Boiling time variation through functional characteristics of boiled red kidney beans

Anita Maya Sutedja[1,*], *Chatarina Yayuk* Trisnawati[1], *Richard* Wang[1], and *Cicih* Sugianti[2]

[1] Department of Food Technology, Faculty of Agricultural Technology, Widya Mandala Surabaya Catholic University, Jl. Dinoyo 42-44 Surabaya, Indonesia
[2] Postharvest Engineering Laboratory, Faculty of Agriculture, Gifu University, 1-1 Yanagido Gifu, Japan

Abstract. Chemical components of red kidney beans, starch and protein, closely related to its functional properties. The starch and protein components are structurally still bound to each other or to other components in a complex structure which can hinder the utilisation of the functional properties of red kidney beans in food products. Utilisation of the red kidney beans based on the functional properties requires preliminary treatment, namely boiling. Boiling time was an important factor that may affect its functional properties. The purpose of this study was to determine the effect of boiling time on the functional properties of boiled red kidney beans. Six levels of boiling time (0, 3, 6, 9, 12 and 15 min) were applied with three replications. Functional properties tested included protein solubility, water absorption, oil absorption, gel formation, foam capacity and stability, emulsion capacity and stability, and moisture content. The results showed that boiling decreased protein solubility, foam capacity, and emulsion capacity of red kidney beans, while the foam stability and emulsion stability increased with the length of boiling time. The absorption of water and oil increased up to 9 min of boiling, while the moisture content increased after 3 min of boiling. **Keywords:** red kidney beans, boiling, boiling time, functional properties

1 Introduction

Red kidney bean (*Phaseolus vulgaris* L) is one of the species of legumes that are commonly consumed in Indonesia, either directly or with other food ingredients. Utilisation of red kidney beans is still less effective and generally used without reviewing their functional properties, both in terms of starch and protein. An understanding of the functional properties will increase the use of red kidney beans in food products such as emulsifiers, foam forming, gelling, water absorption, and oil absorption which greatly affect the functional properties of red beans in their application to food products [1].

The functional properties of red kidney beans are closely related to the protein composition contained. The dominant protein content in beans is globulin, indicating that the main role of protein in legumes is as a storage protein. Red kidney bean protein isolate

* Corresponding author: maya@ukwms.ac.id

reported has the ability to absorb and oil [2]. Water absorption and oil absorption are the determining factors in the application of red kidney bean functional properties. The number of polar and non-polar groups in the chemical structure of a protein also greatly determines the level of protein solubility in a particular solvent.

The functional properties of red kidney beans in food are closely related to its chemical components, such as starch and protein. The starch and protein components of red kidney beans are structurally still bound to each other or to other components in a complex structure. The complex structure is more difficult to dissolve in water or undergo structural conformational changes. This can hinder the utilisation of the functional properties of red beans in food products. Utilisation of the functional properties of red kidney beans requires preliminary treatment to overcome these obstacles.

Heating is one of the most common procedures used in the food processing industry. Boiling is a method of heating foodstuffs using water as a heat conductor and is commonly used by Indonesian people. This is because water is an energy efficient heat transfer medium, easy to apply and flexible for various food processing [3]. Boiling is one of the treatments that can increase the breakdown of complex structures in raw red kidney beans. Boiled red kidney beans make the components of the bean starch-protein complex split, protein denatured, and starch is gelatinized.

Changes in the complex structure in red kidney beans will bring changes in functional properties. Several parameters related functional properties are protein solubility, water absorption, oil absorption, gel formation ability, foam capacity and stability and emulsion capacity and stability. Too long boiling can cause the breakdown of starch granules and protein coagulation so that it can reduce its functional properties of red kidney beans. The potency of boiling in providing the effect of changing the functional properties of red kidney beans underlies the need for research on the effect of boiling time on its functional properties.

2 Material and Methods

1.1 Materials

The materials used in this study were red kidney beans packaged by PT. Pangan Lestari Sidoarjo with the Finna brand.

1.2 Boiled red kidney bean preparation

Raw red kidney beans (300 g) were soaked using water (1500 mL) for 8 h. Then the skin was peeled and sorted to separate the bad condition bean (i.e have black spots). Soaked red kidney beans (50 g) were boiled in boiling water (95°C, 350 mL)) in various boiling times as the treatments. Those were 0, 3, 6, 9, 12, and 15 min. Boiled red kidney beans were drained and cooled. Moisture content and hardness analysis were performed before the bean was crushed. Smooth crushed red kidney beans were prepared for further analysis.

1.3 Protein Solubility Content

Red kidney bean (1g) was dissolved in distilled water (100 mL) and stirred for 10 min. prepared the same sample for various ranges of pH of 2-12 using 1M HCl and 1M NaOH solution. The mixture of samples was centrifuged using centrifuge (Hettich Zentrifugen Universal 320R) at a speed of 5000×g for 10 min then separated the supernatant. The supernatant was used as a soluble protein sample.

Determination of soluble protein content of the Bradford method was carried out through spectrophotometric analysis. Sample (100 ⌈L) was mixed with Bradford's reagent (5 mL), homogenised and incubated for five min. Then the mixture was analysed using a spectrophotometer (Shimadzu UV-1700 Pharmaspec) with a wavelength of 595 nm. Soluble protein content was calculated using the standard curve Bovine Serum Albumin (BSA). The standard curve creation was prepared using a series of standard solutions (0, 2, 4, 6, 8 and 10 ppm) and carried out with the same procedure.

1.4 Water Absorption

Sample (1 g) was dissolved in distilled water (10 mL) and incubated for 30 min. The mixture was homogenised every 10 min then centrifuged (5000×g) for 30 min. Supernatant was separated and the volume was measured. The absorption of water expressed as absorbed volume per 100 g sample.

1.5 Oil Absorption

Sample (1 g) was dissolved in corn oil (10 mL) then incubated at room temperature for 30 min. Homogenise the sample solution with a vortex every 10 min during incubation. Centrifugation of the sample for 15 min at a speed of 5000×g and measured the volume of supernatant. The oil absorption expressed as volume of the supernatant oil per 100 g sample.

1.6 Gel formation

Gel formation analysis was performed according to [4]. Samples were prepared in a various suspension concentration (2–20%) in distilled water. Those samples were heated in a water bath for 1 h at 90°C, then fast cooled and incubated at 4°C for 2 h. Observed the gel formation at a certain concentration of sample which was characterised by the ability of the sample to remain in the tube without falling when the test tube was inverted.

1.7 Foam Capacity and Stability

Sample (2 g) was dissolved in distilled water (100 mL) and beaten using a hand mixer (Kris 932-C) for 5 min. Poured the shaken sample solution into a 250 mL measuring cup and observed the foam formed for 30 sec. The foam capacity was calculated as the increased volume/the initial volume × 100%. The foam stability is observed at 5,10,20,30,40,50,60, 70,80 and 90 min and calculated as final volume at certain time/initial volume × 100%.

1.8 Emulsion Capacity and Stability

Emulsion capacity and stability were performed according to [5] with modification. Sample (8 g) was mixed with distilled water (100 mL) and corn oil (80 mL). Homogenised it using a hand blender (Kris 932-C) for 1 min. Poured the emulsion (10 mL) into a centrifuge tube and centrifuged (8000×g, 15 min). The emulsion capacity was measured as the volume of the formed emulsion phase (mL). Heated the oil-in-water emulsion in a water bath (80°C, 30 min) and cooled at room temperature. Then, centrifuged at a speed of 8,000 g for 15 min. The emulsion stability was measured as the volume of the emulsion phase formed (mL).

2 Result and Discussion

2.1 Protein Solubility

Soluble protein content analysis was carried out using Bradford reagent. The solubility of protein was influenced by the pH of the medium, temperature, the condition of the ionic strength of the material, and the condition of the chemical structure of the material [5-6].

Boiled red kidney bean protein had the lowest level of protein solubility at pH 4 (Figure 1). The dominant protein in red kidney beans is globulin which has an isoelectric point of 4.1–4.6. Globulins will precipitate at pH 4.1 while other proteins such as proteose, prolamin, and albumin are soluble in water, resulting in a decrease in soluble protein levels.

The results also showed that the protein content of boiled kidney beans dissolved in water generally formed a decreasing pattern with increasing boiling time. Boiling has the potential to dissolve red kidney bean protein in water as a heating medium. It will stretch the structure of the starch-protein complex so that the protein is denatured and the starch is gelatinized. The longer boiling time will cause more open quaternary and tertiary protein structures. The opening of the protein structure can cause the hydrophobic groups of the protein to be exposed and come into contact with water. This can reduce the level of water-soluble protein. Starch gelatinization factor also affects protein solubility.

Boiled red kidney bean starch granules for 3 min have started to break and the amylose in the granules comes out. Intramolecular bonds in amylose can trigger the re-association of amylose components after heating at high temperatures [7]. Intramolecular bonds in amylose have the potential to form a matrix structure that can inhibit the interaction of proteins with water so that the solubility decreases to be released and dissolved in water.

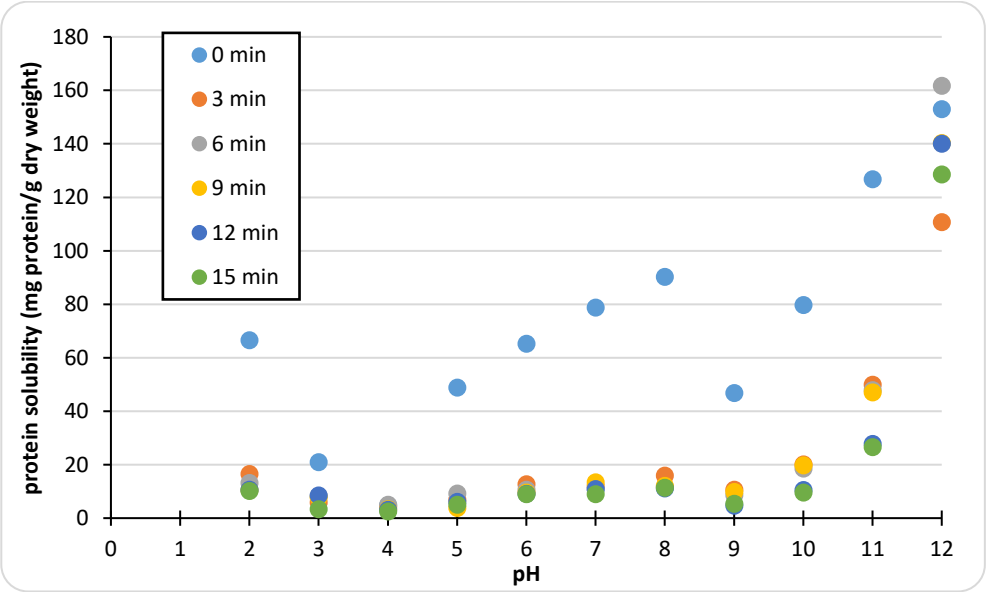

Fig. 1. Effect of boiling time of red kidney bean on soluble protein content in various pH

2.2 Moisture content

The moisture content of boiled red kidney beans in the third minute decreased, then increased at the next boiling time (Figure 2). The measured moisture content is influenced by the starch and protein components in the material because both have the ability to bind water.

Red kidney beans boiled for 3 to 9 min had lower moisture content than control beans. It was due to the strong binding of water by the material due to the gelatinization of starch and some of the hydrophilic groups of the protein which were originally exposed to the outside due to heating then the amount of free water decreases.

The moisture content increased when the boiling time was above 9 min. This was caused by the starch granules starting to break, the longer the boiling time the more starch granules broke, even though at 15 min not all red bean starch granules were broken. Water trapped in starch granules is released out into free water.

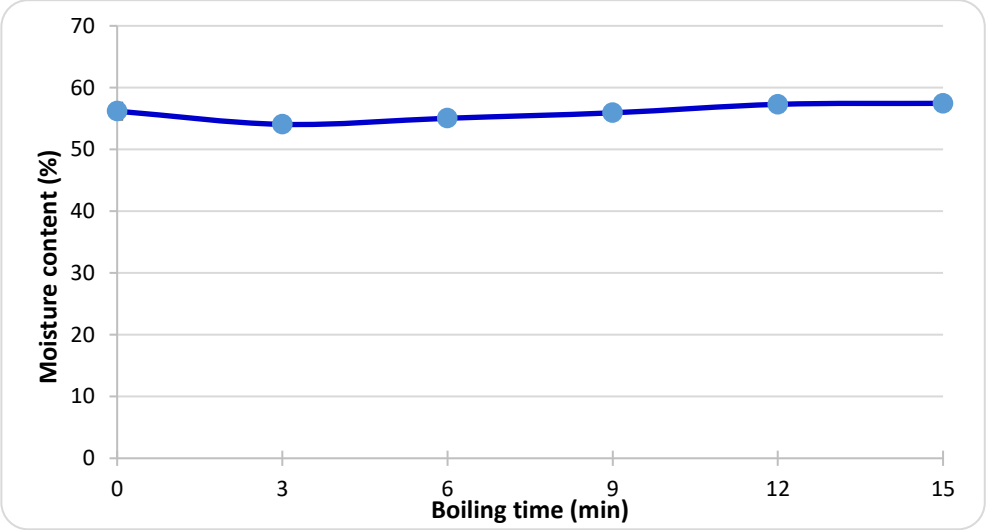

Fig. 2. Effect of boiling time of red kidney bean on moisture content

2.3 Water and Oil Absorption

The water absorption and oil absorption of red kidney beans increased with boiling time up to 9 min and then decreased in the next boiling time (Figure 3a and 3b). The increase in water absorption and oil absorption were caused by the stretching of the starch-protein complex structure due to boiling. Heating can weaken the non-covalent bonds in the secondary and tertiary structures of proteins causing protein denaturation [8].

Heating caused some of the hydrophilic and hydrophobic groups to be exposed so that they interact and bind with water and oil. When in water absorption, the group that plays a role is the hydrophilic group, on the other hand in the oil absorption the role is the hydrophobic group that interacts with the oil. Protein denaturation has reached the threshold at 9 min boiling, all red bean protein structures have been exposed so as to produce maximum water and oil absorption. Proteins also create cavities due to the opening of the protein globular structure so that it can be filled by water.

The size of starch granules that swells due to gelatinization and the presence of empty spaces created by the crystallisation of amylose and the robustness of the starch structure by amylopectin can increase oil absorption, as well as water absorption. Intramolecular hydrogen bonding of amylose can trigger the re-association of amylose components after

heating at high temperatures [7]. This causes the leached amylose to potentially form a matrix that can trap water or oil.

The decrease in water absorption and oil absorption occurred during the next boiling time. This is caused by more protein coagulation and more starch granules are experiencing structural collapse so that the ability to absorb water and oil decreased. It was supported by the water content (Figure 1), where at the time of boiling to 12 and 15 min the amount of free water continues to increase.

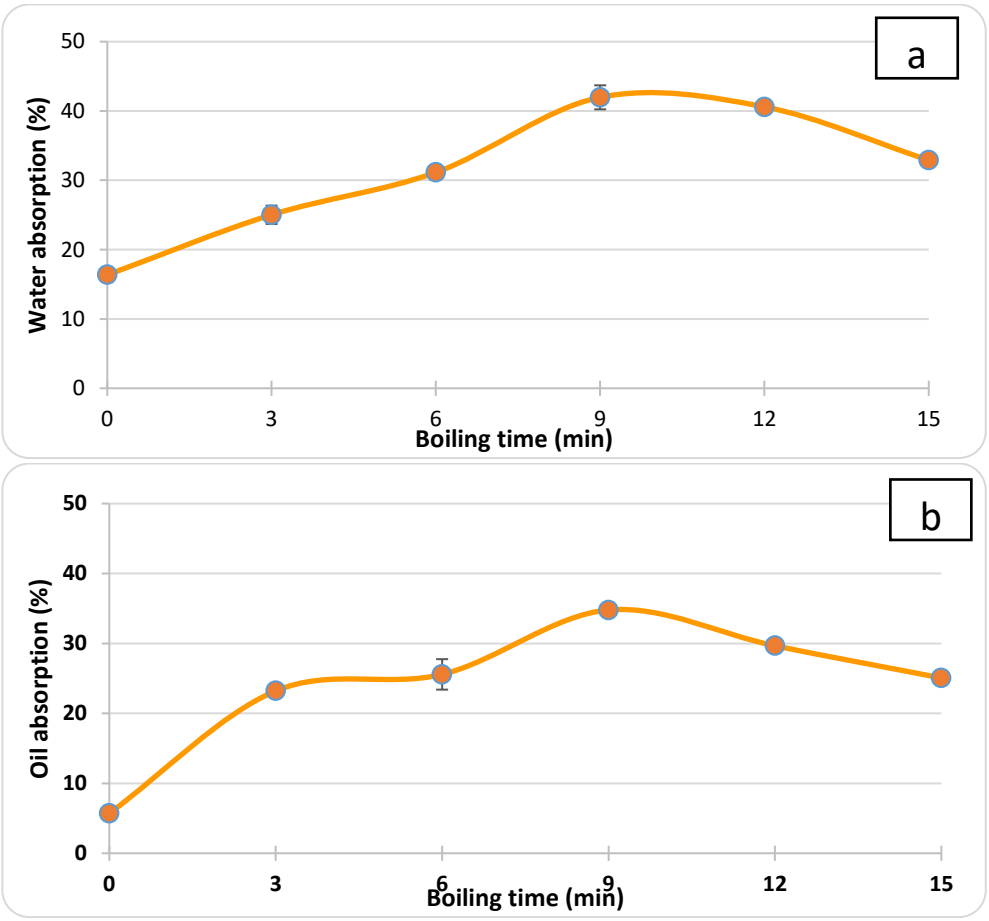

Fig. 3. Effect of boiling time of red kidney bean on water absorption capacity (a) and oil absorption capacity (b)

2.4 Gel formation

Gel formation is a test of the ability of the chemical components of the sample to form a gel [4]. The gel form is formed due to cross-linking between polymer molecules which produces an intermolecular network in a liquid medium [1]. The results showed that no gel could form in all treatments up to the highest sample concentration, which was 20% of the solvent. The gel that did not form indicated that the control red kidney beans and boiled kidney beans did not have sufficient starch and protein to trap all the water up to a concentration of 20%. This

is due to the high-water content of crushed boiled red beans because they have not been dried so that the total amount of solids is low including total starch and protein.

2.5 Foam Capacity and Stability

The capacity and stability of the foam is a test to show the ability to form foam that can be produced by the sample in the solvent through the shaking process as well as the ability to maintain the volume of the foam [4]. The foam capacity is indicated by the observation time of 0 min, while the foam stability is indicated by the pattern of changes in the foam volume between observations (Figure 4).

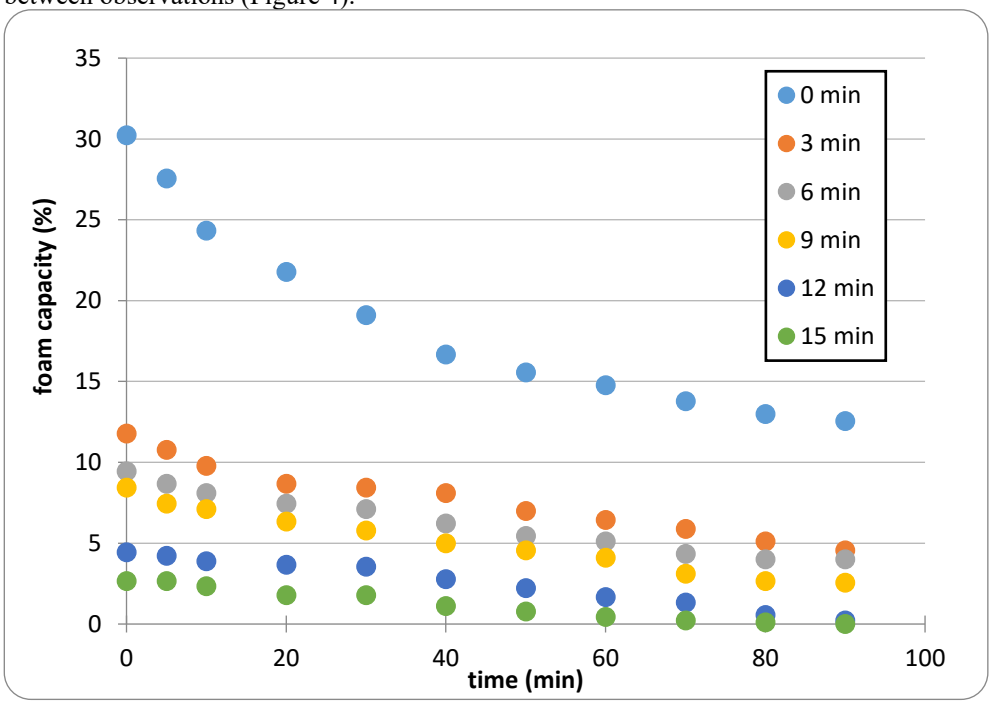

Fig. 4. Foam capacity and stability during 90 min in various boiling time of red kidney bean

The formation of foam is based on the mechanism of dissolved proteins that reach the interface between water and air through the process of diffusion, concentration, and surface tension and undergo changes in the polypeptide structure based on the level of polarity [9]. The polar segment of the protein molecule leads to the water molecule side and the non-polar segment leads to the air particle side. The highest foaming capacity was owned by control red kidney beans and decreased with boiling time (Figure 4a) indicated that with 3 min of boiling, its protein has been excessively denatured so that the hydrophobic groups were exposed to the outside. This resulted in decreased protein solubility and reduced foam capacity.

Foam stability is influenced by the structure of starch and protein in the interface layer. These two components greatly determine the viscoelastic character of the foam, namely the viscous nature of the foam but remains elastic so that it does not collapse easily. Foam formed from boiled red kidney beans gave better stability results because it was able to maintain the foam, as in 3 min of boiling time. This was due to the gelatinization of starch which caused the collapse of the starch granule structure. This component can increase the viscosity of the

foam interface layer formed so that it becomes more resistant to collapse, then caused the stability of the foam to be increased.

2.6 Emulsion Capacity and Stability

Capacity is a test to show the volume of emulsion formed as a result of the emulsification of oil and water by the sample and the ability to maintain the emulsion. Emulsion stability determines the amount of emulsion layer that can still be maintained after the emulsion is heated for a certain time [5].

Emulsion formation is strongly influenced by the presence of protein which has the ability as an emulsifier. This is because proteins have hydrophobic groups that can bind to non-polar lipids and hydrophilic groups that can bind to polar water [10]. Partial denaturation of the protein structure is also required so that the polar and non-polar groups can orient the interface layer between the water and oil fractions and form an emulsion system. The results of the emulsion capacity research are shown in Figure 5a. while the results of the research on the stability of the emulsion to heat are shown in Figure 5b.

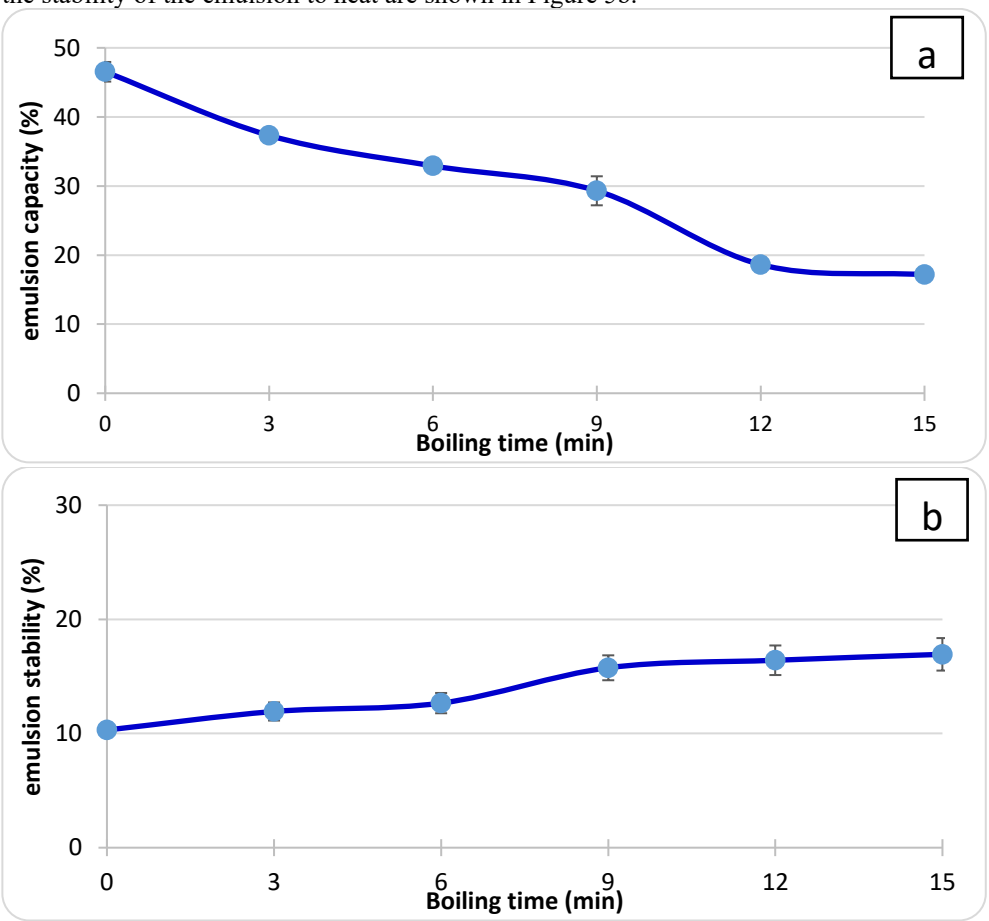

Fig. 5. Emulsion capacity and heat stability of emulsion in various boiling time of red kidney

The highest emulsion capacity was shown in the control red kidney bean sample and continued to decrease in the next boiling time (Figure 5a). The results showed that red beans

without boiling gave maximum results for the emulsion capacity even though partial denaturation had not occurred. The process of shaking the sample in solution with a hand blender for 30 seconds provides mechanical and heat treatment that has the potential to partially denature the sample protein. Boiling treatment that has denatured the sample has the potential to produce samples with excessive denaturation due to the shaking process. This causes the protein solubility to decrease and the formation of the emulsion interface layer becomes increasingly difficult to occur. Changes in emulsion capacity due to boiling produced a different pattern with water and oil absorption increasing up to the 9th minute. This indicates that the role of protein is very dominant in the formation of emulsions, while the absorption of water and oil is mostly influenced by starch.

The stability of the emulsion to heat is carried out because in its application in the next process, boiled red beans will have a high chance of undergoing a heating process, such as in the manufacture of meatballs, sausages, and even cakes. The results of the emulsion stability study showed an increasing pattern from control to boiled red kidney beans for 15 min, inversely with the pattern emulsion capacity (Figure 5b).

The heating process plays a role in denaturing the protein structure. Emulsion by controlling kidney beans is highly dependent on the presence and structure of protein. The protein will be further denatured so that it is excessively denatured and reduces the stability of the emulsion. The emulsion formed by other boiled red beans showed better stability because the protein structure was already coated with gelatinized starch so that the heating effect on the emulsion was not as large as that of the control red kidney bean emulsion.

3 Conclusion

Boiling treatment affects the functional properties of red kidney beans. It affects protein solubility, moisture content, water absorption, oil absorption, foam capacity and stability as well as emulsion capacity and stability, but does not affect the gelling ability of boiled red kidney beans. Protein solubility, foam capacity, and emulsion capacity decreased with boiling time, while foam stability and emulsion stability increased with boiling time. Water absorption and oil absorption increased with boiling time until 9 min, then decreased. Boiling up to 9 min is considered still able to provide good functional characteristics, especially in supporting the formation and stability of the emulsion. The use of these beans in processed food products based on these properties can be an important concern.

4 Acknowledgement

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