Investigation of Physical Stability of Baobab Pulp Emulsion – for Food Application

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Abstract

Background and objectives Baonaise is a novel plant-based savoury sauce. Baonaise sauce base (BSB) is produced from baobab fruit pulp. Creaming and phase separation were observed in Baonasie from previous studies at the company. In this study, concentration, temperature and type of oil, speed and time of mixing were studied to investigate the factors influencing the physical stability of baobab pulp emulsions. Oil content and processing parameters were optimized, while Baonaise with improved physical stability was prototyped.

Methods BSB was made by emulsifying rapeseed oil into liquid baobab base (LBB) which was prepared by soaking baobab pulp in the water bath. Once rapeseed oil was emulsified, the premix of seasonings was added to produce Baonaise. Centrifugal method was used to accelerate the phase separation to evaluate the physical stability of baobab pulp emulsion. Particle size distribution and viscosity were measured to explain the changes in the physical stability, while the morphology of oil droplets was also checked under microscope.

Results When 30%-60% w/w of rapeseed oil was added to LBB by the analytical blender at 22000 rpm for 20 minutes, a comparable centrifugal stability of BSB to the commercial mayonnaise reference was achieved. Besides, incorporating 1%-3% w/w of flaxseed oil had no significant influence on the physical stability of BSB. Finally, Neutral, Sudanese, and Nordic flavours of Baonaise were produced.

Conclusion Higher ratio of oil, greater agitation intensity, prolonged emulsification and addition of mustard could reduce the risk of phase separation of baobab pulp emulsions. Results from this thesis could be used to produce BSB and Baonaise of competitive physical quality to the commercial mayonnaise.

Keywords: baobab fruit pulp, emulsion physical stability, plant-based sauce.

Popular Science Summary

As a cold sauce originated from France, mayonnaise is nowadays one of the most famous sauces worldwide normally made from egg yolks, vegetable oil, salt, vinegar, and mustard. The magic of mixing immiscible liquids into a semi-solid sauce is the formation of an emulsion. The science behind mayonnaise or similar food emulsions is the dispersion of oil in the form of small droplets in the continuous aqueous phase. No specific equipment or technique is needed to make it; by slowly adding the oil while beating the egg yolk and continuously incorporating the other ingredients until thoroughly combined, you will have a light and shiny sauce with a smooth, creamy and thick texture.

You won't be surprised to find a full-fat mayonnaise contains more than 70% of vegetable oil. Although all emulsions are unstable and will eventually break down during prolonged storage, the high amount of oil in mayonnaise contributes to not only its attractive appearance, but a perfect physical stability. However, the concern on the health and the calls for sustainability have put the food producers in the race for alternatives. You might notice that more and more "light mayonnaise" (with reduced fat) and "vegan mayonnaise" have been popping up on the shelves, where egg yolk is usually replaced by modified potato starch, pea protein or soy protein. But, in most cases, the quality of those products is not competitive to the real mayonnaise.

You don't want to see a layer of separate oil on the top of your sauce. Also, you don't too much calories or a long list of additives. Is it possible to have a healthy vegan sauce without compromising to the physical stability?

When looking for an ingredient out of this purpose, baobab (*Adansonia digitata L.*) is listed as a potential candidate which has been approved as a novel ingredient by the EU in 2008. As you might know, the baobab tree is a signature of Africa. The fruit is included in the traditional meals in African, and it has numerous cosmetic and medical applications. It is rich in vitamin C, antioxidant compounds, and dietary fibres, especially pectin, a suggested food emulsifier. Baobab pulp is suitable for extended storage and is convenient for industrial applications as it is naturally dried in shells and light in weight.

Regarding the flavour, nutrition and processing properties, a savoury sauce named Baonaise made from baobab pulp and rapeseed oil was created by Arwa FoodTech AB. However, the very original version of this sauce had a problem of creaming during storage. This thesis is to help to solve this problem. In the preliminary study, a comparison between Baonaise sauce base (BSB) and the commercial references confirmed the need of improving emulsion stability. Then, the investigations into the effect of ingredients and processing parameters were conducted. The stability of the emulsions was evaluated by the centrifugal method, where unstable sauce would be separated into layers while the stable part was still homogenous. At the same time, particle size and rheological behaviours were determined to understand the variations in stability under different treatments. The results show that oil content, mixing speed and time play vital roles in the quality of baobab emulsions, especially the physical stability; baobab emulsions have similar flow behaviours as mayonnaise. Baonaise comes up with three different flavours.

The outcome of this thesis highlighted the needed parameters to produce stable Baonaise, a healthy and plant-based savoury sauce that is promising to satisfy the urgent need from the market.

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BSB – Baonaise sauce base

LBB – Liquid baobab base

RT – Room temperature

SD – Standard deviation

rpm – Revolutions per minute

1 Introduction

1.1 From Mayonnaise to Baonaise

Mayonnaise is one of the most popular and oldest types of sauces globally, and it is a typical application of emulsion in the food industry which has been studied for years (Depree and Savage, 2001). This semi-solid sauce is pale yellow with a thick and creamy texture, while vegetable oil, egg yolk, salt, vinegar and thickeners are widely used as the main ingredients of mayonnaise (Liu, Xu and Guo, 2007). Oil droplets disperse in the aqueous phase making mayonnaise an oil-in-water emulsion (McClements, 2016). Depending on the oil content, mayonnaise could be either full-fat containing 75-80% of oil or low-fat with 30-65% of oil (Depree and Savage, 2001). The high amount of oil contributes to desired firmness; however, it links mayonnaise to some potential health risks such as type 2 diabetes, cardiovascular disease, and cancer. This is why various attempts had been tried to reduce the amount of oil in mayonnaise. (Nikzade, Tehrani and Saadatmand-Tarzjan, 2012; Ghazaei *et al.*, 2015)

Besides health concern, nowadays, more and more aware of sustainability has expanded the market for vegan foods. As an alternative to mayonnaise, a novel vegan sauce named Baonaise has been proposed by Arwa Food Tech, prototyped in Aventure AB, Sweden. This “mayonnaise-like” product is a low pH, semi-solid emulsion made from baobab pulp and rapeseed oil, which is meant to be cold sauce. Notably, with baobab fruit pulp as the flavouring and, more importantly, the emulsifier and stabiliser, Baonaise is considered rich in nutrients with less oil but more dietary fibres, vitamin C and iron, compared to mayonnaise. Moreover, during the previous product development in the company, it was observed that Baonaise had a similar texture to those of full-fat comparable savoury sauces (Aventure AB, 2020). But unlike commercial vegan mayonnaise, no starch-based thickener is needed for Baonaise, thanks to the natural pectin from baobab (Gebauer, El-Siddig and Ebert, 2002). Nevertheless, the nutritional and functional benefits of baobab, which will be discussed in the following chapter, make it a niche market. However, very limited information can be found on the scientific mechanism of its functional properties and specified manufacturing techniques of related food products. Thus, by investigating the baobab emulsion and establishing standard processing parameters, this study is important to the commercialisation of baobab.

1.1.1 Baobab

Baobab (*Adansonia digitata L.*) species belong to the family of Malvaceae, also known as “monkey bread” (Gebauer, El-Siddig and Ebert, 2002; Sidibe and Williams, 2002).

This important indigenous tree is commonly available in the drylands of Africa and also can be found in Jamaica and Australia (Aluko *et al.*, 2016). Baobab fruit capsules contain soft whitish powdery pulp and kidney-shaped seeds (Sidibe and Williams, 2002). Baobab pulp is obtained by cracking the hard outer shell and removing shell and seeds as described in EC regulation No. 258/97. Baobab pulp is naturally dried in a shell, making it suitable for long-term storage, and it is usually grounded and sieved into a fine powder for further applications (Aluko *et al.*, 2016). However, due to the economic and sensory properties concern as from the feedbacks of previous projects on baobab in the company where the powder method was tried by Vincent (2020) and a gritty texture was observed, along with a higher industrial cost on the sieving machine, a soaking method was used in this study to produce liquid baobab base as described in *2.1 Sample preparation*.

Baobab fruit is very nutritious. As stated in EC regulation No. 258/97, there is 2.03 g - 3.24 g of protein in 100g dried baobab pulp. Also, baobab is rich in vitamin C (300mg/100g dw), calcium (655.0mg/100g dw) and antioxidant compounds, according to Gebauer, El-Siddig and Ebert, 2002 and Aluko *et al.*, 2016. Besides, baobab pulp possesses a high amount of dietary fibres from the study of Chadare *et al.* (2009), where the amount varied from 6.0g - 45.1 g in 100 g dried pulp. For polysaccharides, no starch (Gebauer, El-Siddig and Ebert, 2002) but 56.2% of galacturonic acid (Nour *et al.*, 1980) or 2.6% of pectin (Ndabikunze *et al.*, 2011) was found in the pulp. When it comes to the taste, it is said that sugars such as fructose, sucrose and glucose and natural organic acids like citric acid and tartaric acid play essential roles in the flavour, though the amount varies in different planting areas (PhytoTrade Africa, 2006; Aluko *et al.*, 2016).

Studies of baobab primarily focused on agricultural, botanical, biochemical and nutritional aspects (Gebauer, El-Siddig and Ebert, 2002; Chadare *et al.*, 2009) until 2008 when baobab fruit pulp was authorised as a novel food ingredient by the European Union under the EC regulation No. 258/97. Then, more and more research had insight into the functional properties of baobab, especially the thickening, gelling and emulsifying properties from the high amount of pectin in the pulp of baobab. According to Ndabikunze *et al.* (2011), baobab pulp powder was comparable to the commercial pectin in the jam production, where a perfect gelation capacity of baobab pulp was revealed by a nice gel formed at a low concentration of 11%. Likewise, Aluko *et al.* (2016) stated that the emulsification capacity of baobab pulp from Tanzania was from 37.90% to 45.15% regarding different locations, though it was lower than the capacity of egg yolk. Salma Elzen *et al.* (2018) also suggested baobab pulp as an ideal emulsifier in the O/W emulsion as 40.75% to 54.99% of pectin could be extracted from Sudanese baobab pulp. In a previous thesis in the department, Dharukaradhya (2020) made an emulsion from hydrated baobab pulp and rapeseed oil, and the pH was from 3 to 5. However, the mechanism of the emulsifying property of baobab pulp is inadequate.

All the nutrition benefits and functional properties of baobab suggest it a prospective healthy food ingredient. In fact, baobab pulp has been extensively applied in the traditional diet of African, where baobab is known as the contributor to rural revenues and human nutrition (Aluko *et al.*, 2016), let alone its numerous medicinal uses (Chadare *et al.*, 2009). For instance, in Sudan, baobab pulp was used to make a refreshing drink called "gubdi" as a source of protein (Obizoba and Anyika, 1994). Similarly, the Malawian make a sweet and milky drink by boiling the combination of pounded baobab seeds, baobab fruit pulp and water (Wickens, 2008). In addition, baobab pulp is used as a supplement to staple food. For example, farmers from northern Nigeria kneaded baobab pulp in the cold water and strained it through a sieve and use it to dilute thick guinea corn into a thin gruel (NICOL, 1957; PhytoTrade Africa, 2006). Furthermore, thanks to the organic acids, baobab pulp is a perfect alternative to the cream of tartar used in baking (Gruenwald and Galizia, 2005).

It was said that PhytoTrade Africa (2009) had planned to introduce baobab pulp into smoothie and cereal bars. However, baobab is still underutilised when talking about industrial application. This study aims to fill the gap where more investigation on the behaviour of baobab pulp in the food emulsion is needed.

1.1.2 Oil

The emulsion of mayonnaise is formed by gradually adding oil to the aqueous phase with egg yolk under agitation (Depree and Savage, 2001). Oil is critical to the quality of final products. The higher the amount of oil, usually the firmer, smoother and creamier the texture of mayonnaise (McClements, 2016). In some previous studies, it is noticed the relation between the failure of emulsion and less than 50% of oil. For instance, Franco *et al.* (1995) noticed a phase separation after one day when the emulsions with less than 40% oil had been stored at 5 °C. Usually, to prevent creaming, the emulsion requires the help from additives like the emulsifier, e.g., sucrose stearate. In addition, oil has considerable influence not only on viscoelastic behaviours, but on the organoleptic properties (McClements, 2016). Generally, oils with neutral taste such as rapeseed oil and sunflower oil are favourable for mayonnaise. Since rapeseed oil is locally produced, it is used for Baonaise sauce base. According to the food database of Livsmedelsverket (no date), rapeseed oil contains 7.1% of saturated fatty acids, 61.3% of monounsaturated fatty acids and 27.4% of polyunsaturated fatty acids. The density of rapeseed oil is 0.90 $g/m^{3,}$ according to Noureddini, Teoh and Clements (1992).

Sudanese baobab seeds are the by-product of baobab pulp containing 19% of oil (Satti, 2018). Baobab seed oil is edible and is considered a source of unsaturated fatty acids. To be more precise, 31.7%, 37.0% and 31.7% of saturated, monounsaturated and polyunsaturated fatty acids were found in baobab seed oil, respectively (Osman, 2004).

Palmitic, linoleic acid and oleic are the major forms of fatty acids in baobab seed oil (Komane *et al.*, 2017). Recently, the oil produced by cold-pressing baobab seeds was introduced to the cosmetic and food industry (Bamalli *et al.*, 2014). The density of baobab seed oil and rapeseed oil were 0.88 g/m^3 (M *et al.* 2014) and 0.9 g/m^3(Noureddini, Teoh and Clements, 1992), respectively. For sustainability consideration, the baobab seed oil is a candidate of ingredient to substitute rapeseed oil in the production of food emulsion. Compared to rapeseed oil, baobab seed oil has more saturated fatty acids.

Flaxseed oil is pressed from the seeds of the flax plant (*Linum usitatissimum*) which has a high content of lignans, fibre, etc. The density of flaxseed oil is 0.92 g/m^3 to 0.94 g/m^3 (Zhang *et al.*, 2011). Flaxseed oil has recently received increasing attention from the food scientists and public thanks to its nutritional benefits (Goyal *et al.*, 2014). In flaxseed oil, α-linolenic acid, an omega-3 fatty acid, is found around 47% to 70% (Zhang *et al.*, 2011; Martinchik *et al.*, 2012). Numerous evidence indicated the relationship between the consumption of flaxseed oil and the reduction of cardiovascular, obesity, etc. (Roberta, Raffaella and Giuliana, 2014). Hence, a small proportion of flaxseed oil could help to fortify the food products with nutritious polyunsaturated fatty acids.

1.2 Emulsions

1.2.1 Oil-in-water emulsion

Emulsions usually consist of two immiscible liquids such as water and oil, where the discrete droplets compose the dispersed phase while the surrounding substance makes up the continuous phase (McClements, 2016). Food emulsions can be categorised into oil-in-water (O/W) and water-in-oil (W/O) emulsions regarding the phases. Full-fat mayonnaise is a typical O/W emulsion (Walstra, 2003; McClements, 2016). High energy from mechanically stirring breaks oil into smaller droplets to achieve better dispersion of oil in the aqueous phase, with lipoprotein and lecithin in egg yolk stabilising the interface of two phase (Widerström and Öhman, 2017) by adsorbing on the interface and inducing the steric repulsion between oil droplets (Pashley and Karaman, 2005). Beside egg yolk, mustard also contributes to the physical stability (Harrison and Cunningham, 1985). Mucilage in mustard can decrease surface tension; when the interfacial energy between oil and the continuous phase drops, the oil tends to shrink into smaller particles for larger surface area; as the consequence, the physical stability of the emulsion could be increased. (Weber, Taillie and Stauffer, 1974; Cui, Eskin and Biliaderis, 1993).

1.2.2 Instability of mayonnaise and mayonnaise-like emulsions

During the production of mayonnaise, there is a risk of phase inversion where the emulsion converts between O/W and W/O under mechanical agitation during or after the addition of oil (McClements, 2016). Although phase inversion is an essential manufacturing process for butter and margarine, it should be avoided in the production of mayonnaise-like emulsions, Baonaise, in this study. By measuring the texture and particle size, Widerström and Öhman (2017) found that when the oil flow was too fast or the mixing was prolonged too much, there was a phase inversion that broke the mayonnaise, while temperature only had minor influence.

Although Baonaise emulsion is successfully produced, the risk of instability is still there and affect the quality and shelf life of the product. As a matter of fact, all food emulsions are thermodynamically unstable systems and will eventually break down. Therefore, one of the factors that distinguish the quality of emulsions is kinetic stability, and it is used to indicate how fast the emulsion property will change over time (Atkins and de Paula, 2014). Once the emulsion is not stable, several physical movements such as creaming (the rising of oil droplets), flocculation (the aggregation of oil droplets), coalescence (the merging of droplets) and eventually, phase separation may occur as oil droplets aggregate into larger particles resulting in a separation of water and oil phases (McClements, 2016). The creaming/sedimentation velocity (v_{sed}) is affected by the diffence in the mass density of the particle and fluid, the radius of the oil droplets and the dynamic viscosity of the continuous phase (*Equation 1*). Besides, the volume fraction of oil can also influence the creaming velocity (Pashley and Karaman, 2005). Regarding the floccualation, the mechanism is Brownian motions, shear induced flocculation. The coalescence happens due to the interactions between droplets, for instance, van der Waals attraction, electrostatic repulsion, steric repulsion, etc. (McClements, 2016) .

$$v_{sed} = \frac{2\,(\rho_p - \rho_s)g}{9\eta} r^2$$

Equation 1. Stokes' law, where ρ_p, ρ_s, g, η and r refer to the mass density of the particle, mass density of the fluid, gravitational field strength, dynamic viscosity, and radius of the particle.

For full-fat mayonnaise, gravitational separation is not usually seen, as a large amount of dispersed oil traps each other from creaming (Widerström and Öhman, 2017). However, it was noticed in previous projects in the company where the Sudanese Baonaise had a phase separation after two weeks (Aventure AB, 2020), it might be due to the reduction of oil content in Baonaise which weakened the effectiveness of packing of oil droplets in the condensed aqueous phase and thus, led to creaming or phase separation. To achieve better stability, factors affecting the emulsification of baobab and oil phase should be taken into consideration. The ideal physical stability of BSB should be comparable to the selected commercial reference. According to Harrison and Cunningham (1985) and Lowe (2010), except the ratio of the two phases, the formation and stability of mayonnaise were affected by the emulsifier, agitation procedures and the viscosity of the final product.

1.2.2.1 Droplet size

The size of dispersed droplets is an essential parameter for an emulsion system. Usually, the particle size of mayonnaise is in the range of 0.1 μm to 10 μm (McClements, 2016). It is harder to separate smaller droplets from the continuous phase, meaning that the system can be more stable with smaller dispersed particles. Stokes' law predicts that reducing the particle size can enhance the emulsion since the creaming velocity of the droplet is proportional to its squared radius (*Equation 1*). Secondly, a significant impact of particle size on the viscosity of the emulsion also affects the stability. For the purpose of forming an emulsion and/or reducing the size of the droplets in a pre-existing emulsion, mechanical agitation are usually applied (Walstra, 2003; McClements, 2016).

1.2.2.2 Rheological behaviour

Mayonnaise-like emulsions are not pure liquid or pure solid. Instead, they behave partly viscous and partly elastic (Walstra, 2003; McClements, 2016). The so-called viscoelastic materials could instantaneously deform upon stress and instantaneously partly recover to the original form when the force is removed. (Walstra, 2003). Mayonnaise is a pseudoplastic emulsion (Goshawk *et al.*, 1998), and it becomes solid-like elastic under the yield stress (McClements, 2016), which is the minimum force needed to initiate flow (Ma and Barbosa-Cánovas, 1995). In preliminary experiments at the company, it was observed that Baonaise shared a similar texture with mayonnaise (Aventure AB, 2020), indicating that Baonaise could also be shear-thinning.

1.2.2.3 Pectin

As introduced in *1.1.1 Baobab*, baobab pulp is rich in pectin; the thickening and emulsifying activity of pectin make it potential to stabilize the emulsion (Nikzade, Tehrani and Saadatmand-Tarzjan, 2012).

Pectin enhances the stability of emulsions by increasing the viscosity of the continuous phase and forming a gel network; the droplets, therefore, become less movable. However, the composition, structure and other characteristics of pectin in baobab are different based on variations and places, which ends up with various emulsifying activity and emulsion-stabilising capacity. (Ngouémazong *et al.*, 2015)

In 2007, Liu, Xu and Guo mentioned pectin on the list of potential emulsifiers. Lately, Ngouémazong *et al.* (2015) stated that pectin was getting more acceptance as a food emulsifier. In the process of emulsification, pectin tends to adsorb and cover the surface to avoid aggregation of the oil when it is disrupted into fine droplets; over time, more pectin adsorbs on the interface and stabilises the emulsion; the emulsifying activity of pectin mainly comes from its protein moiety, feruloyl, and acetyl groups (Ngouémazong *et al.*, 2015; Kpodo *et al.*, 2018). *Figure 1* shows the pectin adsorption models that illustrate the behavious of pectin during emulsification and instabilizaition.

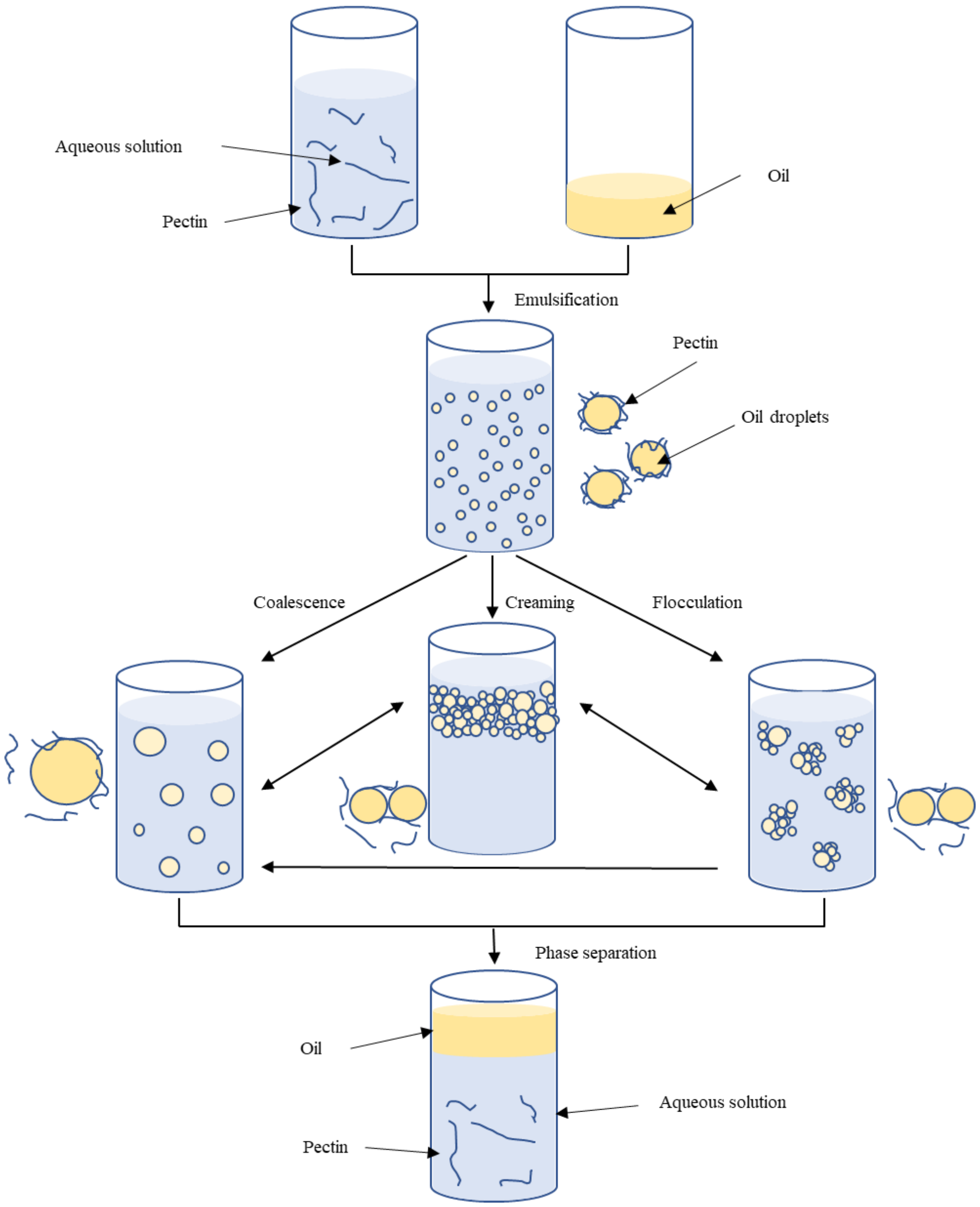


Figure 1. Pectin adsorption model during emulsification and emulsion instability (adapted from Ngouémazong et al., 2015; McClements, 2016).

1.2.2.4 Other possible factors influencing the emulsion stability

Mixing ingredients with different temperatures ended up with undesirable phase inversion in the production of mayonnasie, as mentioned by Widerström and Öhman (2017). Besides, heat from ingredients induces the aggregation of oil droplets (Keeratiurai and

Corredig, 2009; Nikzade, Tehrani and Saadatmand-Tarzjan, 2012). Thus, it might be better to store the ingredients at similar low temperature prior to mixing.

The influence of the mixing method was highlighted by Harrison and Cunningham (1985). It is said that intermittent mixing was better for emulsification than continuous one. However, the investigation on the duration of mixing was missed from their study, giving the freedom for the producer. But, generally, before the breakage of emulsion, longer emulsification and higher intensity of stirring contribute to smaller droplets and result in a more stable emulsion. Ariizumi *et al.* (2017) said that homogeniser speed affected the emulsion microstructure. On the other hand, slow agitation during the first oil addition resulted in low viscosity of mayonnasie (Lowe, 2010).

The storage temperature is also vital. The higher temperature, the greater kinetic energy of particles that increases the droplets velocity. Stimulated movement of oil droplets contributes to higher risk of flocculation. Also, increased temperature leads to decreased viscosity. (McClements, 2016) However, the phase separation could also happen in freezing condition. According to Ghosh and Coupland (2008), when the sauce is thawed, it is significantly destabilised, and in the worst case, it breaks down into the initial phases.

Physical stability is a vital quality of food emulsions. To some extent, it determines the shelf-life of the products. Various methods were applied to evaluate the stability of food emulsions. For instance, Chandra, Singh and Kumari (2015) calculated the emulsion activity percentage of composite flour emulsion as the ratio of the height of the emulsion layer to the total height of the ten times diluted sample in the calibrated centrifuge tubes after centrifugation at 2000 × g for 5 min. While Dharukaradhya (2020) investigated the stability of baobab pulp emulsion by rotational motion for ten days and checked the coalescence and flocculation under microscopy. In this thesis, the suitable method (*2.2.1 Emulsion stability percentage*) was selected based on purpose and sample properties.

1.3 Objectives

The main aim of the thesis is to improve the physical stability the emulsion of baobab fruit pulp and rapeseed oil in the form of Baonaise sauce base (BSB) and the flavoured sauce (Baonaise) by reducing phase separation. The influence of ingredients and processing parameters has been investigated. The following objectives are included to achieve the goal.

- Comparison of the centrifugal stability of BSB with reference commercial mayonnaise products from the local market.
- Determination of the emulsion stability, particle size and apparent viscosity of BSB.
- Investigation on the effect of oil content and mixing parameters.
- Trials on the possibility of incorporating baobab seed oil or flaxseed oil.

- Characterisation of BSB on the flow and viscosity behaviours, and emulsion stability.
- Modification on the recipes of Baonaise to increase the physical stability.

Aiming at longer physical shelf life of Baonaise, several ingredients and production steps have been changed based on the suggestion from Harrison and Cunningham (1985) and Depree and Savage (2001) to study the influence of factors affecting the stability of Baonaise sauce base (BSB). In terms of Baonaise, minor changes in the seasonings were made to obtain comparable physical stability to commercial mayonnaise. Generally, apparent viscosity, particle size distribution and centrifugal stability were determined as hints to evaluate the improvement of the physical shelf-life. Although chemical and microbiological factors can also affect the quality of baobab emulsions, only the physical stability has been discussed in this thesis. The conclusion of this study would be helpful for industrial applications of baobab emulsions to obtain a better quality by avoiding phase separation during storage as much as possible.

2 Materials and methods

2.1 Sample preparation

In general, naturally dried baobab fruit was hydrated and filtrated to produce the liquid baobab base (LBB). Once the oil was emulsified, the emulsion was called Baonaise sauce base (BSB). One of the food product applications of the baobab emulsion, a mayonnaise-like vegan sauce (Baonaise), was made by mixing BSB with seasonings. A flow chart in *Figure 2* illustrates the processing steps of baobab emulsion products.

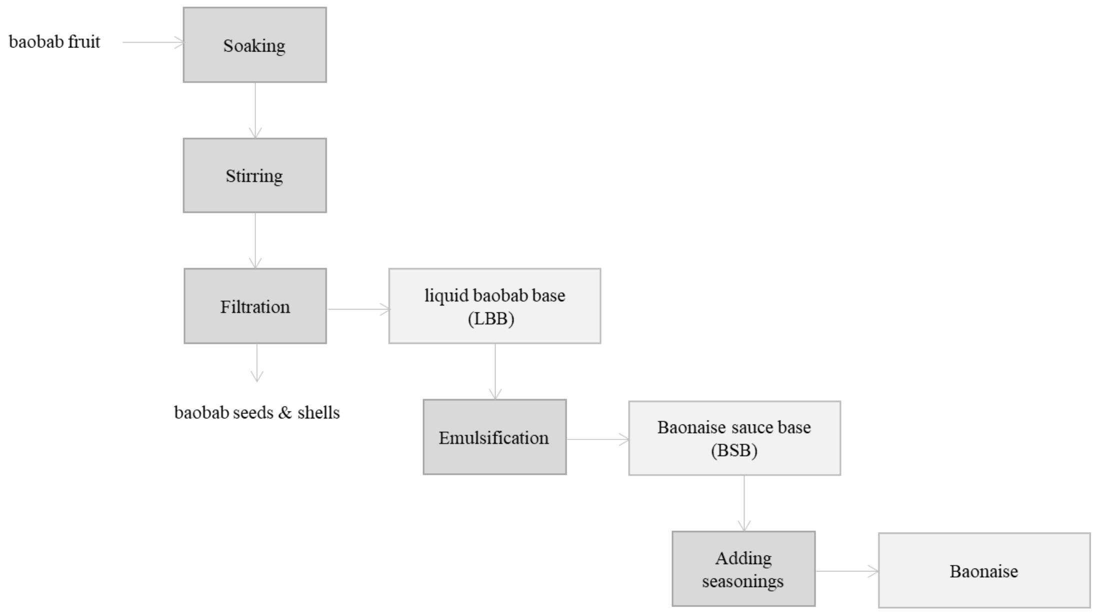


Figure 2. The production of Baonaise.

2.1.1 Preparation of liquid baobab base (LBB)

Liquid baobab base (LBB) was prepared according to the standard operating procedure revised from the internal document (Aventure AB, 2020). Dried baobab fruit (batch 2020, Sudan) was used in this study to prepare LBB. First, baobab fruit was hydrated by water at constant speed in water bath for an hour. After soaking, the mixture was stirred at constant speed for 30 minutes by a turbine vortex blade mounted on CAT agitator rotor R18 (LabTeam Scandinavia AB, Sweden). Next, baobab seeds and shells were separated from LBB through two sieves with different mesh sizes (unspecified). Before producing BSB, LBB should be stored in the fridge with a temperature of around 4 ℃ – 8 ℃.

2.1.2 Preparation of Baonaise sauce base (BSB)

Each time, 400 g of BSB was prepared in a 2000 mL beaker. The only ingredients of BSB were LBB and rapeseed oil (Zeta, Sweden). An analytical blender, Bamix Gastro 350 Pro (Bamix, Switzerland), with two-speed options (17000 rpm and 22000 rpm), was used. Oil was added through a separation funnel at a stable addition rate (approx. 18g/min). BSB samples were stored at 4 ℃ – 8 ℃ for analysis or further applications.

2.2 Analytical methods

2.2.1 Emulsion stability percentage

A 10 g amount of each sample was transferred into a 15 ml centrifuge tube; the work was performed in triplicates. The tubes containing emulsion were centrifugated for 30 minutes at 5000 rpm in Centrifuge 5804 (Eppendorf, Germany) at room temperature to accelerate the phase separation. The emulsion stability presented in percentage was the ratio of the height of the stable BSB emulsion layer to the total sample height in the tubes. The method is adapted from the emulsion stability measurements by Weber, Taillie and Stauffer (1974); Nikzade, Tehrani and Saadatmand-Tarzjan (2012); Chandra, Singh and Kumari (2015). In the original formulation (Aventure AB, 2020), baobab fruit (batch 2019, Sudan) was hydrated to make LBB, while BSB was produced by emulsifying LBB and 30-60% w/w of rapeseed oil.

2.2.2 Optical microscopy

Microstructures of baobab emulsions such as the shape, uniformity and aggregates of oil droplets were observed by biological system microscope CX41 (Olympus, Japan) at a magnification of 60×. Images were captured by the microscopy camera INFINITY1 (Lumenera, Canada).

2.2.3 Particle size

The particle size distribution of oil droplets was determined by light scattering with the help of Mastersizer 2000 (Malvern Instruments, UK). The background was firstly measured, and then samples were added into the water to a concentration of 10% laser obscuration. Triplicates were automatically performed on each sample. Medium size, D (0.5), was used to evaluate the particle size of emulsion droplets.

2.2.4 Viscosity

“Measure_0004 Single shear rate timed” sequence in Kinexus Rheometer (Malvern

Instruments, UK) was runned to measure the viscosity of the BSB under the stable shear rate 50.0 s^{-1} where 24 testing points were recorded within 2 minutes. Apparent viscosity (the ratio of shear stress and shear rate) was used to compare the rheological property of different samples. The viscosity of the sample was calculated as the mean value of the last 12 points where the viscosity became stable. The parallel plate geometries were the same for the two methods where the upper geometry PU40X SC0015 SS (diameter 40 mm) and the lower one PLCS65 C0002 SS were installed with a working gap of 1 mm. A heat exchanger in the equipment helped to set and keep the required temperature during testing. Three measurements were carried out on each sample where the shear rate or apparent viscosity was the mean of experimental values (Izidoro *et al.*, 2008).

2.2.5 Colour

Comparing different colour of the samples and tracking the changes in the colour of the sample during storage, a* (green - red), b*(blue-yellow) and L* (lightness) were measured by the portable spectrophotometer CM-700d (Konica Minolta, Japan). Zero and white calibration were done successively before measurement. The colour difference, ΔE, was calculated according to *Equation 2*. (Vik, 2003)

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Equation 2.Calculation of colour difference.

2.3 Statistical methods

The mean value was calculated to present the results along with a standard deviation that quantifies variability among replicates. In addition, an independent samples t-test and Tukey's multiple comparisons test were performed to compare the significant difference ($p \leq 0.05$) between the intervention and control groups by IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA). The curves with mean values, error bars and significance markers are plotted in Microsoft Excel 2016.

2.4 Experimental design

2.4.1 Comparation of BSB with commercial mayonnaise

To get an idea of the emulsion stability of BSB compared to the commercial mayonnaise, two products from the market, Hellmann's and Garant vegan mayonnaise, were selected to be the references. To understand what had contributed to the difference in the stability, viscosity, particle size distribution and oil droplets morphology were determined.

2.4.2 Formulation and processing optimization of BSB

2.4.2.1 Effect of oil

Firstly, to explore the ideal concentration of rapeseed oil in BSB emulsion, BSB with three different concentrations of rapeseed oil were tried to study the influence of the oil concentration on the emulsion stability.

Secondly, as baobab seeds were the main by-product from the processing of LBB, out of sustainability concern, this study explored the possibility of incorporating baobab seed oil. In the experiment, half of the rapeseed oil was replaced by baobab seed oil (batch 2019, Sudan) to make BSB.

Thirdly, another investigation was made on the flaxseed oil for the purpose of fortifying BSB with polyunsaturated fatty acids. Flaxseed oil (Biofood, Sweden) with a claim of 50% of omega-3 was incorporated either before the addition of rapeseed oil or after the production of BSB.

2.4.2.3 Effect of processing parameters

Based on the original standard operating procedure from Aventure AB, LBB was taken from the fridge right before the production of BSB. To see if the temperature of ingredients could affect emulsion stability, rapeseed oil both from the fridge and the room temperature was tried.

Besides, to understand the influence of agitation intensity on the stability of BSB emulsion, oil was mixed with LBB by Bamix at two different speeds for the same duration.

Finally, to investigate how the mixing time could affect the emulsion stability, the emulsification process by Bamix at 22000 rpm was extended 5 minutes each time.

2.4.3 Characterization of BSB

2.4.3.1 Dry matter

The dry matter of samples was determined by the moisture analyzer MJ33 (Mettler Toledo, Switzerland). First of all, the sample pan was placed in the flat pan holder. When the status of the machine changed to ready for weighing after taring, approximately 2 g of samples were evenly spread on the pan. The measurement started automatically after the sample had been loaded. The moisture content was obtained according to the thermo-gravimetric principle, where the sample was dehydrated to a stable weight. Then, the dry matter was calculated by subtracting the moisture content from 100%.

2.4.3.2 Rheological behaviours

When the production of BSB was optimized, the possible rheological behaviour during processing was investigated. "Measure_0007 Shear stress ramp log" sequence was used to obtain the flow curves of baobab emulsions under the shear stress from 1 to 1000 Pa for 2 minutes with 10 samples per decade. Geometries were the same as described in *2.2.4 Viscosity*. Data from the rheometer was fitted into the Herschel-Bulkley model according to *Equation 3* by Microsoft Excel 2016. This model is commonly used in the case of mayonnaise to adjust the data of shear stress and shear rate (Ma and Barbosa-Cánovas, 1995).

$$\sigma = \sigma_0 + K\gamma^n$$

Equation 3. Herschel-Bulkley model where σ *is the shear stress (Pa),* σ_0 *is the yield stress, K is the consistency coefficient (Pa* s^n*), and n is the flow behaviour index.*

Additionally, different temperatures were applied to BSB to investigate the influence in the rheological behaviours. Target temperatures were chosen based on the similar environment during real-life applications of sauces, e.g., inside the refrigerator (7 °C), during hot summer days (30 °C), served together with warm (50 °C) and hot (80 °C) food.

2.4.3.3 Stability of BSB after pasteurization

BSB products required heat treatment to ensure microbiological safety. To mimic the pasteurization and understand how the physical properties could be affected, BSB was heated in the water bath to hold the centre temperature at 78 °C for 15min. Hellmann's vegan mayonnaise was used as the reference in this study.

2.4.4.4 Stability of BSB during storage

To investigate the stability changes of BSB during storage under different conditions, the samples were stored in the fridge or at room temperature for 2 weeks. Characteristics such as pH, colour, particle sizes, viscosity, and stability% were determined.

2.4.5 Theoretical estimation of the nutritional value of BSB

The nutrient calculation is based on the final formulation of BSB. The nutritional content of baobab pulp is based on the data from PhytoTrade Africa (2009). The nutrient information of rapeseed oil is obtained from the database of the Swedish Food Administration (Livsmedelsverket, no date). All calculations were done by Excel. The theoretical nutritional fact of BSB was compared with commercial references.

2.4.6 Application of BSB

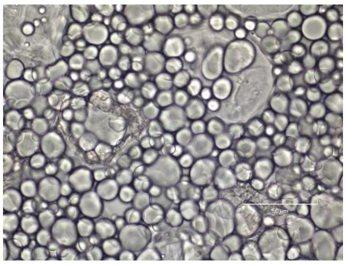
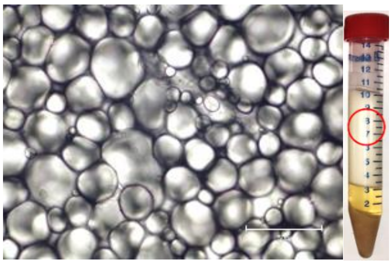
Baonaise is a vegan alternative to mayonnaise made of BSB and seasonings of three different flavours: Neutral, Nordic and Sudanese. Baonaise was produced by adding the premix of seasonings to the BSB under the mixing of Bamix at 22000 rpm for one more minute right after the production of BSB. Baonaise was stored in the fridge overnight. pH, colour, viscosity, microscopy, and stability% were determined. The preliminary trial was performed based on the original formula of Baonaise (Aventure AB, 2020).

3 Results and discussion

3.1 Emulsion stability percentage of BSB

As described in *2.2.1 Emulsion stability percentage*, the centrifugal method was used to accelerate the phase separation process and evaluate the emulsion stability. After centrifugation, BSB were separated into several layers (*Figure 3*).

Microscopy helped to understand the components of each layer and the changes of BSB when the phase separation was speeded up by centrifugal force. From *Figure 3(a)* to *Figure 3(b),* oil droplets aggregated after centrifugation and became larger; while in *Figure 3(c),* the bottom part of the centrifuge tubes consisted of plant cells and a few small oil droplets. It could be concluded that from top to bottom, the layers might be separate oil, BSB, water solution and fibres.

(a)  (b) 

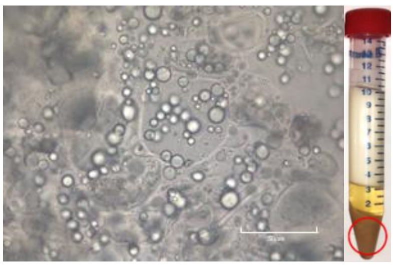
(c) 

Figure 3. Microscopy of (a) BSB before centrifugation, (b) BSB after centrifugation and (c) the bottom layer of the tube after centrifugation at 5000 rpm for 30 minutes under 60× with the scalebar of 50 µm.

3.2 Comparison of BSB with commercial mayonnaise

Information of the references bought from the market and BSB is noted in *Table 1*. BSB consisted of the lowest amount of oil, and it was the only one which contained less-processed plant ingredients.

Table 1. Main ingredients and storage temperatures of BSB and commercial mayonnaise.

	Rapeseed oil (% w/w)	The other main ingredient	Storage temperature
BSB	30-60	baobab pulp	8 °C
Garant vegan mayonnaise	66	pea protein	8 °C
Hellmann's vegan mayonnaise	72	modified corn starch	room temperature

In *Figure 4*, no fibre layer was observed in Hellmann's vegan mayonnaise; meanwhile, there was an apparent phase separation in the centrifuge tube of BSB.

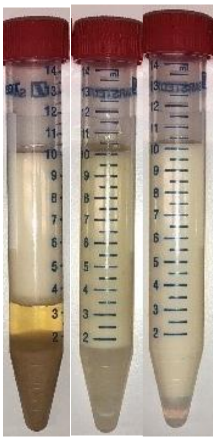


Figure 4. From left to right, Hellmann's vegan mayo, Garant vegan mayo and BSB after centrifugation at 5000 rpm for 30 minutes.

As BSB was designed to be sold in the fridge, this product was supposed to have comparable stability as Garant vegan mayonnaise, which was also a cold sauce. However, to be more competitive, this study aims at better characteristics as close as those of the vegan mayonnaise from Hellmann's, the leading brand in the mayonnaise market. However, BSB made according to the original recipe (Aventure AB, 2020) had significantly lower stability percentages than the commercial products: the stability of BSB, Garant, and Hellmann's vegan mayonnaise after centrifugation were 52.7%(1.0%)^{c}, 68.9%(1.1%)^{b} and 89.0(1.3)^{a}, respectively (values are presented in mean(SD), n=3. Different superscript letters (a-c) suggest an overall statistical difference according to Tukey test where $p \leq 0.05$). Therefore, improvement in the emulsion stability of BSB was needed.

As can be seen in *Table 2*, the viscosity and particle size of BSB were close to Garant vegan mayonnaise but still far from the target, Hellmann's vegan mayonnaise. Noticeably, the results showed the possibility of enhancing the stability of BSB by breaking down the oil droplets into smaller ones while increasing the viscosity of the emulsion.

Table 2. Particle size distribution and viscosity at the shear rate 50 s^{-1} of BSB and the commercial mayonnaise. Values are presented in mean(SD), n=3. Different superscript letters (a and b) in each column suggest an overall statistical difference according to Tukey test where $p \leq 0.05$.

	d(0.5) (μm)	Viscosity (Pa·s)
BSB	$28.73(0.12)^{a}$	$1.56(0.20)^{b}$
Garant vegan mayo	$30.65(2.98)^{a}$	$3.10(0.41)^{ab}$
Hellmann's vegan mayo	$5.55(0.21)^{b}$	$6.79(1.57)^{a}$

However, only comparing the particle size was not enough as BSB contains not only oil droplets. From the microscopy in *Figure 5*, some plant cells and fibres could be seen in BSB but not in the commercial vegan mayonnaise. That result is consistent with the one from *Figure 4*. The droplets size and the degree of uniformity of oil droplets of BSB with rapeseed oil were between two references. Remarkably, the oil droplets in Hellmann's vegan mayonnaise were extremely small.

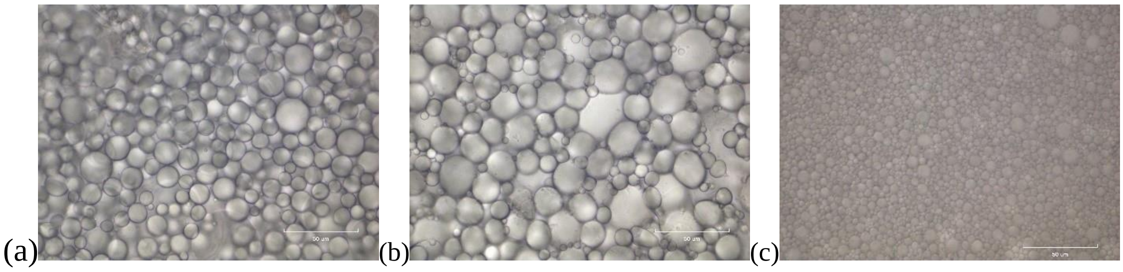


Figure 5. (a) BSB, (b) Garant cold vegan mayo and (c) Hellmann's vegan mayo under 60× microscope with the scalebar of 50 μm.

3.2 Formulation and processing optimization of BSB

3.2.1 Effect of oil content

In *Figure 6*, when the ratio of oil in BSB was higher, the oil droplets became larger and less uniform. *Figure 7* shows the curves of the particle size distribution of BSB with different concentrations of oil. Two peaks could be seen in each curve, where the left one was oil droplets. Agreed with the result of oil droplets morphology in *Figure 6*, the particle size increased with increased oil content.

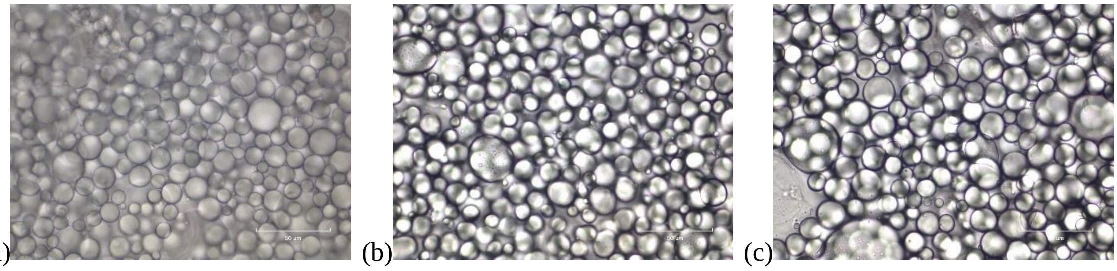

(a) (b) (c)

Figure 6. From (a) to (c), BSB samples with increase amount of rapeseed oil under 60× microscope with the scalebar of 50µm.

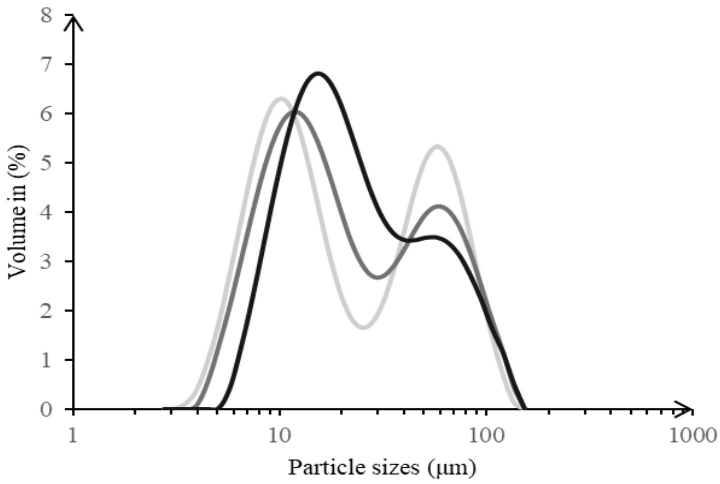


Figure 7. Particle size distribution of BSB with different ratio of rapeseed oil. Triplicates were performed on each sample. The darker colour refers to sample with higher amount of oil.

From *Table 3,* although smaller particle sizes were found in BSB with less oil, the viscosity was significantly lower when less oil was used. The samples were stored in the fridge or at room temperature for two weeks; only the BSB with lowest oil content had a visible phase separation. BSB with lowest oil content had the least stable emulsion after centrifugation, indicating that more rapeseed oil could help to increase the emulsion stability by occupying more volume fraction of the emulsion and increasing the viscosity.

Table 3. Particle size, viscosity at the shear rate 50 s^{-1} and stability percentage of BSB samples with different concentration of rapeseed oil. Values are written in mean(SD), n=3. Different superscript letters (a-c) in each column suggest the overall statistical difference according to Tukey test where $p \leq 0.05$.

Rapeseed oil content (% w/w)	d(0.5) (μm)	Viscosity (Pa·s)	Stability (%)
Lowest (30-40)	18.14(0.51)^{c}	1.03(0.07)^{c}	45.0(0.0)^{c}
Medium (40-50)	19.23(0.51)^{b}	1.57(0.01)^{b}	52.1(0.0)^{b}
Highest (50-60)	22.15(0.16)^{a}	2.46(0.04)^{a}	57.9(0.0)^{a}

The colour was also affected by rapeseed oil. From *Table 4*, a* and b* of the sample with lowest rapeseed oil content was significantly higher while L* was lower than the BSB with highest of oil content. In other words, the lightness of BSB increased while the redness and yellowness decreased with oil. However, these colour differences were not visibly noticeable. The brighter BSB could be explained by more oil used that reflected more light. In addition, sensory results showed that BSB with more oil was creamier, firmer, smoother, but less sour. However, too much oil, the highest oil content in this case, led to an unacceptable oily flavour.

Table 4. Colour of BSB with different amount of rapeseed oil; a (green - red), b*(blue-yellow) and L* (lightness). Values are written in mean(SD), n=3. Different superscript letters (a-b) in each column suggest the overall statistical difference according to Tukey test where $p \leq 0.05$.*

Rapeseed oil)	L*	a*	b*
Lowest	82.09±0.46^{b}	3.66±0.10^{a}	16.41±0.25^{a}
Medium	82.91±0.71^{ab}	2.97±0.06^{b}	14.06±0.59^{b}
Highest	84.35±1.16^{a}	3.09±0.03^{b}	13.81±0.26^{b}

Overall, the high oil content gave the emulsion a better structure where the viscosity and stability were enhanced. Once the volume fraction of the oil droplets goes down, the interactions between triglycerides will be weakened. (Depree and Savage, 2001). However, when more oil is used, more emulsification time will be needed to break down the droplets, and the amount of pectin might not be sufficient to cover oil droplets to prevent them from aggregation. Regarding the organoleptic properties, baobab was occasionally mentioned as possessing sourness but no other tastes (Eltahir and Elsayed, 2019). It acts better as a base and needs to combine with other strong flavour ingredients.

However, as mentioned above, the oily taste dominated if the oil content expanded. For the sake of physical stability, health and sensory, the medium amount of rapeseed oil was used to produce BSB in the subsequent trials.

3.2.2 Possibility of incorporating baobab seed oil or flaxseed oil.

When baobab seed oil was incorporated, unfortunately, the oil mixture started to separate right after mixing. Under the microscope, oil droplets visibly aggregated in a short time. Meanwhile, an appreciable amount of oil was separated after centrifugation, as shown in *Figure 8,* where the emulsion stability dramatically decreased from 2.1%(0.6%, n=3). Therefore, it could be concluded that the mixture of rapeseed oil and baobab seed oil was not emulsified at all with LBB. Moreover, a strong smell from baobab seed oil contributed to an unpleasant odour to the emulsion. No baobab seed oil was used in the subsequent trials of this thesis.

Differences in the microstructure and the interactions between triglycerides molecules contribute to different density, rheology and other physicochemical properties of two types of oils. The ratio of the viscosity of the dispersed phase and the viscosity of the continuous phase affects the particle sizes of oil droplets during homogenization (McClements, 2016). It was observed that the viscosity of baobab seed oil used in this experiment was higher than rapeseed oil. Hence, the agitation used in the experiment might not be strong enough to break the baobab seed oil into small droplets, which might be the reason for the emulsification failure where the droplets aggregated right after mixing. Unfortunately, the study was terminated due to the shortage of baobab seed oil and the density of the one used in this study was not measured. However, the potential of adding baobab seed oil remains. Therefore, it is worth replacing all the rapeseed oil with baobab seed oil instead of using the mixture for further study.

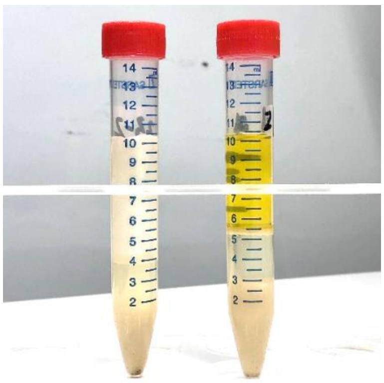


Figure 8. BSB with rapeseed oil and BSB with rapeseed oil and baobab seed oil after centrifugation at 5000 rpm for 30 minutes.

In the study of flaxseed oil, when flaxseed oil was incorporated, smaller particles were formed because of extra mixing time. Nevertheless, the viscosity and centrifugation stability of the emulsion was not affected by flaxseed oil, regarding the results in *Table 5*.

Table 5. Particle size, viscosity at the shear rate 50 s^{-1} and stability percentage of BSB without or with flaxseed oil added before and after the emulsification of rapeseed oil. Values are written in mean(SD), n=3. Different superscript letters (a-c) in each column suggest overall statistical difference according to Tukey test where $p \leq 0.05$.

Samples	d(0.5) (μm)	Viscosity (Pa.s)	Stability (%)
Without flaxseed oil	$26.94(0.02)^{a}$	$4.62(0.13)^{a}$	$79.6(0.5)^{a}$
With flaxseed oil (adding before emulsifying)	$24.49(9.32)^{b}$	$4.60(0.16)^{a}$	$81.3(0.1)^{a}$
With flaxseed oil (adding after emulsifying)	$23.25(0.06)^{c}$	$4.52(0.24)^{a}$	$79.8(1.3)^{a}$

The results indicated that the addition of omega-3 in the form of flaxseed oil could be performed either before or after the emulsification of rapeseed oil, and it would not compromise the desired characteristics. It might be because that the amount of flaxseed oil might be too small (1-3% w/w) to make any significant difference. However, flaxseed oil is known as highly unsaturated, very sensitive and could easily be oxidized. Those issues were not covered in this project; hence, further experiments and research should be done to ensure the chemical stability of the omega-3 fatty acid in the final product.

3.2.3 Effect of oil temperature

The results in *Table 6* indicate no significant difference in the particle size, viscosity, or emulsion stability among BSB samples with oil at different temperatures. The oil temperature in the fridge was not stable and sometimes fluctuated from 7 ℃ to 11 ℃; while the room temperature (RT) varied depending on the outside temperature, it could drop down to 16 ℃ in some days. In other words, the difference in the temperature of the oil from the fridge and LBB was not entirely significant for creating any difference. What is more, the heat produced by Bamix during mixing could warm up the emulsion and reduced the temperature difference between LBB and oil. Thus, despite the possible errors, the oil temperature did not contribute to the emulsion stability.

Table 6. Particle size, viscosity at the shear rate 50 s^{-1} and stability percentage of BSB with rapeseed oil at different temperatures. Values are written in mean(SD), n=3. No significant difference in each column.

Temperature of rapeseed oil	d(0.5) (µm)	Viscosity (Pa·s)	Stability (%)
8 ℃	19.23(0.51)	1.57(0.01)	52.1(0.0)
RT	19.31(0.33)	1.73(0.09)	52.1(0.0)

3.2.4 Effect of the mixing speed and emulsification time

From *Figure 9*, smaller droplets could be seen in the sample made with higher agitation intensity. According to *Table 7* and *Figure 10*, significant improvement in the physical stability of BSB had been achieved by increasing the mixing speed from 17000 rpm to 22000 rpm, thanks to the decreased particle sizes and enhanced consistency.

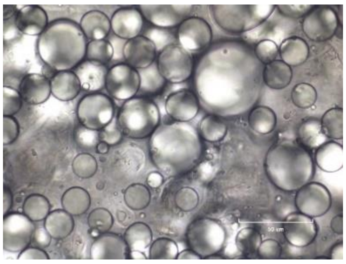
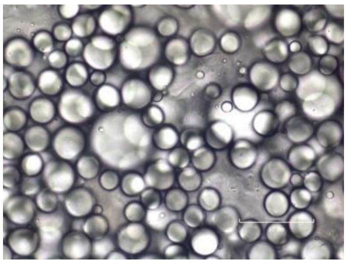
(a)  (b) 

Figure 9. BSB mixed by Bamix at (a) 17000 rpm or (b) 22000 rpm under 60× magnification with the scalebar of 50 µm.

Table 7. Particle size, viscosity at the shear rate 50 s^{-1} and stability percentage of BSB with rapeseed oil at different temperatures. Values are written in mean(SD), n=3. Different superscript letters (a-c) in each column suggest overall statistical difference according to Tukey test where $p \leq 0.05$.

Agitation intensity (rpm)	d(0.5) (µm)	Viscosity (Pa·s)	Stability (%)
17000	38.85(0.05)^{a}	3.97(0.10)^{b}	35.0(0.0)^{b}
22000	34.97(0.03)^{b}	4.30(0.15)^{a}	69.0(0.0)^{a}

Figure 10. BSB mixed by Bamix at 17000 rpm and 22000 rpm after the centrifugation at 5000 rpm for 30 minutes. An apparent oil separation was observed on the BSB with the lower agitation speed.

The other observation was that the prolonged emulsification decreased the particle size and improved the uniformity of oil droplets, as from *Figure 11*. From *Table 8*, the viscosity increased by increasing the mixing time, but the improvement was not significant after 20 minutes. The particle sizes kept decreasing when the mixing time was extended to 25 minutes. From *Figure 12 (a)*, a more stable emulsion remained after centrifugation when the agitation time had been prolonged from 10 minutes to 30 minutes. In other words, the changes in stability were not significant anymore from 20 minutes to 30 minutes, as could be seen in *Figure 12 (b)*. Based on the results, it was decided that each time 400 g of BSB was mixed by Bamix at 22000 rpm for 20 minutes where the stability of BSB became comparable to the reference vegan mayonnaise (*Figure 12 (a)*).

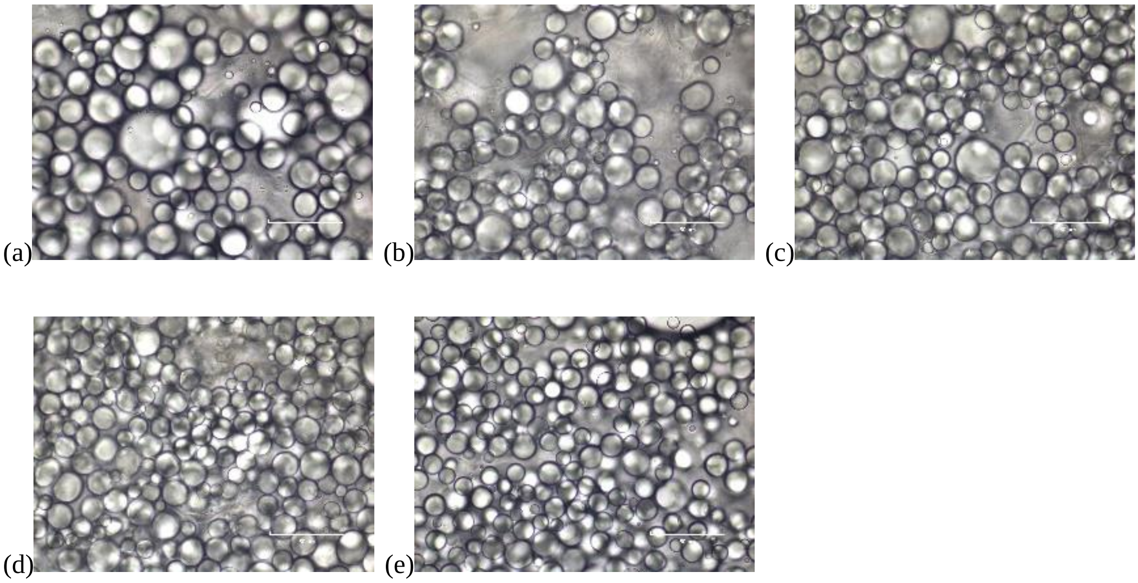
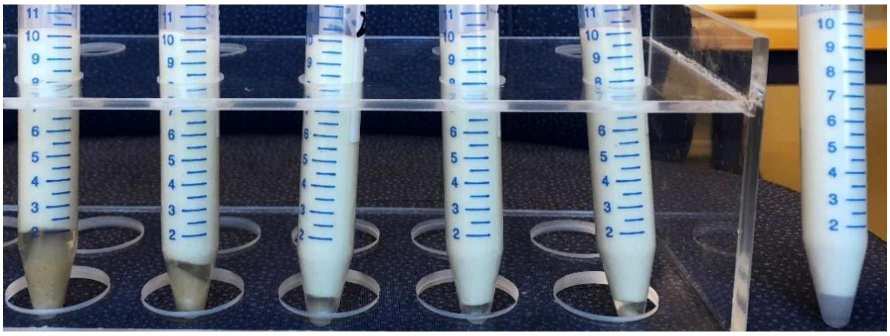


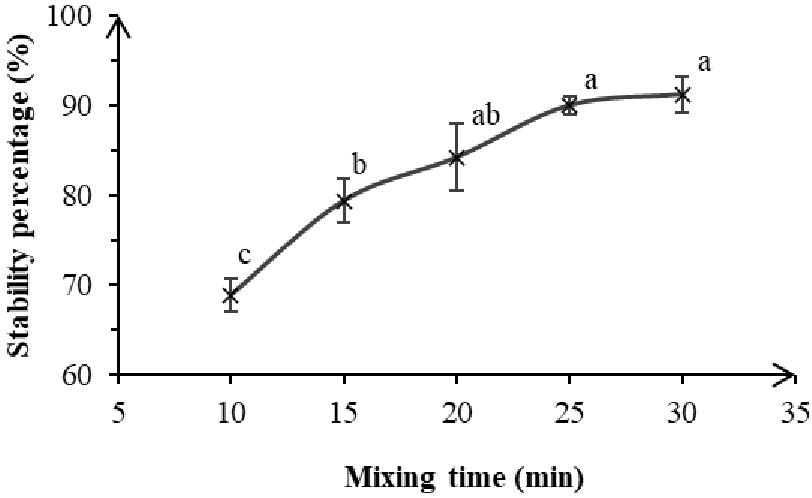
Figure 11. BSB samples mixed by Bamix at 22000 rpm for (a) 10 minutes, (b) 15 minutes, (c) 20 minutes, (d) 25 minutes and (e) 30 minutes under 60× magnification with the scalebar of 50 µm.

Table 8. Particle size, viscosity at the shear rate 50 s^{-1} of BSB with different mixing time. Values are written in mean(SD), n=3. Different superscript letters (a-d) in each column suggest overall statistical difference according to Tukey test where $p \le 0.05$.

Emulsification time (minutes)	d(0.5) (μm)	Viscosity (Pa·s)
10	$34.97(0.03)^{a}$	$4.30(0.15)^{b}$
15	$32.76(0.02)^{b}$	$4.92(0.18)^{ab}$
20	$31.86(0.08)^{c}$	$4.85(0.40)^{ab}$
25	$31.25(0.03)^{d}$	$5.29(0.58)^{a}$
30	$31.04(0.16)^{d}$	$5.71(0.21)^{a}$



(a)



(b)

Figure 12. (a) BSB mixed at 22000 rpm for 10 minutes, 15 minutes, 20 minutes, 25 minutes and 30 minutes and Hellmann's vegan mayo reference (from left to right) after the centrifugation 5000 rpm 30 minutes; (b) Stability percentages of BSB with the different mixing time.

To conclude, the higher speed (22000 rpm) and optimal mixing time (20 minutes) were chosen for mixing. A longer time was not recommended since no significant differences were recorded. From the literature review, pectin acts as an emulsifier as well as a thickener (Ngouémazong *et al.*, 2015); thus, the stability could be improved when more pectin is extracted from baobab cells. Furthermore, prolonged mixing time or increased mixing speed could break more baobab cells and release pectin into the aqueous phase. Consequently, the continuous phase was thickened by pectin, which may contribute to the improved stability of emulsions.

3.3 Characterization of BSB

3.3.1 Properties of LBB and BSB

Determinations on the chemical and physical properties helped to understand the changes from LBB to BSB, and the results could be found in *Table 9*.

Table 9. Characteristics of LBB and BSB. Values are written in mean(SD), n=3. Different superscript letters (a-b) in each raw suggest overall statistical difference according to Tukey test where $p \leq 0.05$.

	LBB	BSB
Dry matter	$14.01(1.03)^{a}$	$54.62(1.35)^{b}$
L*	$44.92(1.07)^{b}$	$72.10(0.37)^{a}$
a*	$1.12(0.41)^{b}$	$2.23(0.01)^{a}$
b*	$9.04(0.66)^{b}$	$15.75(0.07)^{a}$
pH	$3.32(0.03)^{a}$	$3.23(0.05)^{a}$
d(0.5)	$62.17(0.52)^{a}$	$31.96(0.60)^{b}$
Viscosity at $50s^{-1}$	$2.55(0.02)^{b}$	$4.84(0.11)^{a}$

To be more precise, incorporating oil led to a considerable increase in dry matter. The dry matter of LBB varies between different batches of baobab fruit pulp, and the data in *Table 9* was from the dried baobab fruit (batch 2020, Sudan). This batch was the newest batch that would be used in the upcoming production. The sampling problem was pointed out because it created a huge difference in the viscosity of LBB, thus influencing the quality of BSB. In the laboratory, baobab fruit was stored in the boxes. Due to the friction between baobab pulp, there was usually pulp powder at the bottom of the boxes. Thus, it was challenging to take the same amount of pulp from the containers every time.

Therefore, it was suggested that the boxes of baobab should be mixed very evenly before sampling. However, this method did not perfectly solve the problem. The viscosity of LBB still fluctuated. Therefore, the advice to industrial production is to check the dry matter of LBB before producing BSB.

Colour also significantly changed as can be seen from *Table 9*, where BSB was lighter with more redness and yellowness after adding oil to LBB. Baobab pulp is white (Wickens, 2008). Nevertheless, it turned orange after soaking and filtration when producing LBB. It could be due to the fibre inside the pulp and the shells, which has a brownish orange colour (Eltahir and Elsayed, 2019). Moreover, stirring and filtration broke the baobab cells and released enzymes from the cells. The enzymes might be activated by heat from the water and turn the colour of the suspension into a deep brownish orange. However, the effect of enzyme was not included in this study; thus, the actual reactions were not fully understood. Another possible explanation was made on the sugar and amino acids in baobab (Nour, Magboul and Kheiri, 1980) which are exposed to heat treatment, making it possible for the Maillard reaction. Furthermore, according to Lozano and Ibarz (1997), pigments could be destroyed during processing. When the oil was incorporated, oil diluted the continuous phase, making it lighter than LBB. It was also observed that BSB was getting whiter with prolonged emulsification and increased intensity of the mixing. It could be explained by smaller droplets formed by mechanical agitation. The smaller the size of droplets is, the more light is scattered (Olsson et al., 2018).

In *Table 9*, the pH of LBB and BSB were around 3. This agrees with PhytoTrade Africa (2009), where citric acid and tartaric acid were found in baobab pulp.

When it comes to the particle size distribution, the median diameter halved from LBB to BSB. *Figure 13* shows the distribution of particle size. As LBB only contains plant cells and BSB only contains cells and oil, *Figure 13* indicates that the right peak in the BSB sample refers to baobab cells, as it shares the same peak with LBB.

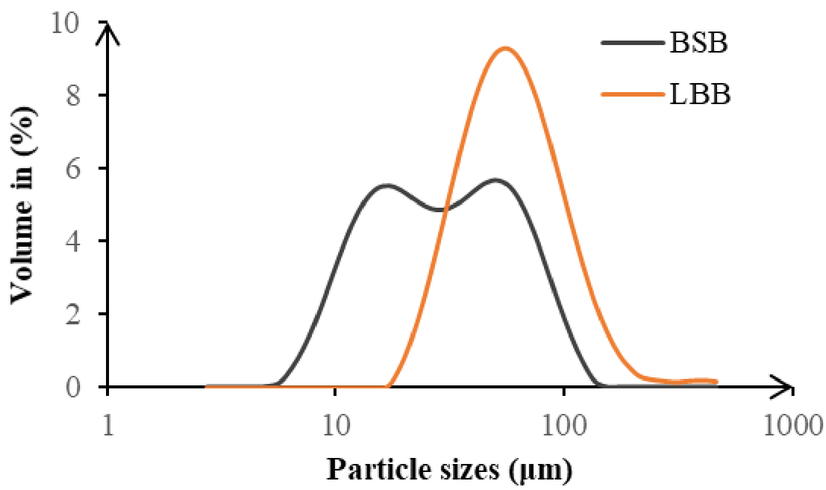


Figure 13. Particle size distribution of LBB and BSB.

Viscosity is also a critical property that influences the quality of baobab emulsions. LBB was fluid and pourable. After rapeseed oil was emulsified, viscosity significantly increased.

Pseudoplastic behaviour was found in BSB with ~~A~~ 30-60% of rapeseed oil by the "shear stress ramp log" sequence according to in *2.4.3.2 Rheological behaviours*. The result was shown in *Figure 14*. From the data analysis, shear stress *vs* shear rate was fitted according to the Herschel-Bulkley model, where the equation was shown in the curve in *Figure 14*. Thus, the yield stress is 1.9 Pa. This information is helpful for industrial applications. Meanwhile, the stress involved during distribution and storage should be lower than the yield stress to minimize instability. Overall, it can be concluded that BSB possesses a viscoelastic property close to mayonnaise which also has yield stress and pseudoplastic behaviour (Izidoro *et al.*, 2008).

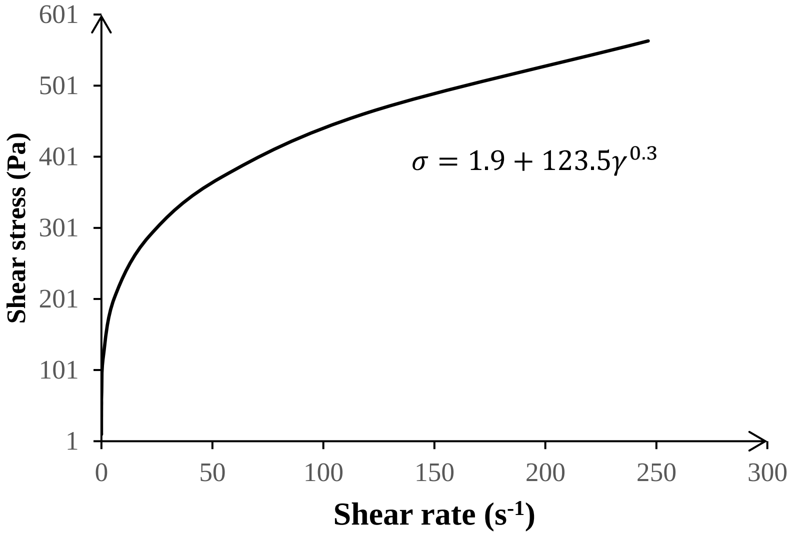


Figure 14. Flow curve of BSB fitted by the Herschel-Bulkley model at 7 ℃ (n=3).

In *Figure 15* below, the higher the temperature, the lower the viscosity. Obviously, the heat affected the microstructure of BSB. As the temperature increased, bigger oil droplets formed and were ready to separate from the emulsion; since then, the continuous phase became less viscous. From the experiment, from 7 ℃ to 30 ℃, it was more fluid, but the consistency remained. Meanwhile, it is observed that BSB became watery when heated above 50 ℃. There was the risk of broken emulsion. However, the turning point was not discovered yet, meaning that more studies on temperature range 50 ℃ to 80 ℃ should be performed.

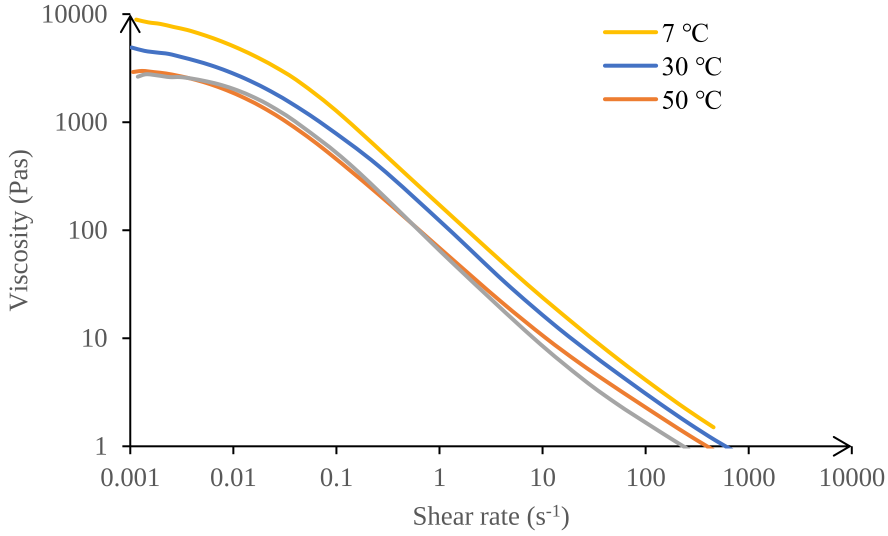


Figure 15. Shear rate vs viscosity of BSB under different temperature (n=2).

3.3.2 Pasteurization stability of BSB

From the microscopy in *Figure 16*, oil droplets of BSB aggregated after heat treatment while the droplets of the reference remained small. According to the physical changes, the stability of BSB after centrifugation was lower (*Table 10*) which might be due to the decreased viscosity. However, the rheological behaviour of the reference did not change too much, as in *Figure 17*.

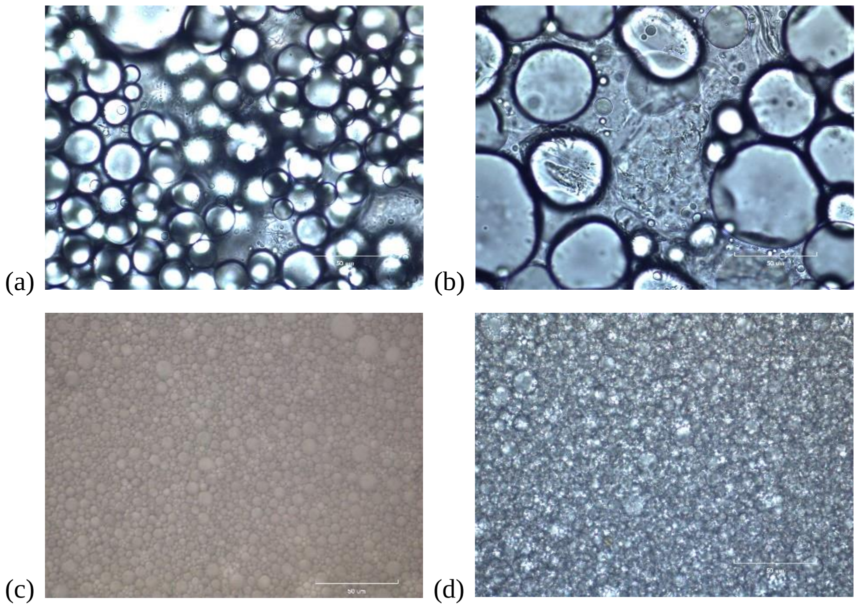


Figure 16. (a) BSB, (b) BSB heated in the water bath at 78 ℃ for 15 minutes and cooled down to 7 ℃, (c) Hellmann's vegan mayo and (d) Hellmann's vegan mayo heated in the water bath at 78 ℃ for 15 minutes and cooled down to 7 ℃ under 60× magnification with the scalebar of 50 µm.

Table 10. Particle size and stability of BSB and the reference mayo before and after heat treatment under 78 ℃ for 15 minutes. Values are written in mean(SD), n=3. Different superscript letters (a-c) in each column suggest overall statistical difference according to Tukey test where p≤0.05.

Samples	d(0.5) (μm)	Stability (%)
BSB	36.24(0.02)^{b}	75.5(1.0)^{b}
pasteurized BSB	52.07(0.11)^{a}	54.4(1.9)^{c}
Hellmann’s vegan mayo	5.55(0.21)^{c}	89.0(1.3)^{a}
pasteurized Hellmann’s vegan mayo	5.54(0.04)^{c}	90.4(0.0)^{a}

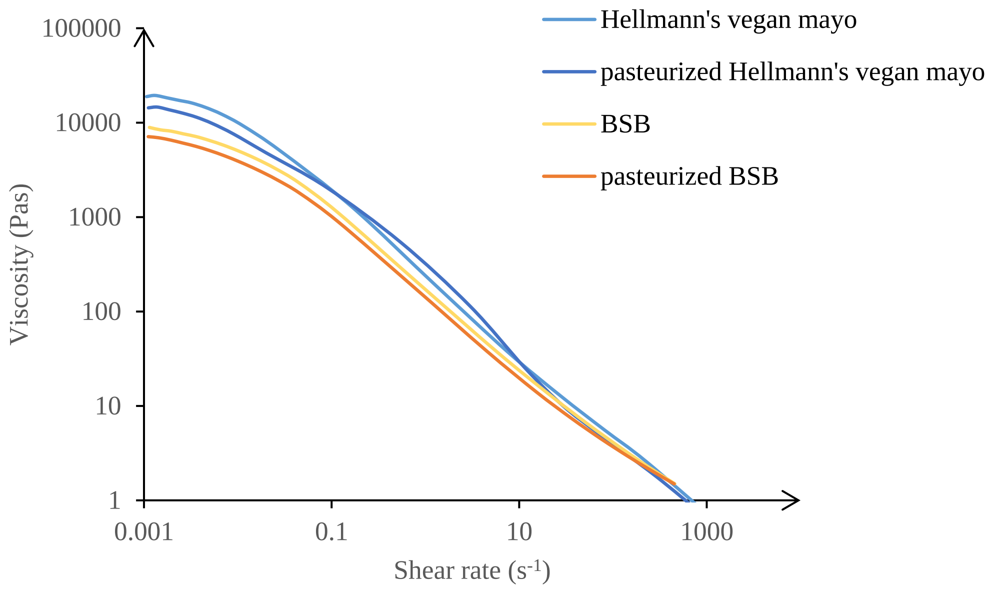


Figure 17. Shear rate vs Viscosity of BSB and Hellmann’s Vegan mayonnaise before and after heat treatment at 78 ℃ for 15 minutes (n=2).

Changes were noticed in the colour of BSB, where the lightness significantly decreased while the redness increased after pasteurization. No difference was observed in the colour of the commercial reference. The colour difference of BSB and Hellmann’s vegan mayonnaise before and after heat treatment were 4.73(1.19, n=3) and 1.41(1.45, n=3). The colour became darker after heat treatment; it might be due to the Maillard reaction.

Table 11. Colour of BSB and Hellmann's Vegan mayonnaise before and after heat treatment at 78 ℃ for 15 minutes. a (green - red), b*(blue-yellow) and L* (lightness). Values are written in mean(SD), n=3. Different superscript letters in each column suggest overall statistical difference according to Tukey test where p≤0.05.*

Samples	L*	a*	b*
BSB	72.70(1.14)^{b}	3.08(0.11)^{b}	17.78(1.08)^{a}
pasteurized BSB	68.00(0.35)^{c}	3.54(0.09)^{a}	18.17 (0.95)^{a}
Hellmann's vegan mayo	84.81(1.28)^{a}	1.91(0.07)^{c}	10.65(0.24)^{b}
pasteurized Hellmann's vegan mayo	86.67(0.94)^{a}	2.05(0.12)^{c}	11.24(0.27)^{b}

The results agrees the literature reviewwhere avoid applying heat on the final product could maintain stability, as heat accelerates physical instability by increasing the droplets mobility (Nikzade, Tehrani and Saadatmand-Tarzjan, 2012). Bigger droplets and a more fluid texture were the evidence of damaged stability by heat. The shear-thinning remained because the emulsion was not wholly disrupted. However, the microbial aspect is out of scope, so the exact duration and temperature have not been explored yet, Hellmann's vegan mayo was not affected too much since it contained starch and stabilizers. To some extent, it highlighted the need for a stabilizer in the BSB. Mustard were added in BSB.

Inspired by Harrison and Cunningham (1985), mustard exerts a stabilizing effect on food emulsions. Two concentrations of mustard were tried where the higher amount of mustard led to smaller droplets (*Figure 18*) and increased stability from 75.4%(0.7%, n=3) to 100.0%(0.0%, n=3). However, BSB with higher amount of mustard powder created an uncomfortable spiciness.

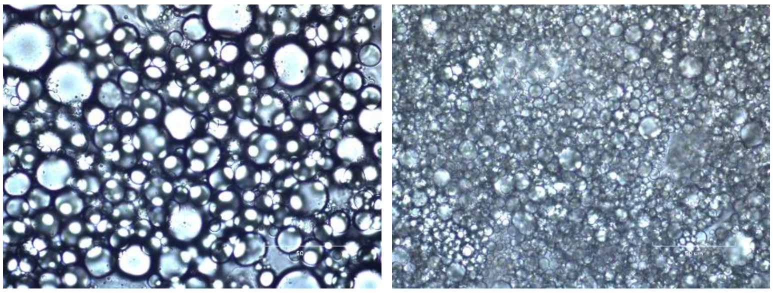


Figure 18. From left to right, BSB with increased amount of mustard after pasteurization at 78 ℃ for 15 minutes.

In some mayonnaise production, raw ingredients such as egg yolk is parteurized, with low pH and high fat content, the commercial mayonnaise is considered microbiologically stable. Similarly, BSB and Baonaise is acidic, if no mustard is supposed to be added, the

LBB can be treated before entering the sterile production line to avoid the heat treatment on the emulsion.

3.3.3 Storage study

The results are shown in *Table 12*. Firstly, the results indicated that storage temperature played a vital role in the physical properties of BSB. When the samples were stored at 4 ℃ - 8 ℃ after one day, higher viscosity and smaller particle size were observed than those stored at room temperature. Regarding the colours, lower lightness was detected in BSB stored at a higher temperature. Secondly, when the cold storage had been prolonged to two weeks, pH dropped down; the oil droplets became larger; a* and b* showed no significant difference while the indicator of lightness, L*, fluctuated from one day to one week, although the changes were not visible by naked eyes. Fortunately, stability and viscosity kept stable in two weeks when BSB was properly stored in the fridge.

Table 12. pH, colour, particle size, viscosity at 50 s^{-1} and stability% of BSB during storage. L means lightness.Values are written in mean(SD), n=3. Different superscript letters in each raw suggest overall statistical difference according to Tukey test where p≤0.05.*

Storage conditions	L*	pH	d(0.5) (μm)	Viscosity (Pas)	Stability (%)
RT, 1day	73.57(0.96)^{b}	3.29(0.01)^{a}	27.21(0.12)^{a}	2.96(0.25)^{b}	73.0(0.5)^{a}
fridge,1day	75.70(0.52)^{a}	3.56(0.04)^{a}	26.76(0.09)^{b}	4.11(0.86)^{a}	74.0(1.7)^{a}
fridge, 2weeks	75.3(0.31)^{b}	3.31(0.00)^{b}	28.60(0.13)^{a}	4.47(0.10)^{a}	85.7(2.6)^{a}

3.3.4 Nutritional value estimation

The nutritional content of 100 g BSB can be found in *Table 13* below. BSB contains 30-60% of fat and 5% of carbohydrates, of which 3 g is dietary fibre. The total energy of 100g BSB was calculated by adding the energy provided by fat, carbohydrate, and protein, and it is 416 kcal per 100 g. Also, since the product contains 3 g of fibre, the BSB can be labelled a nutrition claim as “source of fibre” (European Commission, no date). A comparison with commercial vegan mayonnaise is in *Table 14*. Again, lower calories and higher fibre content can be found in BSB.

Table 13. The nutritional value of 100 g BSB.

Per 100 g	BSB
Energy (kcal)	419
Energy (kJ)	1752
Carbohydrates (g)	5
Protein (g)	0
Fibre (g)	3
Sum of saturated fatty acids (g)	3
Sum of monounsaturated fatty acids (g)	28
Sum of polyunsaturated fatty acids (g)	12
Vitamin C (mg)	7
Iron (mg)	0
Pectin (g)	2
Sodium (mg)	1
Potassium (mg)	139
Calcium (mg)	20
Magnesium (mg)	10
Phosphorus (mg)	4
Copper (mg)	0
Zinc (mg)	0
Manganese (mg)	0

Table 14. Comparison of the nutritional facts of 100g of Hellmann's vegan mayonnaise, Garant vegan mayonnaise and BSB.

Nutritional value Per 100 g	Hellmann's vegan mayonnaise	Garant vegan mayonnaise	BSB
Energy (kcal)	654	550	416
Energy (kJ)	2738	2400	1752
Saturated fat (g)	5.3	4.4	3.2
Carbohydrates (g)	3.9	0.7	5
of which sugars (g)	2.7	0.7	0
Fibre (g)	0	0	3.2
Protein (g)	0.5	0.8	0.2
Salt (g)	1.5	0.9	0

3.4 Application of BSB - Baonaise

In the first trials, except the neutral Baonaise, which had comparable stability and oil droplets morphology under the microscope to the reference commercial product (Hellmann's vegan mayonnaise), both the other two flavours had apparent oil separation after centrifugation. In other words, the stability of Nordic and Sudanese Baonaise needed to be improved. Therefore, mustard was increased for all formulations while the white pepper, parsley and tomato puree were eliminated or reduced from the formulations concerning the flavours and stability issues. Finally, after several times of modification on the certain seasonings, the final formulation of the three different flavours was designed according to the formulations below (*Table 15*).

Table 15. Seasonings of Neutral, Nordic, and Sudanese Baonaise

Neutral Baonaise	% w/w	Nordic Baonaise	% w/w	Sudanese Baonaise	% w/w
BSB	98.5	BSB	95.7	BSB	93.2
Mustard powder	1.0	Mustard powder	1.0	Mustard powder	1.0
Neutral premix	0.5	Nordic premix	3.3	Sudanese premix	5.8

In *Table 16* below, flavoured Baonaise had higher stability and viscosity than BSB, which did not contain any seasonings. Meanwhile, the former had a significant lower particle size. The pH of BSB and sauces were around 3.0 to 3.4. Regarding the colour, it varied based on added seasonings. For example, Sudanese Baonaise was orange and red, while Nordic was green in colour due to dried herbs.

Table 16. pH, colour, particle size, viscosity, and stability% of BSB and Baonaise flavours. a (green - red), b*(blue-yellow) and L* (lightness).*

	L*	a*	b*	pH	d(0.5)	Viscosity (Pas)	Stability (%)
BSB	72.10(0.37)[b]	2.23(0.01)[c]	15.75(0.07)[b]	3.23(0.05)[a]	31.96(0.40)[c]	4.84(0.114)[c]	78.00(1.00)[b]
Neutral	79.16(1.05)[a]	2.15(0.11)[c]	12.26(0.18)[c]	3.00(0.02)[b]	15.77(0.72)[d]	7.851(1.646)[bc]	100.0(0.00)[a]
Nordic	70.58(0.95)[b]	0.27(0.06)[d]	13.51(0.52)[bc]	3.29(0.06)[a]	18.97(1.96)[c]	11.52(1.826)[a]	100.0(0.00)[a]
Sudanese	62.90(0.54)[c]	14.31(0.43)[a]	25.54(0.94)[a]	3.40(0.08)[a]	21.62(0.39)[b]	9.903(0.573)[ab]	100.0(0.00)[a]

The stability of both BSB and Baonaise was improved by the contribution mustard powder. Regarding the mustard powder, the mucilage helped by increasing viscosity, also reducing particle size (Weber, Taillie and Stauffer, 1974). Results in *Table 16* agreed with Weber, Taillie and Stauffer (1974). By adding mustard powder, the stability reached 100%, and particles were smaller by haft size; in correspondence, the viscosity recorded much lower. The fact that surface tension was reduced was the main reason for all those changes. In other words, the property of the surface of the liquid to resist external forces changed with the introduction of other ingredients. Generally, interactions of molecules are high. As surfactants were added in, they absorbed water, and in consequence, they broke these interactions and decreased the force. Besides, the surface tension was responsible for the sphere shape of oil droplets in emulsion; it has the tendency to minimize the wall area. Eventually, the droplet size must be reduced to keep the shape when interactions dropdown. Since the sauces reached the desired stability (100%) and other characteristics such as viscosity particle sizes, no further increase of mustard powder is required; if not, it would end up unspreadable and unsqueezable thick sauce; the taste also altered.

The pH of LBB was naturally low. Accordingly, the pH of BSB was also low since the oil did not affect the final pH. In contrast, the pH of Baonaise was changed. In comparison with BSB, the neutral flavour had lower pH due to the appearance of mustard powder. Weber, Taillie and Stauffer (1974) had mentioned the acidity property of mustard in solution. Regarding Nordic and Sudanese flavours, pH remained despite the mustard; it might be due to the presence of seasonings that shifted to more alkaline.

As mentioned above, baobab pulp contains numerous nutritional and functional compounds. Therefore, applications of BSB are not limited to only the vegan sauce. It is possible to develop a wide range of products such as sorbet, ice cream, filling, nutrition bar, etc. To some extent, industrial jam production usually adds more pectin to obtain the thick structure. But baobab is a pectin-rich fruit; it is absolutely a great ingredient for the jam. Therefore, any food that requires thickeners can consider baobab. Talking about the nutritional value, it should highlight the high amount of vitamin C in baobab fruit. Currently, Arwa FoodTech AB is developing a nutritious drink whose main ingredient is LBB.

4 Conclusion

Baonaise, a novel sauce made of baobab fruit pulp and plant oil was prototyped. It is a savoury sauce for various applications, and it could be a perfect vegan alternative to mayonnaise. The centrifugal stability of the Baonaise sauce base (BSB) was evaluated with a deep look into the particle size distribution and viscosity. The results emphasized the influence of ingredients and processing parameters on the physical stability of the emulsion.

BSB was formed by emulsifying rapeseed oil into liquid baobab base (LBB). Natural pectin in baobab acts as a thickener and emulsifier, where it increases the viscosity of the continuous phase and creates a barrier around the oil droplets to avoid flocculation by immobilizing the droplets. In addition, the microstructure of the emulsion can be altered by reducing particle size and increasing viscosity. Thus, it is possible to improve the stability of BSB by adjusting ingredients and emulsification procedures.

A comparison between BSB and commercial mayonnaises indicated that the baobab pulp has enormous potential to form a mayonnaise-like emulsion, but it required improvement on the stability. Regarding the proportion of ingredients, 30% - 60% of oil was ideal for BSB since the lower amount did not help the stability, while the higher one created undesirable particle size, grittiness and oily flavour. Furthermore, the oil temperature did not significantly affect the stability; hence oil at room temperature was recommended considering the cost. The optimal mixing procedures was 22000 rpm in 20 minutes. Overall, by modifying formula and processing procedures, the physical stability of BSB was very comparable with the commercial products.

The final characterization reveals that BSB is shear-thinning, and can keep the rheological behaviour from 7°C to 80°C. Intense heating accelerates oil aggregation. However, 1% to 3% of mustard helped to maintain the physical stability after heat treatment. From the storage test, unpasteurized BSB was stored for two weeks in the fridge without any dramatic changes in the physical property. For food product applications, the stability of Baonaise was enhanced by modifying the old recipes.

This study has its limitations. For example, the effect of agitation intensity was not fully discovered due to the limit functions of the agitator; oil addition rate effect was not investigated since the lack of speed controlling funnels; shortage of baobab seed oil made it impossible to continue the study of it.

To conclude, baobab fruit pulp is a novel and potential ingredient to produce healthy savoury sauce. The physical stability of baobab emulsion could be improved by prolonging emulsification time, increasing the mixing speed or increasing the ratio of oil within the range of 30-60%.

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Appendices

Figure A1. Baobab fruit; Liquid baobab base (LBB), Baonaise sauce base (BSB) and Baonaise.