriched yogurt consumption Mean±SD	*t*
Total cholesterol (mmol/L)	5.96±1.2	5.54±1.14	2.02
HDL-cholesterol (mmol/L)	**1.29±0.34**	**1.19±0.31**	**2.77***
LDL-cholesterol (mmol/L)	**3.87±1.14**	**3.42±0.93**	**2.44***
Triglyceride (mmol/L)	1.95±1.01	1.95±1.12	–0.01
MDA (nmol/mL)	**5.42±1.48**	**4.12±1.31**	**3.47****
Insulin (pmol/L)	125.32±89.31	102.09±42.78	1.49
OxLDL (U/L)	96.72±33.04	86.99±25.78	1.13
Fasting glucose (mmol/L)	7.35±1.76	7.31±1.87	0.15
Glycosylated hemoglobin (%)	6.20±0.53	6.24±0.85	–0.33

Abbreviations: HDL – high density lipoprotein cholesterol; LDL – low density lipoprotein cholesterol; MDA – serum malondialdehide, OxLDL – oxidized low density lipoproteins; Data are given as mean and standard deviation (SD); * = $p \leq 0.05$, ** = $p < 0.01$;

After the application of inulin enriched yogurt in the diet, serum HDL-cholesterol and LDL-cholesterol decreased significantly. Diet supplementation by inulin enriched yogurt had a tendency towards lowering of the total cholesterol and no effect on the triglyceride concentration. Furthermore, such diet significantly decreased the concentration of MDA ($p \leq 0.01$). No significant effect on glucose concentration was observed.

Comparison of blood pressure and pulse rate in the group of individuals/subjects with metabolic syndrome before and after inulin enriched yogurt supplementation in the diet are given in Table 5.

Table 5. **Comparison of blood pressure and pulse rate in the group of individuals with metabolic syndrome before and after inulin enriched yogurt supplementation in the diet** (n=25)

Indices	Before inulin enriched yogurt consumption Mean±SD	After inulin enriched yogurt consumption Mean±SD	*t*
Systolic blood pressure (mmHg)	**142.12±12.93**	**132.40±11.88**	**3.68*** **
Diastolic blood pressure (mmHg)	**87.20±7.65**	**82.84±6.89**	**2.79** **
Pulse (rate/min)	68.72±4.79	67.12±3.52	1.63

Abbreviation: Data are given as mean and standard deviation (SD); ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Reduction in systolic and diastolic blood pressure ($p<0.001$ and $p<0.01$ respectively) was observed after the completion of the study.

Discussion

F. Russo et al. 2008 reported the effect of inulin on lipid parameters. Significant differences between inulin-enriched pasta diet vs. baseline were found in young healthy volunteers: HDL-cholesterol concentrations increased by 35.9%; total cholesterol/HDL-cholesterol ratio, triglyceride and lipoprotein(a) concentrations decreased by 22.2%, 23.4%, and 16.5% respectively. Letexier et al. 2003 failed to show any significant changes in total, HDL and LDL cholesterol concentrations after inulin supplementation, however a statistically significant decrease in triglycerides levels was observed. Although these studies show a positive effect on lipid profiles with inulin supplementation, evidence that show insignificant changes especially in healthy volunteers are more prevalent (Kelly, 2009). In our study, consumption of yogurt enriched with inulin decreased the concentrations of HDL and LDL cholesterol by 8% and 12% respectively. The effect was minor for HDL cholesterol and did not pass the recommended threshold values that could negatively affect cardiovascular morbidity. Because the comcentration of LDL cholesterol has decreased significanty, this change has a beneficial effect on patients with metabolic syndrome, yet further studies should be done to confirm this data. The present study failed to demonstrate a significant decrease of total cholesterol and triglycerides. This might be due to the moderate dose of inulin used as compared to other studies, using larger dosages (Letexier et al., 2003).

The results of the present study of patients with metabolic syndrome showed a significantly decreased level of MDA (by 24%). Yogurt enriched with inulin decreased the concentration of oxidised LDL from 96.72±33.64 to 86.99±25.78 U/L ($p>0.05$). This difference suggested a trend but was not statistically significant. These results support the administration of inulin as a fibrous substance to the diet of patients with metabolic syndrome and cardiovascular risk. There are only a few articles in the literature addressing the inulin effect on oxidation process in human body. In one of them it is shown that inulin is good scavenger of hydroxyl and superoxide radicals (Stoyanova et al., 2011).

In the present study, a significant reduction of systolic and diastolic blood pressure (7% and 5% respectively) was observed in subjects with metabolic syndrome after inulin supplementation. The results however might have been influenced by certain unintended circumstances, such as differences in the daily diet. According to the findings, these changes are likely to have a beneficial impact on individuals with metabolic syndrome and improve their health.

Even though in some studies inulin was shown as a possible ingredient in lowering glucose concentration, the present study failed to confirm these results. This could be explained by a shorter study time or moderate dose of inulin as opposed to other studies. The present study is in accordance with other studies that failed to show the effect of inulin on glucose concentration (Halvoet et al., 2001; Kelly, 2008). The present study demonstrated new data of the positive effect of inulin supplementation on subjects with metabolic syndrome.

Conclusions

1. Enrichment of yogurt with inulin-type prebiotic improved rheological, syneretic and sensory data (taste, smell and consistency) of the product ($p<0.05$).

2. Subjects with metabolic syndrome showed significantly decreased HDL-cholesterol ($p<0.05$), LDL-cholesterol ($p<0.05$) and MDA ($p<0.01$) after a 28-days period of consumption of inulin enriched yogurt.

3. After inulin enriched yogurt consumption, subjects revealed a reduction in their systolic and diastolic blood pressure ($p<0.001$ and $p<0.01$ respectively).

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