

THE EFFECT OF STORAGE CONDITION ON ANTIOXIDANT ACTIVITY OF PROBIOTICS IN YOGURT DRINKS

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ABSTRACT

Objective: The aim of this study was to investigate the effect of temperature and time of storage on antioxidant activity of probiotics in yogurt drinks.

Methods: The samples used were Yakult, Cimory, YoFit, and BioKul marketed in Medan city. The samples were stored at room and refrigerate temperatures for 2 weeks. Various volumes of samples were withdrawn and mixed with 1 ml of 2,2-diphenyl-1-picrylhydrazil solution and homogenized. Then, it was incubated for 30 min and centrifuged at 5000 rpm for 15 min. The supernatant was collected, and the absorbance was measured at 516 nm by spectrophotometer. The absorbance of different volume of supernatant of each sample was used to construct linear regression equation. Then, the IC₅₀ value was determined.

Results: The present study shows that the higher the temperature and the longer the time of storage decreased the antioxidant activity of yogurt drinks. Yakult exhibited the highest antioxidant activity among the analyzed samples. Before storage, the IC₅₀ value of Yakult was 0.2763 ml, and after stored for 2 weeks, the IC₅₀ value of Yakult was 0.3867 ml at refrigerated and 0.5393 ml at room temperature.

Conclusions: Probiotic in yogurt drinks stored at refrigerated temperature has higher antioxidant activity than stored at room temperature. The IC₅₀ values of yogurt drinks decreased with storage time.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazil, Probiotics, Storage condition, IC₅₀ value.

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INTRODUCTION

Oxidation is an essential process for many living organisms for the production of energy to fuel biological processes. Physiological metabolites such as free radicals and reactive oxygen species (ROS) are formed as a result of respiration in the aerobic organism [1-3]. Formation of free radicals such as superoxide anion radical and hydroxyl radical is unavoidable during respiration, and excessive production can cause damage on lipid, protein, carbohydrate, and nucleic acids [4,5]. Fortunately, human body has developed a number of enzymatic and non-enzymatic defense mechanism which can neutralize the free radicals and oxidant molecules [5,6]. In certain circumstances such as chronic hyperglycemia, this antioxidant defense system fails and not effective enough to totally neutralize free radicals which are known as oxidative stress [2,7]. Therefore, to prevent the damage by oxidative stress, antioxidant compounds contained in fruits, vegetables, and food supplement containing antioxidant is required to reduce oxidative damage [4,6,7].

Probiotic is defined as live microorganisms when administered in adequate amounts, confer a health benefit on the host by improving microbial balance. Probiotic bacteria, especially such as *Lactobacillus* and *Bifidobacterium*, are found to be beneficial in preventing the growth of pathogenic bacteria, reducing inflammation, and inhibiting glycation of protein in hyperglycemia. Some bioactive components are produced by probiotic including antioxidants [7-12]. The antioxidant mechanisms of probiotics could be assigned to scavenge ROS by non-enzymatic and enzymatic antioxidants contained in yogurt drinks [6,13]. Factors such as pH, redox potential, and acidity, buffering capacity, packaging, molecular oxygen, yogurt composition, and storage time and temperature affect the viability of probiotic bacteria during storage and hence antioxidant activity of yogurt drinks [14,15]. The objective of this study was to examine the effect of the temperature

and time of storage on antioxidant activity of probiotics from four plain products of different types/brands of yogurt drinks.

MATERIALS AND METHODS

Materials

Reagents and materials were 2,2-diphenyl-1-picrylhydrazil (DPPH), methanol, Vitamin C, and distilled water. The samples used were plain yogurt products purchased from market Pondok Indah Pasar Buah in Jalan Setia Budi, Medan, Indonesia. The samples (plain products) did not contain flavoring agents of four different type/brand yogurt drinks included Yakult, Cimory, YoFit, and BioKul.

Instruments

Refrigerator, incubator, centrifugation, vortex, analytical balance, and UV-visible Spectrophotometer (Shimadzu UV-1800) and glasswares were used.

Preparation of DPPH solution

50 mg of DPPH was transferred into 50 ml of brown volumetric flask and dissolved in methanol and calibrated up to the marked line. From this solution, 2 ml was transferred into 50 ml volumetric flask and added methanol to volume to get a solution of 40 µg/ml (0.5 mM) [1,16].

Preparation of Vitamin C solution

50 mg of Vitamin C powder was dissolved with methanol in 25 ml brown volumetric flask. From this solution, 2.5 ml was transferred into a 25 ml volumetric flask and added methanol up to the marked line of volumetric flask to make Vitamin C solution with concentration of 200 µg/ml. [1,16].

Procedure

The samples were divided into two groups. Group A samples were stored at cold storage by placing them inside the refrigerator. Group B

samples were stored at the ambient or room temperature. The samples were stored for 2 weeks, and antioxidant activity was determined before storage, after 1 week, and 2 weeks of storage.

Determination of absorbance curve of DPPH solution

The DPPH solution with a concentration of 40 µg/ml was transferred into the cuvette and placed into the spectrophotometer. The absorbance was measured within the wavelength between 400 to 800 nm to get absorbance curve. From this absorbance curve, the maximum absorbance of DHHP solution was found to be 516 nm and used in this study.

DPPH free radical scavenging assay of Vitamin C and tested samples

1 ml of methanolic DPPH solution (40 µg/ml) was transferred into a volumetric flask of 5 ml and added 0.0625 ml Vitamin C solution of 200 µg/ml; the same procedure was carried out with 0.1250 ml, 0.2500 ml, and 0.500 ml of Vitamin C solution. This procedure also applied to each yogurt drink with a series of volumes, namely 0.25 ml, 0.50 ml, 0.75 ml, and 1.0 ml of Yakult, Cimory, YoFit, and BioKul, respectively, then methanol was added to each mixture to the volume of 5 ml (to the marked line). It was well vortexed, and then incubated for 30 min at room temperature. The samples were centrifuged for 15 min at 5000 rpm. The supernatant was collected, and the absorbance of the sample was measured at 516 nm using the spectrophotometer. Negative control was prepared using 1.0 ml of DPPH solution transferred into 5 ml volumetric flask and added methanol to volume without addition of the sample. It was allowed to stand for 30 min at room temperature then the absorbance (A) was measured. The readings were recorded in triplicates. The percentage of radical scavenging activity (RSA) was calculated:

$$RSA(\%) = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$$

Graphs for the effect of storage condition of samples and Vitamin C on DPPH scavenging activity (%) were plotted, and the regression equation determined [1,17].

Determination of IC₅₀ value

The effective volume of Vitamin C and samples required to scavenge DPPH radical by 50% (IC₅₀ value) was obtained from linear regression analysis of dose-response curve obtained by plotting between percentage inhibition and volume (ml). The results obtained from the DPPH scavenging activity were substituted into the regression equation, where the X-axis was the volume of vitamin C, and Y-axis was the percentage of DPPH scavenging activity [17].

Determination of IC₅₀ from regression equation; Y=aX+b

Notes: Y=Percentage of DPPH scavenging activity (IC₅₀, dependent variable); a=Slope/gradient; x=Volume of vitamin C (independent variable); b=intercept.

RESULTS AND DISCUSSION

Absorbance curve of DPPH solution

The absorbance measurement of the DPPH solution in methanol using a UV-visible spectrophotometer and the absorbance curve is presented in Fig. 1.

From Fig. 1, the maximum absorbance of the DPPH solution is at the wavelength of 516 nm and used to determine IC₅₀.

DPPH scavenging activity (%) of yogurt drinks

The effect of storage condition of each sample on DPPH scavenging activity (%) can be seen in Figs. 2-5. The scavenging activity percentage of Vitamin C is shown in Fig. 6.

Regression equation from Figs. 2-6 was used to calculate the IC₅₀ value of each sample. From Figs. 2-5 can be seen that the scavenging

percentage of the all samples decreased with the storage time. The prominent decreased in scavenging percentage can be observed in storage at room temperature compared to the refrigerate temperature.

Figs. 2-6 show that the lower the absorbance value of DPPH solution mixed with sample the higher antioxidant activity of the analyzed samples. This is due to the ability of Vitamin C and bioactive components produced by probiotic to chemically reduce DPPH solution which is used to evaluate the antioxidant activity. Interaction of antioxidant compounds with DPPH is based on the transfer of hydrogen atom to DPPH radical and converts it into DPPH-H. The result of the reduction of DPPH radicals results in color change from a purple color to yellow pale color which indicates the antioxidant activity of the sample [7,18].

Antioxidant activity of Vitamin C and yogurt drinks

The IC₅₀ value of the samples and Vitamin C was determined using linear regression equation obtained by plotting the volume of test samples as X-axis and the scavenging percentage of tested samples as Y-axis. The IC₅₀ value was determined by calculating the volume that required to scavenge 50% of DPPH radicals by the samples. The result of IC₅₀ value can be seen in Fig. 7. From Fig. 7 can be seen that the smaller the volume of tested sample the higher the antioxidant activity indicate that the lower volume needed to scavenge DPPH solution at 50% and stated or called as IC₅₀ and vice versa.

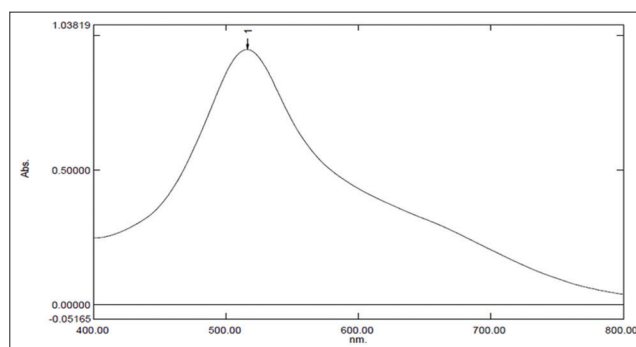


Fig. 1: Absorbance curve of 2,2-diphenyl-1-picrylhydrazil solution in methanol (40 µg/ml)

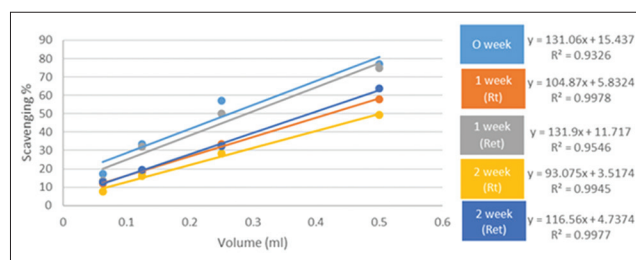


Fig. 2: The effect of storage condition of Yakult on 2,2-diphenyl-1-picrylhydrazil scavenging activity (%). Rt: Room temperature; Ret: Refrigerator temperature

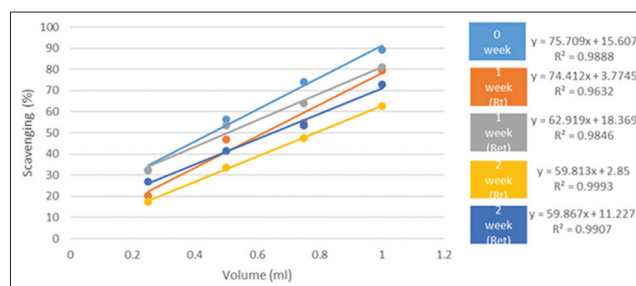


Fig. 3: The effect of storage condition of Cimory on 2,2-diphenyl-1-picrylhydrazil scavenging activity (%). Rt: Room temperature; Ret: Refrigerator temperature

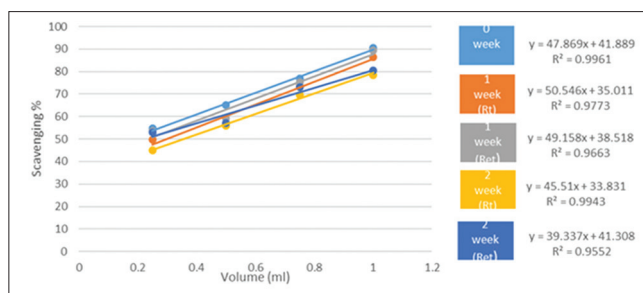


Fig. 4: The effect of storage condition of YoFit on 2,2-diphenyl-1-picrylhydrazil scavenging activity (%). Rt: Room temperature; Ret: Refrigerator temperature

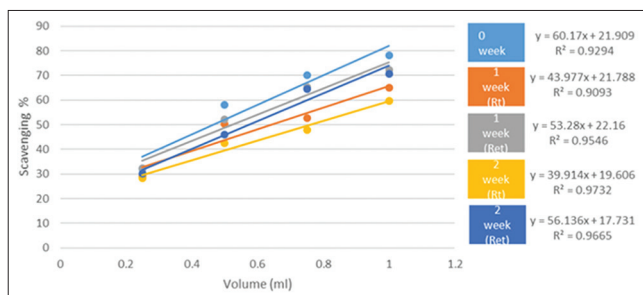


Fig. 5: The effect of storage condition of BioKul on 2,2-diphenyl-1-picrylhydrazil scavenging activity (%). Rt: Room temperature; Ret: Refrigerator temperature

The IC₅₀ value decreased with storage time for both room and refrigerates temperature. Before storage, IC₅₀ value of Yakult (0.2763 ml) is the highest value followed by YoFit, Cimory, and BioKul to scavenge the DPPH radicals by 50%. Even after storage at room and refrigerate temperature, Yakult has the highest IC₅₀ value compared to other samples. Vitamin C with concentration of 2.5 ug/ml as positive control of antioxidant was found to be the strongest antioxidant with IC₅₀ value of 0.1742 ml which is much higher than Yakult [19,20].

Probiotics demonstrate antioxidant activity by secreting enzymes such as superoxide dismutase (SOD) and promote the production of the major non-enzymatic antioxidant and free radical scavenger glutathione (GSH). SOD catalyzes the breakdown of superoxide into hydrogen peroxide and water. Thus, it acts as a central regulator of ROS levels. GSH eliminates radicals such as hydrogen peroxides, and hydroxyl radicals, by collaborating with selenium-dependent GSH peroxidase [16,21]. Probiotics also promote the production of certain antioxidant biomolecules, such as the exopolysaccharides [11]. In addition, antioxidant activity could also be due to metal chelating activity, but the mechanism is still unclear [16,21].

Although Yakult has the shortest expiry date compared to others, Yakult exerted the highest scavenging percentage followed by YoFit, Cimory, and BioKul in both room and refrigerate temperature for 2 weeks. For Yakult, only small volume was withdrawn to react with DPPH solution because Yakult expressed high scavenging percentage in very small volume (0.0625 ml) before and after storage in both room and refrigerate temperature for 2 weeks compared to other samples (Cimory, YoFit, and BioKul). Several factors affecting survival of the probiotic bacteria and thus the antioxidant activities include storage temperature, composition of food, type of packaging materials, moisture content of powders, oxygen content, and exposure to light. [22]. The species and number of probiotic bacteria present in the products could also affect the viability of bacteria during storage and hence the ability to produce the bioactive components including antioxidant peptides [1]. In this study, *Lactobacillus casei* (*L. casei shirota*) is the only probiotic present in Yakult products as mentioned on the label.

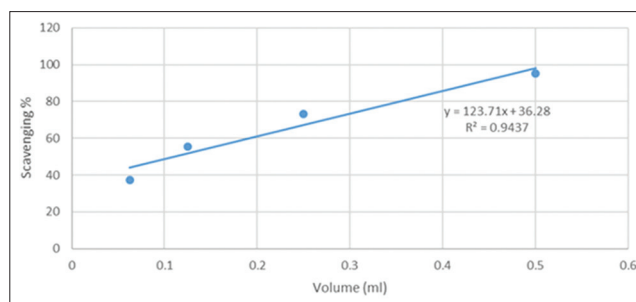


Fig. 6: 2,2-diphenyl-1-picrylhydrazil scavenging activity (%) of Vitamin C 2.5 ug/ml

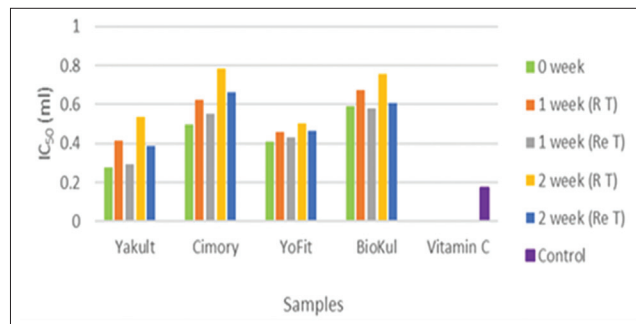


Fig. 7: Antioxidant activity (IC₅₀) of samples and Vitamin C. RT: Room temperature; ReT: Refrigerator temperature

The other samples contain more than one probiotic bacteria as written on the label. Cimory contains two probiotic bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. BioKul contains four probiotics, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium*, and YoFit contains *L. acidophilus* LA-5, *Bifidobacterium* BB12, and *Streptococcus thermophilus*.

The antioxidant activity of probiotic bacteria in yogurt drinks is inversely related to the storage temperature. Food that contain probiotics preferably stored at a temperature of 2–8°C [22,23]. This is because storage above 20°C for prolong duration of time decreases the viability of certain probiotic bacteria such as *L. acidophilus* and *Bifidobacterium lactis*. Thus, the decrease in the viability of bacteria could decrease the antioxidant activity of probiotic bacteria that present in yogurt drinks at room temperature as can be seen from the data that the scavenging percentage decrease for all the samples at 1st and 2nd week of storage. The common probiotics that present in all the samples is *L. acidophilus* and *B. lactis*. Hence, the most suitable temperature for the viability of *L. acidophilus* is 2°C and *B. lactis* is 4°C [23].

Yakult has the shortest expiry followed by Cimory, YoFit, and BioKul with the longest expiry. Addition of growth promoters such as glucose, vitamins, minerals, casein, whey protein, yeast extract, and antioxidants are fortified in dairy products to increase the viability of the probiotics. Oxygen content and redox potential are harmful to the probiotic survival during storage because most of the species are anaerobic and saccharolactic [22]. Free radicals produced from the oxidation of components (e.g., fat and sugar) can be hazardous to the probiotics. Therefore, the level of oxygen during packaging should be as low as possible, because all the tested samples in this study contain sucrose and skim milk powder in their composition, which may produce free radicals during storage; thus, the scavenging activity also decreases on the storage [22,23].

Packaging aspects such as type and thickness of the materials, gas (oxygen, carbon dioxide, and water vapor) and light permeability

may also influence the survival of the probiotics. The surrounding temperature may affect gas permeability through the container and thereby affect the survival of the probiotics. Therefore, the use of plastic films with high oxygen barrier properties or packaging with oxygen absorbers can prolong the survival of probiotics during storage and thereby increase the antioxidant properties [22,23].

CONCLUSION

Storage at refrigerate temperature (4°C–10°C) has higher antioxidant activity compared to the storage at room temperature, and antioxidant activities decrease with time of storage. Yakult has the highest antioxidant activity compared to other tested yogurt drinks but still lower compared with Vitamin C.

AUTHORS CONTRIBUTION

Jansen conceived the study and was in charge of overall direction and planning and supervised the work. Darshieny Nadarason was involved in planning and performed the experiments. Yosy C E Silalahi contributed in the experimental design, collected and analyzed the data, and wrote the manuscript with input from all authors.

CONFLICTS OF INTEREST

No conflicts of interest are associated with this work.

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