STUDY ON EFFECT OF PRESSURE COOKING AND STIR-OIL FRIED FOLLOWED BY PRESSURE COOKING METHOD ON GLYCEMIC INDEX OF SONA MANSULI RICE

by

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Study on Effect of Pressure Cooking and Stir-Oil Fried Followed by Pressure Cooking Method on Glycemic Index of *Sona Mansuli* Rice

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Approval Letter

This *dissertation* entitled *Study on Effect of Pressure Cooking and Stir-Oil Fried Followed by Pressure Cooking Method on Glycemic Index of Sona Mansuli Rice* presented by **Juna Dhungana** has been accepted as the partial fulfilment of the requirement for the **B. Sc. degree in Nutrition and Dietetics**

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Abstract

Rice, a staple food of Nepal, is generally considered as a high Glycemic Index (GI) food. With diabetes rates increasing alarmingly in Nepal, there is an urgent need to find methods of reducing the glycemic impact of rice. This study was aimed to evaluate the effect of cooking methods on the GI of *Sona Mansuli* rice. The test rice was cooked by two methods, pressure cooking and stir-oil fried followed by pressure cooking. The blood glucose response was studied for reference food (glucose) and two test foods (pressure cooked and stir-oil fried followed by pressure cooked rice) in 10 healthy volunteers.

The mean incremental area under the curve values obtained after the ingestion of the reference food (25 g), pressure cooked rice (108.58 g) and stir-oil fried followed by pressure cooking rice (124.22 g) each containing 25 g available carbohydrate were 2475.18 \pm 430.18 mg.min/dL, 1597.93 \pm 257.08 mg.min/dL and 1333.16 \pm 204.71 mg.min/dL respectively. The mean glycemic index of pressure-cooked rice was 64.72 \pm 2.49, whereas the mean GI of stir-oil fried followed by pressure cooked rice was 54.07 \pm 2.57. Similarly, the glycemic load of pressure-cooked rice and stir-oil fried followed by pressure cooked rice as compared to glucose were 35.27 \pm 2.49% and 45.92 \pm 2.44%, respectively. Similarly, the percentage reduction in GI of the stir-oil fried followed by pressure cooking rice as compared to that of pressure-cooked rice was 16.45 \pm 2.32. Based on the present study, it could be concluded that the addition of fat/oils in rice during cooking helps to lower the glycemic index of rice. To sum up, using fat/oils in moderate amount while cooking rice/food could be a strategy to control the blood glucose level.

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Abbreviation	Full form
AD	After Death
AGDP	Agricultural Gross Domestic Product
AOAC	Association of Official American Chemists
BGR	Blood Glucose Response
BMI	Body Mass Index
BV	Biological Value
CV	Coefficient of Variation
DoHS	Department of Health Sciences
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
GI	Glycemic Index
На	Hector
HDL	High-Density Lipoprotein
IAUC	Incremental Area Under Blood Glucose Curve
LDL	Low-Density Lipoprotein
MoAD	Ministry of Agriculture Development
NARC	Nepal Agriculture Research Council
NCD	Non-Communicable Disease
NFC	Nepal Food Corporation Standard
NHRC	Nepal Health Research Council
NIDDM	Non-Insulin Dependent Diabetes Mellitus
PAI	Plasminogen Activator Inhibitor
РВ	Protein Bodies
RS	Resistant starch
TD	True Digestibility
The U.S.	The United States
VDC	Village Development Committee
WHO	World Health Organization

List of Abbreviations

Part I

Introduction

1.1 General introduction

Rice (*Oryza sativa*) is the most important food crop of Nepal and the staple food of more than half of the world's population. It is consumed in several ways in Nepal such as *Bhat* (boiled or steamed raw and parboiled rice), *Khir* (porridge in milk), *Jaulo* (porridge in water), *Chamre/Pulau* (oil or fat stir-fried rice cooked in excess water), *Dhakane* (fats and oil stir-fried rice cooked in excess milk), *Selroti* (deep-fried rice batter) etc. In Nepal rice has both traditional and religious value as its use is famous in occasions like *Annaprasana*, *Dashain*, *Tihar*, *Antyasti*, etc (MoAD, 2015). It is rich in nutrients like complex carbohydrates, vitamins, and minerals. It contains about 76–78% starch and is one of the primary dietary sources of carbohydrate worldwide, including Nepal (Frei *et al.*, 2003).

The term Glycemic Index (GI) is defined as "incremental area under the blood glucose response curve of a portion of carbohydrate from a test food expressed as a percent of the response to the same amount of carbohydrate from a reference food taken by the same subject" (FAO/WHO, 1998). It is calculated numerically under the formula such as (IAUC test/ IAUC reference) x 100 and presents evidence-based index postprandial glycaemia (Rahelić *et al.*, 2011). It is the measure of rise in blood glucose level after the ingestion of food and is important for blood glucose level control especially in case of diabetes, a non-communicable disease (FAO/WHO, 1998).

Sona Mansuli (or *Sona Masuri*, as known with different names in different parts of south Asia (Hossain *et al.*, 2013)), is an improved variety of white, non-aromatic rice was released in 1982 from Andhra Pradesh which matures in 145 days. This variety of rice is one of the most grown and most popular rice variety (48.82%) in the Terai region of Nepal (MoAD, 2015).

Nepali population, including diabetic people, follow different methods of rice cooking. The study done by Rashmi and Urooj (2003) emphasised that the cooking methods; namely, pressure cooking, boiling, straining and steaming influence the nutritionally valuable starch fraction in rice varieties. So there is a need to identify suitable cooking methods with desirable nutritional attributes for different rice varieties. In another study conducted by Kumar *et al.* (2018), showed that the addition of fat/oils showed the GI lowering effect and resistant starch (RS) increasing effect emphasizing the need of identifying and developing rice with high RS and low GI benefitting diabetic patients.

Non-communicable diseases (NCDs) refer to diseases or conditions that occur in, or are known to affect individuals over an extensive period of time and for which there are no known causative agents that are transmitted from one affected individual to another (Daar *et al.*, 2007). Non-communicable diseases prevalence is raising in Nepal due to urbanization and unhealthy lifestyle practices. The World Health Organization (WHO) estimates that the death attributed to NCDs in Nepal have risen from 51% in 2010 to 60% in 2014 (Organization, 2014). Diabetes is one of the major NCD and its estimated pooled prevalence in Nepal is 8.4%, which is an increasing trend (Gyawali *et al.*, 2015). Dietary management, especially carbohydrate intake management is one of the effective ways of managing blood glucose level in the diabetic population.

1.2 Statement of the problem

White polished rice is considered a high glycemic index food (Miller *et al.*, 1992). Majority of Nepali population, including diabetic population, consumes polished white rice as a staple food (MoAD, 2015). Rice being a major source of energy (glucose) in regular Nepali diets, manipulation of digestibility of rice via cooking might be crucial in the management of type -2 diabetes. Moreover, the development of a management tool that does an only minimal alteration to individual default food habit can enhance the acceptability of such intervention (Mohan *et al.*, 2016).

Numerous researches have shown that digestibility (indirectly GI) of medium and high amylose rice can be reduced considerably when heated with certain types of edible fats and oils. Interestingly, several rice-cooking methods in Nepal involve use of fats and oil. Besides, there are not many noticeable scientific publications concerning the glycemic index of Nepalese varieties of rice and study on the effect of cooking methods and its effect on the glycemic index of rice. Thus in the present scenario, the major percentage of death in Nepal are due to NCD (Organization, 2014). Since diabetes is one of them, information on the glycemic index of commonly consumed rice variety and an appropriate home-based cooking method to reduce the GI of rice is more important in case of Nepalese population which prefers rice, a high glycemic index food, as their staple food.

Hence this research aims to study the effect of cooking methods (especially with the addition of fats and oil) on glycemic index of rice using various experimental approaches, involving traditional method, and stir-frying cooking method of rice.

1.3 Objectives

1.3.1 General objective

This objective was to determine the effect of cooking methods on the glycemic index of *Sona Mansuli* rice.

1.3.2 Specific objective

The specific objectives of the study were to:

1) Perform a physico-chemical analysis of Sona Mansuli rice

2) Determine the blood glucose response after ingestion of standard food (glucose).

3) Determine the blood glucose response after ingestion of *Sona Mansuli* rice cooked by pressure cooking and stir-oil fried followed by pressure cooking method.

4) Recommend a better rice cooking method for the diabetic patient without or minimal changes in default food habit.

1.4 Significance of the study

1) This study was conducted to evaluate the effect of different cooking methods in the glycemic index of *Sona Mansuli* rice, which will help both healthy as well as diabetic individuals for the necessary modifications in their food habits.

2) This research study aimed to contribute as a reference in the future for evaluating GI of the different varieties of rice and the effects of the different cooking methods and their role in diabetes management.

1.5 Limitations of the study

1) Only one variety of rice was taken.

2) Other cooking methods were not considered for the study due to time limitation.

Part II

Literature review

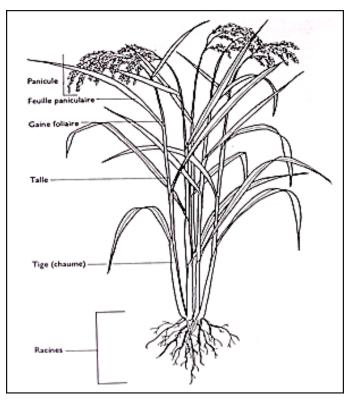
2.1 Rice: an overview

2.1.1 Origin of rice

Rice (*Oryza sativa* L.) is a genus of perennial grass in the Poaceae family. Until 2011 origin of the domesticated rice was a highly contested issue. However, genetic evidence published by the National Academy of Sciences of the United States of America in 2011, testified that all forms of Asian rice, *indica*, and *japonica*, come from a single domestication that occurred 8, 200 – 13, 500 years ago in China (Molina *et al.*, 2011). Another study conducted by (Vaughan *et al.*, 2008), based on a map of rice genome variation further supported this fact and confirmed the domestication of rice occurred in the Pearl River valley region of China. From there, rice spread to South and Southeast Asia (Harris, 1996). Now rice is cultivated across various environments ranging from tropical, semi-tropical, and warm temperature. There are about twenty-three species of rice out of which only two species have been known for their commercial value. These two species are *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice). However, *Oryza glaberrima* is cultivated in the limited areas of South Africa *Oryza sativa*, the most important commercial species of rice is differentiated into three subspecies: *indica, japonica,* and *javanica* based on their commercial production zones (Molina *et al.*, 2011).

2.1.2 Plant structure

Rice is an annual, self-pollinated and semi-aquatic plant. The plants are about 1 meter tall but certain deepwater varieties can elongate up to 5 meters. Rice has a fibrous root system. The rice stem known as culm is hollow and is made up of nodes and internodes. Each node bears a leaf and bud, which may grow into a shoot or tiller. Primary tillers grow out of the main culm. Panicles bearing tillers are known as fertile or productive tillers (Bardenas, 1965).



Source: www.ricehub.org (2019)

Fig.2.1 The Rice plant

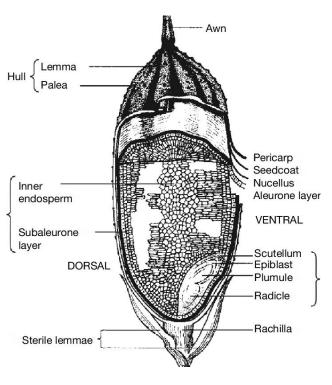
2.1.3 Rice grain structure

The rice grain (rough rice or paddy) consists of an outer protective covering, the hull (husk), and the edible rice caryopsis or fruit (brown, cargo, dehulled, or dehusked rice) (Figure 2.2). Brown rice consists of the outer layers of the pericarp, seed coat, nucellus, and germ or embryo, which are maternal tissues, and the endosperm. The endosperm consists of the aleurone layer and the starchy or inner endosperm. The pigment is confined to the pericarp, but there is a varietal difference in the extent of retention of pigment with the degree of milling. The aleurone layer encloses the embryo (Juliano, 2004).

The inedible hull constitutes 16-28% (mean 20%) of rough rice weight. Brown rice consists of 1-2% pericarp, 4-6% aleurone plus nucellus and seed coat, 1% embryo, 2% scutellum, and 90-91% endosperm. The aleurone and embryo cells are rich in lipid bodies (spherosomes, 0.2-1.5 μ m) and protein bodies (aleurone grains containing inclusions of phytic acid bodies or globoids (1-3 μ m))(Juliano, 2004).

The endosperm cells are thin-walled and packed with amyloplasts containing polyhedral compound starch granules about 3-9 µm in size. Protein occurs mainly in the

form of large (1-2 μ m) and small (0.5-0.8 μ m) spherical protein bodies and crystalline protein bodies (2-4 μ m). Spherical protein bodies (PB I) are rich in prolamin (alcohol-soluble protein), and crystalline protein bodies (PB II) are rich in glutelin (alkali-soluble protein). High night ripening temperature increases PB II/PB I ratio. Spherosomes are present in the sub-aleurone or the two outermost cell layers of rice endosperm (Juliano, 2004).



Source: Juliano (2004)

Fig.2.2 Longitudinal section of rice grain

2.2 Rice in Nepal

2.2.1 Rice production in Nepal

Food grains (rice, maize, wheat, millet, and barley) dominate the country's crop sector accounting for over three-fourths of the gross cultivated area in Nepal (total cultivated area- 3,091,000 ha. These crops - rice, maize, wheat, millet and barley (including buckwheat) account about 42.5, 25.4, 22.6, 8.2 and 1.2% of the total area under food grains, respectively. Similarly, their respective share on total production is about 51.6, 22.9, 21.5 and 4% (MoAD, 2016).

Rice ranks the first among cereal crops in terms of area, production, and livelihood of the people. Nepal is one of the important centres of rice genetic resources. More than 1, 700 rice landraces are reported in Nepal growing from 60 to 3,050 m altitude (Mallick, 1981). Rice samples of 500 years ago are found at *Simraungardh, Bara*. This shows that the commercial production of rice in Nepal has started five hundred years ago. These rice diversities and diversity traits found in Nepalese rice can be commercially utilized in rice breeding program to increase yields and develop biotic and abiotic stress-tolerant rice varieties. Considering this importance of rice diversities, the national gene bank of Nepal Agriculture Research Council (NARC) has conserved more than 3, 980 germplasms collected and received from national and international sources (Nepal-1,141, IRRI-2,672, USA-121, Russia-40, and Africa-6) (MoAD, 2016).

In Nepal, rice is grown in three agro-ecological regions (Terai and Inner Terai- 67 to 900; Mid Hills - 1000 to 1500 masl (meters above sea level); and High Hills - 1500 to 3050 masl) and three major production environments (irrigated, rainfed lowland and upland). Nepal is one of the centres of origin and diversity for Asian rice. The country has over 1,700 landraces of rice (MoAD, 2016).

The rice cultivated in Chhumjul of Jumla (ChhumchourVDC), at an elevation of 3,050 m is the highest place in the world to produce rice (Bhujel et al., 2011) . Jumli Marshi/Kali Marshi, a temperate Javanica variety of indigenous rice, having a cold-tolerant gene, has been cultivated probably since 1300 A.D., and is believed to have been brought by Saint Chandannath Baba, all the way from India. The Terai region, considered the granary of the country, accounts for about 70% of the country's rice output, while the hill produces 26% and the mountain about 4%. However, despite its importance in national food security and the economy, still, rice crop is grown under rainfed conditions in more than 35% of the total rice area. Drought, flooding and insect pests and diseases are major biotic and abiotic constraints to increasing rice yield and production fluctuates over the years depending on the weather conditions and production of rice is seasonal. Besides, rice is produced in different qualities which have varying market values. Rice is transplanted with the onset of the monsoon rain between June and August and harvested from September to November (MoAD, 2015).

The monsoon rain first arrives in the eastern part of the country and gradually advances to the west. The planting and production of the crop are very much dependent on the timing and intensity of the monsoon rain, which is normally active during mid-June until mid-September. In fully irrigated areas, farmers produce two rice crops in a year. Until the early 1980s, Nepal used to export rice to India. In recent years, rice produced in the Terai is being transported to the hills due to improved road infrastructures and market facilities. Likewise, the demand for rice in the hills and mountains are sharply augmented due to increased rice consumption behavior. Furthermore, Nepal has lagged in increasing productivity to fulfill the rice demand of the increasing population. As a result, Nepal gradually turned into a net importer of rice. In 2012/13, Nepal imported rice amounting Rs 9.23 billion (or 259,045 tons). Imports of rice from India alone amounted to Rs, 8.94 billion. At the present context in Nepal, import tariff for processed rice is 8% and unprocessed rice is of 5% (MoAD, 2016).

2.2.2 Rice use in Nepal

As the essential staple food of Nepalese people, rice supplies about 40% of the food calorie intake and contributes nearly 20% to the agricultural gross domestic product (AGDP) and almost 7% to GDP. Rice in Nepal carries unique cultural, religious and traditional values in the society. In Nepalese culture, rice forms an integral part of one's life right from the birth rites to the death rites. *Annaprasana-* the first rice feeding ceremony is observed on or after the fifth or sixth month of a child's birth. This ritualistic ceremony initiates the formal introduction of solid food for the baby. *Dashain*, the biggest Hindu festival in Nepal, is celebrated right after the rice harvesting season. During *Dashain*, elders put *tika* (a mixture of rice, yogurt and vermilion powder) and *Jamara* (paddy grass) on the forehead of younger relatives to bless them with abundance in the upcoming years. Similarly, during *Tihar*, sisters put *tika* on the foreheads of brothers, to ensure their long and safe life. *Pindas* are balls of cooked rice offered to ancestors during Hindu funeral rites (*Antyesti*) and ancestor worship (MoAD, 2015)

Nuwagi, a practice of offering new rice harvest to deities by mixing fresh rice grain with curd and spices on the completion of harvest works is common among rice farming communities in Nepal. Rice is one of the key ingredients in worshipping gods and goddesses across all religions in Nepal. Rice farming symbolizes family and societal cohesion in many parts of the country. In Jumla district where rice is cultivated in the highest altitude (3050 m) in the world, farmers prepare for rice cultivation by soaking paddy seeds in cold water on the 24th of March (12 Chaitra). Because of limited livelihood opportunities, a seasonal labor migration of males from Jumla to India is common. It is a social custom that all migrants are expected to return home before the 24th March of each year. Anyone failing to make it to home on the stipulated period is considered as good as dead (MoAD, 2015).

2.3 Composition of rice

2.3.1 Nutrient composition

According to Juliano (2004) the nutrient content of rice can be described as follows:

a) Energy: The energy content of brown rice and bran is higher than that in milled rice, owing to the higher fat content. The energy digestibility is higher in milled rice than in brown rice owing to the lower dietary fiber and phytic acid levels, as verified by the poor energy digestibility of rice bran.

b) Carbohydrate: The available carbohydrate content is higher in milled rice (77-89 g) per 100 g than brown rice and rice bran. The rice bran has the lowest content of available carbohydrate i.e., 34-62 g per 100 g.

Rice starch has lower amylose contents than other grain starches, such as corn, wheat, and oats. White rice has little to no dietary fiber and higher amylopectin contents, which is believed to be responsible for its rapid starch-hydrolysis rate. Amylose content of rice starch ranges from (0-2%) for waxy varieties too (25%) for normal rice varieties (Juliano, 1979). The relative amounts of amylose and amylopectin have a profound effect on the thermal properties, pasting properties, and enzyme hydrolysis rates of rice starch. The fine amylopectin with a higher proportion of branch-chains DP 12-24 enhances the stability of the crystalline structure, whereas amylopectin with more short branch-chains of DP 6-12 decreases the stability of the crystalline structure (Vandeputte and Delcour, 2004). The long branch-chains can form longer double helixes that help stabilize the starch granule by requiring a higher temperature to disassociate the crystalline lamellar structure. Amylopectin branch-chains than that of other grains (Jane *et al.*, 1999). These structures of

rice amylopectin further increase the starch hydrolysis rates of rice, which lead to higher postprandial blood glucose concentrations (Behall *et al.*, 1988).

Rice starch can form inclusion compounds with lipids known as amylose-lipid complexes, which are responsible for lowering starch hydrolysis rates (Guraya *et al.*, 1997). Reduction in starch hydrolysis rates in rice is associated with increased health benefits by lowering postprandial glucose and insulin responses (Goddard *et al.*, 1984). Amylose and amylopectin contribute to the structural integrity of the starch granule, which affects its gelatinization and pasting properties (Vandeputte *et al.*, 2003). These properties are affected by other components in the rice, such as lipids and proteins. The lipid may form amylose-lipid complexes, whereas proteins provide a barrier for enzymes to hydrolyze (Kaur and Singh, 2000).

c) Protein: Rice has one of the lowest protein contents (7%) among the cereals. The true digestibility (TD) of milled rice protein is also higher than that of brown rice, but the biological value (BV) is lower, resulting in a similar NPU. Bran protein has a lower TD but a higher biological value (BV) than brown and milled rice proteins. The amino acid score corrected for TD in rats proposed by the Food and Agriculture Organization as a protein quality index shows similar values to NPU for the rice proteins. Rice complements legumes (deficient in sulfur amino acids) in amino acid composition for human diets. The solubility fractions of protein are about 15% of albumin-globulin (water-and salt-soluble), 20% prolamin (PB I, alcohol-soluble), and 65% glutelin (PB II, alkali-soluble) in milled rice. Bran proteins are 66-98% albumins. Prolamin is deficient in lysine but rich in sulfur amino acids. The high lysine content of rice protein is due to the low prolamin content. Although cereal proteins are deficient in lysine, rice protein has one of the highest lysine contents among them, corresponding to an amino acid score of 67% in milled rice based on the US Food and Nutrition Board amino acid requirement pattern for the 1-3-year-old child of 5.1% lysine as 100% (Kaur and Singh, 2000).

d) Fat: The crude fat content is higher in the rice bran (15.0-19.7) as compared to brown and milled rice. The crude fat content is 1.6-2.8 g in brown rice and least in white rice (0.3-0.6 g) (Kaur and Singh, 2000). *Sona Mansuli* rice has a fat content of 0.63% while various black rice varieties comprised of significant fat content 2.5-3.5% which is due to fat layer present in bran of the rice. The polishing treatment in rice causes a decrease in the fat content of 86.2% in the black rice (Paiva *et al.*, 2016).

e) Fiber: The total dietary fiber content is relatively high in rice bran (24-29 g). The total dietary fiber of milled rice is least (0.7-2.7 g) as compared to that of brown rice (2.9-4.4 g). The bran layers and embryo are richer in non-starch constituents than the milled (white) rice. The crude fiber content of milled rice is 0.2-0.5 g per 100 g, which is lesser than that of brown rice 0.6-1 g (Juliano, 1979).

f) Vitamins: The major nutritional advantage of brown rice over milled rice is its higher content of B vitamins. Rice has no vitamin A, C, or D. The vitamin E content of whole rice is considerabe.Depending on the rice coluor, the vitamin E distribution in rice is ranked in the following decreasing order: bran > whole grain > endosperm > husk (Goufo and Trindade, 2014).

g) Minerals: Although higher in minerals, bran phytic acid forms complexes with minerals and proteins, reducing their bioavailability. Thus, the available iron (mg per meal) is similar in brown and milled rice meals, but probably higher in brown rice for Zinc (mg per meal) than for milled rice. The kernel of rice consists of phosphate and sulphate of potassium (K), magnesium (Mg) and zinc (Zn). The important minor elements found in rice are iron, magnesium, zinc, calcium etc. Generally, the rice husk has a higher content of mineral matter than the rice kernel. The bran layer of black rice is thicker than any other varieties and accounts for 65% of total ash (Paiva *et al.*, 2014). The Nutrient composition of rice is shown in table 2.1.

	Amounts (per 100 g))	
Property	Brown rice	Milled rice	Rice Bran
Moisture (g)	14.0	14.0	14.0
Energy content (kJ)	1480-1610	1460-1560	1670-1990
Energy content (kcal)	355-385	349-373	399-476
Crude protein (g)	7.1-8.3	6.3-7.1	11.3-14.9
Crude fat (g)	1.6-2.8	0.3-0.6	15.0-19.7
Crude fiber (g)	0.6-1.0	0.2-0.5	7.0-11.4
Total ash (g)	1.0-1.5	0.3-0.8	6.6-9.9
Available carbohydrates (g)	73-87	77-89	34-62
Total dietary fiber (g)	2.9-4.4	0.7-2.7	24-29
Water-insoluble fiber (g)	2.0	0.5	15-27
Sugars (g)	0.8-1.9	0.1-0.5	5.5-6.9

Table 2.1 Comparison of the macro-nutrient composition of brown rice, milled rice, and rice bran.

Source: Juliano (2004)

		Amounts (per 100 g)	
Property	Brown rice	Milled rice	Rice Bran
Thiamin (mg)	0.4-0.6	0.07-0.17	1.2-2.5
Riboflavin (mg)	0.04-0.14	0.02-0.06	0.18-0.43
Niacin (mg)	3.5-6.2	1.3-2.5	27-50
Pantothenic acid (mg)	1.4-1.6	0.8-1.3	20-60
Vitamin B6 (mg)	0.5-0.7	0.1-0.4	3.7
Folate (µg)	16-20	4-9	40-140
Vitamin E (mg)	0.8-2.5	0.1-0.3	3-15
Calcium (mg)	10-50	10-30	30-120
Phosphorus (mg)	0.17-0.43	0.08-0.15	1.1-2.5
Phytic acid (mg)	0.13-0.27	0.02-0.07	0.9-2.2
Iron (mg)	1.4-5.2	0.3-0.8	8.6-43
Zinc (mg)	1.9-2.8	0.8-2.3	4.3-26

Table 2.2 Comparison of the micro-nutrient composition of brown rice, milled rice, and rice bran.

Source: Juliano (2004)

2.4 Sona Mansuli rice

One of the commercial varieties of the white rice (medium grain) is *Sona Mansuli* rice and is easily available in the local markets. The *Sona Mansuli* rice is one of the improved varieties of rice of spring season. The spring season accounts to 92% of the production of

the rice and in the Terai region, 87.9% (93.2% in Eastern Development Region) of the rice cultivated is of improved varieties. Where in the Terai region, 23.03% of *Sona Mansuli* rice i.e., improved variety is being cultivated, which account to the higher percentage of cultivation (MoAD, 2015).

2.5 Cooking methods

Many cooking methods are used to prepare rice for human consumption. Depending on the country of origin and cultural backgrounds, these methods vary widely, but all involve heat moisture process. Rice cooking methods include boiling, steaming, stir-frying, baking, pilaf, and risotto (Bookwalter *et al.*, 1971). These methods impart different textures to rice and are used to create different food eating properties. These different methods also impact starch properties, such as pasting, thermal, starch-hydrolysis rates, and resistant starch contents (Ong and Blanshard, 1995).

2.5.1 Parboiling

Parboiling is a processing treatment that is conducted before milling and is reported to make up to 15% of the worlds milled rice supply (Lamberts *et al.*, 2006). Parboiled rice requires further treatment before it can be eaten. Boiled rice is prepared by heating rice in excess water until the starch has gelatinized, and starch granules become swollen (Bookwalter *et al.*, 1971).

2.5.2 Boiling/steaming

Boiling is a common preparation in most countries and results in a fluffy and dry grain with textural qualities favored by many cultures. Steaming and boiling are used interchangeably, although differences exist between the two methods. In traditional steaming, grains are cooked through water steam by placing it on porous vessels above boiling water in a closed container, or the rice is boiled for a short period and then steamed in a closed container (Bookwalter *et al.*, 1971).

2.5.3 Pilaf method

The pilaf method first cooks rice in oil before adding water. This imparts a nutty flavor due to the initial toasting of the rice grains and results in a firmer texture (Bookwalter *et al.*, 1971).

2.5.4 Risotto

Risotto is an Italian-style of cooking rice; wherein rice is continuously stirred while slowly adding water. This process results in the slow release of rice starch, which develops a creamy consistency (Bookwalter *et al.*, 1971).

2.5.5 Stir-frying

Stir-frying is a chinese cooking technique in which ingredients are fried in a small amount of very hot oil while being stirred in a wok. It is primarily used in Asian countries, although this method is also currently used in the U.S. and European countries due to the globalization of culinary trends. The traditional stir-frying method involves steaming or boiling the rice and storing it at 4°C for 24 h before stir-frying in oil. In this method, food is cooked from the conduction of hot, oiled pan with temperatures between 350 350°F and 400°F. The high heat and oil create a Maillard reaction providing browning and flavor development. Stir-frying results in high quality eating rice and is highly accepted by consumers (Bookwalter *et al.*, 1971).

2.5.6 Pressure cooking

Pressure cooking is the process of cooking food at high pressure, employing water or a water-based cooking liquid, in a sealed vessel known as pressure cooker. High pressure limits boiling, and permits cooking temperatures well above 100 °C (212 °F) to be reached. Pressure cookers work by expelling air from the vessel, and trapping the steam produced from the boiling liquid inside. This raises the internal pressures and permits high cooking temperatures. This, together with high thermal heat transfer from the steam, cooks food far more quickly, often cooking in between half and a quarter the time for conventional boiling. After cooking the steam is released so that the vessel can be opened safely (Baugarten, 1984).

In an ordinary, non-pressurized cooking vessel, the boiling point of water is 100 °C (212 °F) at standard pressure; the temperature of food is limited by the boiling point of water because excess heat causes boiling water to vaporize into steam. In a sealed pressure cooker, the boiling point of water increases as the pressure rises, resulting in subcritical water. At a pressure of 1 bar or approximately 100 kPa (15 psi) above the existing atmospheric pressure, water in a pressure cooker will reach a temperature of 121 °C

(250 °F). The boiling temperature of water (and water-based liquids) is determined by the ambient atmospheric pressure. Pressure cookers always require liquid in order to cook food under pressure. Inside a pressure cooker, once the water (liquid) is boiling and the steam is trapped, the pressure from the steam increases and pushes on the liquid, which increases its boiling temperature. The heat applied to the liquid by the heat source continues to create more steam pressure, and increases the temperature of the liquid. Both the liquid and steam are at the same temperature. Once the selected pressure level is reached, the pressure regulator on the lid releases any excess steam, and the heat can be lowered to maintain the pressure and save energy, since the pressure will increase no further (Baugarten, 1984).

2.5.7 Microwave cooking

A Microwave oven is a multi-utility kitchen appliance that can be used for cooking rice. Microwave heating is gaining popularity over conventional heating owing to its inherent advantage of rapidity and convenience. It provides consistently better nutrition than any other method of cooking and reheating. A microwave oven is also used for cooking rice. Microwave cooking, although not convenient for mass cooking, is very convenient for cooking small quantities, especially in households. Materials containing polar molecules are rapidly heated, when exposed to microwave radiation, due to molecular friction generated by dipolar rotation in the presence of an alternating electric field. Ionic conduction is another important mechanism in microwave heating. Positive and negative ions of dissolved salts in food such as common salt also interact with the electric field by migrating towards the oppositely charged regions of the electrical field and disrupt hydrogen bonds with water to generate additional heat. In foods, the polar molecules, mostly water, interact with microwaves to produce heat (Lakshmi *et al.*, 2007).

2.6 Starch-hydrolysis rates

Rice starch is known to be quickly digested in the human digestion tract. The ability of the digestive enzymes to break down glycosidic bonds to produce glucose for energy use in the body is central to metabolism (Reed *et al.*, 2013). Factors affecting the starch hydrolysis rates are explained as below.

2.6.1 Presence of fats/lipids

Both endogenous and exogenous lipids are known to restrict the ability of the enzyme to hydrolyze starch. Lipid coating of the starch granule may further restrict the accessibility of the starch granule to digestion amylases (Karkalas *et al.*, 1995). Amylose-lipid complex formation has been shown to decrease starch-hydrolysis rate (Reed *et al.*, 2013). The amylose-lipid complex, which is resistant to enzyme-hydrolysis, interacts with amylopectin and further restricts the swelling of the starch granule, leading to a reduction in the enzymatic-hydrolysis rate (Morrison *et al.*, 1993). This rate reduction is also attributed to interactions between amylose molecules and amylopectin, which restricts starch granule swelling and reduces enzyme accessibility to starch molecules (Case *et al.*, 1998).

2.6.2 Cooking

For the cooked cereals, the amount of starch hydrolyzed during the 30-minute incubation ranges from 45% in rolled wheat porridge to 80% in the commercial bread. Cooking greatly increases the rate at which starch can be hydrolyzed by gelatinizing the starch and making it more easily available for enzymatic attack (Snow and O'Dea, 1981).

2.6.3 Particle size

Particle size and surface area to starch ratio are important factors in determining the availability of starch to the hydrolytic enzymes. This is demonstrated clearly for both raw and cooked cereal. The starch in cereal flours hydrolyzes faster than in the corresponding raw rolled cereals. The rolled cereals are a relatively unprocessed form of grain, having been simply flattened rather than finely ground like the flours. They generally have a tightly packed physical form with a low surface area relative to flours, although this can vary depending upon the grain. For example, rolled oats were less compact than the other rolled cereals and were also hydrolyzed faster. This may have also been related to the oats having been steamed and rolled before being packaged. In this sense, the raw rolled oats were cooked, particle size played an important role in determining the rate of starch hydrolysis. For example, the starch in cooked flour (bread) was still hydrolyzed faster than that in cooked rolled wheat (porridge). Similarly, ground rice was hydrolyzed more rapidly than unground rice. In the case of rice, the postprandial glucose and insulin responses to

equal loads of ground and unground white and unpolished (brown) rice correlate extremely closely with the in-vitro rates of starch hydrolysis (Snow and O'Dea, 1981).

2.6.4 Cereal fiber

Cereal fiber does not appear to affect the rate of starch hydrolysis except when it forms a physical barrier limiting access of the hydrolytic enzymes to the starch. This is the case with whole brown rice, which is hydrolyzed more slowly than whole white rice. However, when brown rice is ground it hydrolyzes at the same rate as ground white rice despite the presence of the same amount of fiber. Similarly, most of the whole grain flours with their full complement of cereal fiber hydrolyze at the same rate as white wheat flour. These results are consistent with the in vivo findings that cereal fiber does not reduce postprandial glucose and insulin responses to carbohydrate. Cereal fibers are quite different physically and functionally from the viscous fibers, such as guar gum, which have been shown to have marked effects on rates of absorption of carbohydrate in vivo (Snow and O'Dea, 1981).

2.7 Available carbohydrate portion

Not all carbohydrate is available for digestion (i.e. broken down into mono-saccharides and absorbed), so only the available, or glycemic portion of carbohydrate is measured and used (FAO/WHO, 1998). Note that fructose is the exception as it is relatively non-glycemic (Englyst *et al.*, 2003). Available carbohydrate can be defined as 'total carbohydrate less dietary fiber' and determined 'by difference' (FAO/WHO, 1998). This method involves measuring the food components of water, fat, protein, ash and total dietary fiber, summing the values and subtracting the sum from 100; the remainder is then taken as the available carbohydrate content of the food (AOAC, 1990). The method has inaccuracies because each separate test for the components is associated with error and the errors accumulate in the summation. Additionally, the leftover fraction may also contain a small amount of non-available carbohydrate amounts of sugars and starch directly, although this also involves several separate determinations and the summation of monosaccharide fractions (Weinmann, 1947).

The GI is defined as the "incremental area under the blood glucose response curve of a portion of carbohydrate from a test food expressed as a percent of the response to the same amount of carbohydrate from a reference food taken by the same subject" (FAO/WHO, 1998).

The food may be classified into one of three categories depending on its mean GI value. Foods are classified as low GI if the GI value is less than 55; moderate GI if between 55-69; and high GI if greater than 70 (Brand-Miller *et al.*, 2003). The categories are arbitrary and do not necessarily relate to healthy food choices. For example, foods with high sugar, high-fat content such as ice-cream, have a low GI, whereas many fruits have a medium GI (Atkinson *et al.*, 2008). The values of GI and GL of some common foods are shown in table 2.3.

GI	Serving	Carbohydrate*	GL per
(Glucose=100)	Size	per Serving (g)	Serving
82	1	30	25
	medium		
76	1 cup	11	8
71	1 large	14	10
	slice		
66	1 cup	53	35
63	2 tsp	10	6
62	2 oz	40	25
58	1 Tbsp	17	10
58	¹∕₂ cup	19	11
55	1 cup	24	13
50	1 cup	42	20
46	1 cup	44	20
42	1	11	5
	medium		
39	1	15	6
	medium		
	(Glucose=100) 82 76 71 66 63 62 58 58 58 58 55 50 46 40 42	(Glucose=100) Size 82 1 76 1 cup 76 1 cup 71 1 large 82 1 66 1 cup 63 2 tsp 62 2 oz 58 1 Tbsp 55 1 cup 50 1 cup 50 1 cup 46 1 cup 42 1 39 1	(Glucose=100)Sizeper Serving (g) 82 1 30 medium 11 76 1 cup 11 76 1 cup 14 76 1 cup 53 66 1 cup 53 63 2 tsp 10 62 $2 oz$ 40 58 1 Tbsp 17 58 $\frac{1}{2}$ cup 19 55 1 cup 24 50 1 cup 42 46 1 cup 44 42 1 11 $medium$ 39 1 39 1 15

Table 2.3 Table showing the glycemic index and glycemic loads of some common foods.

Source: Atkinson (2008)

2.9 Glycemic load

The GL refers to the cumulative exposure to postprandial glycemia, as a measure of insulin demand, over a specified period. It does not take into account the pattern of loading within the specified time, i.e., few high– glycemic impact meals versus frequent meals of low glycemic impact. It is calculated indirectly as the product of the average GI of carbohydrate foods consumed and the total carbohydrate intake over a specified period (Monro and Shaw, 2008).

The GL was calculated by using formula suggested by Atkinson et al. (2008)

GL= (GI x amount (g) of available carbohydrate per serving)/100

2.10 Factors affecting the glycemic index

Different factors are found to be responsible for affecting the glycemic index of foods, including rice. Some of the important factors that affect the glycemic index of food are described under the following headings;

2.10.1 Rate of ingestion

Sipping 50 g of glucose slowly over several hour periods produces a much smaller increase in blood glucose than the rapid intake of the same amount. Eating three apples takes more than 15 minutes, whereas their juice can be consumed in 1.5 minutes. As the rate of ingestion increases, the glycemic index also increases (Shrilaxmi, 2014).

2.10.2 Food components

1. Protein and fat: Foods rich in protein and fat like ice cream, groundnut, and milk have a low glycemic index. However, they are not recommended for diabetes because they have a high calorific value. Pulses which have high protein are low in the glycemic index compared to cereals (Shrilaxmi, 2014).

2. Complex carbohydrates: Starches have low glycemic index compared to sugar. As the fruit ripens, starch gets converted to glucose and glycemic index increases. The glycemic index of starch is affected by the proportion of amylose to amylopectin in the grains. The higher proportion of amylopectin, the higher the glycemic index because amylopectin, which is made up of branched- starch molecules is more easily hydrolyzed in the gut. Some starches are more slowly digested not only because of amylose content but also because they are protected in structure for enzymes to act (Shrilaxmi, 2014).

Foods with soluble fiber such as beans have a low glycemic index. The extent to which the fiber in a particular food is responsible for its glycemic index is a subject of much debate (Shrilaxmi, 2014).

3. Acidity: an increase in the acidity of a meal can significantly lower its GI. Increasing the amount of vinegar in a meal, for instance, will affect the glucose response. The addition of sourdough bread to a meal can result in different GI's, depending on its content of organic acids. These foods affect the glucose response at least partially by slowing gastric emptying (Shrilaxmi, 2014).

2.10.3 Processing

Milling and grinding Starch particles within an intact grain are less accessible to digesting enzymes. Starches from milled particles that have had their cell walls removed are more easily accessible and therefore digested more quickly. Cooking Increases the digestibility of the starch and therefore GI of some foods. Organic acids formed during the fermentation of some bread delays gastric emptying (Brand *et al.*, 1985).

Starch encased in its seed coat or coarsely ground is not efficiently hydrolyzed to glucose because digestive enzymes are prevented from reaching the starch. Starch granules subjected to moist heat and subsequent cooling becomes dense and less available to enzyme action. Thus the physical form, as well as food processing and cooking methods, influence the enzyme availability of dietary carbohydrate (Shrilaxmi, 2014).

2.11 Methods of testing blood glucose level

Blood glucose tests can be performed at home, used in blood glucose monitoring for illness that have already been diagnosed medically or in laboratory to diagnose illness. At present there is no satisfactory substitute to glucose measurement. Alternatives, such as measurements of glycated haemoglobin, glycated proteins and 1,5-anhydroglucitol, although specific, are too insensitive to reliably detect lesser degrees of glycaemic disturbances. There are many methods available for measuring blood glucose, ranging from visually-read test-strips to sophisticated automated methods. Some of the home testing methods include;

- a. Finger prick type of glucose meter
- b. Continuous glucose monitor

Similarly, laboratory tests methods for the determination of blood glucose level include methods such as;

- a. Fasting blood sugar (FBS), fasting plasma glucose (FPG)
- b. Glucose tolerance test
- c. Postprandial glucose test
- d. Random glucose test

For all the methods of testing, precision and accuracy are required. If portable meters are to be used, they should be checked under a full quality assurance programme and a coefficient of variation >5% should not be accepted. When automated procedures are used, care must be taken to minimize the risk of errors in sample identification (Khatib, 2006).

2.12 Glucometer

A glucometer is a blood glucose level testing device specially made for the self-monitoring of blood glucose levels. Self-monitoring of blood glucose is an important component of modern therapy for diabetes mellitus. It has been recommended for people with diabetes and their health care professionals in order to achieve a specific level of glycemic control and to prevent hypoglycemia. The goal of self-monitoring of blood glucose levels is to collect detailed information about blood glucose levels at many time points to enable maintenance of a more constant glucose level by more precise regimens. Glucometer consists of a small, palm-sized, battery-powered meter about 5 cm x 9 cm and 1.5 cm thick, with a display screen about 3 cm x 4 cm. The test strips are inserted into a small opening on the top or bottom edge. The shape of the strip is specially designed so that it cannot be inserted incorrectly. The electrochemistry of the strip is the key to the operation of the glucose sensor (Sherman, 2006).

Working principle: The glucometer set consisted of a digital meter device and strip. The blood is pricked using lancet and is applied in the strip, which is then inserted into glucometer for a reading of glucose response.

On each strip, there are about 10 layers, including a stiff plastic base plate, and other layers containing chemicals or acting as spacers. Of the 10 layers, there was a layer

containing two electrodes (silver). There was also a layer of the immobilized enzyme, glucose oxidase, and another layer containing microcrystalline potassium ferricyanide, [K3Fe(CN)6]. These layers were suitably separated by the spacers to allow a small amount of blood to enter. When an end of a strip is touched to the droplet of blood (usually on a fingertip), the blood flows in by capillary action. A "beep" sounds, signalling that testing has begun. The glucose in the blood sample reacts with the glucose oxidase to form gluconic acid, which then reacts with ferricyanide to form ferrocyanide. The electrode oxidizes the ferrocyanide, and this generates a current directly proportional to the glucose concentration (Sherman, 2006).

A study conducted by Lin et al. (2010), indicated that the use of glucometers might be used in determining the IAUC, GI and rank GI value of food, however the recommendations was made that the result on that study does not necessarily apply to other glucometers. Similarly, the study done by Hettiaratchi et al. (2012) is also favours the agreement made by Lin et al. (2010). Chiu and Stewart (2013), had an use of glucometer (Onetouch) to study the effect of cooking method on resistant starch content of white rice and subsequent postprandial glucose response and appetite in humans. Similarly, Afaghi et al. (2007), also had an use of finger prick blood glucometer in their study to determine the GI of meals. The Study conducted by Chlup et al. (2004) has also mentioned the use of glucometer for monitoring the blood glucose response for determination of GI of selected foods. Similary, a study conducted by Henry et al. (2006), also had use of glucometer to study the glycemic index values for commercially available potatoes in great Britain. In another study conducted by Fasanmade and Anyakudo (2007), had also mentioned the use of glucometer for the determination of GI of selected nigerian flour meal products in male type 2 diabetic subjects. Therefore, based on all these studies referred, it could be understood that the glucometer can be used as the means of measuring the blood glucose response and for the determination of GI of foods.

2.13 Intervention studies involving the glycemic index

White rice elicits a relatively large glycemic response and is thus associated with exacerbating impaired glucose tolerance. It contributes a large glycemic load (GL) to the diets of those in countries where it is the main staple due to both the large quantity eaten and its greater GI. These countries incidentally are also where diabetes rates are markedly increasing. Therefore, devising ways and means of reducing the glycemic response of rice

is imperative to reduce the risk of developing the metabolic syndrome. A good understanding of all the factors affecting the GI of rice is essential to achieve this (Kaur et al., 2016). This study concluded on the remarks that rice being the main constituent in the diets of a large population segment in the world. Whilst it contributes a large GL to the diet and thereby potentially promotes impaired glucose tolerance (notably in countries and communities accustomed to rice consumption), advocacy to reduce consumption may not be a sustainable solution. Reducing the GI of rice may be the more practical approach.

A study conducted by Jeevetha *et al.* (2014) showed that there was a negative relationship between amylose content and GI value, which indicated that rice with low amylose content has high GI values, thus producing high postprandial glucose responses associated with increased risk of metabolic diseases such as type 2 diabetes.

In another study conducted by Kumar *et al.* (2018), showed that amylose content and resistant starch content of foods are important factors that influence starch digestibility and reduce the glycemic response by controlling the release of glucose in the small intestine. Furthermore, the study also found that pulses and fat reduce the GI individually and that the GI lowering effect was more pronounced when pulses or fat were added separately and that the impact on GI was not additive when both fat and pulses were included together with rice. The magnitude of GI lowering was more pronounced when oils/fats were added during cooking, compared to when it was added to the cooked rice. This study suggested that oil/fat might be added to rice during cooking, to lower its GI value and the rice cooked in such a manner would be better suited to diabetics.

In managing the diets of diabetic patients, the major objectives are to reduce hyperglycemia, prevent hypoglycemic episodes in insulin-treated diabetes, and reduce the risk of complications, particularly cardiovascular disease. From the evidence among nondiabetics, the consumption of slowly absorbed carbohydrates that produce lower peaks in blood glucose appears to be an advantage in maintaining glycemic control. Although this has been an area fraught with controversy, a review of randomized trials among persons with diabetes suggests that the consumption of low- rather than high-glycemic-index carbohydrates is advantageous (Willett *et al.*, 2002).

One of the major criticisms of the GI concept is that it lacks clinical application in those with chronic diseases such as diabetes and cardiovascular disease (Alfenas and Mattes, 2005). In contrast to this view, significant improvements in disease markers have been seen in those following lower GI diets in intervention studies by De Natale *et al.* (2009) and many reviews are also in favor of a low GI diet (Jenkins *et al.*, 2002).

In a crossover study conducted by Ludwig *et al.* (1999) where twelve teenage boys fed with different test meals which had a low, medium and a high GI (high and medium GI meals desined to have similar macronutrient composition, fiber content, and palatability, and all the meals for each subject had equal energy content), showed that the high-GI meal resulted in higher serum insulin levels, lower plasma glucagon levels, lower post absorptive plasma glucose and serum fatty acids levels, and elevation in plasma epinephrine as compared with the low-GI meal. It means that the rapid absorption of glucose after consumption of high-GI meals induced a sequence of hormonal and metabolic changes that promoted excessive food intake in obese subjects.

2.14 Benefits of low glycemic diets

2.14.1 Blood glucose management

The starch in high GI diets is rapidly digested and absorbed, resulting in hyperglycemia, which is followed by a hypoglycemic event. Constant fluctuations in blood glucose generate high stress on regulatory mechanisms of glucose homeostasis (Ludwig, 2003). Studies have shown that high post-prandial glucose levels are associated with decreased levels of serum antioxidants, increasing oxidative damage risk (Ceriello *et al.*, 1998). It has long been advised that diabetic patients stringently control blood glucose levels to reduce future complications. Studies have shown that subjects with decreased glucose tolerance have exaggerated postprandial glucose responses (Crapo *et al.*, 1980).

Consuming slowly digestible carbohydrates has been shown to reduce post-prandial glucose levels, decreasing the rise in gut hormones such as insulin surges (Jenkins *et al.*, 2002). Another phenomenon is the second meal effect. It has been shown that a low GI meal in the morning can improve glucose tolerance in the following meal (Jenkins *et al.*, 1981).

Diabetes Mellitus (DM) is one of the most common chronic disease, with an overall prevalence of approximately 2%. International Diabetes Federation (IDF) has estimated 200 million people around the world have diabetes and by 2025 it is expected to increase to

333 million and double by 2030. In US, Type 1 diabetes mellitus approximately account for 10% and type 2, 85-90% of all known cases of DM. Untreated Dm causes much morbidity and mortality due to its devastating late complications involving micro-vascular and macro-vascular structures. Nepal has highest prevalence of prediabetes among SAARC countries. The Prevalance of diabetes is around 15% among people aged more than 20 years and 19% among people age 40 years and above. One in ten Nepalese are found to have DM (Maskey *et al.*, 2011).

Low glycemic diets were first proposed as a mechanism to help people with diabetes control their blood glucose levels. Many studies have found that with the intake of low glycemic diets, both glycosylated serum proteins and glycosylated haemoglobin (HbA1) are decreased (Jenkins *et al.*, 1988) . HbA1 is another indicator of plasma glucose concentrations, usually over a 12-week time, note red blood cells have a 120-day life cycle and if exposed to glucose, remain glycosylated until apoptosis. Studies have also shown a correlation between increasing β -cell function and low glycemic diets (Esfahani *et al.*, 2009). The inclusion of low glycemic foods may increase insulin sensitivity by avoiding major fluctuations in blood glucose levels (Thomas and Elliott, 2009). According to the study done by Jenkins *et al.* (2002), improvements in disease markers can be gained from dietary GI changes

2.14.2 Weight management

Dietary evolution plays a major role in today's obesity and diet-related health problems. Obesity is known to contribute to hyper-insulinemia, an overproduction of insulin by β -cells of the pancreas, as a response to decreased insulin sensitivity FAO/WHO (1998). Low GI diets are beneficial to obesity-related disease by helping to control glucose release, insulin response and satiety (Cheftel, 1986). Positive correlations have been observed between the consumption of low GI diets and body weight regulation through increased satiety and insulin sensitivity (Jenkins *et al.*, 2002). Human research has shown that consumption of food with a high postprandial glucose response is coupled with the greater subsequent intake (Ludwig, 2003). Animal research by Rand *et al.* (2004), supports this theory and it is documented that cats fed at *Libitum* had significantly higher energy consumption with rice-based diet, which elicited a higher glucose response, than with a sorghum/corn-based diet, 140 kcal/feeding versus 71 kcal/feeding, respectively. This

implies that the reduced glucose response of a feed will increase the satiety of an animal, which is intrinsic to the starch source itself.

2.14.3 Cardiovascular disease

A meta-analysis on the effects of low GI compared to high GI diets on markers for lipid metabolism showed that an average difference of 22 dietary GI units resulted in a significant mean change in total cholesterol concentration of -0.33 mmol/L (Opperman *et al.*, 2004). Prolonged periods of high insulin have been correlated with an increase in blood pressure, triglyceride level and a decrease in high-density lipoprotein (HDL) cholesterol, all of which are predisposing factors to cardiovascular disease (O'Keefe *et al.*, 2008). Decreasing low-density lipoprotein (LDL) cholesterol has also been correlated with decreasing the risk for cardiovascular disease mortality and morbidity (Wolever and Jenkins 1986).

Jenkins *et al.* (1985) have shown that a one-month low GI diet therapy in hyper lipidemic patients significantly decreased serum cholesterol and triglycerides, thus decreasing their cardiovascular disease risk. A study by Wolever *et al.* (1992) showed that a low GI diet in diabetics significantly reduced PAI-1, by 58%. Plasminogen activator inhibitor-1 (PAI-1) is used as an indicator of impaired fibrinolysis. Fibrinolysis is the ability to break down a fibrin clot caused by coagulation, and an increased level of PAI-1 is a substantial risk factor for coronary heart disease. Researchers have hypothesized that this is due to a low GI diet having an improved metabolic profile with lowered insulin concentrations (Leeds, 2002).

2.14.4 Cancer

Insulin and insulin-like growth factors have been correlated with cancers such as those of the colon, breast, and prostate (Jenkins *et al.*, 2002). Researchers are hypothesizing that high GI diets and sedentary lifestyles may be associated with an increased risk of cancer (Giovannucci, 2001). Insulin, being an anabolic hormone, causes protein accretion, and since cancer is the excessive proliferation of diseased cells, it is hypothesized that there is a correlation between high insulin levels, insulin resistance and cancer (Jenkins *et al.*, 2002). Low GI diets are proposed to reduce post-prandial insulinemic responses, thus lowering insulin's risk in cancer cell production. However, there have been conflicting reports as

found that GI or glycemic load showed no association with prostate, lung and or ovarian adenomas or cancers (Flood *et al.*, 2006).

Part III

Materials and methods

3.1 Materials and equipment

3.1.1 Materials

1. Rice (Sona Mansuli) was bought from the local market of Bardibas, Mahottari, Nepal.

2. Glucose powder (Dabur glucose-D, Dabur Nepal. Pvt. Ltd., Bara Nepal) was bought from the local market of Dharan.

3. Refined sunflower oil used to make stir-oil fried rice (*chamre*) was also bought from the local market of Dharan (Cello Refined Sunflower Oil, Manufactured by, Bagmati Oil Industries, Katahari, Morang, Nepal).

4. For the cooking of rice, the pressure cooker of 2 Liter size (Diamond, India) was bought from the local market of Dharan.

3.1.2 Chemicals

1. The enzyme Diastase (1,4- α -D-Glucan-glucanohydrolase; α -Amylase) with reference number (REF: RM638-100G) manufactured by HIMedia Laboratories Pvt. Ltd., India.

2. Other chemical used

- a) Acetic acid
- b) Sodium acetate
- c) Lead acetate
- d) Potassium oxalate
- e) Hydrochloric acid
- f) Sodium hydroxide
- g) Methyl red
- h) Methylene blue
- i) Carrez I and II
- j) Fehling's solution
- k) Phenolpthalein indicator
- l) Dextrose standard
- m) Acetone
- n) Petroleum ether

*All the chemicals used were of analytical grade and were obtained from the laboratory of Central Campus of Technology (CCT, Dharan)

3.1.3 Equipments

- a) Incubator (Victolab, India)
- b) Electric balance (Phoenix instrument, 620g)
- c) Hot air oven (Victolab, India)
- d) Measuring cylinder
- e) Refrigerator
- f) Glasswares (Erleynmeyer flask, Soxhlet apparatus, Distillation flask, Dessicator, Beaker, Volumetric flask, conical flask, Burette, Petri dish, Porcelain basin, separating funnel, pipette etc.)
- g) Hot water bath
- h) Filter paper
- i) pH meter
- j) Bunsen burner
- k) Muffle furnace
- l) Crucible
- m) Stadiometer (locally made by Central Campus of Technology)
- n) Weighing balance (Seca-scale, Manufactured by: Microlife Co.)
- o) Glucometer: In order to measure the blood glucose concentration of the participating volunteers, blood glucose monitoring system (Glucometer, Clever Chek, TD-4239, TaiDoc Technology Corporation, 24888, New Taipei City, Taiwan) was bought from the Surgical House Dharan.

3.2 Standardization of glucometer

For the standardization of glucometer, initially, 5 volunteers were taken under study. The blood samples of those volunteers were taken and tested in clinical laboratory and glucometer for the blood sugar level before and after ingestion of 25 g glucose. The variation obtained was considered while standardizing the Glucometer.

3.3 Methods

3.3.1 Research design

The research design used in this study was analytical research (quantitative research method) design as there was a comparison in the glycemic index of both the pressure cooked rice and the stir-oil fried followed by pressure cooked rice. The research design also included clinical trial as volunteers were selected and were used clinically to obtain their blood glucose response.

3.3.2 Analysis of physical properties of rice

Physical characterisation of collected sample for length, breadth, length to breadth ratio, bulk density and appearance were performed as following:

1. Determination of Length

Length of ten grains from each sample was determined using a slide caliper. The reading of the main scale and vernier scales were recorded. The length of each rice grain was computed by the following equation.

Length (mm) = main scale reading + vernier scale reading \times vernier constant

2. Determination of breadth

The breadth of ten grains from each sample was determined using a slide caliper. Each grain was placed between the two jaws of slide caliper along its breadth and fixed. Then reading of the main scale and vernier scale was recorded. The breadth of the rice grain was computed by the following equation.

Breadth (mm) = main scale reading + vernier scale reading \times vernier constant

3. Determination of length/breadth (l/b) ratio

The l/b ratio of rice grain was calculated by using the following formula;

Length / breadth ratio =
$$\frac{Average \ lenght \ of \ rice \ grain \ (mm)}{Average \ breadth \ of \ rice \ grain \ (mm)}$$

4. Determination of 1000 kernel weights

Thousand kernel were counted from the rice sample and weighed in an electronic balance (MP series electronic balance, Sanghai Hengping Scientific Instrument Co. LTD., China, Weighing capacity 200 g; readability: ± 0.01 g) and were expressed in grams.

5. Determination of extraneous matters in rice

For the determination of the quality of rice, 10 g of rice sample was screened for pest infestations, pebbles, and other extraneous matters. The weight of extraneous matters was taken. The quality of rice was then determined by using the following equation,

Extraneous matters in rice (%) = $\frac{Wt \ of \ undesirable \ matters}{Wt \ of \ Sample} \times 100$

3.3.3 Chemical analysis of rice sample

Chemical compositions (moisture, ash, protein, fat, and crude fiber contents) of *Sona Mansuli* rice was determined as mentioned below. Total carbohydrate content were determined by the indirect method, (subtracting the sum of all other contents from 100 g sample) described by Weinmann (1947).

3.3.3.1 Determination of moisture content

The determination of the moisture content was done by Hot-air oven method as given by AOAC (2004).

3.3.3.2 Determination of fat content

The determination of the fat content was done by Solvent extraction method as given by AOAC (2004).

3.3.3.3 Determination of crude fiber

The determination of the crude fiber content was done as given by AOAC (2004).

3.3.3.4 Determination of protein

The determination of protein content was done by Kjeldahl method as given by AOAC (2004).

3.3.3.5 Determination of ash

The determination of ash content was done by Dry-ashing method as given by AOAC (2004).

3.3.3.6 Determination of total available carbohydrate.

The total available carbohydrate content of the test rice sample was determined by the method described by Weinmann (1947) with some modifications.

1. Preparation of Buffer Solution: A buffer solution of pH 4.45 was prepared by mixing three volume parts of 0.2 N acetic acid with two volume parts of 0.2 N sodium acetate solution. The buffer solution was prepared fresh every time whenever required.

2. Procedure: Fifty gram of cooked rice each of pressure cooked and stir-oil fried followed by pressure cooked rice was taken in a clean dried beaker. Fifty ml of water was added into each rice containing beaker and was grounded in the mixture. Grounded samples (1.94 g of pressure-cooked rice and 2.40 g of stir-oil fried followed by pressure cooked rice) were kept in a separate reagent bottle. Three gram of diastase (1,4- α -D-Glucanglucanohydrolase; α -Amylase) enzyme and 10 ml of buffer solution and 20 ml of water were also added into the rice sample taken in those reagent bottles and was well shaken before incubating at 37±1°C for 44 h. After incubation, both of the samples were kept in room temperature and 75 mg of lead acetate was added into each reagent bottle, mixed well and was allowed to precipitate. The precipitate was then filtered through filter paper (Whatman No.42). The filtrate of both pressure cooked and stir-oil fried followed by pressure cooked rice sample was separately collected in a beaker containing 150 mg of potassium oxalate and was shaken well. Both of the filtrates were placed in a beaker with stopper and kept in room temperature for 4 h and again the filtration was done. Fifteen milliliter of the second filtrate of each sample was taken for acid hydrolysis.

For the acid hydrolysis, 0.75 ml of 25% HCL was mixed into 15 ml of each sample taken. Both samples were heated in a boiling water bath for 30 min attaching the flask to a reflux condenser and allowed to cool in room temperature. 1 to 2 drops of methyl red was added into the mixture and to neutralize it 25% of NaOH was added dropwise till light orange color appeared. Volume was made up to 50 ml for each sample of pressure cooked and stir-oil fried followed by pressure cooked rice. After the volume was made up, 25 ml of each sample were taken for reducing sugar determination by using Lane and Enyon

method (AOAC, 2004). For the blank sample, except the addition of rice samples, all the other processes were the same. The determination of available carbohydrate for blank, pressure cooked rice and stir-oil fried followed by pressure cooked rice was carried out simultaneously.

3.3.3.7 Determination of reducing sugar

The determination of reducing sugar was done by Lane and Eynon method as given by AOAC (2004).

3.3.3.8 Determination of glycemic index of rice

The glycemic index of *Sona Mansuli* rice was determined by following the protocol provided by FAO/WHO (1998). A brief overview of the protocol to measure GI was as follows;

- 1. Selected individuals were asked for overnight fasting of 12 hours.
- 2. Two baseline blood glucose levels of the volunteers were measured before the test food was ingested.
- 3. A food portion containing 25 g available carbohydrate was given to the participants.
- 4. Blood samples were taken to test blood glucose levels over a 2 h period for nondiabetic participants. Samples were collected at 0, 15, 30, 45, 60, 90, 120 minutes after the test food was consumed.
- 5. Capillary blood was used to determine blood glucose concentration; Capillary blood was preferred because it had been found to be less variable.

Calculating the glycemic index

The GI was calculated using the given formula as described by Wolever (1990):

$$GI = \frac{\text{Incremental area under blood glucose response curve (IAUC) for a food}}{\text{IAUC for reference food}} \times 100$$

The final glycemic index for each test food was calculated as the mean of the respective GI of the ten individuals.

The area under the curve was calculated as the incremental area under the blood glucose response curve (IAUC), ignoring the area beneath the fasting concentration. This was

calculated geometrically by applying the trapezoid rule. In case of blood glucose value fell below the baseline, only the area above the fasting was included. The area obtained from the curve was obtained by the sum of the areas of the triangles and trapezoids (FAO/WHO, 1998).

3.4 Cooking methods

Initially, a survey was conducted for the optimization of cooking time, ratio of water and rice, and amount of cooking oil (for stir-oil frying) for both pressure cooking and stir-oil fried followed by pressure cooking method. The survey method adopted in the survey was simple random sampling. Three wards were selected by simple random sampling. The survey was carried out in three wards (11, 14 and 15) of Dharan. Sixty households in total were surveyed during the survey in which 20 households from each ward were selected. Housewives were surveyed in most common methods they use for cooking rice. From the survey results, it was found that the most common method they used was pressure cooking. Cooking methods of rice by pressure cooking and stir-oil fried followed by pressure cooking process was surveyed among housewives for cooking time and for rice-water ratio. The overall response from the respondents were analyzed, and then both the pressure cooking and stir-oil frying followed by pressure cooking of rice was standardized in the the laboratory of Technology. setting Central Campus of After the standardization/optimization of both the cooking methods, the optimized process were adopted for cooking rice during the survey period.

3.4.3.1 Pressure cooking

Pressure cooked rice was prepared by cooking the rice in the commercial pressure cooker. Two hundred and fifty gram (250 g) of rice was cooked using 375 ml of water (2:3). The cooking time for pressure cooking was 9 ± 2 minutes. The gas was turned off and the gas inside the pressure cooker cooked was allowed to release by itself in normal temperature by allowing the pressure cooker to cool for 45 minutes.

3.4.3.2 Stir-oil frying followed by pressure cooking

For stir-oil frying, the cooking time was 12 ± 1 minutes including 4 minutes of frying of rice in oil. The stir-oil fried followed by pressure cooked rice (*chamre*) was prepared in two stages. Firstly, the pressure cooker was heated for 1 minute and 20 ml of refined sunflower oil was heated in the cooker. The rice (250 g) was poured into a pressure cooker and was fried for 4 minutes. 375 ml of water was then added to fried rice and was cooked under pressure for 6 minutes. After 6 minutes of cooking the gas was turned off and the cooked stir-oil fried followed by pressure cooked rice was allowed to cool by itself in normal temperature for 45 minutes.

For both of the cooking methods applied in this study, the heat was maintained constant.

3.5 Statistical method

3.5.1 Study population

Inclusion criteria was of normal body weight, within normal body mass index (BMI) range, non-chronic smokers, non-chronic alcoholics, with no any notable disease history, and age of 18-49 years were involved in the determination of the GI of the *Sona Mansuli* rice. The selection of volunteers or participants in the study was based on the guidelines provided by FAO/WHO entitled "Glycemic index: Methodology" (FAO/WHO, 1998). For this study, 10 students of Central Campus of Technology were taken as volunteers. Those volunteers taken were 5 male and five female.

3.5.2 Study variables

The variables taken in this study were the methods of cooking rice, i.e., pressure cooking and stir-oil fried followed by pressure cooking. The response analysed was postprandial blood glucose response in the volunteers as affected by variation on cooking methods.

3.6 Validity and reliability

To maintain the validity and reliability of the study, blood glucose measuring method's precision was ascertained. Glucometer was chosen for blood glucose measurement. The precision of the method chosen was evaluated. Ideally, glucometer reading with a CV > 3% should not be used for scientific purpose (Brouns *et al.*, 2005).

For validitating the glucometer use for the research, 5 samples of 5 individuals were taken from glucometer and coefficient of variation calculated as 1.24%. Since the results given by glucometer was within the acceptable criteria, Clever Chek glucometer was used for the research.

Also the complete glycemic index determination procedure followed strictly in this research was based on procedure given by FAO/WHO (1998).

3.7 Data collection

Initially, all the selected subjects were informed and oriented about the aim and purpose of the study. They were also counseled about the roles and conditions to be followed during their involvement in study period.

On the previous day of blood glucose tests, the volunteers were recommended to have a uniform meal pattern and no intake of alcohols and unusual foods before test day. They were also recommended not to have vigorous exercises except regular normal exercises and to have sound sleep of 8 hours at night. Subjects were on fast for 11 hours, from the previous evening before examination.

On the test day, after the volunteers attended the lab, they were requested to wash their hands with soap and water and to rest for 15 min. The fasting blood glucose of all the subjects were measured and all subjects were fed reference food (25 g glucose), dissolved in 250 ml of water and both test foods ie., pressure cooked rice (108.58 g) and stir-oil fried followed by pressure cooked rice (124.22 g) containing 25 g of available carbohydrates with 250 ml of water on different days of 2 days interval. Foods were consumed within 10 min. The fingers of subjects were washed with alcohol (70%) swab before pricking with lancet for capillary blood glucose measurement. The subjects carried out self-monitoring blood glucose measurement seven times in total: at fasting, and at 15, 30, 45, 60, 90, and 120 min by using glucometer (Clever-Chek) and every reading of individual volunteer were recorded. Next test was then performed in 2 days interval allowing for the wash-out period. Glucose response curve was made based on blood glucose levels of volunteers during fasting, 15, 30, 45, 60, 90, and 120 minutes after consumption of the foods. The area under the curve was calculated geometrically using trapezoid method (the area under fasting conditions was ignored).

3.8 Data analysis

Preliminary data analysis was done using Microsoft Excel (2010). Both one way and two way analysis of variance (ANOVA) followed by the least significant difference test at 5% significance level (LSD 5%) was carried out using IBM SPSS V 20 to describe the effect

of rice cooking methods (Pressure cooking and Stir-oil fried followed by pressure cooking) on GI of *Sona Mansuli* rice.

3.9 Logistical and ethical considerations

Ethical approval was taken from the Nepal Health Research Council (NHRC), under the government of Nepal for conducting the research. Written permission was taken from the Department of Nutrition and Dietetics, Central Campus of Technology to conduct the research. Written Consent was also taken from each volunteer to obtain the blood samples and other necessary information. The purpose of the study was informed to participants prior to the interview. Privacy and confidentiality of the respondents were maintained throughout and after the study period.

3.10 Research design framework

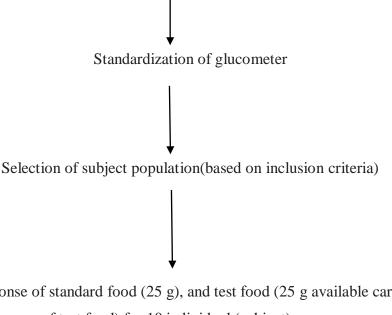
Selection of the most common variety of rice (Sona Mansuli) grown in Bardibas, Nepal

Physico-chemical properties of rice variety (length, breadth, l/b ratio, 1000 kernel weight, extraneous matter, moisture content, protein, fat, crude fiber, total available carbohydrate) determination.

Survey on procedure for cooking the rice through different methods

- a. Pressure cooking
- b. Stir-oil fried followed by pressure cooking *(Chamre)*

(cooking time, temperature, amount of water to be added, method of cooking were also surveyed and optimized)



Blood glucose response of standard food (25 g), and test food (25 g available carbohydrate of test food) for 10 individual (subject)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Subject 1	Standard food	Standard food	Pressure cooked rice	Pressure cooked rice	Stir-oil fried followed by pressure cooked rice	Stir-oil fried followed by pressure cooked rice

••

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	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Subject 10	Standard food	Standard food	Pressure cooked rice	Pressure cooked rice	Stir-oil fried followed by pressure cooked rice	Stir-oil fried followed by pressure cooked rice

Calculation of IAUC, GI and GL of cooked rice

Statistical analysis to find the effect of cooking method on GI of rice

Part IV

Results and discussion

The rice sample was analyzed for physico-chemical properties. For the determination of GI, the selected volunteers were given 25 g of glucose and the amount of cooked rice (pressure cooked and stir-oil fried followed by pressure cooking) equivalent to 25 g of available carbohydrate. Rice samples were given to volunteers on 2 days interval. The glucose responses over the test period recorded in duplicate and values were plotted in the graph to determine the glycemic index of glucose, pressure-cooker cooked, and stir-oil fried rice followed by pressure cooking.

4.1 Physical properties of Sona Mansuli rice

The physical properties of *Sona Mansuli* rice measured during this study are tabulated in Table 4.1.

Parameters	Values
1000 kernel weight (g)	16.35± 0.67*
l/b ratio	2.66± 0.15*
Extraneous matters (in 250 g sample rice) (%)	$0.41 \pm 0.03*$

Table 4.1 Physical properties of Sona Mansuli rice

*The values are means \pm standard deviation of the triplicate measurements and standard deviation.

The 1000 kernel weight and l/b ratio of the rice sample were 16.35 g and 2.66, respectively. According to Prakash (2019), the 1000 kernel weight and l/b ratio of *Sona Mansuli* rice were 19.53 g and 3.25, respectively. As the percentage of extraneous matter determines the quality of rice, the rice used in this study was of superior quality as it contained only 0.41% of extraneous matters. The maximum tolerable limit of extraneous matter in *Sona Mansuli* is 0.50% (NFC, 2062).

4.2 Chemical compositions of *Sona Mansuli* rice.

The proximate composition of rice as obtained in the variety *Sona Mansuli* is tabulated in Table 4.2.

Parameters	Values
Moisture content	13.53 ± 0.11
Crude protein	$6.31 \pm 0.13*$
Total ash	$0.5 \pm 0.1*$
Fat	$0.67 \pm 0.09*$
Crude fiber	$0.57 \pm 0.20*$
Total carbohydrate	$78.06 \pm 0.42*$

Table 4.2 Chemical properties of Sona Mansuli rice

*All values were expressed in wet percentage basis. The values are means of the triplicate measurements and standard deviation.

The moisture content of *Sona Mansuli* rice was found 13.53%. The maximum tolerable limit for moisture content in case of rice is 14% (NFC, 2062). The crude protein, total ash, fat, crude fiber and total carbohydrate of *Sona Mansuli* was 6.31%, 0.5%, 0.67%, 0.57%, 78.06% respectively. In a study conducted by Shobana *et al.* (2012), showed that the moisture content, protein, fat and total carbohydrate content of *Sona Mansuli* (termed as *Sona Masuri* in her research) rice was found to be 11.4%, 5.5%, 0.22% and 79.7% respectively, the values obtained were nearly similar to that obtained in this research.

According to USDA, the moisture content, crude protein, crude fat, crude fiber and total carbohydrate of white rice should be 11.62%, 7.13%, 0.66%, 1.3%, and 79.95% respectively on a dry basis (David *et al.*, 2016). These results are near to USDA specification, which indicates that the rice variety used was of good quality. According to the study done by Rashmi and Urooj (2003), the protein content of *Sona Mansuli* rice was 6.7%, which is similar to the findings of this study. The ash and fat content was 1.7% and 1.8% respectively, which was slightly more than the findings of this study.

4.2.1 Determination of available carbohydrate

The determination of available carbohydrate content for both test foods (pressure cooked rice and Stir-oil fried followed by pressure cooked rice) was carried out prior to the measurement of blood glucose response of the subjects. The amount of both the test foods, containing 25 gram of available carbohydrate was evaluated in order to find the effect of pressure cooked and stir-oil fried followed by pressure cooked rice on GI of *Sona Mansuli* rice. The amount of pressure cooked rice and stir-oil fried followed by pressure cooked rice by pressure cooked rice on GI of *Sona Mansuli* rice. The amount of pressure cooked rice and stir-oil fried followed by pressure cooked rice on GI of *Sona Mansuli* rice containing 25 g available carbohydrate was 108.58 g and 124.22 g respectively.

4.3 Anthropometric parameters of the volunteers

The anthropometric characteristics of the subjects who volunteered in this research are tabulated in Table 4.3.

Gender	Age (Y)	Weight (kg)	Height (cm)	BMI (kg/m ²)
Male (n=5)	22.8±2.58	62±3.16	168.6±3.13	21.80±0.86
Female (n=5)	20.4 ± 0.54	51.4±2.70	157.6±5.63	20.76±1.93
Total (n=10)	21.6±1.56	56.7±2.93	163.1±4.40	21.28±1.39

Table 4.3 Anthropometric characteristics of subject participants

The values are mean \pm standard deviation of samples.

The average age of the participants was 21.6 ± 1.56 years. The average age for male participants was 22.8 ± 2.58 years, and for females, it was 20.4 ± 0.54 . The average BMI of volunteers were 21.28 ± 1.39 Kg/m². The average BMI of male and female participants was 21.80 ± 0.86 20.76 ± 1.93 kg/m², respectively. All the individuals met the inclusion criteria needed for the study.

4.4 Effect of cooking methods on blood glucose level and IAUC value

4.4.1 Blood glucose level (mg/dL)

The mean blood glucose level (mg/dL) of 10 volunteers after consumption of glucose, pressure cooked rice, and stir-oil fried followed by pressure cooked rice at different time interval are tabulated in Table 4.4.

Time	Glucose (mg/dL)	Pressure cooked rice	Stir- oil fried followed
		(mg/dL)	by pressure cooked rice
			(mg/dL)
0	84.4±7.02	84.2±6.44	85.9±9.40
15	116.3±10.96	99.1±9.54	94.4±10.16
30	143.75±8.78	115.5±9.46	103.25±9.94
45	124.5±11.89	105.7±15.69	101.3±9.97
60	106.15±14.34	97.05±10.47	96.25±9.42
90	82.8±13.16	90.55±6.83	96.8±10.09
120	75.35±9.60	90.25±6.26	96.1±10.23

Table 4.4 Periodic change in blood glucose (mg/dL) in volunteers after consumption of reference food (glucose), pressure cooked rice and stir-oil fried followed by pressure cooked rice.

The values are presented as mean \pm standard deviation and are means of duplicate readings.

The mean blood glucose concentration above baseline was compared for reference food (glucose), pressure cooked rice, and stir-oil fried followed by pressure cooked rice at the time intervals of 0, 15, 30, 45, 60, 90, 120 minutes (Table 4.4). The mean blood glucose concentration was nearly the same at 0 minutes for all food types. At 30 minutes, the blood glucose concentration rose to its peak value for all the test foods. A study conducted by Greffeuille *et al.* (2015) was in favour with the results of this study where the peak blood glucose response was obtained at 30 minutes after the ingestion of 50 g glucose used as reference food, and the three types of pasta used as test foods. The mean peak blood glucose concentration of all volunteers at 30 minutes was 143.75±8.78 mg/dL, 115.5±9.46 mg/dL and 103.25±9.94 mg/dL for glucose, pressure cooked, and stir-oil fried followed by pressure cooked rice, respectively (Table 4.4). Peak glucose level is very crucial for type-2 diabetes. A frequent high level of blood glucose in such patients can cause many complications, such as cardiovascular diseases (Collaboration, 2010). Similarly, high blood glucose might put the patients always at a higher risk of death (Balkau *et al.*, 1998). Therefore, the results indicated that the stir-oil fried followed by pressure cooked rice

consumption helps in minimum fluctuations in peak blood glucose level as compared to that of pressure-cooked rice and hence might be helpful to minimize the risk of various cardiovascular diseases and mortality too.

 Table 4.5 Blood glucose responses (mg/dL) of volunteers at 30 minutes after the ingestion of reference and test foods.

Volunteer	Glucose	Pressure cooked rice	Stir-oil fried followed by pressure cooked rice (mg/dL)
1	132.5±3.53 ^a	105.5±0.70 ^b	89.5±6.36 ^b
2	137.5±3.53 ^a	117.5 ± 0.70^{b}	91±4.24 ^c
3	134.5±0.70 ^a	118.5±2.12 ^{ab}	$95{\pm}14.14^{b}$
4	143.5±2.12 ^a	110 ± 1.41^{b}	106 ± 2.82^{b}
5	156.5 ± 2.12^{a}	124±2.82 ^b	109±1.41 ^c
6	153.5±3.53 ^a	131.5±9.19 ^{ab}	113.5±6.36 ^b
7	152.5±3.53 ^a	112±4.24 ^b	110±5.65 ^b
8	135±2.82 ^a	111±2.82 ^b	110.5 ± 2.12^{b}
9	148±5.65 ^a	101 ^b	97 ± 1.41^{b}
10	144±2.82 ^a	124 ± 1.41^{b}	111±5.65 ^b
Total mean	143.5±3.035	115.5±2.54	103.25±5.015

*The values expressed are a mean \pm standard deviation. Figures of the same superscript are not significantly different and figures of different superscript are significantly different as presented in each row in the table.

Analysis of variance showed a significant effect (p < 0.05) of food type on blood glucose level at 30 minutes after consumption of test foods. In most of the volunteers, blood glucose level at 30 minutes was significantly higher in glucose-fed condition than rice fed condition (Table 4.5).

Glucose transportation into the blood is a single step process, whereas rice needs to be digested first by the enzyme before transportation into the blood. Change (rise and fall) in the blood glucose concentration with time was lesser when stir-oil fried followed by pressure cooked rice was fed to volunteer as compared to the glucose and pressure cooked rice (Fig 4.1). The blood glucose level of the volunteers rose and dropped rapidly with time when glucose was fed. In contrast, blood glucose level rose and dropped slowly when pressure-cooked, and stir-oil fried followed by pressure cooked rice was served. Also, it is noticeable in Fig 4.1 and Appendix F that the blood glucose level decreased much slower for stir-oil fried followed by pressure cooked rice and stir-oil fried followed by pressure cooked rice. This indicated an immediate hypoglycemic effect of pressure-cooked rice and stir-oil fried followed by pressure cooked rice fed conditions than glucose fed condition could be the longer time needed for enzymes to break down the complex starch structure present in rice.

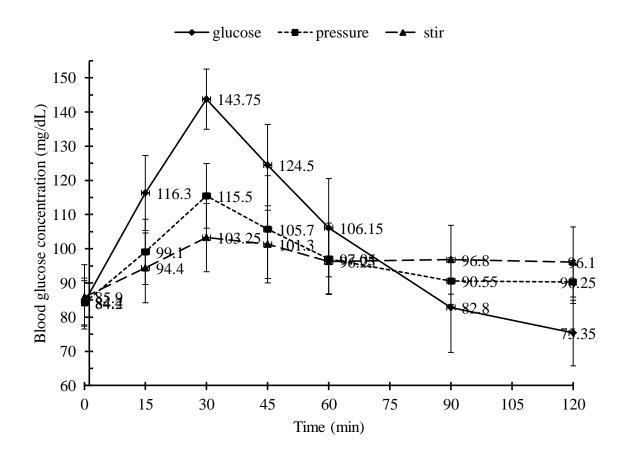


Fig.4. 1 Mean glycemic response curve affected by glucose and test foods

Interestingly, an increase and decrease in blood glucose level in each volunteer was slower when fed stir-oil fried followed by pressure cooked rice than pressure cooked rice. At 120 minutes after consumption of test food, the blood glucose level in each volunteer was higher for stir-oil fried followed by pressure cooked rice than pressure cooked rice (fig 4.1). This indicates much slower digestion of stir-oil fried followed by pressure cooked rice than pressure cooked rice. Such slow release of glucose in the human body makes stiroil fried followed by pressure cooked rice more ideal for type two diabetic patients who need moderate blood glucose level in the body. Stir-oil fried followed by pressure cooking of rice with fats and oil helped to form a complex between amylose and fat called amylolipid complex. A similar result was obtained by Latge et al. (1994) in which, the amylolipid complex was found to demonstrate a hypoglycemic effect on the human body. In addition, the study conducted by Reed et al. (2013) also showed that rapid drying of the rice kernel during stir-frying followed by the gelatinization of rice starch makes the rice texture harder even after pressure cooking. As such, cooked rice disintegrates as hard granules while chewing rather than as paste as in pressure cooked rice. Harder the food structure, lesser will be the enzymatic activity. This phenomenon could be another reason for the slower increase and decrease in blood glucose level in volunteers when fed with stir-oil fried followed by pressure cooked rice than pressure cooked rice alone.

4.4.2 IAUC values

The mean IAUC values of various test foods for each volunteer are tabulated in Table 4.6. Analysis of variance showed a significant effect (p<0.05) of food type on IAUC values of each volunteer. The IAUC values presented in Table 4.6 showed that the incremental area under the blood glucose level curve was always the largest for glucose fed condition followed by pressure cooked rice and stir-oil fried followed by pressure cooked rice in each volunteer. Besides, a wide variation in IAUC values of the same test food was observed among the volunteers. However, each volunteer demonstrated a similar proportional difference on the IAUC values of test foods.

Volunteer	Glucose	Pressure cooked rice	Stir-oil fried followed by
			pressure cooked rice
1	1870±65.1 ^a	1298.5±33.2 ^b	1072.5±21.2 ^c
2	2906.4±101.3 ^a	1908.8±26.5 ^b	1492.5±42.4 ^c
3	2660.9±66.3 ^a	1762.5±31.8 ^b	1526.3±37.1 ^c
4	2754.2±34.3 ^a	1833.8±15.9 ^b	1563.8±26.5 ^c
5	2520.5±21.9 ^a	1601.3±26.5 ^b	1331.3±15.9 ^c
6	3245.6±112.4 ^a	1961.3±37.1 ^b	1616.3±26.5 °
7	2391.69±39.7 ^a	1548.8±15.9 ^b	1286.3±15.9 ^c
8	1958.6±24.9 ^a	1258.3±23.6 ^b	1076.3±26.5 °
9	2183.4±43.7 ^a	1402.5±21.2 ^b	1181.3±25.5 ^c
10	2260.5±29.7 ^a	1403.5±29.4 ^b	1185±31.8 ^c
Total mean	2475.18±430.18	1597.93±257.08	1333.16±204.71

Table 4.6 The IAUC values (mg.min/dL) for volunteers for glucose, pressure cooked, and stir-oil fried followed by pressure cooked rice

*The values expressed are a mean \pm standard deviation. Figures of the same superscript are not significantly different and figures of different superscript are significantly different as presented in table in each row.

As the GI is defined as the incremental area under the curve for the blood glucose response after consumption of a food relative to that produced by a reference food given in an equivalent carbohydrate amount (Jenkins *et al.*, 1981), which means that the GI values depends upon the area of the blood glucose response over the specific time period for test and reference food. Therefore, the small IAUC values for the test food signifies that the GI of that food will be smaller. In this study, the mean IAUC value of pressure-cooked rice (1597.93±257.08 mg.min/dL) was higher than the mean IAUC values of stir-oil fried followed by pressure cooked rice (1333.16±204.71 mg.min/dL) thus resulting in the higher value of GI for pressure-cooked rice than that of stir-oil fried followed by pressure cooked rice than that of stir-oil fried followed by pressure cooked rice than that of stir-oil fried followed by pressure cooked rice (Table 4.7). According to the study done by Lavin and Read (1995), consumption of foods with a lower glycaemic response has been promoted to have several

putative health benefits including promotion of long-term weight loss by influencing satiety and food intake and reduced glucose and insulin responses. In reference to this study, the consumption of stir-oil fried followed by pressure cooked rice having a low glycemic response as compared to the pressure cooked rice might be one of the benefits to promote long term weight loss.

4.5 Effect of cooking methods on GI and GL

4.5.1 GI value of test foods

Glycemic index, a numerical index, refers to the glucose-raising ability of carbohydrate. In this study, the GI values of the pressure cooked rice and stir-oil fried followed by pressure cooked rice ranged from 60.43 to 69.44 and 49.79 to 57.35, respectively (Annex: O). The overall mean value of the glycemic index was lower for stir-oil fried followed by pressure cooked rice than pressure cooked rice as shown in Table 4.7. Stir-oil fried followed by pressure cooking of rice in oil before pressure cooking reduced the GI by 13.40% to 21.81% (Annex: O).

Parameters	Glucose	Pressure cooked rice	Stir-oil fried followed by pressure cooked rice
GI	100	64.72±2.49	54.07±2.57
GI classification	High	Medium	Low
GL	25	16.18±0.62	13.51±0.64
GL classification	High	Medium	Medium

Table 4.7 Mean GI and GL values of tests foods

The values expressed are a mean \pm standard deviation.

The GI values are classified as a low (<55), medium (55-69) or high (\geq 70) (Brand-Miller *et al.*, 2003) Interestingly, as it can be seen in Table 4.7, the pressure cooked rice, which had medium GI (64.72±2.49) showed low GI (54.07±2.57) when rice was stir-fried with oil before pressure cooking. It was found that the pressure cooked *Sona Mansuli* rice was the food that fell into the category of medium GI foods whereas when a same variety of rice was stir-oil fried followed by pressure cooking, it showed the characteristic of low GI food as the hypoglycemic effect of stir-fried rice was more pronounced than the pressure cooked

rice. A study done by Shobana *et al.* (2012), in which *Sona Mansuli*, variety of rice was cooked in a rice cooker (cooked with rice to water ratio of 1:3.5) were examined, found that the GI of these three varieties was 72.0 ± 4.5 . Whereas, the result of the present study showed that the GI of pressure cooked and stir-oil fried followed by pressure cooking of *Sona Mansuli* rice (cooked with rice to water ratio of 2:3) was 64.72 ± 2.49 and 54.07 ± 2.57 respectively. These results might demonstrate that a simple modification in the cooking method of rice could significantly decrease the GI of the rice. This strategy could be used to plan a diet of type 2 diabetic patient. This results might be in favor with the study conducted by Björck *et al.* (1994), which stated that many factors such as food form, particle size, cooking, processing and starch structure affect the GI of food. The study done by Kaur *et al.* (2016) also stated that rice is generally considered a high GI food; however this depends on varietal, compositional, cooking (water absorption and volume absorption during cooking, with the hardness of boiled rice and cooking time), and accompaniment factors.

As the selection of low-GI foods has been advocated in healthy eating guidelines for people with diabetes, thus it appears that oil/fat may be added to rice during cooking to lower its GI value. Rice cooked in such a manner would be better for individuals for having the modifications in a healthy diet for healthy living. Furthermore, *Sona Mansuli* rice cooked in this manner would also be better suited to people with diabetes.

Glycemic load corresponds to the amount of available carbohydrate capable of increasing the blood glucose level. In this study, the glycemic load of the pressure cooked and stir-oil fried followed by pressure cooked rice varied from 15.56 to 18.80 and 12.87 to 14.15, respectively (Table 4.7). Analysis of variance of the means of GL values indicated a significant effect (p<0.05) of cooking method on GL of the rice. Stir-oil fried followed by pressure cooking of rice significantly (p<0.05) reduced the GL of the rice. This phenomenon indicates that stir-oil frying before pressure cooking helped decrease the available carbohydrate of the rice.

	aluaasa	Pressure cooked rice	Stir-oil fried followed by
Volunteer	glucose	Pressure cooked rice	pressure cooked rice
1	100 ^a	69.44±1.77 ^b	57.35±1.14 ^c
2	100^{a}	65.67 ± 0.91^{b}	51.35±1.45 ^c
3	100^{a}	$66.24{\pm}1.19^{b}$	57.35±1.39 ^c
4	100^{a}	66.57 ± 0.57^{b}	56.77±0.96 ^c
5	100^{a}	$63.52{\pm}1.05^{b}$	52.81±0.63 ^c
6	100^{a}	60.43 ± 1.14^{b}	49.79±0.83 ^c
7	100 ^a	64.76 ± 0.66^{b}	53.77±0.67 ^c
8	100 ^a	$64.24{\pm}1.20^{b}$	54.9±1.41 ^c
9	100 ^a	64.23 ± 0.95^{b}	54.08±1.24 ^c
10	100^{a}	$62.10{\pm}1.27^{b}$	52.43±1.40 ^c

Table 4.8 GI values of glucose, pressure cooked rice and stir-oil fried followed by pressure

 cooked rice in volunteers

*The values expressed are a mean \pm standard deviation. Figures of the same superscript are not significantly different and figures of different superscript are significantly different

Analysis of variance indicated a significant effect (p<0.05) of food type on GI values of each volunteer. The results of this study showed a hypoglycemic effect of rice when compared to glucose. Besides, stir frying of rice in oil before pressure cooking significantly (p<0.05) decreased the GI value as compared to pressure cooked rice alone (Table 4.8). The results of this study are in agreement with Kumar *et al.* (2018) where authors stated that the addition of fat on rice during cooking reduced the GI. The same author also mentioned that magnitude of GI lowering was more pronounced when fats/oils were added during cooking, compared to when it was added to the cooked rice. A noticeable variation in proportional changes in glucose level pattern was observed among the volunteers in this study. This could be attributed to variations in chewing habit and body physiology of the individual volunteer as the study done by Ranawana *et al.* (2014), also favours with this statement that the rate of chewing also affects the GI of food .

4.6 Effect of gender and cooking methods on peak blood glucose level

Peak blood glucose level corresponds to the maximum blood glucose level reached after the consumption of food. Annex G depicts a gender-dependent effect of cooking methods (food type) on the blood glucose level. The blood glucose level of each volunteer peaked in 30 minutes after the consumption of test foods. Analysis of variance indicated no significant (p< 0.05) effect of gender on peak blood glucose level of the individual volunteer (Table 4.6). However, as discussed above, cooking methods (types of foods) had a significant effect on the peak blood glucose level.

In both the pressure cooked and stir-oil fried followed by pressure cooked rice, the peak value rose to its peak at 30 minutes after the consumption of food. However, the mean peak value for the stir-oil fried followed by pressure cooked rice was less than the mean peak value of pressure-cooked rice. The possible reason behind the lesser value of peak blood glucose for stir-oil fried followed by pressure cooked rice might be due to the addition of oils during stir-frying of rice causes rapid drying of rice granules which inhibits the complete gelatinization process during pressure cooking making starch less digestibe as compared to that of pressure-cooked rice alone. Cummings and Englyst (1995), has stated that when starch granules are fully gelatinized and dispersed, starch becomes easily digestible. Thus the high peak value of pressure cooked rice in this study might be due to the more gelatinization and dispersion of starch granules of pressure cooked rice as compared to stir-oil fried followed by pressure cooked rice. Similarly, the study conducted by Behall et al. (1988), stated that lipid-amylose complexes have been shown to be digested more slowly than starch alone and this might favour this present study result that the stir-oil fried followed by pressure cooked rice had lower peak value as compared to that of pressure cooked rice.

4.7 Effect of gender and cooking methods on IAUC value

A two-way ANOVA showed a significant (p<0.05) effect of cooking methods and gender on the IAUC values. In each type of test foods, IAUC value for male was always lower than those of female (Annex: G). A higher IAUC value corresponds to a condition when a blood glucose level over a fixed time is relatively higher in the human body. It is worth mentioning that IAUC values of pressure cooked and stir-oil fried followed by pressure cooked rice was higher for female than male. Such a trend might indicate that females were likely to digest rice better than male. However, such better digestibility in female could be attributed to chewing ability, digestive system, body physiology, and a combination of all. In a study conducted by Soeters *et al.* (2007), showed that the plasma glucose levels were lower in women as compared to men after 38 h of fasting which was in favour with the result of the present study where the fasting glucose of females were always lower than those for male.

4.8 Effect of gender and cooking methods on glycemic index and glycemic load

On analyzing the effect of gender on GI and GL, analysis of variance indicated that there was no significant (p<0.05) effect of the gender on the glycemic index and glycemic load of the test foods. The results suggested that the lowering of glycemic index and glycemic load by changing the cooking methods is equally applicable for both genders. This study showed that the mean GI of pressure cooked and stir-oil fried followed by pressure cooked rice was almost similar in the case of both genders (male and female). A recommendation by Brouns *et al.* (2005), also suggested that no differences had been observed in glycemic response between males and females. The results also signifies that stir-oil frying of rice before pressure cooking can be recommended for both gender. Stir-fat frying of rice before pressure cooking is a widely used method to make an everyday dish which is very similar to a local dish called *Chamre*.

Parameter	Male	Female				
Mean IAUC of glucose	2245±234.1	2705.2±449.38				
Mean IAUC of pressure cooked rice	1450.90±117.09	1744.91±266.91				
Mean IAUC of stir-oil fried followed by	1218±96.9	1455±205.70				
pressure cooked rice						
Mean GI of pressure cooked rice	64.8±2.77	64.62±2.51				
Mean GI of stir-oil fried followed by	54.15±1.95	54.04±3.33				
pressure cooked rice						

Table 4.9 Mean IAUC and GI values in male and female

The values expressed in Table 4.9 are a mean \pm standard deviation.

	Female			Male		
Time	glucose	pressure	Stir-oil	glucose	pressure	Stir-oil
(min)	(mg/dL)	(mg/dL)	fried	(mg/dL)	(mg/dL)	fried
			(mg/dL)			(mg/dL)
0	81.8±7.2	81.9±6.6	85±10.3	87±6.09	86.5±5.66	86.8±8.81
15	116±7.8	96.710.13	94±11.5	116.4±13.8	101.5±8.7	94.8±9.18
30	140.8±7.7	117.7±8.75	103.2±10.7	146.7±9.13	113.3±10.07	103.3±9.67
45	127.8±12.13	106.3±13.68	103±10.19	121.2±11.29	105.1±18.21	99.6±9.9
60	110.3±16.7	94.7±11.6	96.4±10.4	102±10.7	99.4±9.11	96.1±8.9
90	79.2±10.86	89.9±7.8	96.610.78	86.4±14.8	91.2±5.95	97±9.93
120	72.7±11.1	88±5.86	96.3±9.36	78±7.46	92.5±6.09	95.9±11.56

Table 4.10 The gender-based mean blood glucose (mg/dL) of 10 healthy volunteers after consumption of reference food, pressure cooked rice and stir-oil fried followed by pressure cooked rice.

The values expressed in Table 4.10 are a mean \pm standard deviation. The time was measured in minutes.

Table 4.10 illustrates that the fasting blood glucose concentration of female subjects were less than that of male subjects all conditions. It means that the blood glucose value of males was higher than that of females in fasting state. The study conducted by Soeters *et al.* (2007), also favoured the present study result, which showed that the plasma glucose levels were lower in women as compared to men after 38 h of fasting. Whereas, the peak blood glucose value was obtained at 30 minutes for both males and females. It showed that the time at which the blood glucose value rose to its peak was same for both the genders. A study conducted by Greffeuille *et al.* (2015) was in favour of results of this study where the peak blood glucose response was obtained at 30 minutes for both genders.

Part V

Conclusion and recommendations

5.1 Conclusion

In the present work, the effect of cooking methods on glycemic responses of the rice (*Sona Mansuli*) was studied. Various parameters related to glycemic response were compared among the standard food (glucose), direct pressure cooked rice, and stir-oil fried rice followed by pressure cooking. The following points could be concluded from the results obtained,

1. The physico-chemical properties of *Sona Mansuli* rice was examined. The 1000 kernel weight, l/b ratio and the amount of extraneous matters were found to be 16.35 ± 0.67 , 2.66 ± 0.15 and 0.41 ± 0.03 % respectively. The moisture content, crude protein, total ash, fat, crude fiber and total carbohydrate of *Sona Mansuli* rice used in this study were found to be 13.53 ± 0.11 , 6.31 ± 0.13 , 0.5 ± 0.1 , 0.67 ± 0.09 , 0.57 ± 0.20 and 78.06 ± 0.42 % respectively.

2. The mean IAUC obtained for stir-oil fried rice followed by pressure cooked rice was smaller than that of pressure cooked rice alone when fed at different times to the volunteers. The mean blood glucose concentration of reference food (glucose) and both test foods (pressure cooked and stir-oil fried followed by pressure cooked rice) rose to peak at 30 minutes after the ingestion of those foods. The peak blood glucose concentration at 30 minutes for glucose, pressure cooked rice and stir-fried rice were 143.75 ± 8.78 mg/dL, 115.5 ± 9.46 mg/dL and 103.25 ± 9.94 mg/dL respectively.

3. The GI of pressure cooked rice and stir-oil fried followed by pressure cooking of *Sona Mansuli* rice was found to be 64.72 ± 2.49 and 54.07 ± 2.57 respectively. It means that the GI of pressure cooked rice was under medium category and that of stir-oil fried followed by pressure cooked rice was under low category under GI classification. The GL of pressure cooked rice and stir-oil fried followed by pressure cooked rice was found to be 16.18 ± 0.62 and 13.51 ± 0.64 respectively, which both values lied under medium category under GL classification.

4. Glycemic index and glycemic load of the rice (*Sona Mansuli*) could be reduced to an acceptable level by stir-oil frying of rice before pressure cooking.

5. The ingestion of rice cooked with fats/oils has a positive hypoglycemic effect than cooked by pressure cooking method

5.2 Recommendations

The followings are the recommendations for future research studies.

1) The stir-oil frying followed by pressure cooking of might be beneficial to lower the glycemic index of Sona Mansuli rice more significantly than pressure cooking alone.

2) Further studies with different varieties of rice, different cooking methods and with applications of other various types of edible fats/oils can be carried out.

3) This test can be carried out in diabetic subjects in order to see the hypoglycemic effect of stir-fat fried *Sona Mansuli* rice.

Summary

Rice (Oryza sativa) is the most important food crop in Nepal and the staple food of more than half of the world's population. Rice has both nutritional and religious significance in the context of Nepal's population. Among different varieties of rice cultivated in Nepal, *Sona Mansuli* is one of the primarily grown varieties in most productive land in Nepal (Terai region).

Rice being a significant source of energy in regular Nepali diet, manipulation of digestibility of rice through cooking might become one of the beneficial steps towards controlling some epidemic like diabetes by diet modification. This study was based on evaluating the effect of different cooking methods in the glycemic index of *Sona Mansuli* rice. The selected variant of rice was cooked through two methods, which included pressure cooking and stir-oil frying followed by pressure cooking. Both cooked rice demonstrated a lower GI value (pressure cooked: 64.72 ± 2.49 , stir-oil fried followed by pressure cooking 54.04 ± 2.57) than the reference food (glucose).

The result showed that the GI value of pressure-cooked Sona Mansuli rice was medium and was low for the stir-oil fried followed by pressure cooked rice. Whereas, the GL of both pressure cooked and stir-oil fried followed by pressure cooked rice was medium (16.18±0.62 and 13.51±0.64 respectively). The gender-wise GI values for both male and female were almost similar to both pressure cooked and stir-oil fried followed by pressure cooked rice. In comparison between pressure cooking and stir-frying, the stir-oil frying before pressure cooking showed more hypoglycemic effect in the subjects. The possible reason for the hypoglycemic effect of stir-oil fried followed by pressure cooked rice might be the formation of amylose-lipid complexes, which were responsible for lowering the starch hydrolysis rates, as supported by various literature reviewed during the research. In the present study, modification of rice cooking method, for example, stir-oil frying before cooking, demonstrated a significant decrease in glycemic responses. GI and GL were decreased significantly when rice was stir fried in cooking oil before pressure cooking. In addition, the results also demonstrated that the stir-oil fried followed by pressure cooked rice digested much slower than the regular cooked rice. Therefore, stir-oil fried followed by pressure cooked rice could be beneficial for both healthy populations as well as people with diabetes to improve their eating habits and ultimately for the betterment of their health.

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Appendices

A. NHRC Approval



Ref: Approval of thesis proposal entitled Study on effect of cooking method on glycemic index of Sona mansuli rice

Dear Ms. Dhungana,

It is my pleasure to inform you that the above-mentioned proposal submitted on 20 September 2018 [Reg. no. 624/2018] has been approved by Nepal Health Research Council (NHRC) National Ethical Guidelines for Health Research in Nepal, Standard Operating Procedures Section 'C' point no. 6.3 through Expedited Review Procedures.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **February 2019**.

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and submit progress report in between and full or summary report upon completion.

As per your thesis proposal, the total research budget is NRs 21,000 and accordingly the processing fee amounts to NRs 1,000. It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

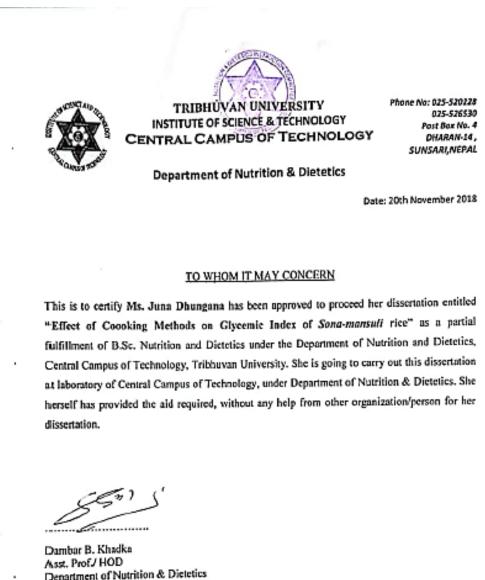
Thanking you,

Dean

Nirbhay Kumar Sharma Deputy Chief Admin Officer

> Tel: +977 1 4254220, Fax: +977 1 4252469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

B. Approval from College/Department of Nutrition and Dietetics



Asst. Prof HOD Department of Nutrition & Dietetics Central Campus of Technolgy, TU Dharan CHAIRMAN

Untrition & Dicteties Instruction Consiler.

C. Consent Form

Namaste!

I Miss. Juna Dhungana, a graduate student in the Department of Nutrition and Dietetics conducting a dissertation work for the award of bachelor's degree in Nutrition and Dietetics. The topic for the study is "study on effect of pressure cooking and stir-oil fried followed by pressure cooking method on glycemic index of sona mansuli rice".

I would like to tell you that this is solely for a dissertation procedure, that your participation is voluntary and you reserve the full right to withdraw from the study at any time without having to give a reason. Confidentiality will be maintained and only be shared for academic purposes.

I hereby give consent to participate in the above study. I am also aware that I can withdraw this consent at any later date if I wish to. This consent form being signed voluntarily indicates participate in the study until I decide otherwise. I understand that I will receive a signed and dated copy of this form.

I consent to:

- Attending the glycemic index facility on 14 days following an overnight fast
- Consuming a test food

• Providing eight blood samples obtained by finger pricking over two hours on each glycemic index test day.

- I know that:
- \checkmark The data may be published but my name will not be disclosed
- ✓ My participation is voluntary
- \checkmark I am free to withdraw from the project at any time without any disadvantage
- \checkmark I agree to take part in this project
- \checkmark I have signed this consent form before my participation in the study.

Signature of Participant:

.....

Date:

Mobile no.:

I hereby state the study procedures were explained in the detail and all questions were fully and clearly answered to the above-mentioned participant /his/her relative. In case of any infections or any causes to disease condition due to taking part in research or due to ingestion of test food, I will be responsible and hence, I would be providing all the medical expenses and compensations.

Researcher's sign:..... Date: E-mail: junadhungana01@gmail.com Phone Number: 9860075060

D. Participant Questionnaire

(Note: In cases of boxes given below, please tick $\sqrt{}$ for the correct options).

Name:	ID No
Gender: male	female
Postal address:	
Email address: (if app	plicable)
Telephone numbers:	(Work/Home/Mobile)
Date of birth:	Age:
Height:	Weight: BMI:
smoker?	ker, past smoker, current cigarette smoker, cigar smoker or pipe Yes No
Frequency of smokin	g (if applicable): (Please Specify)
	gnosed with diabetes mellitus, heart disease, stroke, cardiovascular uses of the digestive system?
	Yes No
1 2 3	rent medicines, dose and frequency:
Please list current sup	oplements, brand, and frequency:
Are you Married? Are you Pregnant	Yes No Second Se
Do you have any foo	d allergies?
	Yes No
Please list for any for	od allergies you have (if applicable):

1. _____

2. _____ 3. _____ Others: _____

Please indicate to which ethnic group you belong:_____

Please indicate your Religion: _____

E. Blood Glucose Monitoring Form

To be filled by researcher

ID No. _____

Cooking Method	Blood	l Glucose	Respo	nse at (U	nit mg/dL)	Remarks
	Time	Day 1	Day 2	Day 3	Day 4	
Reference food (Glucose 25g)	0 min 15 min					
	30 min					
	45 min					
	60 min					
	90 min					
	120 min					
Pressure cooking	0 min					
	15 min					
	30 min					
	45 min					
	60 min					

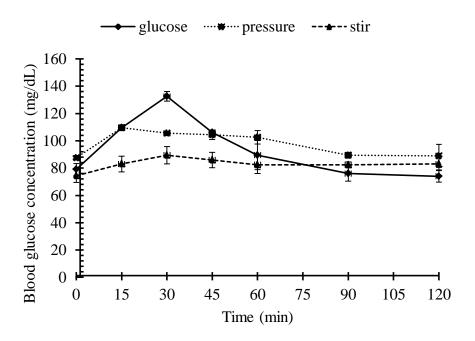
-			 	
	90			
	min			
	120			
	min			
Q.(: '1				
Stir-oil	0			
fried followed	min			
by	15			
pressure	min			
cooking	30			
	min			
	45			
	min			
	60			
	min			
	90			
	min			
	120			
	min			

(Note: The blood samples for reference food, pressured cooked rice and stir-oil fried followed by pressure cooked rice will be collected on different days. The gap of 2 days is kept for Blood Glucose Response (BGR) in order to maintain the wash out period of mentioned foods from body)

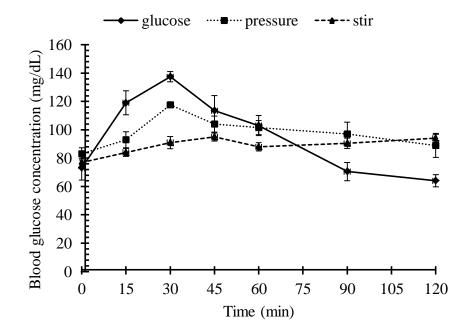
Sign of participant

Sign of Researcher

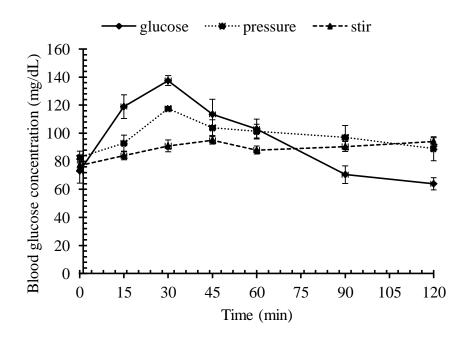
F. Blood glucose response curve subject wise



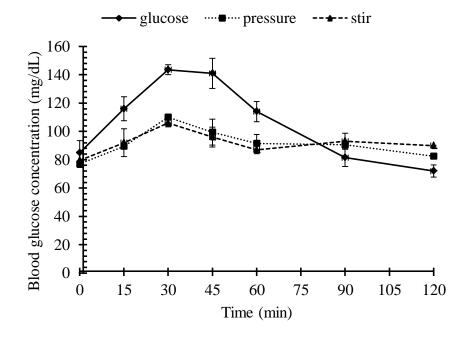
Graph 1.Mean blood glucose response curve for Subject 1



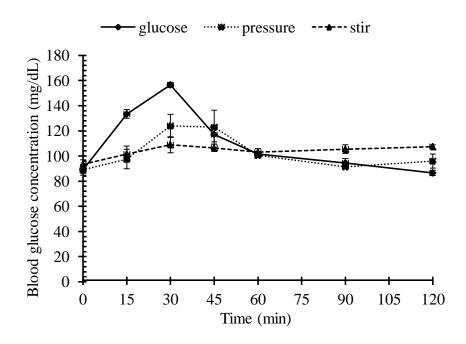
Graph 2. Mean blood glucose response curve for subject 2



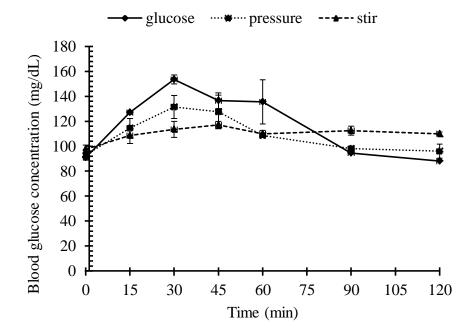
Graph 3. Mean blood glucose concentration for subject 3



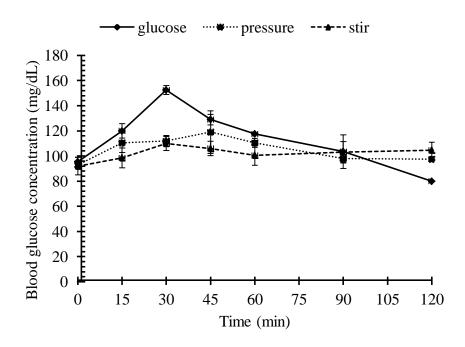
Graph 4. Mean blood glucose response curve for subject 4



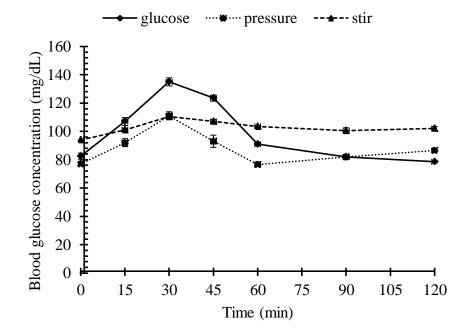
Graph 5. Mean blood glucose response curve for subject 5



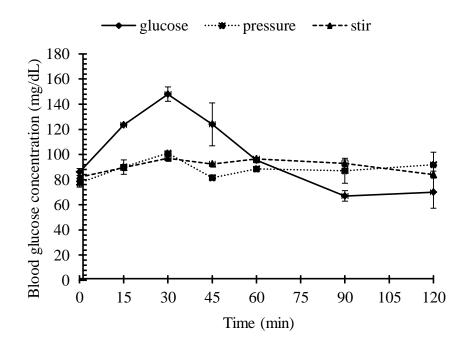
Graph 6. Mean blood glucose curve for subject 6



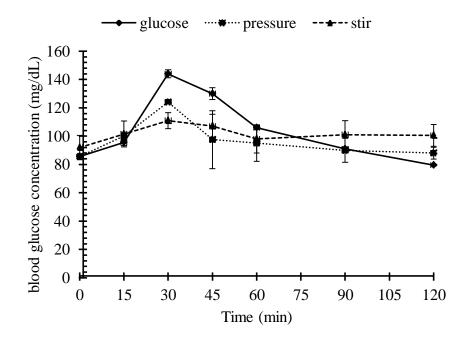
Graph 7. Mean blood glucose response curve for subject 6



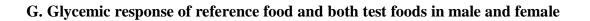
Graph 8. Mean blood glucose response curve for subject 8



Graph 9 Mean blood glucose response curve for subject 9



Graph 10. Mean blood glucose response curve for subject 10



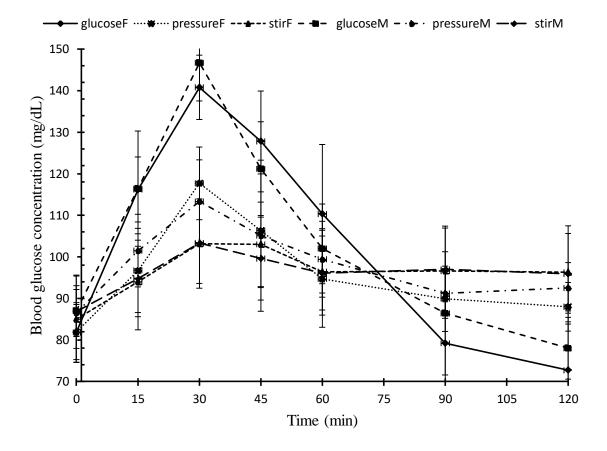


Fig. Glycemic response of reference food and both test foods in male and female

H. IAUC and ANOVA Table of Subjects

1. Subject 1

Source	DF	Adj SS	Adj	F-	P-
			MS	Value	Value
Cooking	2	675819	337910	175.33	0.001
method					
Error	3	5782	1927		
Total	5	681601			

2. Subject 2

Source	DF	Adj SS	Adj MS	F-	P-
				Value	Value
Cooking	2	2111789	1055894	248.32	0.000
method					
Error	3	12756	4252		
Total	5	2124545			

Source	DF	Adj SS	Adj	F-	P-
			MS	Value	Value
Cooking	2	1433714	716857	317.17	0.000
method					
Error	3	6780	2260		
Total	5	1440494			

Source	DF	Adj SS	Adj	F-	P-
			MS	Value	Value
Cooking	2	1558270	779135	1097.4	0
method					
Error	3	2130	710		
Total	5	1560400			

5. Subject 5

Source	DF	Adj SS	Adj	F-	Р-
			MS	Value	Value
Cooking	2	1554824	777412	1623.27	0
method					
Error	3	1437	479		
Total	5	1556261			

Source	DF	Adj SS	Adj MS	F-	Р-
				Value	Value
Cooking	2	2948733	1474366	300.8	0
method					
Error	3	14704	4901		
Total	5	2963437			

Source	DF	Adj SS	Adj	F-	Р-
			MS	Value	Value
Cooking	2	1334288	667144	960.48	0
method					
Error	3	2084	695		
Total	5	1336372			

8. Subject 8

Source	DF	Adj SS	Adj	F-	Р-
			MS	Value	Value
Cooking	2	868122	434061	691.85	0
method					
Error	3	1882	627		
Total	5	870004			

Source	DF	Adj SS	Adj	F-	Р-
			MS	Value	Value
Cooking	2	1108707	554354	543	0
method					
Error	3	3063	1021		
Total	5	1111770			

Source	DF	Adj SS	Adj	F-	P-
			MS	Value	Value
Cooking	2	1292594	646297	702.55	0
method					
Error	3	2760	920		
Total	5	1295354			

I. Blood Glucose 30 minutes only and ANOVA Table

1. Subject 1

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	1889.33	944.67	52.97	0.005
method						
Error		3	53.5	17.83		
Total		5	1942.83			

2. Subject 2

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	2176.33	1088.17	105.31	0.002
method						
Error		3	31	10.33		
Total		5	2207.33			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	1579	789.5	11.55	0.039
method						
Error		3	205	68.33		
Total		5	1784			

Source	DF	Adj SS	Adj	F-	P-
			MS	Value	Value
Cooking	2	1696.33	848.167	175.48	0.001
method					
Error	3	14.5	4.833		
Total	5	1710.83			

5. Subject 5

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking	,	2	2358.33	1179.17	243.97	0.000
method						
Error		3	14.5	4.83		
Total	1	5	2372.83			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	1605.3	802.67	17.51	0.022
method						
Error		3	137.5	45.83		
Total		5	1742.8			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	2300.33	1150.17	55.21	0.004
method						
Error		3	62.5	20.83		
Total		5	2362.83			

8. Subject 8

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	784.33	392.167	57.39	0.004
method						
Error		3	20.5	6.833		
Total		5	804.83			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	3217.33	1608.67	141.94	0.00
method						
Error		3	34	11.33		
Total		5	3251.33			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	1105.33	552.67	39.48	0.00
method						
Error		3	42	14		
Total		5	1147.33			

J. GL and ANOVA Table

1. Subject 1

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	120.781	60.3905	651.97	0.000
method						
Error		3	0.278	0.0926		
Total		5	121.059			

2. Subject 2

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	156.26	78.1299	1269.61	0.000
method						
Error		3	0.185	0.0615		
Total		5	156.444			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	126.564	63.2818	901.74	0.000
method						
Error		3	0.211	0.0702		
Total		5	126.774			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	128.403	64.2015	2433.33	0.000
method						
Error		3	0.079	0.0264		
Total	:	5	128.482			

5. Subject 5

Source	DF	Adj SS		Adj	F-	Р-
				MS	Value	Value
Cooking		2	152.981	76.4907	2437.79	0.000
method						
Error		3	0.094	0.0314		
Total		5	153.076			

DF		Adj SS	Adj	F-	P-
			MS	Value	Value
	2	175.002	87.5008	2091.24	0.000
	3	0.126	0.0418		
	5	175.127			
	DF	2	2 175.002 3 0.126	MS 2 175.002 87.5008 3 0.126 0.0418	MS Value 2 175.002 87.5008 2091.24 3 0.126 0.0418

Source	DF		Adj SS	Adj	F-	P-
				MS	Value	Value
Cooking	2	2	145.803	72.9016	3918.35	0.000
method						
Error	3	3	0.056	0.0186		
Total	5	5	145.859			

8. Subject 8

Source	DF		Adj SS	Adj	F-	P-
				MS	Value	Value
Cooking	4	2	141.668	70.8338	986.94	0.000
method						
Error	3	3	0.215	0.0718		
Total	4	5	141.883			

Source	DF		Adj SS	Adj	F-	P-
				MS	Value	Value
Cooking		2	145.476	72.7379	1419.25	0.000
method						
Error		3	0.154	0.05313		
Total		5	145.63			

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Cooking		2	158.046	79.0232	1048.37	0.000
method						
Error		3	0.226	0.0754		
Total		5	158.273			

K. IAUC versus Gender, Cooking methods

Source	DF		Adj SS	Adj MS	F-	Р-
					Value	Value
Gender		1	331798	331798	1181.87	0.000
Cooking method		2	2858701	1429350	5091.36	0.000
Gender*Cooking		2	25597	12799	45.59	0.000
Error		6	1684	281		
Total		11	3217781			

L. 30 m inutes versus Gender, Cooking methods

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Gender		1	0.85	0.85	0.15	0.715
Cooking method		2	3451.17	1725.58	295.48	0.000
Gender*Cooking		2	53.33	26.66	4.57	0.062
Error		6	35.04	5.84		
Total]	11	3540.39			

Source	DF		Adj SS	Adj	F-	Р-
			-	MS	Value	Value
Gender		1	0.02	0.02	0.06	0.82
Cooking method		2	4623.39	2311.69	7808.5	0.000
Gender*Cooking		2	0.02	0.01	0.03	0.972
Error		6	1.78	0.3		
Total		11	4625.2			

M. GI versus Gender, Cooking methods

N. GL versus Gender, Cooking Methods

Source	DF		Adj SS	Adj	F-	Р-
				MS	Value	Value
Gender		1	0.000	0.000	0.000	0.957
Cooking method		2	288.292	144.146	8868.04	0.000
Gender*Cooking		2	0.001	0.000	0.02	0.981
Error		6	0.098	0.016		
Total	1	1	288.391			

O. Percentage reduction in GI of test food of subjects

Subject	GI of	GI of	GI of Stir-oil	%reduction in GI of stir-oil	
	glucose	Pressure	fried followed	fried followed by pressure	
		cooked	by pressure	cooked rice as compared to	
		rice	cooked rice	pressure cooked rice	
1	100	69.43	57.35	17.40	
2	100	65.67	51.35	21.81	
3	100	66.23	57.36	13.40	
4	100	66.58	56.77	14.72	
5	100	63.53	52.81	16.86	
6	100	60.42	49.79	17.59	
7	100	64.75	53.78	16.94	
8	100	64.24	54.95	14.46	
9	100	64.23	54.10	15.77	
10	100	62.08	52.42	15.56	
	mean	64.72	54.07	16.45	
	SD	2.49	2.57	2.32	

Table. Percentage reduction in GI of test foods of subjects

P. List of Plates



P 1. Survey on cooking recipe of pressure cooked and stir-oil fried rice



P 2. Pressure Cooking of Sona Mansuli rice



P 3. Stir-oil frying of Sona Mansuli rice in pressure cooker



P 4. Measurement of pH of a buffer solution



P 5. Incubation of rice samples and blank



P 6. Performing the filtration of rice samples



P 7. Performing acid hydrolysis connected with reflux condenser



P 8. Performing lab work



P 9. Male volunteers eating the cooked sample rice



P 10. Measurement of Blood glucose response