

The Effect of Beetroot (*Beta vulgaris* L.) Ice Cream on Haemoglobin and Total Protein Levels on Teenage Girl

Tiar Lince Bakara¹, Rumida², Urbanus Sihotang³, Ginta Siahaan^{4*}

^{1,2,3,4} Department of Nutrition, Medan Health Polytechnic of Ministry of Health, Indonesia

*Email: ginzsiahaan@gmail.com

Abstract

Background: Adolescents having diet problems are usually underweight and anemic. The problem of anemia can be overcome by giving ice cream as an additional food and is very popular among young girls. Aim: This study aims to know the effect of beetroot ice cream on haemoglobin (Hb) and total protein levels among adolescent girls. **Method:** The study followed a quasi-experimental pre and post-test design. There were total 35 people recruited in the study and they were divided into 2 groups; one was given ice cream with addition of beetroot and the other group was given plain ice cream. Each group was given 100 gm of ice cream for 30 days during school hours. Data analysis was carried out by using paired and unpaired t-test with data that were normally distributed. **Results:** The results showed that the average Hb level before treatment was 11.09 and that after treatment was 13.49. The average total protein in blood before and after treatment was 5.86 and 6.73 respectively. On the other hand, in the control group, the average Hb level was 11.23 before treatment and 11.78 after treatment. Whereas, the average total protein content before giving ice cream to them was 5.45 and that after giving ice cream was 6.41. **Conclusion:** Beetroot ice cream can affect both Hb and total protein levels, while the regular ice cream only affects the total protein levels.

Keywords: Ice Cream, Beetroot, Total Protein, Haemoglobin

BACKGROUND

Adolescence is the age of transition from childhood to adulthood and many changes occur during this period due to increased muscle mass, fat tissue in the body and hormonal changes. These changes affect the nutritional needs and intake of nutrients in adolescents. Advances in technology and westernization has caused changes in lifestyles and mindsets in society, especially among young women who tend to like fast food. Moreover, many young women also indulge themselves into strict diets and do not follow the rules recommended by nutritional standards.^[1] This is because they want to look more attractive with a proportional, slim and tall body. In addition, there are also many young women who follow the western lifestyle by eating fast food which is high in fat, high in simple carbohydrates and low in fiber.^[2] Such lifestyle causes both under and overweight problems in young women.^[3] A poor diet accompanied by menstruation can cause young women to be anemic and underweight. A study showed that 8.7% of adolescent girls aged 13-15 years and 8.1% of adolescent girls aged

16-18 years were underweight and had poor nutritional status. Moreover, 18.4% of these young women were anemic. Meanwhile, the anemia rate in adolescent girls in Indonesia is 24%.^[4]

In addition to blood albumin and anthropometric measurements (weight and height), the level of Hb and total protein can also determine whether young woman are experiencing a lack of macro and micro nutrients or not. Young women having low levels of Hb and total blood protein can experience a decrease in endurance, get tired easily and cannot concentrate at school. Studies have shown that people with a BMI below 18.5 and Hb below 12 g/dl are suspected to have a deficiency of both macro and micro nutrients.^[5,6] Provision of additional foods containing folic acid, iron, vitamin C

Address for Correspondence: Department of Nutrition, Medan Health Polytechnic of Ministry of Health, Indonesia
Email: ginzsiahaan@gmail.com

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and potassium can reduce the risk of being underweight and anemic in adolescent girls.^[7,8]

Additional food in form of modified ice cream with the addition of beetroots is expected to prevent or improve anemia and total protein status of adolescent girls.^[9] Because beetroots are high in folic acid, iron, protein, vitamin C and magnesium, therefore, its ice cream has a higher nutritional value than ice cream sold on the street by traditional ice cream vendors. According to the North Sumatera University Food Chemical Analysis Laboratory, 100gm of beetroot ice cream contains energy (410.74 kcal), carbohydrates (82.41gm), protein(15.43gm) and vitamin C (175.03mg).

Ice cream is a dairy product that is quite popular among various groups including children, teenagers and adults.^[10] Ice cream can be made from various ingredients which are easy to find and are also affordable.^[11,12] According to a study^[13], growth rate of domestic ice cream market continues to increase at least 20% every year. In addition to food diversification, the use of beetroots in the manufacturing of ice cream can increase marketability by changing the color to become more attractive from natural ingredients. Moreover, young women are more accustomed to consuming vegetables and fruits high in Zn, folic acid and Fe which are especially needed for the formation of hemoglobin during menstruation. Besides these advantages, beetroots are easily available in traditional markets on affordable prices.^[14,15] This study aims to check the effect of beetroot ice cream on Hb and blood protein levels among adolescent girls.

METHODS

This research is a quasi-experimental study with pre and post test design. The study was carried out on female students at Parulian 1 High School and UISU Medan High School from September 30 – October 31, 2017.

The sample size was determined using the following formula^[16]:

The sampling technique used in this study was simple random sampling. Total 70 students were recruited in the study, of which 35 were included in experimental group who were given beet ice cream whereas 35 were in control group who were given ice cream without beetroot. The former group belonged to Parulian 1 High School and the latter group was from UISU High School Medan.

Average age, Hb levels and total protein of participants were noted followed by editing, coding, cleaning and tabulating of the data. Total 1.5 - 2 cc of blood was taken from the left arm of each research participant.

Hb was examined by *Cyanmethemoglobin* with the help of *Spectrofotometry photometer 4010 Mannheim Boehringer* having an accuracy of 0.01 mg/dl. Whereas, the total blood protein was measured by using the biuret method with the help of spectrophotometer. The blood collection was assisted by a health analyst and later was examined at the North Sumatra Regional Health Laboratory (LABKESDASU).

The provision of ice cream with the addition of beetroots and ice cream without beetroots was carried out for 30 consecutive days. Based on the habit of consuming ice cream in the community per meal and also on the Indonesian Food Consumption Table, 100gm (8 scoops of ice cream) was given to each student.^[17] Ice cream was given directly by the researcher and consumed by the students during break time at school around 9.30 WIB every day. While during holidays, students came to school to take ice cream. The consumption process was carefully supervised by the researcher.

Data analysis was carried out using univariate and bivariate methods. Based on the normality test using Kolmogrov–Smirnov test, the data was found to be normally distributed. The changes in Hb and total protein level before and after giving ice cream to the treatment and control groups were analyzed by the paired t-test, while changes in the indicator of Hb and total protein between groups were examined using the unpaired t-test. The ethical approval for the study was obtained from the Ethics Committee (No: 036/KEPK/POLTEKKES KEMENKES MEDAN/2017).

Table 1: Nutritional content of 100gm ice cream with and without the addition of beetroots

Indicator	Original Ice Cream	% RDA	Beetroot Ice Cream	% RDA
Carbohydrate (gm)	20.6	6.8	82.4	27.1
Fat (gm)	12.15	17.8	12.72	18.1
Protein (gm)	4	6.1	9.4	14.4
Zinc (mg)	0.7	7.7	4.2	46.6
Fe (mg)	0.3	2	8.95	59.6
Energy (kcal)	207	9.8	410.7	19.5

Source: Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Brawijaya University in 2019

RESULTS

1. Characteristics of Samples

Sample characteristics based on age and class can be seen in table 2. Total 57% of the students in treatment group from Parulian 1 High School and 43% in control group from UISU Medan High School were 15 years old. Whereas, most of the students in both groups were in class ten.

Table 2: Sample Distribution Based on Characteristics

General Indicator	Parulian 1 High School		UISU Medan High School	
	n	%	n	%
Age				
14	3	9	7	20
15	20	57	15	43
16	12	34	13	37
Class				
X	21	60	22	63
XI	14	40	13	37

2. Nutrient intake before and after giving beetroot ice cream

24-hour Food Recall was carried out by interview technique by filling out a form to find out the food consumed in 24 hours for two consecutive days. Then the recall data was edited and the nutritional intake assessment results were calculated using the Nutri Survey Program. The average nutrient intake before and after giving ice cream to the treatment group and control group can be seen in table 3.

Table 3. Sample Distribution Based on Nutrient Intake

Nutrients	RDA (2019)	Treatment							Control						
		Before			After			p value	Before			After			p value
		Mean	SD	%RDA	Mean	SD	%RDA		Mean	SD	%RDA	Mean	SD	%RDA	
Energy (kcal)	2100	1885	2.17	89.7%	1918	2.02	91.3%	0.078	1832	4.9	87.2%	1894	5.01	90.1%	0.063
Carbohydrate (gm)	300	276.4	10.3	92.1%	281.2	9.58	93.7%	0.123	269.9	8.98	89.9%	278.1	7.97	92.7%	0.054
Protein (gm)	65	44.7	2.43	68.7%	46.3	2.32	71.2%	0.077	41.6	1.89	64%	43.2	2.02	66.4%	0.082
Fat (gm)	70	58.4	4.08	83.4%	59.9	4.34	85.5%	0.125	57.3	3.97	81.8%	58.1	4.13	83%	0.167
Zinc (mg)	9	5.01	1.08	55.6%	6.12	1.12	68%	0.052	5.02	2.1	55.7%	5.87	2.24	65.2%	0.082
Vitamin C (mg)	75	68.2	2.10	90.9%	70.1	3.01	93.4%	0.180	66.8	2.98	89%	68.1	3.15	90.8%	0.270
Iron (mg)	15	6.2	3.17	41.3%	7.7	4.01	51.3%	0.067	6.3	2.88	42%	7.1	3.02	47.3%	0.098

The recall results showed that the homogeneity in terms of the nutrients consumed is not significantly different ($P > 0.05$), so that the administration of ice cream can be used as an indicator to see the levels of Hb and total blood protein.

3. Hb and Total Blood Protein Levels Before and After Ice cream Intake

The average level of Hb and total protein before and after treatment (ice cream with beetroot) and their levels before and after in the control group (ice cream without beetroots) can be seen in table 4.

Table 4. Mean and SD of Hb Levels and Total Blood Protein

Variable	Treatment		Control		p value
	Mean ± SD	p value	Mean ± SD	p value	
Hb before	11.09 ± 2.13	0.001*	11.23 ± 2.60	0.062*	0.991**
Hb after	13.49 ± 1.52		11.78 ± 2.45		0.036**
Hb difference	2.4 ± 2.78		0.55 ± 15.12		0.035**
Total Blood Protein before	5.86 ± 1.68	0.037*	5.45 ± 1.50	0.040*	0.723**
Total Blood Protein after	6.73 ± 1.89		6.41 ± 2.23		0.617**
Total Blood Protein difference	0.87 ± 0.58		0.96 ± 1.12		0.078**

*) Paired t-test
**) Unpaired t-test

Table 4 shows significant difference in the level of Hb before and after treatment ($p = 0.01$). Similar results were obtained for total protein level also ($p = 0.037$). In case of

the control group, there was no change in Hb levels before and after giving ice cream ($p = 0.062$), while significant change was observed for total protein level ($p = 0.040$). In addition, the difference in Hb levels before and after ice cream was given to the treatment group was 2.4 g/dL, while in the control group the difference was 0.55 g/dL. Based on the unpaired t-test, significant difference in Hb level was observed between the treatment and control groups ($p = 0.035$). On the other hand, the difference in total protein level before and after ice cream intake was 0.87 mg/dL in the treatment group and 0.96 mg/dL in the control group. Moreover, unpaired t-test showed no difference in total blood protein between the two groups.

4. Anemia and Total Protein Status

The normal Hb level in young women is ≥ 12 gm/dL. Before treatment, 68% of the students had anemia, whereas only 8% were left anemic after giving beetroot ice cream to them for 30 days. For the examination of total protein levels there was a change from low levels of 51% to 29%, where the normal total protein level is (≥ 6.4 mg/dL). Giving ice cream without beetroot (control), there was also a change in the status of Hb levels with anemia from 49% to 31%. Meanwhile, the examination of the total protein content of low levels showed a change from 43% to 31%.

Table 5: Distribution of Samples based on the Status of Anemia and Total Protein

Indicator	Treatment Group				Control Group			
	Before		After		Before		After	
	n	%	n	%	n	%	n	%
Anemia	24	68	3	8	17	49	11	31
Not Anemia	11	31	32	91	18	51	24	68
Abnormal Total Protein	18	51	10	29	15	43	11	31
Normal Total Protein	17	49	25	71	20	57	24	68

DISCUSSION

1. Characteristics of Samples

The age range of the sample in this study was between 14-16 years old and they were in class X and XI of Senior High School. Usually, adolescent girls aged 14-16 years have experienced the menstrual process which is likely to lose a lot of blood. Hence during menstruation, it is recommended to consume nutritious food so that young women can avoid anemia.^[1]

2. Beetroot Ice Cream

Ice cream is a favorite food for all age groups, from children to adults. Ice cream is a frozen milk product made from milk, sugar, cream, butter, eggs and stabilizer which has a delicious and savory taste with a soft texture. Nowadays, ice cream is often made with additional ingredients to improve the commercial aspect, aesthetics and nutritional fortification. Commercial ice cream often uses synthetic dyes which can harm the health of consumers. Beetroot, apart from its nutritional value, can also be used as a natural coloring agent in the presence of anthoxianin bioactive substances so they can enhance the aesthetic value of ice cream more attractively. Beetroot extracts of liquid consistency are easier to blend with the basic ingredients of ice cream. Ice cream with various nutritional contents can actually increase immunity, stimulate the dopamine hormone in the brain, provide energy for activities, and help the growth of bones and teeth in children.^[13,18,19]

3. Effect of Beetroot Ice Cream on Hb Levels

In the present study, a change in Hb levels was observed in the treatment and control groups, but based on the paired t-test, this difference was significant only in the treatment group ($p = 0.001$). Ice cream with beetroots turned out to have a contribution in the formation of Hb. The nutritional content in beetroot includes iron, vitamin C, amino acids (tryptophan, lysine, folic acid and Zn).^[20,21]

Iron, folic acid and vitamin B12 are the main nutrients that form Hb and vitamin C helps to absorb iron.^[5] Iron is the main component that plays an important role in the formation of blood (hemopoiesis), the hemoglobin

molecule. Iron stores in the body (ferritin and hemosiderin) are found in the liver (30%), bone marrow (30%), and the rest is stored in the spleen and muscles. Absorption of iron minerals in non-heme form can also increase fourfold in the presence of vitamin C.^[22] Beetroot contains 2-4 times more vitamin C than oranges, hence it can increase the production of erythrocyte cells by mobilizing iron stores in the tissues in the form of hemosiderin. Vitamin C also helps release iron from transferrin in the plasma so it can be incorporated into tissue ferritin. In addition, the protein content of beetroot and that of milk in ice cream can help transport iron and help in the conversion of ferri (Fe^{3+}) into ferrous (Fe^{2+}) so that it can be easily absorbed.^[14,23] Insufficient protein intake results in inhibition of iron transport and leads to iron deficiency. Protein in the form of transferrin and ferritin is a transport to carry out iron absorption that occurs in the small intestine which is then transported to the bone marrow where Hb synthesis occurs.^[22]

Beetroot also contains Zn which is also a heme-forming mineral and is also found in red blood cells. Zn minerals can interact directly with Fe which acts as a co-enzyme of the amino acid levulinic acid (ALA). This enzyme has a role in forming heme when it is in the cytosol of bone marrow cells.^[5] Vegetables in the form of tubers such as beetroot also contain vitamin B9 which circulates directly as polyglutamate in red blood cell reserves. Due to vitamin B9 deficiency, the proliferation of erythrocyte formation in the bone marrow can be suppressed.^[2] Vitamin B9 is also needed in various biochemical reactions of the body which are involved in the transfer of one carbon unit in protein amino acid interconversion or in the formation of DNA precursors in the erythropoiesis system (the process of forming red blood cells in the kidneys).^[5] The increase in Hb levels can also occur because 100gm of beetroot contains 10.2% vitamin C and 34% folic acid, which are nutritional elements that can accelerate the formation of Hb.^[15]

These results are in accordance with another study^[21] which reported an increase in Hb levels from 9.8 gm/dl to 11.9 gm/dl after consuming beetroots. Another group of researchers gave colored fruits such as tomatoes, dragon fruit, red guava to people with HIV and observed significant differences in Hb level.^[22] Similarly, another study reported an increase in Hb level from 0.6 to 0.8 gm/dl when 500 ml of beetroot juice was given for 7 days.^[20]

4. Effect of Beetroot Ice Cream on Total Protein Levels

The results showed a change in total protein levels in both control and treatment groups ($p < 0.05$). Ice cream given to research participants was prepared with milk flour and sweetened condensed milk. In addition to this, beetroot was also added to the ice cream for treatment

group.^[24] Amino acids in milk have high bioavailability because milk comes from animal sources. Beetroots also contain amino acids in the form of tryptophan and lysine, although the bioavailability value is lower than animal protein.^[24] Protein requirements are based on the minimum requirements needed to maintain nitrogen balance. The amount of protein needs is adjusted to the state of the human body which is adapted to its physiological processes. During adolescence, the need for protein increases and apart from the need for growth and development, protein is also needed to avoid the gluconogenesis process due to the diet of teenagers who want their bodies to be thin.^[2,25]

Total blood protein describes the occurrence of protein catabolism in the body in short term due to low intake of protein. Abnormal total blood protein can trigger inflammation in a person causing weight loss and if it continues, it can cause malnutrition.^[26,27] Total protein in blood, liver and other organs has a half-life of 2.5-10 days.^[5,28] In the present study, giving ice cream with and without the addition of beetroot for 30 days was able to increase the total protein levels. These results are in accordance with another study which reported that adequate protein consumption in people suffering from illness has made a positive contribution in increasing the total blood protein level thereby reducing the risk of infection.^[22] Another study showed positive effect of consuming ice cream on children's growth because milk used in making ice cream contained insulin like growth factor-1 (IGF-1) which is a hormone for growth and development of children.^[29] The formation of hormones and protein reserves in the blood comes from adequate protein intake, which is characterized by high blood proteins such as albumin and total protein.^[30]

In addition, beetroots also contain anti-oxidants and bioactive substances that can strengthen the liver, especially during menstruation when the protein content in ice cream and beetroot can be maximized by the liver to synthesize blood proteins.^[22] Around 19.5% and 9.8% of the energy is produced by ice cream with and without the addition of beetroot respectively. The energy produced is sufficient to help the body's maintenance process so that a young woman does not experience energy deficiency through the gluconeogenesis process. Instead, the energy helps the formation of body tissues, the process of growth and development by increasing total blood protein. The absorption of Zn in the body is strongly influenced by vitamin C intake, which is found in beetroots. Appropriation of ice cream with the addition of beetroot can increase the intake of Zn which improves the rate of protein metabolism so that there is an increase in the formation of proteins in the plasma including albumin and total protein. In addition, Zn also has a role in maintaining pancreatic function in secreting protease enzymes which are needed in the gastrointestinal tract

to metabolize protein. Increased protease enzymes and the activity of enzymes related to protein metabolism affect the metabolic rate in the liver so that total plasma protein levels will also increase.^[31] Increased levels of total protein are suspected to come from milk and the Zn content in beetroots can also increase the formation of albumin which automatically increases total protein.^[32]

CONCLUSIONS

Beetroot ice cream, when compared with the ice cream without beetroot, can increase hemoglobin levels. However, total blood protein levels are increased by the ice cream irrespective of the addition of beetroots.

SUGGESTION

Beetroots should be used more often by people in everyday life, because it is an alternative that can not only increase the aesthetic value of ice cream but also, the total protein content and blood Hb level can be increased.

LIMITATIONS AND WEAKNESS

1. The research time to examine Hb levels should be at least 3 months, because the formation of Hb occurs in 90 – 120 days.
2. Ice cream has an easy to melt texture, so it is necessary to use a tool that can maintain the consistency of the ice cream (temperature and texture).

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