EFFECT OF DIFFERENT PROTEINS FROM MILK AND SOY BEAN ON HYPERGLYCEMIA

By

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B.Sc. Agric. Sci. (Dairy Sci. & Tech.), Ain Shams Univ., 2002

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Dairy Science)

Department of Dairy Science
Faculty of Agriculture
Cairo University
EGYPT

2016

APPROVAL SHEET

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ABSTRACT

The target of this study was a trial to find some natural materials used as a treatment for diabetes mellitus type 2. The first part of this study was dealing with the practical experiment to find the difference between the effect of whey protein concentrate (WPC) and soy protein concentrate (SPC) as natural proteins in comparison with pharmaceutical material when dealing with diabetes disorders with streptozotocin (STZ). The second part was producing yoghurt fortified with these protein concentrates (WPC), (SPC) and their mixture 1:1. The yoghurt treatments were analyzed for chemical , physical and sensory properties. Obtained results showed that oral administration of rats with WPC, SPC., or their mixture (1:1) induced a significant increase in the body weight matched with a significant reduction in the fasting blood glucose level , reflecting the hypoglycemic potential of both WPC and SPC similar to the effect of the pharmaceutical drug (Diamicron®). SPC recorded a higher hypoglycemic effect than WPC did. Standardized buffalo's yoghurt (4% fat) was manufactured in four treatments. First treatment without any addition as a control, second, third and forth treatments were fortified with WPC, SPC and their mixture 1:1 respectivelly. The best properties were found with WPC treatments.
Key words: Streptozotocin (STZ) - whey protein concentrate (WPC) – soy protein concentrate (SPC)- pharmaceutical drug (Diamicron®).

DEDICATION

I dedicate this work to whom my heart felt thanks; to my sisters ***Rania Nour El-Din*** *and* ***Nermeen Nour El-Din*** *and their children for their patience, help, and all the support they lovely offered along the period of my post graduation.*

ACKNOWLEDGEMENT

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Prof. Dr. Moneir Mahmoud El-Abd*** *Professor of Dairy Science for his kind and worth supervision and planning, great thanks for his useful advice,valuable helpless and kind encouragement, Also I would like to expressing great thanks to* ***Dr. Essam Mohamed Mohamed Hamad*** *Lecturer of Dairy Science, Faculty of Agriculture . Also* ***Dr.Eman Tawfik Abd El-Aziz*** *for her kind supervision, Head Research of Dairy Researche Dept., Agricultural Research Centre (ARC).*

I extend my sincere thanks and gratitude to ***Prof. Dr. Alaa Azouz Salama****, Professor and Director of Food Technology Research Institute, Agricultural Research Centre, for his support and standing next to me until I finished this work; so, fully thanks to him . and I would like to express my appreciation to my dearest* ***Dr. Jihan Mohamed Kassem****, associate professor of Dairy Science, Dairy Department, National Research Centre, for her help of this study and kind advices. I also, thank her for support and encouragement.*

To my teacher, ***Dr. Khaled G. Abdel-Wahhab*** *associate professor of medical physiology, medical devision, National Research Centre, deep thanks and gratitude for his help and support in the biological part. I also thank him very much for his kind teaching me along this study. I really appreciate his valuable feedback, and his worth advices.*

Sincere thanks to ***Dr. Laila Khaled Hassan,*** *Researcher of Dairy Science, Dairy Department,* ***Dr. Fayza Mohamed Assem,*** *Professor, and* ***Dr. Reham Shehab El-Nemr,*** *Researcher of Histochemistry, Pathology Department, NRC,Giza.* ***Dr. Suhila Ahmed Saad,*** *Researcher of dairy science, Dairy Department and* ***Dr. Afaf Omar Serag,*** *associate professor, ARC,Giza. Finally, grateful appreciation is also extended to all staff members of Faculty of Agriculture Cairo University.*

LIST OF ABBREVIATIONS

LDL- Cholesterol	Low density lipoprotein cholesterol
HDL- Cholesterol	High density lipoprotein cholesterol
MDA	Malondialdehyde
GSH	Glutathione in Tissue Homogenate
NO	Nitric Oxide
TAC	Total antioxidant capacity
ASAT	Aspartate Amino Transferase
ALAT	Alanine Amino Transferase
WPC	Whey protein concentrate
SPC	Soy protein concentrate
MIX.	Mixture of whey and soy protein concentrates |(1:1)
STZ	Streptozotocin
DM	Diabetes mellitus
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non insulin dependent diabetes mellitus
L*:	Value represent darkness from black (0) to white (100).
a*:	Value represent color ranging from red (+) to green (-)
b*:	Value represent yellow (+) to blue (-)
TPA	Texture profile analysis
IU/L	mIU/mL
TG	Triglycerides

CONTENTS

INTRODUCTION

Diabetes was first recognized around 1500 B.C. by the ancient Egyptians, who considered it a rare condition in which a person urinated excessively and lost weight. The term diabetes mellitus, reflecting the fact that the urine of those affected had a sweet taste, was first used by the Greek physician Aretaeus, who lived from about 80 to 138 A.C. It was not until 1776, however, that Matthew Dobson actually measured the concentration of glucose in the urine of such patients and found it to be increased (Dobson,1976).

American Diabetes Association, (2014) was defined diabetes mellitus as a complex, chronic illness requiring continuous medical care with multi-factorial risk reduction strategies beyond glycemic control.

The International Diabetes Federation (IDF) estimated that 371 million person had diabetes. That number is projected to rise to 552 million (or 1 in 10 adults) by 2030, which equates to 3 new cases per second (IDF, 2013). People with diabetes are over 3 times more likely to be hospitalized with cardiovascular disease, 12 times more likely to be hospitalized with (end-stage renal disease) ESRD and over 20 times more likely to be hospitalized for a non-traumatic lower limb amputation, compared to the general population. Also, people with diabetes were 3 times more likely to require hospital admission in the preceding year with longer lengths of stay. Therefore, the impact of diabetes is significant not only for individuals but also for their families and for society as a whole (Public Health Agency of Canada, 2011).

Individuals with diabetes have a life expectancy that can be shortened by as much as 15 years, with up to 75% dying of macro-vascular complications (Davies *et al*., 2004).

Dietary proteins, in particular, have been studied extensively during recent years, and accumulating evidence supports that a high proportion of dietary energy from protein increases weight loss and prevents weight (re)gain (Skov *et al*., 1999; Weigle *et al*., 2005 and Larsen *et al*., 2010). Proteins also have unique characteristics related to its source, content of amino acids, and absorption kinetics. It is, therefore, speculated that proteins from different sources have diverse metabolic effects (Gilbert *et al*., 2011), and some evidence exists that different protein sources differ in their satiating capacity (Hall *et al*., 2003; Pal and Ellis, 2010 and Acheson *et al*., 2011).

Proteins vary in their ability to decrease postprandial hyperglycemia, but the mechanisms responsible for these effects are poorly defined (Gannon *et al*., 2003). Milk serum proteins are defined as substances that remain soluble in milk serum (Luhovyy *et al*., 2007). These proteins are naturally formed during the production of cheese and account for 20% of the all protein in milk (Pal *et al*., 2010), such as β-lactoglobulins, α-lactalbumin, immunoglobulins, lactoferrin, lactoperoxidase, glycomacropeptide, bovine serum albumin and other proteins (Barnett *et al*., and 2008 Hulmi *et al*., 2010).

Therefore, milk serum proteins perform several functions, such as mineral absorption, improvement of protein synthesis, sensitivity to hormones, and decreased blood glucose and lipid levels (Gunnarsson *et*

al., 2006; Mortensen *et al*., 2009, Petersen *et al*., 2009; Pal *et al*., 2010 and Sousa *et al*., 2012).

Milk proteins have been also shown to stimulate insulin secretion (Liljeberg-Elmstahl and Bjorck, 2001). However, whey proteins prove to be more insulin-tropic, compared with caseins or other animal and plant proteins (Pal and Ellis, 2010). The addition of whey-based protein reduced postprandial hyperglycemia in a dose-dependent manner, when added to a drink of 50 g glucose (Petersen *et al*., 2009). This dose-dependent effect of whey protein was also achieved when acutely applying different amounts of whey protein before or together with high carbohydrate test meals. Quantities higher than 20 g per serving led to pronounced effects in lowering blood glucose and increased insulin levels (Akhavan *et al*., 2010).

On the other side, soybean is becoming the most important vegetable source of proteins. The increased acceptance of soy proteins is due to soybean many fold qualities, good functional properties in food applications, high nutritional value, availability and low cost. The major soybean storage proteins referred as glycinin, ß-conglycinin and globulins, and the functional properties of soy based protein products (such as flour, concentrates and isolates) are reflected on their composition and the structure (Barać *et al.,* 2004). As known that soybean contains phytoestrogen (isoflavones), which are plant components that interact with mammalian endocrine systems. Furthermore, soybean products are good source of high-quality amino acids, contains no cholesterol, is low in saturated fat and it has been shown to reduce glycemia, to ameliorate glucose tolerance and insulin

tests. In 2000, the American Heart Association (AHA), the Nutrition Committee concluded that it is prudent to recommend including soy protein foods in a diet low in saturated fat and cholesterol (Erdman, 2000). Beneficial effects of soybean were demonstrated in many studies where soybean consumption prevented some types of cancer, reduced risk of osteoporosis, ameliorated chronic renal disease, exhibited anti-atherosclerotic activity, and decreased the risk of coronary disease (Omoni and Aluko, 2005 and Reinwald and Weaver, 2010).

Management of diabetes and insulin resistance syndrome involves strategies including modifications in lifestyle, physical activity, and a balanced diet (Schrezenmeir and Jagla, 2000), leading to an overall reduction in energy intake; a decrease in the consumption of saturated fats, trans fats, and cholesterol; and an increase in the consumption of low-fat dairy products, vegetables, and grains (Tremblay and Gilbert, 2009).

So, the target of this study was a trail to find some natural materials used for diabetes mellitus type II. The first part was concerned with the practical experiments to find the difference between whey protein concentrate (WPC) and soy protein concentrate (SPC) as natural proteins with different sources, compared to pharmaceutical drug when dealing with diabetes disorder; therefore, we can conducted the best protein compared with medicinal drug, which gives the most effectiveness we need. The second part was producing yogurt as an example of dairy products that fortified with these proteins to give an application point of view.

REVIEW OF LITERATURES

1. Definition of Diabetes (D M)

Diabetes can be defined as an inability to produce or optimally respond to insulin. It has been classified into many types, which differ in their clinical presentation. (Schrezenmeir and Jagla, 2000).

"Prediabetes" is a practical and convenient term referring to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or a glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications (American Diabetes Association, 2012).

While King *et al*. (1998) claimed that diabetes mellitus (DM) is a chronic debilitating condition that is rapidly increasing in prevalence worldwide, as a consequence of increases in obesity, changing patterns of diet and physical activity, and ageing populations. The World Health Organization estimated that 154 million person in the world had DM at the beginning of the 21stcentury. DM is a metabolic disorder characterized by hyperglycemia. The hyperglycemia is caused as a consequence of a deficiency in insulin in type I diabetes (TI D), and is a feature of late type II diabetes (TII D) along with insulin resistance.

TII D is significantly more prevalent than TI D. Molecular patho-physiological mechanisms that precede hyperglycemia, or are observed with the clinical symptoms of DM, include, among others, alterations in lipid and amino acid metabolism (Oresic *et al*., 2008 and Wang *et al*., 2011), changes in hormone levels (including insulin and

adiponectin), increases in adipokine levels and alterations in copper metabolism (Cooper *et al*., 2005; Li *et al*., 2009 and Stuart *et al*., 2012).

On the other hand, diabetic nephropathy is one of the most devastating complications in patients with diabetes. In diabetic nephropathy, damage to the kidneys occurs as a consequence of hyperglycemia, which induces damage in blood vessels leading to several phenomena, including impaired blood flow. Features of diabetic nephropathy include increased excretion of protein in the urine, increased blood pressure and declining kidney function. Severe diabetic nephropathy can lead to kidney failure and end-stage renal disease (ESRD), during which individuals must rely on hemodialysis, peritoneal dialysis or kidney transplantation to survive. The natural history of diabetic nephropathy includes several stages, starting with apparent normality in the first few years after diagnosis, followed by incipient nephropathy [characterized by the presence of small amounts of protein in the urine, known as micro-albuminuria], then by overt clinical nephropathy leading to progressive renal failure (Gross *et al*., 2005). However, diabetes is still the most important cause of ESRD in industrialized countries (Finne *et al*.,2005). Moreover, nephropathy is a clinical diagnosis based upon the finding of proteinuria in a patient with diabetes and in whom there is no evidence of urinary infection (Al-Amoudi, 2013).

2. Classification of diabetes

Diabetes can be classified into different clinical categories:

a. Type I diabetes

Type I diabetes, an autoimmune condition, is characterized by the destruction of pancreatic β-cells, which results in reduced or complete loss of insulin secretion. So, type 1 diabetic patients often present with acute symptoms of diabetes and markedly elevated blood glucose levels, and some cases are diagnosed with life threatening ketoacidosis (Schrezenmeir and Jagla, 2000). The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet *et al.*, 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey *et al.*, 1998).

Willis *et al.*(1996) illustrated that individuals with this form of Type I diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis.

Markers of immune destruction, including islet cell auto-antibodies, and/or auto-antibodies to insulin, and auto-antibodies to glutamic acid de-carboxylase (GAD) are present in 85–90 % of individuals with Type I diabetes mellitus when fasting diabetic hyper-glycaemia is initially detected (Verge *et al.*, 1996). The peak incidence of this form of Type I diabetes occurs in childhood and adolescence, but the onset may occur at any age, ranging from childhood to the ninth decade of life (Mølbak *et al.*, 1994). There is a genetic predisposition to auto-immune destruction of β-cells, and it is also related to environmental factors that are still poorly defined. Although