The Development of Pet Food Using Cricket Meal

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Abstract

In the context that insect protein is increasingly considered as one of the major sources of alternative proteins in the pet food industry, there is still a lack of research on using cricket as the sole or major protein source in dog foods, let alone commercialised industrial level mass production of such dog foods. Therefore, the study objective was to develop a series of novel dog foods using cricket flour as the alternative protein source. The whole project consisted of three stages, formula design, baking test, and acceptability test. During the first stage, the formulas were targeted to meet the nutrient requirements of the Adult Maintenance category of the AAFCO (The Association of American Feed Control Officials) Dog Food Nutrient Profiles. A series of three dog food formulas were developed with the adoption of cricket flour as the major protein source, including one complete (CF as complet dog food formula 1, containing 34% cricket flour) and two treat (TF1 as treat formula 1, with 17% cricket flour and TF2 as treat formula 2, with 25% cricket flour) formulas. Based on calculation and comparison, it was shown that all complied with the minimum/maximum nutrient requirement of the Adult Maintenance part of AAFCO Dog Food Nutrient Profiles. A formulation tool was also built to facilitate the estimation of the nutrient levels, which was based on the Microsoft Excel software and the nutrient profile data of USDA. It was demonstrated that using cricket flour in the formulation of dog foods is feasible due to the high nutrient levels of both essential amino acids and other nutrients such as essential fatty acids and certain minerals. In the second stage, the study objective was to evaluate the feasibility of producing a series of cricked-flour-based, gluten-free dog food formulas, including the formability, the determination of time-temperature range, and the control of water activity (Aw). The three formulas were tested under different oven temperatures (150°C, 120°C, and 100°C respectively). All the formulas showed good formability with solid and well-formed pellets after baking. Due to the different compositions, the optimum baking time at constant oven temperature varied between the three formulas, with a pattern that the baking time was inversely proportional to the oven temperature. The Aw value of the three formulas was effectively controlled through a proper combination of oven temperature and baking time. The third stage was designed as a preliminary palatability test. An acceptance test was conducted to testify the acceptance of the tested formulas. Eight dogs were randomly chosen from the Centre for Canine Nutrition. It was shown, both the complete and the two treat dog food formulas got a 100% acceptancy. A comparison of palatability between the two treats was also conducted. TF2 gained a slightly higher preference; 71.43% of dogs chose to approach TF2 first, 85.71% of dogs started eating

from TF2 first, and 57.14% of dogs finished consuming TF2 first. The result indicated, that using cricket flour in dog foods has a highly promising future.

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1 Introduction

Given the ever-increasing number of household companion dogs, the trend of today's consumers to move into more natural dog foods and the environmental pressure of producing enough meat-sourced protein to feed the growing human population; the seeking of alternative proteins for companion dogs has become more important than ever. In the case of insect proteins, on one hand, insects have already gained lots of interest from academic society, industrial players and consumers. While on the other hand, the current research related to the direct practice of adopting insects in dog food formulas are still scarce. At the time this research was conducted, there were still less than 10 published papers on the direct use of insects in dog food formulations.

The major goal of this research was to develop a series of insect-protein-based, gluten-free dog foods and test their feasibility through baking trials and the basic acceptance tests. The second goal was to provide some insights and information to those industrial players who may adopt baking technology on insect-based dog food products in the near future. Hence this thesis comprises eight chapters that cover a literature review and the three stages of the project, the design of formulas, the baking test, and the acceptability (palatability) test.

Chapter 2 is the literature review which comprises two major parts. The first part covers the basic nutrient requirements of dogs such as energy and nutrient composition and the key functions of the major nutrients such as dietary fat and essential fatty acids (EFA), protein and essential amino acids, and briefly mentions minerals and vitamins. The second part puts the insect's nutrient value in the context of dog food formulation, including both house crickets (*Acheta domesticus*) and black soldier fly (*Hermetia illucens*). In this part, the current advances of using insects as dog food ingredients are reviewed and the results showing the feasibility of replacing meat proteins with insect proteins are presented.

Chapter 3 focuses on the topic of formula design including a brief review of the ingredients that were used to replace wheat in traditional dog food formulas (lentils, pumpkin, sweet potato, flaxseed and oatmeal), the major formulas and the nutritional spreadsheet that were used to predict nutrient levels. In this chapter, one complete and balanced and three treat formulas were proposed for the later baking tests.

Chapter 4 describes the materials and methods that were used in the baking tests, including raw materials used, baking process, determination of water activity and moisture content. This

chapter also includes the preliminary trial which was conducted to test the basic formability of the cricket-meal-enhanced formula.

Chapters 5 and 6 relate to baking trials on the designed dog food formula (Chapter 5 on the complete formula and Chapter 6 on the treat formulas). Twenty trials at four temperature levels (200°C, 150°C, 120°C and 100°C) were conducted to define the optimum baking temperature-time combination and influences of this on the Aw (water activity) value with different levels of glycerine. The results were positive and consistent with the conclusions that had been drawn in the literature review.

Chapter 7 reports on the acceptance testing which is also the basic test of palatability. A comparison between the two selected treats formula was conducted at the same time. The results showed a 100% overall acceptance on all the three tested formulas and a slightly higher acceptance of treat formula 2 in comparison to treat formula 1.

Chapter 8 outlines the main conclusions of the project and recommendations to dog food manufacturers. The results were reviewed to provide more general conclusions on baking conditions of cricket-based formulas. The limitations of the baking experiments were also discussed here, i.e. the difference between the oven in the lab and the pieces of equipment used in pet food plants.

2 Literature Review

2.1 Dogs and their basic nutrition requirements

Domestic dogs are classified as *Canis lupus familiaris* and belong to the family Canidae (Animal Diversity Web, 2020). Previous archaeological research has shown that the domestication of dogs can be traced back to the Middle Stone Age (around 10,000 B.C. to 1,200 B.C.), while fossil evidence has revealed an even earlier linkage between dogs and humans in the Old Stone Age (around 14,000 years ago) (Clutton-Brock, 2012). From the angle of animal nutrition, two notable features should be mentioned. On one hand, although modern companion dogs share many common characteristics with their ancestor, the gray wolf (*Canis lupus*), they are facultative carnivores that can thrive on a wide variety of foodstuffs of both animal and plant origin. Secondly, even though there are huge morphological differences between breeds, dogs have similar biological, health needs, and the same basic nutritional requirements which means a similar diet with different portion sizes can meet the needs of most companion dogs (Cohen & Diaz, 2013; McNamara, 2006).

2.1.1 Energy intake and balance of nutrient composites

Besides water, energy is the most important constituent in a diet, as dogs need the energy to meet the body requirements for normal growth, maintenance, reproduction, and physical activity. Energy is a property that fats, carbohydrates, and proteins contribute to diets (NRC, 2006). Like all animals, dogs need a constant inclusion of energy to survive. Energy deficiency in diets will lead to weight loss and decreases in both fat and stores of lean body tissue, while chronic excessive energy intake normally leads to obesity which may further induce diabetes (NRC, 2006).

2.1.2 Determining energy requirements

The gross energy (GE) of a diet is defined as the total chemical energy released from the complete combustion (oxidation) during direct calorimetry. Digestible energy (DE) is calculated as GE minus faecal energy. Metabolisable energy (ME) is determined as DE minus the energy losses in urine and through fermentation gasses. As energy loss in fermentation gases are almost neglectable in the dog, it is accurate to calculate ME as DE minus faecal energy. Net energy (NE) is defined as ME minus heat loss during the transformation from ME to NE. Today, the determination of dietary energy is mainly based on DE and ME systems. ME can be determined through actual feeding trials but such methods are both highly time-consuming and costly, hence normally it is estimated with mathematical formulas (Case et al.,

2010). The currently used formula to predict ME of dog foods was given by AAFCO (2019) :

$$ME = (3.5\ x\ g\ progein) + (8.5\ x\ g\ fat) + (3.5x\ g\ NFE) \quad \text{eq (1)}$$

Nb. NFE is the abbreviation of nitrogen-free extract which is a measure of soluble carbohydrates.

To determine the energy requirements of dogs, NRC (2006) provides the following formulas:

$$ME_{Inactive\ adult\ dog} = 95\ x\ W_{kg}^{0.75} \quad \text{eq (2)}$$

$$ME_{Active\ adult\ dog} = 130\ x\ W_{kg}^{0.75} \quad \text{eq (3)}$$

* $W_{kg}^{0.75}$represents metabolic body weight calculated as body weight to the power of 0.75.

2.2 Nutrients and nutrition

A nutrient is defined as a compound or substance needed to support the maintenance, growth, development, lactation, reproduction, and health of animals (Wu, 2018). Dogs, like all animals, need six major categories of nutrients including water, proteins, fats, carbohydrates, vitamins, and minerals (Case et al., 2010). Another key concept is essential nutrients, which represent those nutrients that animals cannot synthesise or synthesise sufficient quantities of for physiological functions. Essential nutrients normally refer to 10 amino acids, 7 macro minerals and 9 microminerals (trace)， 13 vitamins, and 2 fatty acids. In this thesis, I will focus on protein and fat and mention the other categories when necessary.

2.3 Dietary Fat in Dog Food

As an important component of the companion canine foods, dietary fat is part of a broader group of compounds known as lipids. In the diet, fats serve as the most concentrated energy source. The gross energy (GE) of fat is 9.4 kcal/g which is more than double the GE of carbohydrates (4.15 kcal/g) and much higher than protein (5.65 kcal/g). In addition to it's high energy density, fat is also highly digestible. The total tract apparent digestibility of most fats in dog food is typically higher than 90% (Algya et al., 2018; Cargo-Froom et al., 2019; Kilburn, Allenspach, et al., 2020). Dietary fat also serves as the provider of EFAs, enhances food palatability, and acts as the carrier of fat-soluble vitamins. Essential fatty acids and their derivative long-chain polyunsaturated fatty acids (PUFAs) fulfill crucial roles during the different life stages of dogs; for example docosahexaenoic acid (DHA) is critical for the early

neurological development of dogs, and the PUFAs are the precursors for eicosanoids synthesis (Case et al., 2010b). Further discussion on EFAs and PUFAs will be made in the following sections.

2.3.1 EFAs Essentiality and the Biochemical Basis

Essential fatty acids (EFAs) are those that an animal cannot synthesise for itself and must obtain from its food (Sadava et al., 2009). Chemically all EFAs are polyunsaturated, straight-chain hydrocarbons. For dogs, two EFAs are biologically essential, α-linolenic acid (ALA) an n-3 (ω-3) fatty and linoleic acid (LA) an n-6 (ω-6) fatty acid. ALA is the parental form of several other important ω-3 PUFAs including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). While LA is the parental form of the major ω-6 PUFAs including arachidonic acid (AA) and γ-linolenic acid (Mehler et al., 2016).

Dogs, like most mammals, can synthesise monosaturated 16-, and 18-carbon fatty acids from glucose or amino acids together with acetyl coenzyme A (acetyl-CoA). The synthesis of PUFAs is accomplished by inserting double bonds into the monosaturated fatty acid molecules with the participation of certain specified desaturase enzymes. Dogs are incapable of synthesising ω-3 and ω-6 PUFAs via Δ15 and Δ12 desaturases hence cannot synthesise ALA and LA *de novo*. This is the biochemical basis of the essentiality of fatty acids. In contrast, if enough ALA and LA are provided in the diet, dogs can synthesise EPA and DHA from ALA, and GLA, AA from LA. Hence the LA and ALA are normally regarded as the essential fatty acids for dogs; in the meantime, the long-chain derivatives of the 18-carbon EFAs such as DHA and EPA are typically defined as conditionally essential fatty acids due to their reliance on sufficient dietary ALA and GLA levels and their role in crucial biological functions in neurological tissue and the body's immune and inflammatory reactions (NRC 2006).

2.3.2 EFAs Functions and Dietary Fat Requirement

All EFAs and conditionally essential fatty acids have important roles in the dog, the major ones include;

1) maintaining cell membrane stability, structure, and functionalities such as epidermal water barrier and transport, and metabolic regulation;

2) facilitating cell membrane protein conformational changes through lipid-protein interactions;

3) As precursors of the important 20 carbon metabolites known as eicosanoids such as

prostaglandins, leukotrienes, prostacyclins, and thromboxanes;

4) Influencing brain and retinal development during the neonatal and prenatal stages in the dog (Bauer, 2016; NRC 2006).

According to the current published *Dog and Cat Food Nutrient Profiles* by the Association of American Feed Control Officials (AAFCO, 2019), the minimum crude fat requirements are 8.5% for growth and reproduction, and 5.5% for adult maintenance, all calculated on a dry matter (DM) basis and presuming a caloric density of 4,000 kcal ME/kg. Besides the recommended minimum total fat level, AAFCO also specify a minimum LA content 1.3% for growth and reproduction and 1.1% for adult maintenance. For ALA, AAFCO defines a minimum of 0.08% for growth and reproduction, but no specific value for adult dog maintenance (AAFCO, 2019). This is noted that there should be enough ω-3 fatty acids in the diet so that a maximum ω-6:ω-3 fatty acid ratio determined as 30:1 by AAFCO is not exceeded. I will put more emphasis on the ω-6:ω-3 ratio in the following section.

One functional aspect is of both great importance and intensive interest is the ω-6:ω-3 fatty acid ratio. Besides providing fluidity and structural integrity to cell membranes, cell membrane fatty acids are also important for cell function regulation. Most importantly, AA, GLA, EPA, and DHA are precursors of eicosanoids which are a group of bioactive fatty acyl-derived metabolites that are typically short-lived and have local hormone-like effects (Case et al., 2010b). Eicosanoids are important mediators of inflammatory responses, immunoregulation, and epidermal cell proliferation, so different combinations of ω-6 and ω-3 fatty acids in the diet will affect the inflammatory reactions and the overall health status of dogs. There are several factors behind this phenomenon:

1) the amount and relative proportion of ω-6 and ω-3 fatty acids in the cell membrane phospholipids can be modified by diet;

2) the metabolism of ω-6 and ω-3 fatty acids forms different families of eicosanoids and the two fatty acids compete directly for the same enzyme systems and metabolic pathways;

3) ω-6 and ω-3 fatty acids are also in competition for the incorporation into the cell membrane, and

4) the amount of synthesis of different families of eicosanoids, are limited by how much and what type of PUFAs are available when they are being released from the cell membrane (NRC, 2006).

There are four major types of eicosanoids which are prostaglandins, leukotrienes, prostacyclins, and thromboxanes. The ω-6 PUFAs produce 2- and 4-series eicosanoids while ω-3 PUFAs produce 3- and 5-series eicosanoids. As shown in Figure.1, those eicosanoids are synthesised with the involvement of both lipoxygenase (LO) and cyclooxygenase (CO). Under the LO pathway AA (ω-6) is metabolised into leukotriene B_4 (LTB_4) and EPA (ω-3) is metabolised into leukotriene B_5 (LTB_5) while under the CO pathway AA produces prostaglandin E_2 (PGE_2), and EPA produces prostaglandin E_3 (PGE_3). Generally, it is believed that increasing the amount of ω-3 fatty acids in dog food can increase the synthesis of less inflammatory metabolites and decrease the production and activity of the pro-inflammatory eicosanoids. This can be explained that;

1) compared to ω-6 originated eicosanoids, the ω-3 originated ones are less inflammatory (less/non-aggregatory and non-vasodilatory);

2) the existence of a direct competition for incorporation into the cell membrane and enzyme systems, and

3) the 15-hydroxyeicosapentaenoic acid, which is produced during the EPA metabolism, can inhibit the potency of the more pro-inflammatory LTB_4. One study conducted by Moreau et al. (2013) proved this effect. The researchers fed a fish-based diet rich in ω-3 fatty acids (1.47% DM) to dogs afflicted by osteoarthritis for 13 weeks. At the end of the study, the dogs' locomotor disability and the performance in daily activities have been improved. Similar results were obtained in other studies (Combarros et al., 2020; Mehler et al., 2016).

Therefore, when formulating dog foods, it is important to take into account both the absolute amount and the relative ratios of the ω-6 and ω-3 fatty acids. The current AAFCO maximum ratio for ω-6:ω-3 is 30:1 which is calculated from (LA+AA)/(ALA+DPA+DHA).

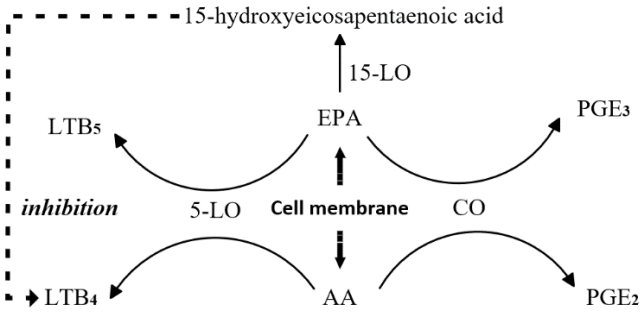


Figure 2-1 Schematic illustration of eicosanoids synthesis from ω-6 and ω-3 fatty acids (from Nutrient Requirements of Dogs and Cats, NRC 2006, p91).

2.3.3 Deficiency and Excess of Fat in the Diet

A deficiency of fat in a diet can lead to a shortage of both total energy and essential fatty acids. Under such circumstances, the effect of deficiency on total energy could be amplified given low fat levels will normally lead to low palatability hence decrease the dog's total food intake (Case et al., 2010).

The deficiency of EFAs is mainly manifested in the skin and hair coat. Due to the important role that LA has in maintaining the normal function of the epidermal water barrier, dogs deficient in LA show a dry and dull coat, hair loss, skin lesions, and stunted wound healing. All the above signs can be found after 2-3 months if LA deficient foods are fed continuously. Without dietary intervention, the dogs' skin will become pruritic, greasy, and susceptible to infection (Case et al., 2010; NRC, 2006).

In addition to skin and hair coat lesions, an EFA deficient diet could cause other clinical symptoms including decreased growth rate, reproductive abnormalities, immunological abnormalities, and degenerative changes in other organs, etc. A deficiency of ω-3 fatty acids could lead to decreased visual acuity, retinal abnormalities, nervous system abnormalities, and reduction in learning and memory (Abdulmajeed et al., 2014; Angrimani et al., 2017; Wąsik et al., 2017).

The consumption of excess fat is also potentially harmful to dog health. Although dogs can digest and assimilate a high-fat diet, there are also negative impacts on their health. In the short term, steatorrhea and diarrhea could occur, while longer term, due to the high palatability and energy density dogs could become obese. Another possible side effect of a high-fat diet is the potential deficiencies of other essential nutrients if the diet is not properly balanced between energy density and them. Lastly, overconsumption of LCPUFAs may lead to increased Vitamin E requirements due to Vitamin E being more easily oxidised than LCPUFA ((Case et al., 2010).

2.4 Dietary Protein in Dog Food

Proteins are complex organic compounds that consist of amino acids linked by peptide bonds. In addition to containing carbon, hydrogen, and oxygen like carbohydrates and fats, they all contain nitrogen and some of them contain sulphur (McDonald, 2011). Proteins are basic building blocks of organisms and are the nutrients in the highest concentration in muscle tissues of dogs. In the body, proteins perform many important functions including acting;

1) As structural components of cell membranes, and existing in muscle, skin, hair, nails,

tendons, cartilage, and ligaments, 2) as enzymes that take part in the essential biochemical reactions and the digestion and metabolism of dietary nutrients,

3) as hormones that are critical to the survival of animals are proteins, i.e. insulin and glucagon, which are a pair of substances that regulate a dogs' blood glucose level,

4) as important carriers of substances such as oxygen, iron, and vitamins, and important in regulating the acid-base balance,

5) as immune antibodies which are vital to protecting animals against specific infections and

6) as an energy source i.e., when the diet is insufficient on fats and carbohydrates (Pond et al., 2005).

1.1.1 Protein Requirement of Dogs

The proteins existing in the body are actually in a constant state of flux, rather than in a static state. All proteins in the body tissues will eventually be metabolised and replaced, although the turnover rates of different tissues are quite different. Hence a regular inclusion of protein through the diet is essential to maintain a normal level of metabolic processes and to support tissue maintenance and growth. Two facts need to be known;

1) the animal body is capable of synthesising new proteins from amino acids given that the levels of essential amino acids are sufficient and

2) at the cell level, there is no difference between the amino acids from different pathways, whether they are synthesised de novo, supplied as amino acid units from the diet, or provided in the diet as intact proteins (Case et al., 2010).

As the basic building blocks of proteins, amino acids are typically classified into two categories, the essential (indispensable) amino acids, and the non-essential (dispensable) amino acids. Among the over 200 amino acids that have been found in naturally occurring biological materials, but only about 20 are found as protein components, and 10 are known as essential amino acids for the dog. Essential amino acids are those that cannot be synthesised by animals' body tissues or cannot be produced sufficiently to meet the metabolic needs, whereas nonessential amino acids are those that can be synthesised sufficiently by body tissues (McDonald, 2011). Essential amino acids vary from species to species, as shown in Table 2.1, dogs need 10 amino acids, cats need 11, and human need only 9.

Table 2.1 Essential amino acids for humans, dogs, and cats.

Essential Amino Acids	Human	Dog	Cat
Histidine	√	√	√
Isoleucine	√	√	√
Leucine	√	√	√
Lysine	√	√	√
Methionine	√	√	√
Phenylalanine	√	√	√
Threonine	√	√	√
Tryptophan	√	√	√
Valine	√	√	√
Arginine		√	√
Taurine			√

2.4.1.1 Determination of protein requirement

The requirement for dietary protein is to meet the dogs' need for essential amino acids and to provide enough non-essential amino acids for the synthesis of tissue protein and to provide nitrogen (N) for the synthesis of non-essential amino acids and other N-incorporated compounds (Case et al., 2010). According to the current AAFCO dog food nutrient profiles, the requirement is expressed as a minimum requirement for both crude protein (22.5% for growth and reproduction and 18% for adult maintenance, on DM basis) and for each essential amino acid. Table 2.2 shows the current AAFCO minimum requirement for all the essential amino acids.

Table 2.2 AAFCO dog food nutrient profiles based on dry matter.

Nutrients	**Units DM Basis**	**Growth & Reproduction Minimum**	**Adult Maintenance Minimum**	**Adult Maintenance Maximum**
Crude Protein	%	22.5	18.0	
Arginine	%	1.00	0.51	
Histidine	%	0.44	0.19	
Isoleucine	%	0.71	0.38	
Leucine	%	1.29	0.68	
Lysine	%	0.90	0.63	
Methionine	%	0.35	0.33	
Methionine-cystine	%	0.70	0.65	
Phenylalanine	%	0.83	0.45	
Phenylalanine-tyrosine	%	1.30	0.74	
Threonine	%	1.04	0.48	
Tryptophan	%	0.20	0.16	
Valine	%	0.68	0.49	

(from AAFCO official publication 2021).

As Table 2.2 shows, in addition to the minimum required levels of methionine and phenylalanine, AAFCO also defines a minimum required levels for methionine plus cysteine and phenylalanine plus tyrosine. The reason for this is although cysteine is not an indispensable amino acid, it is only synthesised from methionine and supplies around one-half of the total sulphur amino acid needs, hence the minimum requirement of methionine is expressed both individually and in combination with cysteine. In the case of phenylalanine, tyrosine can only be produced from phenylalanine, and tyrosine spares around one half of the dogs' total need for phenylalanine given that there is enough tyrosine present in the diet. Hence it is reasonable to present the minimum requirement of phenylalanine as both the phenylalanine individually and as the sum of phenylalanine and tyrosine (NRC, 2006).

For the last three decades, researchers and dog food practitioners have adopted two systems to determine dogs' dietary protein requirements, nitrogen balance, and growth rates. Nitrogen balance methods are based on the fact that all protein molecules averagely contain about 16% nitrogen (Case et al., 2010). It is practically calculated as:

$$Nitrogen\ balance = Nitrogen_{intake} - Nitrogen_{excretion} \quad \text{eq (4)}$$

Whereas net protein utilisation is usually adopted to calculate the growth rate (Bawa et al., 2020a). Net protein utilisation measures the efficiency of the target protein on dogs' body N accumulation by comparing the dogs' body N resulting from test protein with that resulting from the dogs fed with a protein-free ration for the same length of time (Pond et al., 2005), as expressed below:

$$NPU = (Body\ N_{test\ protein} - Body\ N_{protein-free\ diet}) / Total\ N_{intake} \quad eq(5)$$

Zero balance is deemed as a reflection of the body's N equilibrium state when the N loss is fully compensated by N intake, hence is regarded as an indirect measure of the whole-body N turnover. Accordingly, a positive N balance occurs when N intake exceeds N excretion; a negative N balance is a state when N intake is less than the N excretion. Nitrogen equilibrium (zero balance) is the state of healthy normal adult animals during maintenance, hence is used for studies of the protein requirements of adult maintenance dogs. For dog growth and reproduction, maximum positive N balance and growth rate are used to measure the adequacy level of dietary proteins (Case et al., 2010).

Although the zero N balance methods have been widely used to measure companion animal

protein requirements, the method itself has certain limitations, such as;

1) Zero N balance is incapable of showing the requirements of certain individual amino acids,

2) The requirements derived from the zero N balance may not be able to reflect the needs to support optimum health status and performance, hence can normally be used as the minimum levels and

3) N balance methods, in general, cannot reflect the presence and the degree of protein degradation, synthesis, and oxidation in response to different levels of protein intake or different combinations of essential amino acids. In regard to this issue, the current advances to elucidate such changes known as metabolomics adopts more advanced technology such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) to study the metabolome mechanics of companion animals including dogs, as summarised by Carlos et al. (2020).

2.4.1.2 Influencing factors of protein requirement

The protein requirement of specific dogs is influenced by many factors. On one hand, dietary factors influencing the dogs' N balance include protein digestibility, protein quality, amino acid profile, and the energy density of the diet. On the other hand, the dogs' physiological state, activity level, and current nutrition status will also affect their protein requirement.

A dogs' protein requirement is inversely correlated to protein digestibility and protein quality. As digestibility increases the total protein requirement decreases. As for protein quality, it is normally referred to as biological value (BV), the higher the protein quality (BV) the lower the protein level is required. We will discuss protein quality in more detail latter in the literature review.

Energy density affects potential protein requirements in two ways with the common basis that dogs tend to meet their energy needs first. If the nonprotein calories such as fat and carbohydrates are deficient, the body will use proteins as an energy source; so protein is used to synthesise body tissues only if there are enough nonprotein energy resources in the diet. The second aspect lies in the fact that when the energy density of the diet increases the proportion of protein should also be increased to balance the effect of dogs consuming less food to meet requirements (Case et al., 2010).

2.4.1.3 Protein Deficiency and Excess

A general protein deficiency causes symptoms such as retarded growth, brain development and learning abilities in young dogs, and leads to weakened work performance, blunted

reproductive capabilities, and decreased lean body mass in adult animals (Humbert et al., 2001; Laflamme, 2008).

Concerning EAAs deficiency, signs of deficiency for each EAA are quite specific and different among the EAAs. Table 2.3 shows the EAAs and the corresponding deficiency symptoms.

Table 2.3 Essential amino acids and the corresponding deficiencies.

Essential AA	Possible signs of deficiency
Arginine	Emesis associated with hyperammonemia Excessive salivation and muscle tremors Elevated urinary orotic acid excretion Cataracts
Histidine	Weight loss and negative nitrogen balance Diet aversion, lacking energy, and reduced activity Decreases in plasma histidine, hemoglobin, and albumin Decreases in muscle histidine and carnosine
Isoleucine	Bodyweight loss and negative nitrogen balance Depression in food intake
Leucine	Bodyweight loss and negative nitrogen balance Depression in food intake
Lysine	Bodyweight loss and negative nitrogen balance Depression in food intake
Methionine and Cysteine	Immediate decrease in food intake Severe weight loss Dermatitis, swelling, and reddening of the skin

*Modified from *Nutrient Requirements of Dogs and Cats* (NRC, 2006).

When protein is consumed in excess in dogs, and diets are energy deficient, the excess protein is used as an energy source by the body. In contrast, when nonprotein energy sources are sufficient, the excessive protein and amino acids are metabolised into fat and glycogen, and N is excreted as urea in urine because the body cannot store excess amino acids (Case et al., 2010b). One concern related to excess protein intake is whether the metabolism of excessive protein will exert an extra burden on the kidney and eventually lead to chronic renal disease. Although historically it was believed that excessive protein was detrimental to kidney function (Martin et al., 2005), no studies support this effect. Actually, in aging dogs, an increased protein intake is suggested to compensate for the lean body mass reduction caused by aging (Frantz et al., 2007; Lees et al., 2005; Wakshlag et al., 2002).

2.4.2 Evaluation of the nutritional value of protein

As previously mentioned, the degree to which a protein can be utilised is restricted by both the digestibility and the protein quality. I will discuss these two topics further in this section.

2.4.2.1 Digestibility

Digestibility or digestion coefficient is defined as the proportion of a nutrient that is not excreted in the faeces and therefore is assumed to be absorbed by the animal (McDonald, 2011). The protein digestibility can be calculated as:

$$Digestibility = (N_{intake} - N_{faecal})/N_{intake} \quad \text{eq (6)}$$

It should be noted that above value is normally considered as apparent digestibility due to the inflow of endogenous amino acids into the intestinal tract via saliva, stomach secretions, bile, small-intestinal secretions, pancreatic secretions, epithelial cells sloughed off from the digestive tract, and the intestinal microorganisms. Hence the apparent digestibility calculation will result in an underestimation of the true digestibility of the tested protein. In contrast, the true digestibility is calculated taking into account the basal endogenous amino acid flow which is measured normally with a cornstarch-based nitrogen-free diet (Wu, 2018). Once the basal endogenous amino acid flow is determined, the true digestibility can be calculated as:

$$True\ digestibility = (N_{intake} - N_{faecal} - N_{basal})/N_{intake} \quad eq\ (7)$$

2.4.2.2 Protein quality

The true utilisation of a feed protein is determined not only from the amount being absorbed but also it's utilisation after absorption. Biological value (BV) is a major criteria used to assess protein utilisation after absorption, and BV is defined as the percentage absorbed from the gastrointestinal tract and is used for productive functions (Pond et al., 2005). Biological value is calculated as:

Biological value = (N_{intake} - N_{faecal} –$N_{urinary}$) / (N_{intake} –N_{faecal}). eq (8)

$$Biological\ value = (N_{intake} - N_{faecal} - N_{urinary})/(N_{intake} - N_{faecal}) \quad \text{eq (9)}$$

Other evaluation methods include net protein utilisation, protein efficiency ratio (PER), essential amino acid index (EAAI), and chemical score, as shown in Table 4.4.

Table 4.4 Protein quality determination equations.

Biological value (BV)	$(N_{intake} - N_{faecal} - N_{urinary})/(N_{intake} - N_{faecal})$
Net protein utilization	$BV\ x\ Digestion\ coefficient$
Protein efficiency ratio (PER)	$Weight\ gained\ (g)/Protein\ intake$

Total essential amino acid content (E/T)	$N\ from\ EAAs/Toptal\ N\ of\ the\ protein$
Essential amino acid index (EAAI)	$Total\ EAAs\ of\ test\ protein/Same\ EAAs\ in\ reference\ prot$
Chemical Score	$Limiting\ AA\ in\ test\ protein/Same\ AA\ in\ reference\ protei$

Modified from *Canine and Feline nutrition* (Case et al., 2010 p23).

2.4.3 Traditional and novel resources of diet proteins (dog food)

Proteins in dog foods can be of both animal and plant origin. Generally, animal proteins (except for gelatin), are superior to plant proteins in both digestibility and amino acid profile, and hence have higher nutritional value. In comparison to animal proteins, the amino acid profiles of plant proteins are not as balanced as animal proteins, i.e. soybean protein is lacking sulphur amino acids whereas corn proteins typically lack lysine and tryptophan. Hence in commercial dog food formulations, a variety of protein sources are mixed to leverage the complementary effect (Pond et al., 2005). In addition, under the context of the population explosion and the urgent sustainability requirements, novel new proteins have gained increasing amounts of attention such as fish by-products and insects (Gasco, et al., 2020; Bosch et al., 2014; Nikoletta, 2019). I will discuss insect protein in detail later in this literature review.

2.5 Vitamins and minerals

Vitamins are a diverse group of dietary organic constituents that are indispensable to the metabolic processes for the growth and sustenance of life. Vitamins can be classified into two broad categories, fat-soluble vitamins, and water-soluble vitamins (Hynd, 2019). Appendix A summarises the essential vitamins and their functions, and signs of deficiency.

The animal body contains around 60 minerals, 16 of which are defined as essential minerals and they are categorised into macrominerals and microminerals. The macrominerals are required at levels of g/day and typically diets contain g/kg or percentages of them on a DM basis, while the microminerals are required at levels of milligrams or micrograms per day and typically feeds contain mg/kg or parts per million. Table 1.5. shows the mineral requirements of dogs per the AAFCO nutritional profiles.

2.6 Insect Protein and Fat (Crickets and Black Soldier Fly)

It is projected that the planet will need to sustain 9.8 billion people by 2050 and 11.2 billion by 2100 (UN DESA,2017), and the demand for more resource-intensive proteins such as milk and

meat is expected to increase at an even higher rate. The current rapid population growth and the increasing demand have exerted unprecedented pressure on the food supply system and natural resources (Dossey et al., 2016). In addition, companion animals have become part of human society and the number of companion animals continues to grow. In the case of dogs, about 34% of New Zealand households own a dog and the total pet dog population is 851,000 (NZPFMA,2020), while in the US, 38.4% of households own a total of 7.68 million dogs (APPA, 2019), and in EU, the total number of pet dogs has reached to 66.4 million in 2017 (FEDIAF, 2018). Although it is hard to conclude that pets are directly competing with humans for food resources as dogs typically consume by-products of human food, but in a broader view, food production for companion animals ultimately consumes natural resources required for human food production, such as land and water. As shown in the study of Okin (2017), in the US, the total dietary energy consumed by dogs and cats equals around one fifth that of humans. So, when considering the recent "humanisation" trend in the pet food industry it is reasonable to conclude that companion animals might, at least to some extent, compete for food resources with humans.

In short, to cope with the imbalance between increasing demand and limited resources, the global food supply system must find new ways and new resources for protein productions of both human and pet foods. Insects protein is one of the most promising alternatives as insects are abundant in nature, high in nutrient value, and more environmentally sustainable than traditionally farmed animals such as swine, cows, and chickens (Sogari et al., 2019).

2.6.1 The nutrient value of insects

Research has been carried out to determine the chemical and nutrient composition of insects (Do et al., 2019; 2020; Ewald et al., 2020; Finke, 2002, 2013a, 2015; Gasco et al., 2019), for this review we will mainly focus on house crickets (*Acheta domesticus*) and black soldier fly (BSF, *Hermetia illucens*), and refer to other species when necessary. Table 5.5 compares the nutrient composition of crickets and BSF with the nutrient requirements defined by AAFCO for growth and reproduction and adult maintenance of dogs.

Table 5.5 **AAFCO dog food nutrient profiles based on dry matter.**

Nutrients	Units DM Basis	Growth & Reproduction Minimum	Adult Maintenance Minimum	Maximum	Crickets (adult) *Acheta domesticus*	Crickets (nymph) *Acheta domesticus*	Black soldier fly (prepupae) *Hermetia illucens*
Crude Protein	%	22.5	18.0		66.6	67.2	45.1
Arginine	%	1.0	0.51		4.06	4.10	3.2
Histidine	%	0.44	0.19		1.56	1.48	1.5
Isoleucine	%	0.71	0.38		3.05	2.88	2.0
Leucine	%	1.29	0.68		6.66	6.42	3.1
Lysine	%	0.90	0.63		3.57	3.62	3.1
Methionine	%	0.35	0.33		0.97	0.87	0.9
Methionine-cystine	%	0.70	0.65		1.53	1.44	1.1
Phenylalanine	%	0.83	0.45		2.11	1.88	1.9
Phenylalanine- tyrosine	%	1.30	0.74		5.36	5.59	5.1
Threonine	%	1.04	0.48		2.40	2.40	1.8
Tryptophan	%	0.20	0.16		0.42	0.35	0.8
Valine	%	0.68	0.49		3.47	3.32	3.3
Crude Fat	%	8.5	5.5		22.1	14.4	36.1
Linoleic acid	%	1.3	1.1		7.4	4.8	4.4
alpha-Linolenic acid	%	0.08	ND[a]		0.2	0.2	0.2
Eicosapentaenoic + Docosahexaenoic acid	%	0.05	ND[a]		0	0	0
(Linoleic + Arachidonic):(alph a-Linolenic + Eicosapentaenoic + Docosahexaenoic) acid Ratio			30:1		~38:1[b]	~28:1[b]	~26:1[b]

Minerals							
Calcium	%	1.2	0.5	1.8	0.1	0.1	2.4
Phosphorus	%	1.0	0.4	1.6	1.0	1.1	0.9
Ca:P ratio		1:1	1:1	2:1	~ 1:7	~ 1:9	~ 1:2.6
Potassium	%	0.6	0.6		1.1	1.5	1.2
Sodium	%	0.3	0.08		0.4	0.2	0.2
Chloride	%	0.45	0.12		0.7	1.0	0.3
Magnesium	%	0.06	0.06		0.1	0.1	0.4
Iron [f]	mg/kg	88	40		62.7	92.6	171.6
Copper [g]	mg/kg	12.4	7.3		20.1	22.3	10.4
Manganese	mg/kg	7.2	5.0		37.3	38.9	159.3
Zinc	mg/kg	100	80		217.9	296.9	144.8
Iodine	mg/kg	1.0	1.0	11	0.7	1.2	0.7
Selenium	mg/kg	0.35	0.35	2	0.6	0.4	0.8
Vitamins & Other							
Vitamin A	IU/kg	5000	5000	250000	< 3,247[c]	< 3,247[c]	< 2575[c]
Vitamin D	IU/kg	500	500	3000	< 831[c]	< 831[c]	464[d]
Vitamin E	IU/kg	50	50		63.96	41.92	24
Thiamine	mg/kg	2.25	2.25		1.30	0.87	19.85
Riboflavin	mg/kg	5.2	5.2		110.71	41.48	41.75
Pantothenic acid	mg/kg	12	12		74.68	114.85	99.23
Niacin	mg/kg	13.6	13.6		124.68	143.23	182.99
Pyridoxine	mg/kg	1.5	1.5		7.47	7.42	15.49
Folic acid	mg/kg	0.216	0.216		4.87	6.33	6.96
Vitamin B_{12}	mg/kg	0.028	0.028		0.17	0.38	143.81
Choline	mg/kg	1360	1360		4931.82	4777.29	2835.05

a. Refer to the original AAFCO comment, that no minimum requirement but must meet the maximum ω-6 to ω-3 ratio.
b. Calculated as LA/ALA.
c. Value below the detection limit of the assay used in the experiments (1,000 IU vitamin A/kg, and 256 IU vitamin D/kg).
d. The value of Vitamin D_3.

2.6.2 The nutritional profile of house cricket (*Acheta domesticus*)

House cricket (*Acheta domesticus*) species belongs to the order Orthoptera and are one of the most widely commercially reared insects (Halloran et al., 2018). House crickets are hemimetabolic hence the whole life cycle includes three life stages as eggs, nymphs, and adults (Huis & Tomberlin, 2017).

As is the case of most insects, the nutritional composition of house crickets varies across different development stages and is affected by the diet fed (Bawa et al., 2020a; Finke, 2015; Oonincx et al.,2020). Also, the nutritional value of crickets is affected by the preparation and processing methods such as roasting, drying, and extrusion which are used in the preparation of crickets and pet food processing (Nyangena et al., 2020; Ottoboni et al., 2018; Poelaert et al., 2018). Another factor that has recently gained a lot of attention is the killing methods of insects which can also affect the nutritional composition and digestibility of house cricket (Singh et al., 2020).

2.6.2.1 Cricket protein in the context of dog nutrition

As shown in Table 1.5, house crickets (nymphs and adults) are a good source of protein and contain sufficient quantities to meet AAFCO recommendations. While adult crickets contain slightly lower total protein levels than cricket nymphs, 66.6%, and 67.2% respectively on a DM basis, both are substantially higher than the minimum protein levels required by AAFCO.

The amino acid profile of crickets shows levels of all the essential amino acids that exceed the minimum AAFCO requirements (Table 1.5). When compared with AAFCO requirements for dogs, the first limiting amino acid of crickets appears to be tryptophan or the sulphur amino acids methionine-cystine. For adult crickets, the first limiting amino acid is tryptophan in the AAFCO growth & reproduction dog nutrient profiles while methionine-cystine is the first limiting amino acid in the case of adult maintenance dog nutrient profiles. As for cricket nymphs, tryptophan is the first limiting amino acid for both growth & reproduction and adult maintenance dog profiles. In short, cricket protein has both sufficient total protein content and a complete amino acid profile to be a potential protein source for dog food. Digestibility as another key factor will be discussed later in this review.

2.6.2.2 Fat and fatty acid composition

As shown in Table 1.5, crickets contain sufficient levels of dietary fat (22.1% in adult crickets and 14.4% in nymph crickets on a DM basis) to meet AAFCO minimum requirements for dogs (8.5% for growth & reproduction, 5.5% for adult maintenance).

As for fatty acid composition, crickets contain sufficient levels of the essential fatty acids, linoleic acid (LA, ω-6) and linolenic acid (ALA, ω-3), to meet AAFCO minimum requirements for dogs. The ω-6 fatty acid to ω-3 fatty acid ratio of cricket nymphs (28:1) is slightly lower than the maximum requirement of AAFCO (30:1), while cricket adults' ratio (38:1) is higher than the maximum AAFCO requirement. There is no arachidonic acid (AA, 20:4n-6), docosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) found in crickets. However, the fatty acid composition of crickets can be manipulated by feeding different diets and it is feasible to increase the ω-3 fatty acid level and get an optimal ω-6/ω-3 ratio, through feeding ω-3 fatty acid enhanced food scraps. Research conducted by Dennis G.A.B. Oonincx et al. (2020), fed house crickets, along with other two insect species, diets enhanced with ω-3 (ALA) enriched flaxseed oil (control diet, 1%, 2%, 4%). The result showed that for every one percent increase in flaxseed oil a 2.3% ~ 2.7% increase ALA level was recorded in the crickets. This is consistent with the research conducted by Bawa et al.(2020) and Finke (2015).

2.6.2.3 Chitin, vitamins, and minerals

Chitin as the main component of the exoskeleton of insects and this common fibre form is a derivative of glucose, N-acetyl-d-glucosamine linked by β-1-4 bonds (Van Huis et al., 2013). Chitin has gained a lot of interest in recent years due to its prebiotic potential and other possible physiochemical functions (Kipkoech et al., 2018). Research on cricket chitin conducted by Lokman et al. (2019) showed that broilers that were fed a diet containing cricket chitin at an inclusion level of 0.5 g/kg diet showed improvement in organ characteristics, carcass quality, and growth performance. Finke et al. (2007) estimated the chitin levels of the house cricket adults and nymphs are 67.6 mg/kg and 81.5 mg/kg respectively.

As for vitamins, house crickets are deficient in vitamin A, vitamin D, and vitamin B_1 in comparison to AAFCO minimum requirements for dogs. The levels of all other vitamins are sufficient. On the mineral side, house crickets are deficient in calcium (11% of the minimum requirement for growth & reproduction in dogs and 24% of the adult maintenance requirements of dogs), although the Ca:P ratios are good (1:7 of adult crickets and 1:9 of nymph crickets). Also, adult crickets are deficient in iron when compared the levels required for growth & reproduction in dogs (71% of the required minimum level), while nymph crickets meet AAFCO minimum maintenance requirement (105% of the required minimum level). Levels of the other minerals meet AAFCO reference criteria.

2.6.3 The nutritional profile of black soldier fly (*Hermetia illucens*)

Black soldier fly (Hermetia illucens) is an insect species that belongs to the Diptera order and is another widely commercially farmed species (Halloran et al., 2018). BSF are holometabolous, hence lifespan includes four distinguishable stages as eggs, larvae, pupae, and adults (Huis & Tomberlin, 2017).

Similar to crickets, the nutritional composition and value of BSF depends on the developmental stage, diet fed (Danieli et al., 2019; Ewald et al., 2020; Meneguz et al., 2018; Mohamad et al., 2020; Mohd-Noor et al., 2017), preparation and processing methods (Nyangena et al., 2020; Ottoboni et al., 2018), and killing methods (Caligiani et al., 2019; Zhen et al., 2020).

2.6.3.1 Black soldier fly protein in the context of dog nutrition

As depicted in Table 1.5, BSF are a good source of protein and meet the AAFCO requirements for protein for dogs. The crude protein content of BSF prepupae is 45.1% which is substantially higher than AAFCO minimum requirement for protein (22.5% for growth and reproduction dogs and 18% for adult maintenance dogs on a DM basis).

The amino acid analysis is also shown in Table 1.5. The amino acid profile of BSF is well suited for inclusion in dog food and is a good source of essential amino acids. BSF contains all of the essential amino acids the dog requires and in sufficient quantities to meet AAFCO minimum requirements. The first limiting amino acid of BSF is threonine for the growth & reproduction profiles of dogs and the sulphur amino acid methionine-cysteine for adult maintenance profiles of dogs.

2.6.3.2 Fat and fatty acid composition

As shown in Table 1.5, BSF contains 36.1% dietary fat which is over four times higher than the AAFCO minimum requirements for dogs (8.5% for growth & reproduction dogs, 5.5% for adult maintenance dogs). The crude fat level of BSF is substantially higher than that of house crickets.

In the case of fatty acid composition, BSF contains sufficient quantities of the essential fatty acids, LA and ALA, to meet AAFCO minimum requirements for dogs. The ω-6 fatty acid to ω-3 fatty acid ratio of BSF (~26:1) is lower than the maximum AAFCO ratio (30:1). There were no AA, DPA, and DHA found in BSF, although like house crickets, it is possible to manipulate the level of ω-3 fatty acids and optimise the ω-6/ω-3 ratio through feeding an enhanced diet (Danieli et al., 2019; Ewald et al., 2020; Liland et al., 2017; Meneguz et al., 2018; Mohamad et al., 2020; Mohd-Noor et al., 2018; Mohd-Noor et al., 2017; Spranghers et al.,

2017).

2.6.3.3 Chitin, vitamins, and minerals

The chitin level of BSF is higher than house crickets and reaches 21.0 g/kg as estimated by Finke (2013). As for vitamins, BSF is deficient in vitamins A, D, and E, but abundant in vitamin B_1 (thiamine). Other than that, BSF can provide sufficient amounts of other vitamins in comparison to the AAFCO minimum vitamin requirements. On the mineral side, BSF unlike house crickets, are abundant in calcium (2.4%) and can meet the AAFCO minimum calcium requirements for dogs (1.2% for growth & reproduction dogs and 0.5% for adult maintenance dogs) on a DM basis. The Ca:P ratio of BSF (~2.6:1) is slightly high in comparison to the AAFCO requirements (minimum 1:1 and maximum 2:1 for both grow & reproduction and adult maintenance). While phosphorus content (0.9%) is slightly deficient when compared to the growth and reproduction nutrient profiles of dogs (1.0%) on a DM basis, so supplementation is required.

2.6.4 Research on insects as supplementary to companion dog food

Although insects have been widely researched and practically applied to the industrial sector of livestock feeding (Gasco et al., 2020a; Gasco et al., 2020b; Sogari et al., 2019), only recently has the pet food industry been considered to be the next arena for insect-enhanced foods (Beynen, 2018; Bosch et al., 2013; 2014; McCusker et al., 2014). In the case of direct application for companion dog food, only a few studies on the topic are readily available, however, all indicate that insect-based dog food could be a promising prospect.

Kilburn et al. (2020) studied using cricket (*Gryllodes sigillatus*) meal to feed adult dogs. In the 29-day feeding trial, 32 adult Beagles were randomly fed with one of the four diets containing different levels of cricket meal (0%, 8%, 16%, and 24%) that was substituted for a chicken meal on a protein level basis to evaluate the apparent total tract digestibility (ATTD) and potential health effects. The results revealed that the ATTDs of dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CF), total dietary fibre (TDF), and gross energy (GE) decreased with the increased level of cricket meal. As the authors indicated, although the ATTDs decreased (DM from 88.9% to 83.9%, OM from 91.5% to 90.0%, CP from 88.2% to 82.1%, fat from 96.4% to 94.8%, GE from 92.4% to 88.3%, and TDF from 57.5% to 46.3%, as level of inclusion increased from 0% and 24% cricket meal), the cricket-supplemented diets stilled showed a high digestibility (> 80%). In addition all blood parameters remained within healthy ranges, indicating no negative health effects on the participating dogs. The research

showed that cricket meal can be considered an optional protein resource for dog foods.

As for BSF, Lei et al. (2019) conducted a 6-week feeding trial with 9 healthy female Beagles fed with one of the three grain-based diets of different levels of defatted BSF meal (0%, 1%, and 2%) to test the effects of feeding BSF-supplemented foods on dogs. Escherichia coli lipopolysaccharide (LPS) was intraperitoneally injected (100 ng/kg body weight) at the end of week 6 before a complete blood count was carried out. The result indicated that the BSF meal inclusion led to a linear increase in the ATTDs of DM (71.97% at 0% BSF, 74.55% at 1% BSF, and 75.21% at 2% BSF level) and CP (73.16% at 0% BSF, 77.06% at 1% BSF, and 78.51% at 2% BSF level) but did not affect the ATTD of CF. At the end of the feeding period, a linear increase ($P<0.05$) was observed on glutathione peroxidase (GPx) level while a linear decrease ($P<0.05$) was recorded with increasing the BSF level at 6 hours after the LPS injection. In addition, a quadratic increase was observed in the concentrations of superoxide dismutase and GPx. Therefore, the author concluded that the inclusion of BSF meal in the diet may lead to beneficial effects such as improvement in the digestibility of DM and CP and have anti-inflammatory and anti-oxidative effects in Beagles. The ATTD results are consistent with the research conducted by Kröger et al. (2020) who compared diets with either BSF meal (200g/kg) or lamb meal and showed similar trends in ATTDs of DM, CP, CF, and observed no adverse signs and also an effect on immunological measurements.

2.7 Sustainability

As reviewed by Sykes et al. (2020) the estimated total number of companion dogs in the world is between 700 million to 1 billion at an average ownership rate of ~120 dogs per 1000 people. In the context of these ever-increasing numbers, the environmental impacts of owning companion dogs have become unprecedentedly prominent in the modern world. Yavor et al. (2020) conducted a life cycle assessment (LCA) study to quantify the average environmental impacts of dogs (based on the assumption of a 15kg body weight and a 13-year lifespan) and the results revealed that companion dogs can cause remarkable impacts to the environment, i.e., an average dog annually emits around 630 kg CO_2 equivalent greenhouse gas (GHG) which equals around 7% of the emission of an EU citizen. Martens et al. (2019) and Su (2018) calculated (based on the consumption of dry dog food) that the dietary ecological paw print (EPP, biologically productive land used) of dogs in China was around 22.5~114.8 million hectares which equal to the dietary ecological footprint (EF) of around 37.5~191.3 million Chinese people and the annual emission of GHGs were around 72.3~252.3 million tons. In Japan, dogs' dietary EPP was around 3.4~22.7 million hectares which equal the dietary EF of

around 2.4~15.9 million Japanese people, and the emission of dietary GHG was around 1.3~8.6 million tons which equal to the dietary GHG emission of about 0.6~3.96 million Japanese people.

As the main contribution to most impact categories over the dog's life is caused by pet food (Yavor et al., 2020), to mitigate the environmental impacts of dog ownership, using insects to replace traditional animal-sourced protein in dog food is of great interest because insects production has lower environmental impacts, and compared to traditional livestock, insects show a higher feed efficiency, less land usage and water consumption, less GHG emissions, higher reproduction rate, and can be raised with bio-wastes (Dossey et al., 2016). As reviewed by van Huis and Oonincx (2017), in comparison to poultry, insects have a better feed efficiency (lower dietary protein to edible body mass ratio) i.e., black soldier flies have a 1.8~2.3:1 feed conversion rate, and cockroaches are 1.1~1.96:1, both of which are lower than poultry (conversion rate around 3.0:1), while yellow mealworm has a conversion rate of 2.2~4.5:1 which is closer to poultry. As for GHG emissions, Oonincx and De Boer (2012) showed that GHG from rearing insects (2~122 g/kg mass gain) is much lower than that of beef cattle (2850 g/kg mass gain) and in the lower range of swine (80~1130 g/kg mass gain). Also, insects can be reared with a wide variety of bio-wastes which make insects more environmentally friendly (Gasco, et al., 2020b; Pinotti et al., 2019; Varelas, 2019). Energy consumption, as in the case of black soldier fly, is the most significant concern, mainly due to the drying process which alone contributes to the various impact categories with values ranging from 48.9% for terrestrial ecotoxicity to 26.6% for ozone layer depletion (van Huis & Oonincx, 2017).

3 Formula design

3.1 Introduction

Although the nutritional profile of the adult house crickets (*Acheta domesticus*) is affected by many factors such as diet fed, preparation, and processing methods, research shows a generally high level of crude protein and fat. In addition, the amino acid composition of cricket protein and fatty acids composition of cricket fat are also highly promising (Finke, 2013b, 2015; Oonincx et al.,2020).

From the viewpoint of nutrient adequacy, AAFCO establishes two categories, the adult maintenance and growth and reproduction category, with the latter including gestating and lactating dogs. The two categories are different on the minimum/maximum levels of some ingredients, ie. the growth category requires a minimum 25% (DM basis) crude protein level while the adult and maintenance category requires an 18% minimum crude protein level. The difference generally reflects the variation of nutrient requirement of dogs at different life stages. This study was conducted to develop formulas for the adult maintenance category.

Another aspect from the regulation side which is related to formula design is the product type. For the formulas labeled as complete and balanced foods, there are minimum requirements according to the profile, because the “complete and balanced” statement means that the normal dog's healthy and physiology are maintained only if the provision of the diet can meet it consistently. On the other hand, those dog foods designed for intermittent or supplemental feeding only don’t have to meet the minimum levels presented in the profile such as dog snacks and treats (AAFCO, 2019).

Energy density is another key parameter of dog foods. According to AAFCO’s Regulation F9 (of the Model Regulations for Pet Food and Specialty), the energy density value in the Dog Food Nutrients Profiles is calculated based on the 4,000 kcal ME/kg. For those formulations with an energy density higher than 4,000 kcal ME/kg the energy density must be corrected accordingly. While it is not required to correct the energy density when it is lower than 4,000 kcal ME/kg (AAFCO, 2019).

The last major concern of this study is gluten-free design. The demand for gluten-free products has increased because of the incidence of celiac disease (German et al., 2003) and other gluten-associated allergies. Therefore it is necessary to avoid the use of gluten-contained raw materials such as wheat flour to keep away from such disorder (Naqash et al., 2017).

This project was to develop a series of novel dog food formulas using cricket flour as the alternative protein source. To meet the desired amount of nutrient intake, the formulations (diets) must contain adequate levels of each nutrient. It was shown that given the high level of protein content in cricket flour, the design of high-quality dog food using cricket flour as the major alternative protein source was feasible.

3.2 Major Ingredients

3.2.1 Lentil

Lentils (*Lens culinaris Medik.*) are now mainly used for human food and are popular among vegetarians. Although variations exist between different genotypes and those cultivated under different environmental conditions, Lentils are widely perceived as a healthy food ingredient with a high content of protein, fibres, and minerals. As reviewed by Bhatty (1988), lentils (Canadian-grown) have an average nutrient composition of 28.6% crude protein, 4.9% crude fibre, 44.3% starch, and 63.1% total carbohydrates (all on a DM basis). Therefore, lentils can be added into the formula in combination with cricket flour as an alternative source of protein.

3.2.2 Pumpkin

Pumpkin (*Cucurbita*) is an edible, heat-sensitive plant, which has diversified food uses as it is a high-quality source of essential macro- and micro-nutrients, amino acids, vitamins, antioxidants, and bioactive compounds including carotenoids, flavonoids and tocopherols. As reviewed by Kaur et al. (2019), pumpkin pulp contains about 66% carbohydrates and 3% crude protein while pumpkin seeds contain about 39.25% crude protein, 16.84% crude fat, and 27.83% oil (all on a DM basis). Therefore, in this study, pumpkin was used as an important source of a variety of carbohydrates, dietary fibres, amino acids, vitamins, and minerals.

3.2.3 Sweet potato

Sweet potato (*Ipomoea batatas*) belongs to the Convolvulaceae family. Although carbohydrates are the predominant component, the roots of the sweet potato are also relatively rich in dietary fibre, spramins, ophosphoros, beta carotene, phenolic acids, and anthocyanins. The unique composition of sweet potato contributes to their various health benefits, such as antioxidative, hepatoprotective, anti-inflammatory, antitumor, antidiabetic, antimicrobial, antiobesity, antiaging effects (Wang et al., 2016). Therefore, in this study, the sweet potato was chosen to be a major carbohydrates source.

3.2.4 Flaxseed

Flaxseed is the seed of flax (*Linum usitatissimum*) which is also called linseed. As reviewed

by Bekhit et al. (2018), the whole flaxseed contains 30–41% fat, 20–35% dietary fibre, 20–30% protein, 4–8% moisture, 3–4% ash, and 1% simple sugars. Flaxseed also has been studied as a functional food since it is rich in ω-3 fatty acid α-linolenic acid (ALA) (52% of total fatty acids) and of phenolic compounds known as lignans (>500 μg g^{-1}, as-is basis) (Oomah & Mazza, 2000). In this study, flaxseed was added as an important alternative source of protein, and as an ingredient to balance the ω-6 to ω-3 fatty acids ratio. Flaxseed oil was also added to one formula (complete dog food maintenance formula) to increase the amount of ω-3 fatty acids.

3.2.5 Oat meal

Oats (*Avena sativa* L.) are an important feed grain, primarily being used in the form of rolled oat groats or oat flour. The research conducted by Sterna et al. (2016) showed that the crude protein content of naked oats is 14.61-16.81%, with 9.49-10.83% crude fat and 21.08-24.86% dietary fibre. Oat grains are rich in biologically significant substances and their consumption in the human diet is beneficial. In this study, oatmeal was used as an alternative protein resource and an important provider of dietary fibre.

3.3 Formulation and Nutritional Spreadsheet

A spreadsheet was created using Microsoft Excel to develop formulations and calculate the nutritional components for a given formulation. For the nutrient information data was downloaded from the USDA official website (USDA, 2021) and the data of the selected food ingredients were sorted out with Access and then imported into Excel for use.

3.3.1 Major formulas

1) Calorie content was calculated as below modified Atwater formula (AAFCO, 2019).

$$ME\ (kcal/kg) = 10[(3.5\ x\ CP) + (8.5\ x\ CF) + (3.5\ x\ NFE)] \quad eq\ (10)$$

ME = metabolizable energy

CP = crude protein (as-fed basis)

CF = crude fat (as-fed basis)

2) DM and As-fed inter conversion

% Dry matter = 100% - % Water, hence eq (11)

$$\%\ Dry\ matter = 100\% - \%\ Water, hence \quad eq\ (11)$$

$$\%\, nutrient\ (dry) = [\%\, nutrient\ (as\ fed)/\%\, dry\ matter] \times 100 \quad eq\ (12)$$

All the indicating nutrient levels of each formula ingredient were summed and compared with the minimum/maximum requirement of the AAFCO Dog Food Profile. Then adjustments were made through the trial and error method till a proper ingredient composition of the selected formula was achieved.

3.4 Complete Formula

The complete dog food formula was designed to meet the nutrient requirement of the adult maintenance category designated by AAFCO (2019). Since the formula was developed as "complete and balanced" dog food, it must meet the requirement accordingly. The formulation is shown in Table 3.1

Table 3.1 Complete Formula.

Ingredient	Percentage (DM)
Cricket Flour	34%
Pumpkin Powder	25%
Lentil Powder	13%
Flaxseed Meal	11%
Peanut Butter	4%
Vegetable Glycerin	3%
Coconut Oil	2%
Flaxseed Oil	2%
Molasses	3%
Sallt	0.50%

Below is the estimated nutrient level after calculation.

Insect Meal	g	Percentage
Cricket Adult	340	34.0%
Cricket Nymph		
Black Soldier Fly		

Animal Meat	g	Percentage
Chicken Meal		
Beef Meal		
Lamb Meal		
Salmon Meal		

Carbohydrates & Mics Plants		
Pumpkin	250	25.0%
Carrots		
Sweet Potato		
Lentils	130	13.0%
Oatmeal		
Yellow Peas		
Flax Seed Meal	110	11.0%
Barley		
Peanut Butter	40	4.0%
Vegetable Glycerin	30	3.0%

Tapioca Starch		
Molasses	30	3.0%
Garlic		
Rosemary		
Cinnamon		
Vinegar		
Wheat Flour		
Rice Flour		
Corn Flour		
Agar (Veg gelatin)		

Animal Fat	Percentage
Poultry Fat	
Lard	
Beef Tallow	

Fish Oil	Percentage
Herring	
Menhaden	
Salmon Oil	
Cod liver	

Plant Oil	Percentage
Canola	
Babassu	
Coconut	2.0%
Palm	
Seasame	
Flaxseed	2.0%
Safflower (high oleic)	

	g	Percentage
Olive		
Palm Kernel		
Corn		
Safflower		
Soybean		
Sunflower (high oleic)		
Sunflower		

Natural Flavor	20	2%

Salt & Suppemer	10.0	1.0%

Per Kg (1000g)

Raw Material Weigh	0	1000	1000
Energy Density (kcal/kg)		Proper	3165.28

	Deviation	Min / Max	Actual
Total Protein (g)	139.96	169.08	309.04
Arginine (g)	12.77	4.79	17.57
Histidine (g)	4.79	1.78	6.57
Isoleucine (g)	8.90	3.57	12.47
Leucine (g)	19.55	6.39	25.94
Lysine (g)	9.76	5.92	15.68
Methionine (g)	0.73	3.10	3.83
Methionine-cystine	0.10	6.11	6.21
Phenylalanine (g)	5.30	4.23	9.53
Phenylalanine- tyros	15.48	6.95	22.43
Threonine (g)	5.48	4.51	9.99
Trytophan (g)	0.52	1.50	2.02
Valine (g)	9.59	4.60	14.19
Crude Fat (g)	128.70	51.66	180.36
Linoleic acid (g)	17.72	10.33	28.06
alpha-Linolenic acid (g)		ND	20.47
Eicosapentaenoic + Docosahexa		ND	0.00
(LA+AA)/(ALA+EPA+I	**Proper**	<=30:1	1.37
Minerals			
Calcium (g)	(3.40)	4.70	1.30
Phosphorus (g)	1.28	3.76	5.04
Ca:P ratio	**Too low**	1:1 ~ 2:1	0.26
Total Carbohydrates (g)			351.46

Final Product Moisture	14.77	%
Final Product Weight	1102	g

	Deviation	Min / Max	Actual
Potassium (g)	9.67	5.64	15.31
Sodium (g)	0.72	0.75	1.47
Chloride (g)	1.23	1.13	2.35
Magnesium (g)	0.29	0.56	0.85
Iron (mg)	24.04	37.57	61.61
Copper (mg)	4.63	6.86	11.49
Manganese (m	13.46	4.70	18.15
Zinc (mg)	12.13	75.15	87.27
Iodine (mg)	(0.70)	0.94	0.24
Selenium (mg)	(0.11)	0.33	0.22
Vitamins & Other			
Vitamin A (IU)	223381	4697	228077
Vitamin D (IU)	(469.67)	469.67	0.00
Vitamin E (IU)	462.74	46.97	509.71
Thiamine (mg)	0.86	2.11	2.97
Riboflavin (mg	35.59	4.88	40.47
Pantothenic ac	24.83	11.27	36.10
Niacin (mg)	54.34	12.78	67.12
Pyridoxine (mg	3.82	1.41	5.23
Folic acid (mg)	1.43	0.20	1.64
Vitamin B12 (m	0.03	0.03	0.06
Choline (mg)	754.48	1277.51	2031.99
Total Moisture		14.77%	0.00

Crude Protein (%)	**28.04**	Insect Ratio	72.38%	Insect Protein Content	65.8%
Crude Fat (%)	**16.36**	Insect Ratio	41.14%	Protein content of other ingredients	13.5%
Crude Fiber (%)	**7.93**	Insect Ratio	29.97%		
Moisture (%)	**14.77**				

Figure 3.1 Estimation of nutrient level of the complete dog food formula 1.

As shown in Figure 3.1, the formula was deficient in Vitamin D, calcium, iodine, and selenium. To overcome these deficiencies a vitamin and mineral premix is commonly added to pet food formulations. The premix is added at a low rate usually around 1-2% of the formulation and has little to no impact on the processing and palatability of the product. The addition of a premix would be added in the mass production phase.

3.5 Treats Formulations

Three treat formulas were developed based on different levels of cricket flour (see Table 3.2).

Table 3.2 Treat dog food formulas.

Ingredient	Percentage		
	Formula 1	Formula 2	Formula 3
Cricket flour	16.0%	25.0%	13.0%
Sweet potato	52.0%		
Lentil flour		24.0%	
Oat meal	20.0%		9.5%
Pumpkin powder		26.0%	53.0%
Flaxseed meal		13.0%	
Carrot powder			12.5%
Tapioca starch	1.0%		1.0%
Peanut butter		2.0%	
Canola oil	3.0%		4.0%
Agar	1.0%		1.0%
Molasses	1.0%	3.0%	1.0%
Coconut oil		2.0%	
Glycerin	1.0%		2.0%
Salt	0.5%	0.5%	0.5%

3.6 Conclusions

It was shown that from the nutrient side, cricket flour could be used to provide the nutritional requirements for dogs as an alternative to meat proteins. The next part of the study was conducted to find out the feasibility of making baked pet food products based on the developed formulas.

4 Materials, Methods and Preliminary Trial

4.1 Introduction

Baking with cricket flour has brought great challenges to both bakery technologists and scientific researchers. As for bakery products, current published scientific papers mainly focus on using cricket flour to enhance the nutrient value of various products from traditional wheat-based bread to non-gluten biscuits, and on the influences of adding cricket flour on the technical properties of bakery foods. Some recently published research conducted by Kowalczewski et al. (2021) comprehensively showed that cricket flour enrichment of a non-gluten bread considerably increased its nutrient value. During the experiment, the starch was replaced by cricket flour at three different levels (2%, 4%, and 6%) which gave significantly increased protein levels (by 2, 4, and 7 times respectively), as well as increased levels of minerals such as Cu, P, and Zn in comparison to the reference bread. The results were consistent with other research (Bawa et al., 2020b; Nissen et al., 2020; Osimani et al., 2018). Regarding the influences of adding cricket flour on the technical properties, Cappelli et al. (2020) assessed the effects of cricket flour on dough rheological and bread characteristics. A gradient replacement of wheat flour (5%, 10%, and 15%) was adopted and compared with the reference bread. Results of the farinograph showed a slight increasing trend in flour water absorption with increased cricket flour level, an increase of dough stability, and a reduction in the degree of softening at 15% cricket flour level. While alveograph tests showed a trend of increased dough tenacity and P/L ratio (the ratio between the maximum overpressure and the length of the curve with a higher level indicating a more inextensible and resistant dough, and vice versa), together with a decreased dough extensibility and index of swelling as the level of cricked flour increased. The cricket flour-enriched bread showed decreased bread volume in comparison with the reference bread. Other research on bread products (Kowalczewski et al., 2019; Machado & Thys, 2019; Osimani et al., 2018) also presented similar results. While research conducted by Biro et al. (2020) indicated that adoption of cricket flour (at 3.2%, 6.7%, and 9.7% level, DM basis) had no significant changes in the texture of the tested oat biscuits.

The aforementioned research are different from the products to be tested in the current work in three aspects. First, the levels of cricket flour are lower (<10%) in comparison with the current designed formulas (34% for the complete formula, 17%, 25% respectively for the two treats formulas). Second, cricket flour was added as a non-major ingredient and a supplement source of protein. Third, the existing research concentrated on air-leavened products while the present testing formulas were based on non-leaven bakery. Therefore, it is necessary to design the

experiments based on established fundamental baking theories. In the context of my research, I concentrated on three major aspects, structure formation, colour development, and flavour and aroma development. A series of complex chemical reactions and physical changes take place during the baking process. As for structure and texture formation, protein and starch are the two major structure builders in baked products. On one hand, as temperature increases, proteins denature then coagulate to form a rigid structure which contributes to set up the final shape and size of bakery products. The starting temperature of protein coagulation is around 70°C. On the other hand, starch gelatinisation occurs under heat and the presence of water causes the dough to thicken and take on the final shape (Hui et al., 2008). The gelatinisation temperature is affected by both the nature of the starches and the presence of other ingredients such as sugars, fats, and salts. Generally, the gelatinisation occurs at around 60°C and can be completed at around 95°C in the presence of enough water (Figoni, 2010). With respect to colour and flavour development, two categories of complex chemical reactions are most important, Maillard reaction and caramelisation which are also called browning reactions. The Maillard reaction that gives a food a brown colour and its distinctive flavour arises where reducing sugars amino acids and, proteins, and/or other nitrogen-containing compounds are heated together, while caramelisation describes a complex group of reactions that occur when carbohydrates, particularly sucrose and reducing sugars, are heated directly (Parkin, 2017). Usually, browning takes place when the water activity (Aw) decreases to 0.4–0.7 and the temperature exceeds 105–120°C (Purlis, 2010).

Water activity (Aw) is an important parameter for the end product. Aw affects the growth and proliferation of microorganisms in baked goods hence it is very important to food stability and will considerably affect the product's shelf life. Microorganisms including bacteria, yeast, and mould which require a water population with molecular mobility close to that of free water, so their growth in a food material takes place only when $Aw > 0.7$. When $Aw < 0.6$, no microbial proliferation will happen (Parkin, 2017).

The aim of the experiment was to provide a reference and guidance for future mass production of the newly developed dog formulas. Hence the first goal was to evaluate the formability of the three cricket-flour-based dog food formulas. The second goal was to define the optimum time and temperature range with which the formulated dog foods can be baked.

4.2 Raw materials

Cricket flour was purchased from a local store that imported the product from a Thailand

producer. Pumpkin powder, flaxseed meal, lentil flour, oatmeal, peanut butter, molasses, agar, canola oil, coconut oil, and salt were purchased at a local grocery store.

4.3 Baking Process

The baking experiments were modified from the process of producing traditional non-leavened bakery goods. The whole process consisted of four separate steps including mixing, kneading and shaping, oven baking, and cooling. For the purpose of the new product development, variations were made to each step with experiment results and development goals at each stage outlined and discussed. Below are the common steps.

4.3.1 Blending and Mixing

The mixing of ingredients occurred in three stages:

(1) All the dry materials listed except for salt in the test formula including cricket flour, pumpkin powder, flaxseed meal, lentil flour, oatmeal, and agar were weighed and pre-mixed in a plastic container for better homogeneity in the final product.

(2) All the liquid and semi-liquid materials listed in the test formula were pre-mixed in a stainless mixing bowl including peanut butter, molasses, canola oil, and coconut oil. Water was prepared with the proper amount of salt dissolved in it, which was then added to the oils and semi-liquid mixtures and stirred to make a homogenised suspension.

(3) The liquids were added to the dry-material mixture for final mixing. The material was gently stirred and hand mixed for about 2 minutes then the dough was removed to a chopping board for kneading and shaping.

4.3.2 Kneading and shaping

Hand kneading was carried out for 3-4 minutes till the dough became soft and extensible. The dough then was rolled out with a hard wood rolling pin to form a sheet with an approximate 1.0 cm thickness. The sheet then was cut into 1.0 x 1.0 cm cube shape for baking.

4.3.3 Baking

The oven was preheated to the required temperature for 30 minutes. The samples were loaded on to an aluminum baking plate with holes and placed in the middle rack of the oven for a predetermined time.

4.3.4 Cooling

After baking (the procedure of each baking trial is described in detail in later chapters), the

product was cooled for 20 minutes then the cubes were loaded into an airtight plastic container and stored at ambient temperature (around 20°C) overnight. The samples were then ground for the determination of Aw and moisture content.

4.4 Determination of water activity

The water activity was conducted using AQULAB 4 TE. The moisture content was conducted using the CONTHERM OVEN 240 oven. Data were then recorded for further analysis.

4.5 Determination of moisture content

1) Dishes were dried in the oven for 1 hour and then allowed to cool in a desiccator.

2) Samples were ground into powder with a ceramic mortar and pestle.

3) Dishes were weighed and then about 2 g of ground powder was placed into the dish and weighed.

4) The dish was placed into an oven at 120°C for 3 hours.

5) The dishes were removed from the oven and cooled in a desiccator for 20 minutes.

6) The cooled dishes were weighed and the moisture content was calculated based on the weight that was lost during drying.

All sample tests were performed in triplicate and the average value was calculated and recorded for further analysis.

4.6 Preliminary Trial

A preliminary trial was conducted in order to develop and test methods and establish some idea of the temperature and time required for baking. A simple formula was developed based on traditional commercially baked dog food that was wheat flour based and included a protein and fat source. Usually, the protein source is a dried meat and bone meal from by-products of the meat industry. For this case, cricket meal was used as the protein source as this is a major objective of this project. Canola oil was used as the fat source. The sweet potato was added to the formula as this was an ingredient of interest for the final formulation. The formulation used is given in Table 3.1. The weight of the ingredients less water was summed to 100% and then water is a fixed percentage. For example, in Table 3.1 the cricket flour, wheat flour, canola oil and Sweet Potato represent 100% (DM) and water is added a weight that is 44.9% of DM. A baking temperature of 200°C was used. Two baking times were used 21 minutes and 10

minutes.

Table 4.1 Preliminary trial formula.

Ingredient	Actual Weight (g)	Percentage (DM)
Cricket Flour	20.1	40.1%
Wheat Flour	22.5	44.9%
Canola Oil	2.5	5.0%
Sweet Potato	5	10.0%
Water	22.5	44.9%

Figure 4.1Results of trial 1.

In trial one, with a 21-minute cooking time the product was found to be burnt and overcooked as seen in Figure 4.1. Therefore, the second test was performed using a baking time of 10 minutes and this produced a good result as seen in Figure 4.2. The product had good colour was not over baked and was solid, typical of baked pet food products. The water activity was determined and found to be 0.65 at 20°C which is typical of baked pet foods and will ensure stability against microbial growth.

Figure 4.2 Results of trial 2 baked at 200°C.

The trial showed that cricket flour could be easily substituted for meat and bone meal that is traditionally used as a protein source in pet food. The general methods for mixing and baking were successful. While the baking temperature of 200°C was successful when limited to 10 minutes it was decided in further trials that lower temperatures and longer cooking times could be considered as this would afford a greater margin of error in baking time and prevent excessive drying of the crust.

For further experiments it was desired to replace the wheat with other grain-free products such as pumpkin, sweet potato as a 'grain free' claim has market appeal in the pet food market. Achieving the desired texture and establishing baking conditions would be a challenge.

5 Complete dog food formula

5.1 Introduction

The desired product attributes include: a water activity below 0.65 which is determined numerically through the water activity meter (described in Section 4.4), a good texture that is firm enough to hold together but not excessively hard or overly brittle, and a desirable colour that has a good tint of browning without being burnt. The palatability of the product to dogs was scheduled to be tested after the baking experiments were completed.

The aim at this stage of the project was to establish the proper controlled values of the key parameters including the oven temperature, the baking time, and the level of added water in the mixture before baking.

It is noted that different ovens have different rates of heat transfer. So even at the same set temperature, different ovens will require slightly different baking times the achieve the same results due to their differences in geometry and the modes of heating. The work here was designed to provide some generalised values for oven temperature and baking times that could be used as guidelines for commercial-scale production. Another key parameter that will affect the final moisture content and water activity along with baking time and temperature is the amount of added water. The water that is added to form the dough is generally baked out during baking so that the moisture content of the final product is close to the initial moisture content of the raw materials. Hence the most efficient way to produce the product is to minimise the use of water which in turn minimises the energy consumption in baking to dry it out. The challenge is to add sufficient water for mixing and dough formation while avoiding excessive water that could cause the dough to slump and not hold its shape sufficiently.

Nine trials were conducted and grouped into three blocks. Section 4.2 comprises the five baking trials conducted at 150°C, Section 4.3 includes trials based on formulas with a gradual increase in glycerine levels (3%, 4%, and 5%) to evaluate the effect on the final water activity, and section 4.4 includes three trials conducted at 120°C and 100°C.

5.2 Baking trials at 150°C

Baking trials were conducted at 150°C with recipes developed to provide a complete and balanced formula with cricket flour as the main source of protein. The initial temperature chosen was 150°C as this is a mid-range baking temperature. Five trials were completed, and these were largely trial and error and completed in sequential order with the results from initial trials used to modify and eventually establish suitable baking parameters in subsequent ones. The formulations are presented in Table 4.1 where the ingredients (less water) are summed to 100% and water was added as a percentage of the other ingredients. For example in Trial one 57 g of water was added per 100 g of the other ingredients as is.

As shown in Table 5.1 the differences between Trials 2-5 including a reduction in water and inclusion of vegetable glycerine in an effort to control water activity. A reduction in pumpkin powder and lentils between Trials 1-2 and Trials 3-5 as these were identified as higher cost ingredients and to compensate for these reductions the flaxseed meal was increased for Trials 3-5.

5.2.1 Test Formulas

Table 5.1 Test formulas of the complete formula tests.

Ingredient	Percentage (As-is)				
	Trial One	Trial Two	Trial Three	Trial Four	Trial Five
Cricket Flour	32.0%	31.1%	34.5%	34.5%	34.5%
Pumpkin Powder	36.6%	35.7%	25.4%	25.4%	25.4%
Lentil Flour	18.0%	15.6%	13.2%	13.2%	13.2%
Flaxseed meal	2.1%	2.0%	13.2%	13.2%	13.2%
Peanut Butter	4.1%	4.0%	4.1%	4.1%	4.1%
Molasses	3.1%	3.0%	3.0%	3.0%	3.0%
Coconut oil	2.1%	4.0%	2.0%	2.0%	2.0%
Flaxseed oil	2.1%	2.0%	2.0%	2.0%	2.0%
Vegetable Glycerin	0.0%	2.0%	2.0%	2.0%	2.0%
Salt	0.0%	0.6%	0.6%	0.6%	0.6%
Subtotal	100.0%	100.0%	100.0%	100.0%	100.0%
Water (1)	57.0%	37.6%	37.6%	47.4%	47.4%

(1) The water amount is calculated as the ratio of water to all ingredients less water.

5.2.2 Summary Results and Discussion of 150°C Trials

For Trial 1 the first batch was baked at 150°C and inspected every three minutes which revealed a baking time based on surface colour of between 18 and 21 minutes. The second batch was baked for 20 minutes and found to be visually appealing with good colour, some browning but no burning. However, the measured water activity was found to be 0.749 at 20°C and this is significantly higher than the required 0.65. It was also observed that the water addition made the dough excessively wet, hence the amount of water added could be reduced. It was also observed that the underside of the material was very pale in colour while the top side of the dough sheet was a darker colour at the end of baking indicating a marked difference in baking between the top and bottom of the product.

Given the high water activity for Trial One, the water addition was reduced and the humectant vegetable glycerin was added at 2%. A new baking tray was made and is shown in Figure 5.1. The new baking tray was perforated rather than solid and would enable greater moisture transfer from the bottom surface. This was used for all further baking trials.

Figure 5.1 The new baking tray for better bottom baking.

It was found that a reduction in the amount of water added resulted in better dough formation that was easy to shape and cut. Baking was carried out for 20 and 22 minutes and observations are shown in Table 5.2. Baking at 22 minutes produced a product that was darker and may be considered slightly overbaked. The water activity of both of the samples was less than 0.65. Hence a recommended baking time of 20 minutes at 150°C with a water addition of 37.6g /100g of ingredients was adopted.

Table 5.2 Observations for Trial 2.

	Observations	Water Activity
20 minutes	Good colour	0.64 @ 20°C
22 minutes	Dark colour	0.63 @ 20°C

Because of cost considerations, it was decided to reduce the level of pumpkin powder and increase the flaxseed meal for Trial 3. In order to meet AAFCO requirements, levels of lentil flour were slightly decreased and flax seed meal was increased. Two batches of diet for Trial 3 were baked; one observed after 18 minutes of baking, and one observed after 20 minutes of baking. For both batches, it was observed that the dough was excessively dry and formed a brittle sheet that was easily fractured. It was found that after both 18 and 20 minutes of baking the products were very dark colour and over baked. As the products were very dry, the water activity was not measured as the products were clearly not suitable. The results demonstrated that changing the dry ingredients affected the water requirements. In order to adjust this, additional water was added for Trial 4.

The water addition for Trial 4 was increased to 47.4 g /100 g of ingredients compared with 37.6 g / 100 g ingredients for Trial 3. The increased water addition resulted in a significantly improved dough that was elastic and easy to sheet and cut. Batches were cooked at 150°C for 17 and 18 minutes. However both were still found to be excessively dark and overcooked, with the product baked for 17 minutes being slightly better.

Trial 5 was a repeat of Trial 4 using the same formulation but batches were baked for 15 minutes and 17 minutes at 150°C. Observations for these are given in Table 5.3 and show that at 15 minutes the product is underbaked with a high water activity and moisture content. Baking for 17 minutes showed good colour, lower moisture content and lower water activity than baking for 15 minutes as would be expected. However, the water activity was slightly higher than the required 0.65 or less.

Table 5.3 Observations for Trial 5.

	Observations	Moisture Content	Water Activity
15 minutes	Light colour	16.78%	0.702 @ 20°C
17 minutes	Good colour	14.77%	0.666 @ 20°C

As the water addition for Trials 4 and 5 were ideal for dough formation, and a baking time of 17 minutes at 150°C was ideal for colour it was decided to investigate increasing the amount of glycerin. Glycerin is a humectant and therefore its addition lowers the water activity of a product with the same moisture content.

5.3 Trial 6: Effect of increasing glycerin level on the final Aw value:

Based on the results of the 5th trial and to further lower the water activity, increasing the glycerin level was tested. Here glycerin was used as a humectant to bind water molecules to lower the water activity while maintaining the same moisture content in the product. This trial was conducted to demonstrate the effect of a gradient of glycerin levels on the Aw performance of the final products.

5.3.1 Methods

Three formulas with 3%, 4%, and 5% glycerin were tested. The same amount of flaxseed meal was deducted from the formula as the glycerin added. At each glycerin level, three baking times (15, 16, and 17 minutes) were tested for comparison. Water addition was maintained at 57.40% of the total of the ingredients.

5.3.2 Test Formula

Table 5.4 Complete Formula with gradient glycerin level.

Ingredient	Percentage (As-is)		
	3% Glycerin	4% Glycerin	5% Glycerin
Cricket Flour	35%	35%	35%
Pumpkin Powder	26%	26%	26%
Lentil Flour	13%	13%	13%
Flaxseedf Meal	11%	10%	9%
Peanut Butter	4%	4%	4%
Vegetable Glycerin	**3%**	**4%**	**5%**
Coconut Oil	2%	2%	2%
Flaxseed Oil	2%	2%	2%
Molasses	3%	3%	3%
Salt	1%	1%	1%
Subtotal	100%	100%	100%
Water (1)	57.40%	57.40%	57.40%

(1) The water amount is calculated as the ratio of water to all ingredients less water.

5.3.3 Results and conclusion

As shown in Figures 5.2 and 5.3 (the raw data is included in Appendix 1.1), there was no obvious correlation between the water activity and glycerin level in the samples with the same baking time. From the data the relationship between moisture content and glycerin level was also not clear.

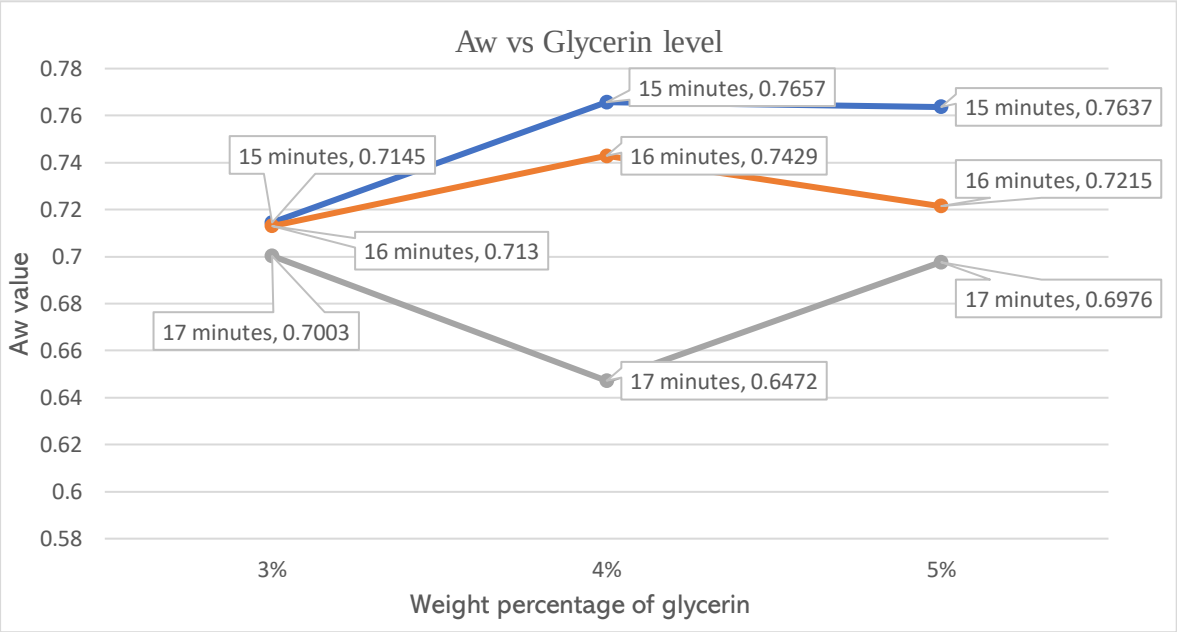


Figure 5.2 Effects of a gradient of glycerin levels on Aw value.

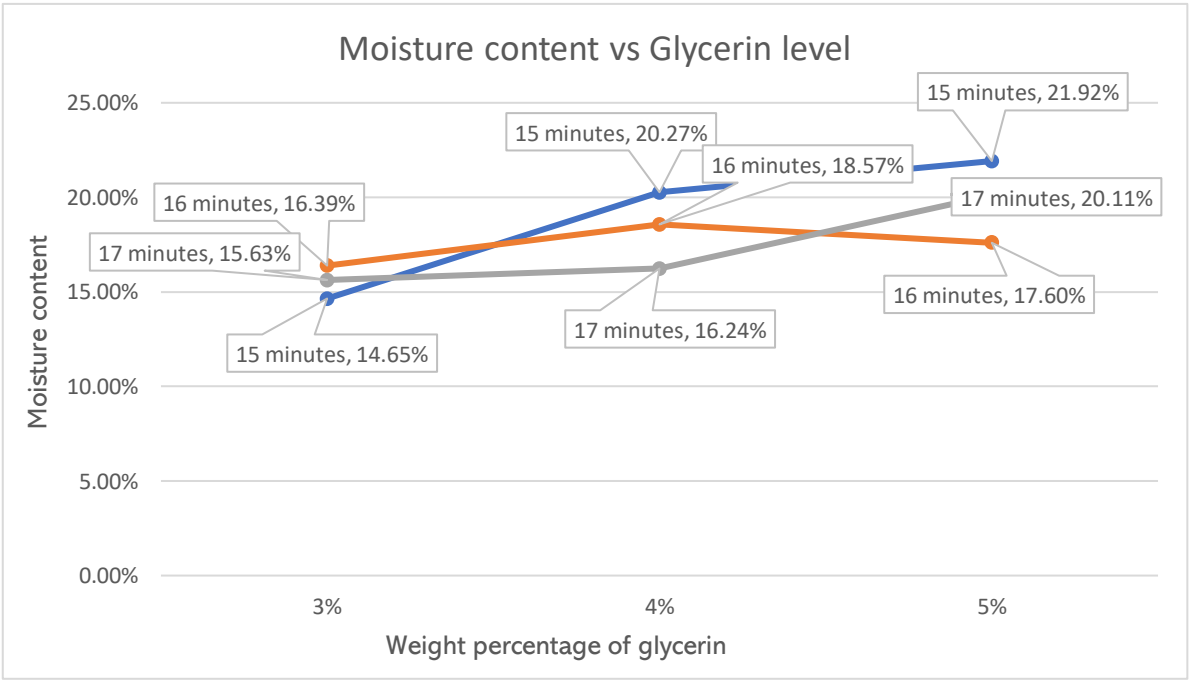


Figure 5.3 Effects of a gradient of glycerin levels on moisture content.

The results showed that glycerin is not very effective at reducing water activity. The baking time had a greater effect on the water activity than the use of glycerin. The result also showed the increased water led to a more expandable dough, hence the 57.4% water level was used in the next trials, but it was decided that lower temperatures would be used to reduce the possibility of burning, with longer baking times in order to reduce the moisture content and water activity.

5.4 Baking trials at 120℃ and 100℃

5.4.1 Trial 7 and 8: Baking trials at 120℃:

Trial 7 was aimed to verify the feasibility of baking the samples at 120°C and find out the relationship between the water activity and the baking time, in order to estimate a time range within which the desired Aw level can be reached.

Trial 8 was aimed to further narrow down the range of the baking time. Three baking times (44, 47, and 50 minutes) were tested and the water activity was assessed after cooling overnight.

*The formula selected to use was the first one in the 6th test (3% glycerin).

5.4.1.1 Methods

For Trial 7, a continuous drying curve was obtained. The dough was cut into small pieces (batch A) and samples (3-4 pieces) were taken out every 3 minutes for the water activity test. When the curve was obtained a second batch (batch B) was baked according to a time range based on the result of the batch A (51 minutes) and was tested against water activity. For trial 8, the baking time was chosen as 44, 47, and 50 minutes to further narrow down the baking time.

5.4.1.2 Results of A batch.

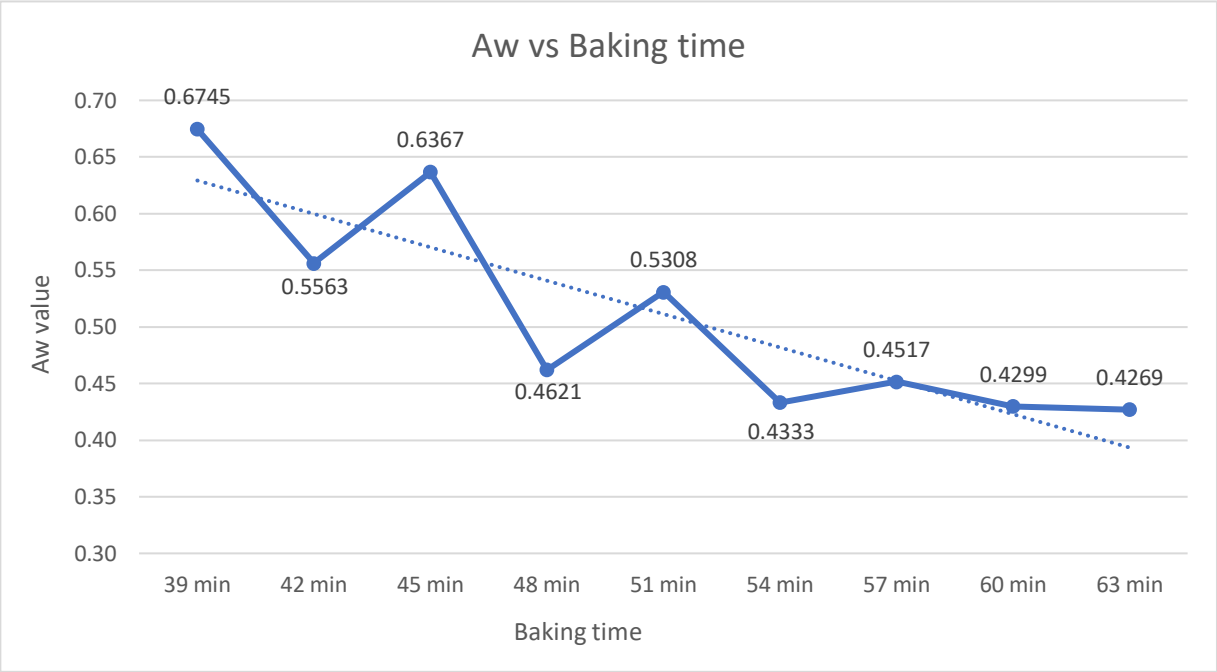


Figure 5.4 Aw levels against baking time (Batch A).

The results of the continuous baking for Batch A are given in Figure 5.4. This shows a decline in water activity from 0.6745 at 39 minutes to 0.4269 at 63 minutes. The measured water activity did fluctuate and increased at some time intervals as different pieces were taken from the batch as water activity is a destructive method, however the overall trend is downward. The fluctuations are likely the result of variability of the individual pieces not being representative of the entire sample. Hence the check using Batch B and removing at the estimated baking time.

Despite the fluctuations as shown in Figure 4.3, to get a Aw value less than 0.6, the time range can be roughly estimated as any time after 45 minutes. To ensure a sufficinelty low water activity given the uncertainty of results in Batch A, a baking time of 51 minutes for the batch B was tested. A comparison could then be made of the 51-minute sample between batch A and batch B. The Batch B sample baked for 51 minutes, had a water activity of 0.3428 at 20.01°C. This is lower than Batch A and could be due to the oven being opened throughout the baking to take samples for Batch A.

Conclusion

The trend line in Figure 4.3 shows that the Aw level was negatively related to the baking time. From Figure 4.3, the optimum range of baking time could be estimated as between 45~55

minutes. The Aw value of batch B at 51 minutes was much lower than the batch A sample at the same time. This will be discussed in the final part of the discussion.

In Trial 8, the cube-shaped samples made from the same dough was split into three batches, then were baked consecutively. The result was shown in Table 5.5.

Table 5.5 Baking Time vs Water Activity in Trial 8.

44 minutes	47 minutes	50 minutes
0.6399 @ 20.09 °C	0.4200 @ 20.10 °C	0.2686 @ 20.04 °C

Taking into account both the water activity level and the colour change (that the 50-min sample was a bit overcooked), it was estimated that at the 120°C oven temperature, the optimum baking time was around 47 ± 3 minutes.

5.4.2 Trial 9: Baking trials at 100°C

This test aimed to try a lower baking temperature (100°C) and observe the colour and water activity changes of the product.

The same formula as used in trial 7 and 8 was used in this trial. The dough was rolled out into a sheet and cut into small, diced pieces and split into three batches. The first batch (Batch A) was baked, and samples (3-4 pieces) were taken out every 3 minutes from 58 minutes to 109 minutes of baking for a water activity test. The second batch (Batch B) and the third batch (Batch C) were baked for 79 and 88 minutes respectively and were also tested for water activity.

5.4.2.1 Results.

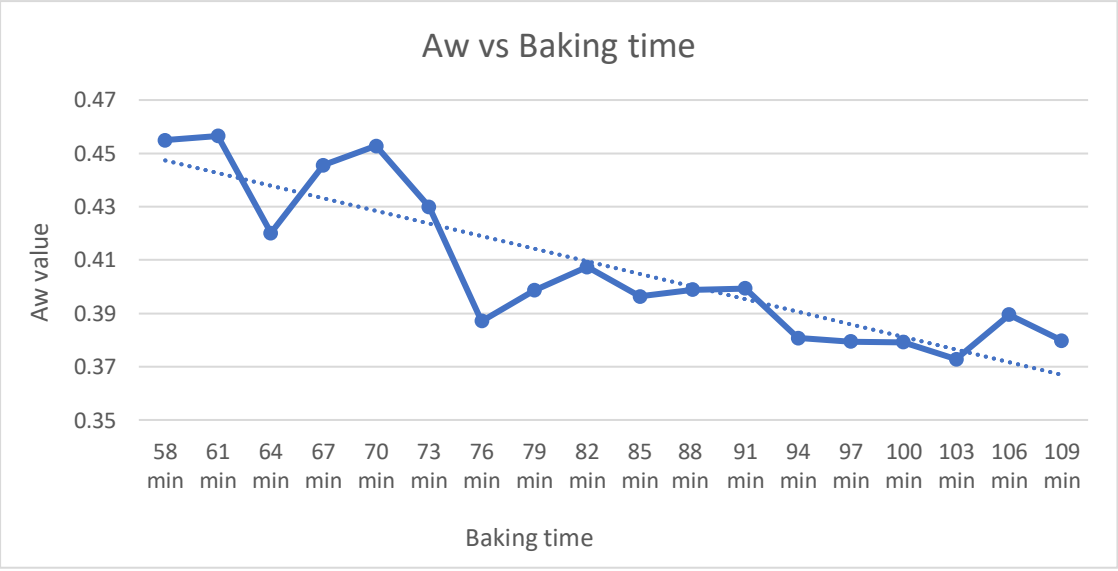


Figure 5.5 Aw values over time for Batch A (*raw data is in Appendix C 1.2).

There was a general decline in water activity with baking time although there is an increase for some time intervals. This is likely due to the fluctuation of the oven temperature together with the different places in the oven of certain samples. Acceptable water activity is found after 58 minutes, however, the colour development was found to be poor.

In the consecutive test, 79 and 88 minutes were arbitrarily chosen as the middle range of selected baking times. The Aw value of batch B (79 minutes baking) was 0.3533 at 20.02°C. The Aw value of batch C (88 minutes baking) was 0.3201 at 20.05°C. The water activity for both Batches (B and C) was lower than the value of the sample with the equivalent times in Batch A (Figure 5.5). This could be due to the oven door being opened to take samples every three minutes to gather the data for Batch A.

Sufficient colour changes at 100°C oven temperature even after 88 minutes of baking was not observed. The reason was that the browning reactions that give colour to bakery products, namely the Maillard reaction and caramelisation, normally happen above 120°C.

Conclusion and discussion

Table 5.6 Baking time vs oven temperature

Oven Temperature	Estimated baking time	Comments
150°C	15 - 18 minutes	35.5% pumpkin level
	19 - 25 minutes	25% pumpkin level
120°C	44 - 50 minutes	3% glycerin level

*The 100°C results are not included as the baking time is too long to be practical in commercial production.

The estimated optimal baking times at different oven temperatures are shown in Table 5.6.At 150°C the estimated range was 15-18 minutes for the formula with 25% pumpkin powder and 19-25 minutes for the formula with 35.5% pumpkin powder. At 120°C, using 3% glycerin the estimated range was 44-50 minutes. The 100°C baking test showed sufficient reduction in water activity but failed to develop adequate colour and is not recommended.

6 Development of Treats

6.1 Introduction

This part of the project aimed to establish the proper controlled values of the key production parameters: including the oven temperature, baking time, and the level of added water in the mixture before baking but focused on the treats formula.

The desired product attributes are the same as that were defined in Chapter 4 which includes both water activity less than 0.65 and a desired texture. The palatability of the tested dog treats was conducted together with the complete dog food and will be discussed in the following chapter.

Three treat formulas were tested and two of them were retained. Eleven trials were carried out using the two treat formulas (6 using treat dog food formula 1 (TF1) and 5 using treat dog food formula 2 (TF2)) and were grouped into two sections accordingly. The major difference between TF1 and TF2 is that TF1 comprised sweet potato and oatmeal as the major carbohydrate sources while TF2 used lentil flour, pumpkin powder, and flaxseed meal as the major carbohydrate sources.

6.2 Treats dog food Formula 1

Six baking trials were conducted using recipes developed as dog treats adopting cricket flour as the major protein source and the sweet potato and oat meal as the major carbohydrate source. The total six trials were completed at 200°C, 150°C, and 100°C and arranged in sequential order with results used from the earlier trials to establish the suitable baking parameters. The formulations are presented in Table 6.1 where the ingredients (less water) are summed to 100% and water is added as a percentage of the other ingredients.

Trial one and trial two were conducted at 200°C (with a 10- and 20-minute baking time) as the preliminary test to observe the ability of the formulation to form a sheeted dough that holds its shape. Trial three was conducted at 150°C and comprised two parts. The first part was a 10-minute continuously baking test while Part 2 was conducted using the samples produced in the first part to narrow down the possible range of an optimum baking time. The samples were taken out every three minutes and visually inspected to observe the colour changes. Trial four was a baking test at 150°C with 25- and 27-minute baking times. Trial five was a baking test at 150°C with 29- and 31-minute baking times. Trial six was designed to test the feasibility of

baking at 100°C.

Table 6.1 Test formulas of TF1.

Ingredient	Percentage (As-is)			
	Trial One/Two	Trial Three	Trial Four/Five	Trial Six
Cricket Flour	17.5%	17.5%	17.4%	17.4%
Sweet Potato	53.5%	53.6%	53.3%	53.2%
Oat Meal	20.6%	20.6%	20.5%	20.4%
Tapioca Starch	1.0%	1.0%	1.0%	1.0%
Canola Oil	3.1%	3.1%	3.1%	3.1%
Molasses	1.0%	1.1%	1.0%	1.0%
Vegetable Glycerin	2.1%	2.1%	2.0%	2.2%
Agar	1.2%	1.0%	1.0%	1.0%
Salt	0.0%	0.0%	0.6%	0.6%
Subtotal	100.0%	100.0%	100.0%	100.0%
Water(1)	56%	59%	59%	59%

(1) The water amount is calculated as the ratio of water to all ingredients less water.

6.2.1 Summary Results and Discussion of 200°C Trials

The sample from trial one was clearly undercooked and had an undeveloped structure, slightly changed colour, and high moisture content (as the center part of the sample was still very wet). Hence trial two was conducted at a longer 20-minute baking time. The result indicated that the sample at this baking time was overcooked (pictures of both trials one and two are included in the Appendix). The dough formation was not good as it was slimey and very difficult to sheet and form a good cubic shape.

Table 6.1 Observations for 200°C Trial 1 & 2.

	Observations	Dough formability
10 minutes	Undercooked	bad dough formability
15 minutes	Overcooked	bad dough formability

Based on the above results and the positive results from the trials conducted on the complete dog food formula one (Chapter 5), the further trials were conducted at 150°C and less water was added (as shown in table 6.1) to optimise the dough formability.

6.2.2 Summary Results and Discussion of 150°C Trials

The third trial was conducted in two consecutive steps. In the first step, a 10-minute baking test was conducted. As predicted, the sample was clearly undercooked. Then a second test was

conducted (using the sample produced in the first step) at 150°C and inspected every three minutes. The result showed that a possible optimum baking time was approximately 25 minutes.

In the fourth trial, two fixed-time tests (one of 25 and the other 27 minutes) were conducted at 150°C. For the purpose of comparison, the samples used in each baking test were prepared from the same dough. As shown in Table 5.3, the 25-minute sample and the 27-minute sample were in almost the same colour, with water activity values of 0.7549 and 0.6807 respectively, which were both out of specifications (target ≤ 0.65). Hence the next step was conducting trials adopting longer baking time to further lower the water activity

Table 6.3 Observations for 150°C Trial 4 & 5.

Baking Time	Observations	Moisture Content	Water Activity
25 minutes	Well-developed medium brown colour	N/A(1)	0.7549 @ 20.10°C
27 minutes	Very similar to the 25-minute sample	N/A(1)	0.6807 @ 19.99°C
29 minutes	Medium-dark brown	10.14%	0.5504 @ 20.06 °C
31 minutes	Medium-dark brown	9.15%	0.4901 @ 19.97 °C

(1) As the water activity is out of specifications, the moisture content was not tested.

Trial five was conducted at 150°C with baking for 29 and 31 minutes respectively. As shown in Table 6.3, both samples were within the required water activity range. By taking into account that a shorter baking time leads to less power consumption, 29 minutes was confirmed as the optimum baking time.

6.2.3 100°C Trials

This test was aimed to try a lower baking temperature to observe the colour and water activity changes when samples were baked using an oven temperature of 100°C.

The cubes of samples from the same dough were split into three batches. The first batch (Batch A) was baked, and samples (3-4 cubes) were taken out every 3 minutes from 72 minutes of baking for a water activity test. The second batch (Batch B) was baked continuously for 96 minutes and was tested for water activity. The third batch (Batch C) was baked continuously for 105 minutes and was tested for water activity.

Aw changes over time of Batch A

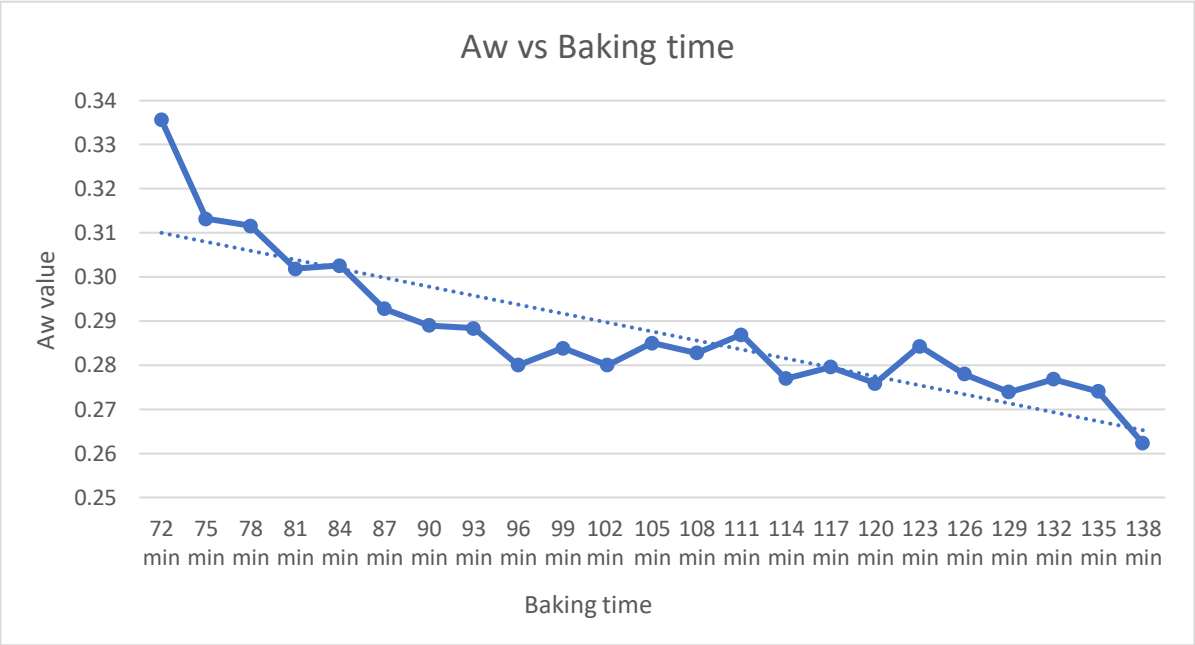


Figure 6.1 Aw values versus baking time for batch A (*raw data is in Appendix C 1.3).

Table 6.4 Water activity values of baches B and C.

96 minutes (B batch)	105 minutes (C batch)
0.2314 @ 19.99 °C	0.183 @ 19.97 °C

6.2.3.1 Results and conclusion

Again, sufficient colour changes at 100 °C oven temperature even after 105 minutes of baking was not observed. This was consistent with the test of the complete dog food formula (100 °C) test. The Aw test showed that the Aw value decreased over time which means the required Aw value could be reached given enough baking time. As for batches B and C, the Aw values were lower than the samples which were taken out during the batch A test with a shorter baking time. This is discussed further in the final discussion.

6.3 Treats dog food Formula 2

Five baking trials were conducted using recipes developed for dog treats based on adopting cricket flour as the major protein source and the lentil flour, pumpkin powder, and flaxseed meal as the major carbohydrate sources. Five trials were completed at 150°C and 100°C and are described in sequential order with results used from the earlier trials used to establish revised baking parameters. The formulations are presented in Table 6.5 where the ingredients (less water) are summed to 100% and water is added as a percentage of the other ingredients (as previously described in Chapter 4.2).

In Trial one, the sample was separated into two batches. The first batch was baked at 150°C and inspected every three minutes and revealed an optimal baking time based on the surface colour of around 22 minutes. The second batch was then tested at 150°C for 22 minutes. Trial two was conducted to further narrow down the baking time and compared two times (20 and 22 minutes). Trial three was a three-hour drying test to test the effect of oven drying on the final product's water activity. Trial four was a 40-minute drying test based on the result of trial three. Trial five was carried out at 100°C to test the feasibility of using a lower baking temperature.

Table 6.5 Test formulas of TF2.

Ingredient	Percentage (As-is)			
	Trial One	Trial Two/Three(1)	Trial Four	Trial Five
Cricket Flour	25.8%	25.6%	25.6%	25.6%
Lentil Flour	24.7%	24.6%	24.6%	24.6%
Pumpkin Powder	26.8%	26.6%	26.6%	26.6%
Flaxseed meal	13.4%	13.3%	13.3%	13.3%
Peanut Butter	4.1%	4.1%	4.1%	4.1%
Molasses	3.1%	3.1%	3.1%	3.1%
Coconut Oil	2.1%	2.0%	2.0%	2.0%
Salt		0.6%	0.6%	0.6%
Subtotal	100.0%	100.0%	100.0%	100.0%
Water(2)	59.1%	58.8%	58.8%	58.7%

(1) Trial 3 was conducted with samples from Trial 2
(2) The water amount is calculated as the ratio of water to all ingredients less water.

6.3.1 Summary Results and Discussion of 150°C Trials

In the first batch of trial one, the optimised temperature was narrowed down to between 20 ~ 25 minutes. Hence the second batch was baked at 150°C for 22 minutes. The result showed the 22-minute sample had an Aw value of 0.7469 as shown in Table 6.6, which was too high. Hence the second trial was conducted using a longer baking time (24 and 26 minutes). The water activity values were determined as 0.8202 and 0.7608 for the 24- and 26-minute samples respectively.

Table 6.6 Observations for 150°C Trial 1 & 2.

	Observations	Moisture Content	Water Activity
Trial 1 22 minutes	Well developed medium brown colour	N/A(1)	0.7469 @ 20.11°C
Trial 2 24 minutes	Good medium brown colour	N/A(1)	0.8202 @ 20.01°C
Trial 2 26 minutes	Slightly overcooked with deep colour	N/A(1)	0.7608 @ 20.04 °C

(1) As the water activity is out of specification., the moisture content was not tested.

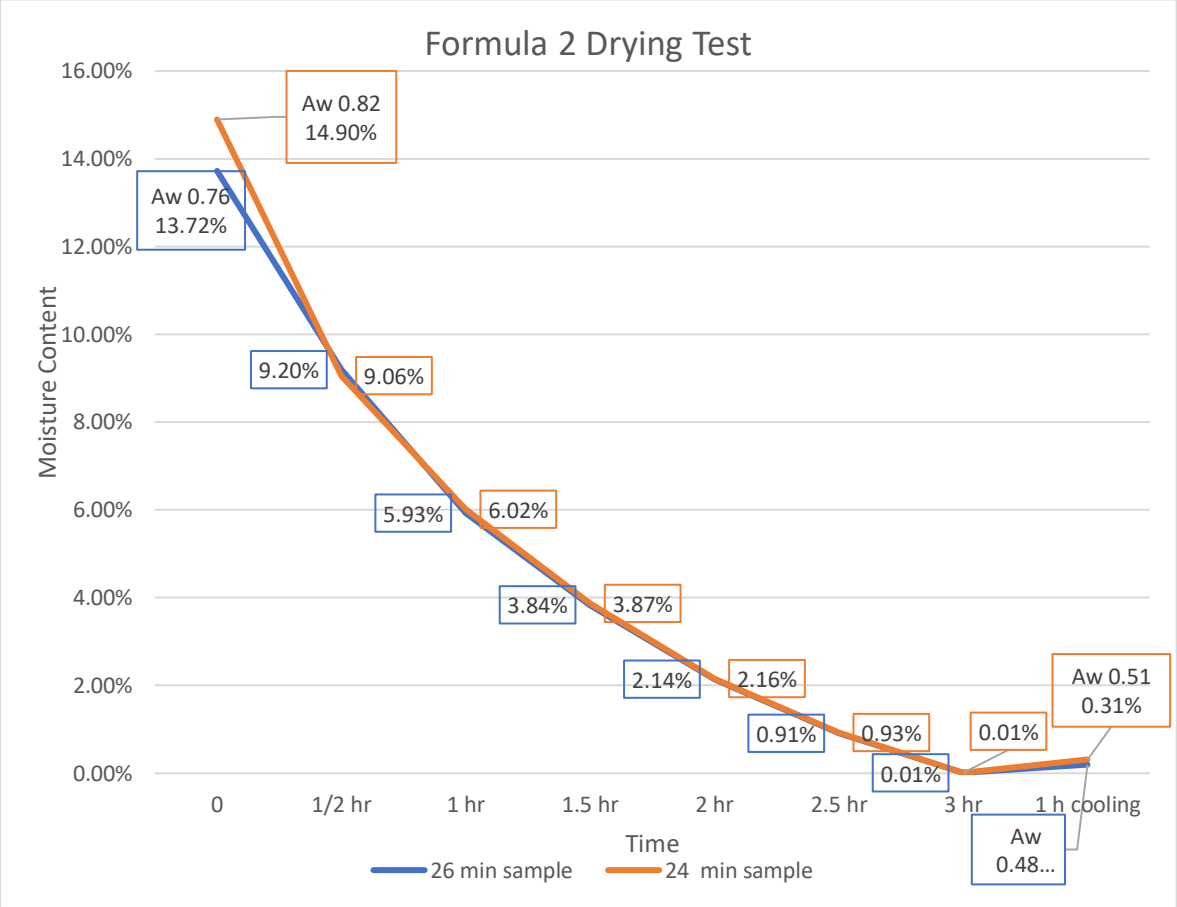
Given the 26-minute sample showed a deep brown color and was slightly overcooked, it was concluded that there was a limitation when increasing baking time at the constant temperature which could be caused by the limitation of water evaporation speed in the tested environment. Therefore trial three was conducted as a drying test to find out the potential optimum drying time.

6.3.2 Trial three, Drying test

Based on the results from the trial two, this drying test was conducted to determine/estimate the drying time for Treat Formula 2. The samples were from Trial two.

A modified Air-Oven method was adopted. In the first step, the aluminium moisture dishes were accurately weighed then the samples were put on each dish and were quickly reweighed. In the second step, the dishes (with contents) were placed into the oven, the air oven was set at 50°C. In the third step, the dishes were taken out every 30 minutes and quickly weighed. In step four, the Aw values were determined.

6.3.2.1 Drying curve



6.3.2.2 Results and conclusion

From figure 6.2, it was estimated the minimum drying time required was between 30 ~ 60 minutes.

Based on this result, a 40-minute drying test following a baking test with a 24-minute baking time was then planned.

6.3.3 Trial four, the test of baking followed by 40 minutes of drying

This test was conducted to verify the effect of a 40-minute period of drying after the 24-minute baking time.

The sample was baked at 150°C for 24 minutes. After baking, the samples were split into two batches. The first batch (batch A) was kept for the Aw and moisture content test. The second batch (batch B) was dried at 50°C for 40 minutes before being cooled for Aw and moisture content test.

As shown in Table 6.7, it was proved that drying after baking can effectively reach the target Aw level. The water activity dropped from 0.7469 to 0.6580 and the moisture content decreased from 17.06% to 13.46%.

Table 6.7 Results of the 40 minutes drying test.

	Observations	Moisture Content	Water Activity
24 minutes	Well-developed medium brown colour	17.06%	0.7745 @ 20.10°C
After 40 min drying	No apparent colour change	13.46%	0.6580 @ 20.06 °C

6.3.4 Baking trials at 100°C

The aim of this test was to try a lower baking temperature (100 °C) to observe any changes in the colour and water activity.

The cube-shaped samples were split into three batches. The first batch (Batch A) was baked, and samples (3-4 pieces) were taken out every 3 minutes from 58 minutes to 127 minutes of baking for a water activity test. The second batch (Batch B) was baked for 97 minutes and was tested for water activity. The third batch (Batch C) was baked for 106 minutes and was tested for water activity.

6.3.4.1 Aw changes over time of A batch

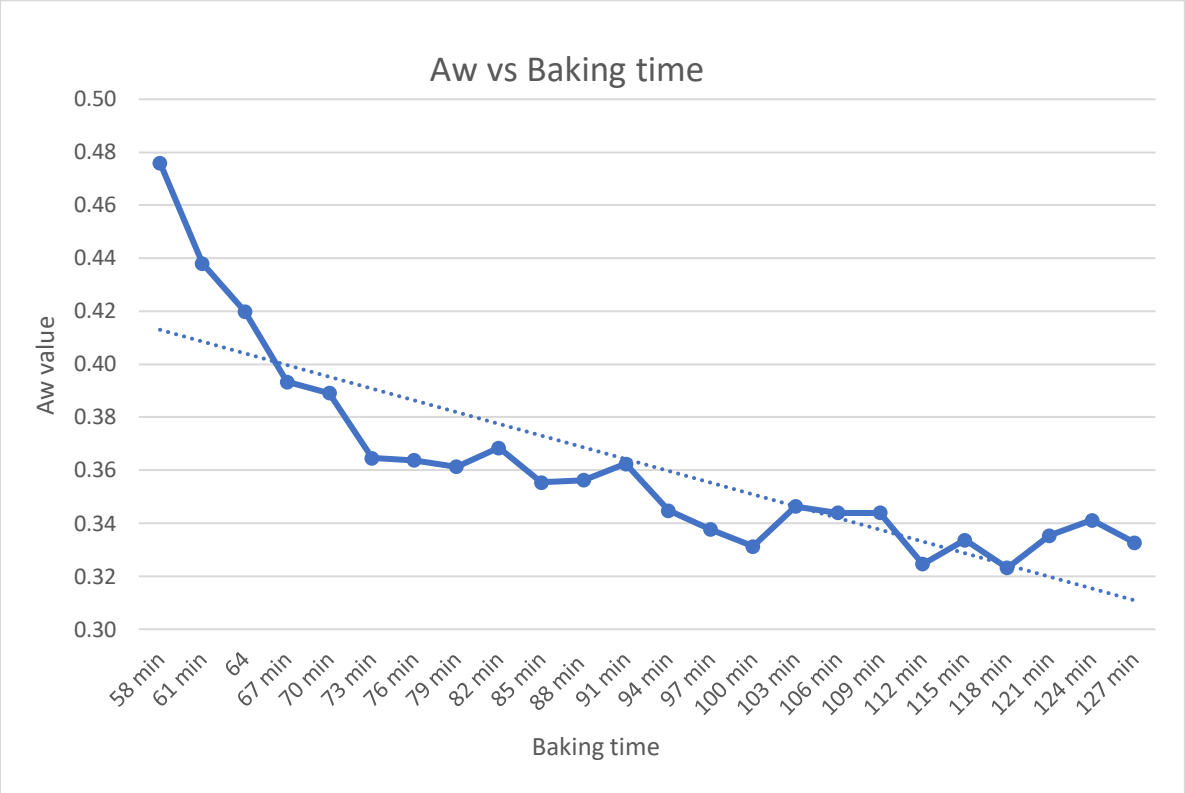


Figure 6-31 Aw values over time for batch A (*raw data is in Appendix C 1.5).

Table 6.8 Water activity values of batches B and C.

97 minutes (batch B)	106 minutes (batch C)
0.2386 @ 19.97 °C	0.2255 @ 19.97 °C

6.3.4.2 Results and conclusion

During the test, little colour change was observed at 100°C oven temperature. This was consistent with the previous tests. While the Aw test showed that the Aw values decreased over time indicating that the target Aw value could be reached given enough time. In this trial, it was shown that the target Aw level was achieved in less than one hour, although the colour changes were not visually obvious. Regarding Batches B and C, the Aw values were lower than the samples which were taken out during the Batch A test at the same baking time. This is discussed in the final discussion.

6.4 Discussion and conclusion

6.4.1 Treat formula 1

The estimated optimal baking time is shown in Figure 6-4. At 150 °C the estimated range was 27-31 minutes.

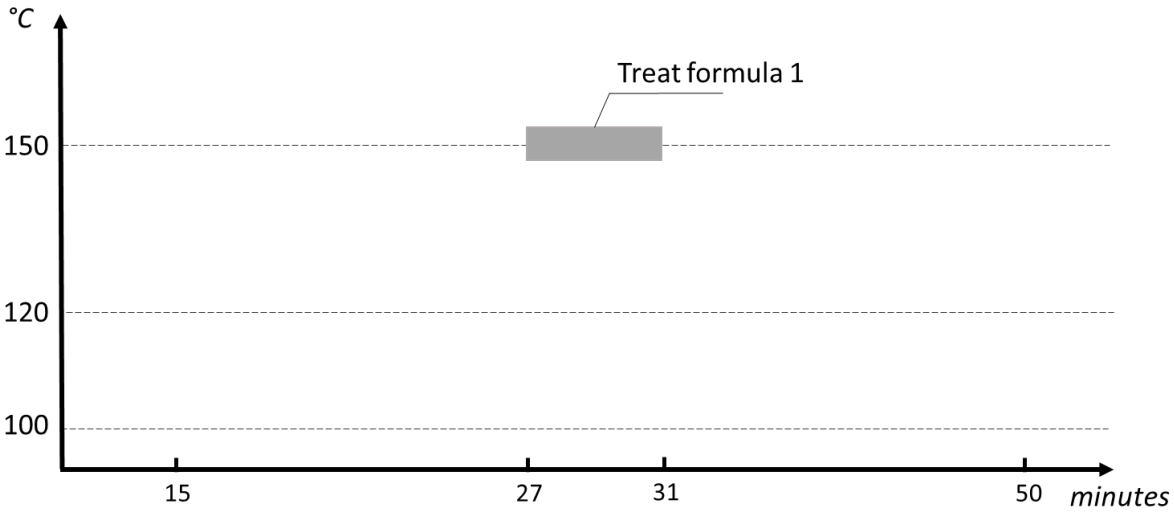


Figure 6-4 Baking time vs oven temperature (TF1).

*The width of the bar indicates the estimated range of baking time.

** Vertical axis represents baking temperature; horizontal axis represents baking time.

In the Trial three, the 25- and 27-minute baking times were tested, the resulting Aw values were 0.75 and 0.68 respectively. Given the target Aw was <0.65 and possible fluctuations of the actual temperatures occurred in the oven, the optimal baking time is likely above 27 minutes. In the Trial four, a 29- and 31-minute baking time was tested, and the resulting Aw values were 0.55 and 0.49 respectively. By taking into account that the 31-minute sample was slightly overcooked, the upper limit to the baking time was estimated as 31 minutes.

6.4.2 Treat formula 2

The estimated optimal baking time is shown in Figure 6-5. At 150 °C the estimated range was 21-30 minutes baking plus oven drying.

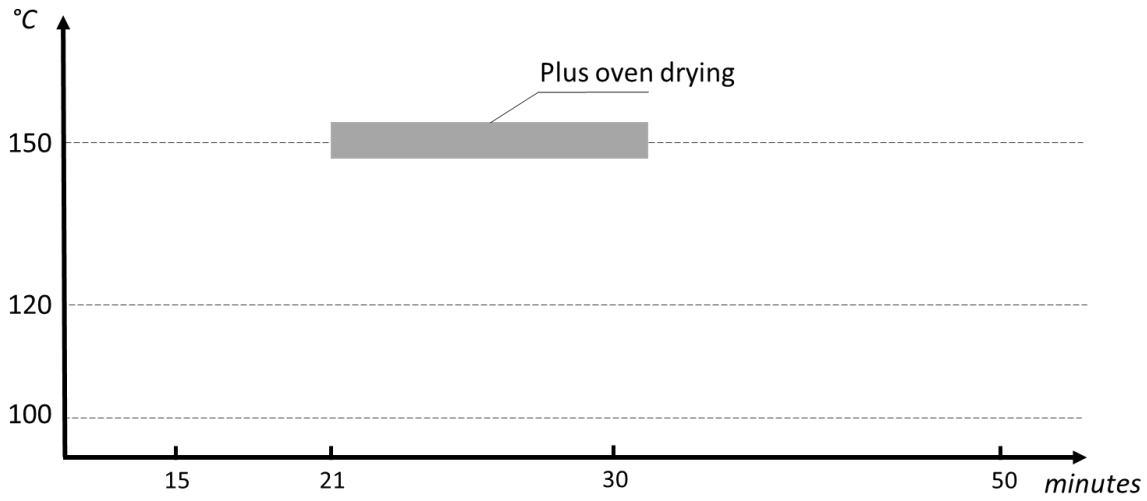


Figure 6-5 Baking time vs oven temperature (TF2).

*The width of the bar indicates the estimated range of baking time.

** Vertical axis represents baking temperature; horizontal axis represents baking time.

7 Acceptancy Test

The acceptancy test aimed to test the basic acceptance of the tested formulas by a panel of dogs.

7.1 Experimental design

7.1.1 The complete dog food formula

Eight dogs were randomly selected to be provided with a bowl of the samples. The reaction of each dog to the diet were observed, recorded, and split into three categories, including whether they smelled the sample first (SF), had difficulty in eating it (DE), or refused it (R).

7.1.2 The treat Formula 1 and treat Formula 2

Eight dogs were randomly selected to be provided two bowls of samples. Each bowl contained one type of treat sample and the two bowls were placed randomly. Again, the reaction of each dog to the two diets was observed, recorded, and split into three categories, including which sample that smelled first (SF), eat first (EF), finished first (FF).

7.2 Sample Preparation

The Samples were prepared as described in Chapter 3. Around 100 g of each formula were prepared the day before the testing.

7.3 Results and Conclusion

7.3.1 Acceptancy of the complete formula

As shown in Table 8.1, there is no refusal hence it was concluded the acceptance of the complete formula was 100%.

Table8.1 Acceptancy of the complete formula.

SF	DE	R	Acceptance
8	1	0	100

7.3.2 Acceptance of the treat formula 1 and the treat formula 2

As shown in Table 8.2, the acceptance was also 100%.

Table8.2 Acceptance of treat formula 1 and treat formula 2.

FA		FE		FF		Acceptance
TF1	TF2	TF1	TF2	TF1	TF2	Total finished %
2	5	1	6	3	4	100

A comparison was made to compare the dog's preference to the two formulas. As shown in

Table 8.3, the dogs showed a slightly higher preference to treat Formula 2. According to the results, 71% of dogs chose to approach Formula 2 first, 86% of dogs started eating from the bowl containing Formula 2 first, and 57% of dogs finished the Formula 2 first.

Table8.3 Preference between treat formula 1 and 2.

First Approach		First Eaten		First Finished	
TF1	TF2	TF1	TF2	TF1	TF2
28.57%	71.43%	14.29%	85.71%	42.86%	57.14%

`

7.3.3 Discussion

From the results shown above, it could be concluded that the acceptance of the three newly developed formulas was good.

In a more general viewpoint, a comparison with the normal dog foods in the marketplace is a good way to further define their acceptance or palatability. On one hand, such a comparison can show if the transition from the traditional dog foods to the novel ones is feasible or if there will be some difficulty for dogs. On the other hand, this is a bit of a dilemma because dogs can exhibit food preferences that may have been conditioned by previous dietary experiences. Such kinds of preferences can be shown towards a particular flavour or a certain type of product form (Case et al., 2010a). Hence it is of great importance that the acceptance of the novel pet food is not confused with the conditioned diet preference.

8 Conclusions and Recommendations

8.1 General discussion

It was demonstrated that cricket flour can be used in petfood products as a substitute of the main protein source. When substituted in place of meat and bone meal (a traditional protein source) into a baked petfood formula a product of good colour and low water activity was easily obtained. However when wheat was removed to achieve a grain free status achieving the right level of water addition rate, baking time and temperature, to achieve the desired formability of the dough, water activity and colour required some trial and error. Different parameters were needed for the different formulas.

Baking temperatures of 120-150°C produced favourable results with baking times ranging from 15 to 50 minutes depending on formulation and temperature used. A baking temperature of 200°C tended to have very short drying times, but there was the risk of burning. While baking at 100°C resulted in insufficient colour development. As is summarised by Hui et al. (2008), during the normal biscuit baking process, both protein denaturation/coagulation and starch gelatinisation happen below 100°C therefore the development of the biscuit structure can happen below 100°C. During bread baking, a core temperature of 94–96°C for bread is enough for either the microbiological or the structural needs, while the colour development starts from around 100-120°C.

Although it is technically feasible to bake the samples at 100°C there are two concerns. First, at this temperature, it is very difficult to obtain the desired surface colour. The second, the cooking time is too long which is not cost-effective from a commercial angle.

8.1.1 Limitation to increasing the baking time

There is a limitation on increasing the baking time at lower temperatures. As shown in the TF2 second test, water evaporation could not occur fast enough so that before the desired Aw level was reached, the crust had been over-baked.

8.1.2 About drying test

In Trials 3 and 4 of the TF2, the Aw value was lowered through extra oven drying. There was one fact been noticed in the three formulas 100°C tests, the Aw values of batches B and C were lower than the samples which were taken out during the batch A test at the same baking time. This phenomenon could be explained that during the long waiting time (between 1 hour to over 2 hours), part of the water had evaporated which led to the lower Aw value in the final products. This means that the drying process can be done before or after baking. If the shaped dough

pieces are kept in the open air for some time this may solve the high Aw level issue, which is a good option because there is no extra energy consumption. But there are also concerns such as stock space occupation, or the possibility of potential microorganism proliferation, etc. In short, if drying is needed to lower the Aw level, after-baking drying is a more practical method, while pre-baking drying could be another option.

All of the formulations that were tested using dogs were found to be acceptable.

8.2 Conclusion, Recommendation and Limitation

The above test results showed the general feasibility of using cricket meal as the alternative protein source in dog foods. Cricket meal has a high protein level and good amino acid profile. The diets were also found to be sufficient in terms of fat and fatty acid requirements. However in order to meet the minimum requirements defined by AAFCO, a premix of vitamins and minerals is necessary to gain nutrient balanced formulas (i.e. meet vitamin D, calcium, iodine, selenium levels etc). Given the reported lower impact on the environment for crickets compared with animal protein production, insect meal offers a feasible alternative.

8.2.1 Processing characteristics

Results showed the formability of replacing wheat from the traditional formulas with alternative raw materials such as pumpkin, sweet potato, lentils etc. Also the work showed it is feasible to control the Aw level through the right combination of the oven temperature and the baking time. Well, sometimes an extra stage of drying is necessary to reach a desirable low Aw.

8.2.2 Recommendations on oven temperature and baking time

The general pattern is that the water activity level is negatively correlated with oven temperature and baking time.

For the complete dog food formula, at 150°C oven temperature and 35.5% pumpkin level, the recommended baking time is 15-18 minutes, at 25% pumpkin level the recommended baking time is 19-25 minutes, and at 120°C oven temperature and 3% glycerin level the recommended baking time is 44-50 minutes. In commercial production, due to the different types of baking equipment, the baking time should be adjusted accordingly.

For treat formulas at 150°C oven temperature, the recommended baking time of treat formula 1 is 27-31 minutes. For treat formula 2, the recommended baking time is 21-30 minutes plus a stage of oven drying after baking at 50°C of 30-60 minutes (a 40-minute drying test was

conducted as described in Chapter 6).

8.2.3 Major limitations

The major limitation of the experiment was oven temperature fluctuation. The oven used in the experiment is an analogue-controlled traditional kitchen oven. The temperature fluctuations were reflected in the Aw value of the samples. Better results would be obtained if the weight loss on drying could be monitored continuously during baking without having to remove any sample. However, the results obtained will be useful for providing guidelines for baking and drying of formulations on other equipment.

9 References

AAFCO. (2019). *AAFCO Official Publication*. Champaign, IL USA Champaign, IL USA

Abdulmajeed, R., Ramadeen, A., Masse, S., Foomany, F. H., Balasundaram, K., Hu, X., Nanthakumar, K., Dorian, P., & Umapathy, K. (2014). The effects of long chain polyunsaturated fatty acids on local activation properties in dogs vulnerable to atrial fibrillation. 2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society,

Affairs, U. D. o. E. a. S. (2017). World Population Prospects: The 2017 Revision. https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html

Algya, K. M., Cross, T. W. L., Leuck, K. N., Kastner, M. E., Baba, T., Lye, L., de Godoy, M. R. C., & Swanson, K. S. (2018). Apparent total-tract macronutrient digestibility, serum chemistry, urinalysis, and fecal characteristics, metabolites and microbiota of adult dogs fed extruded, mildly cooked, and raw diets [Article]. *Journal of Animal Science*, *96*(9), 3670-3683. https://doi.org/10.1093/jas/sky235

Angrimani, D. S. R., Nichi, M., Losano, J. D. A., Lucio, C. F., Veiga, G. A. L., Franco, M. V. J., Vannucchi, C. I. J. J. o. a. s., & biotechnology. (2017). Fatty acid content in epididymal fluid and spermatozoa during sperm maturation in dogs. *8*(1), 1-8.

Animal Diversity Web. (2020). *Canis lupus familiaris*. https://animaldiversity.org/accounts/Canis_lupus_familiaris/

Association, A. P. P. (2019). *2019-2020 APPA National Pet Owners Survey* https://www.americanpetproducts.org/press_industrytrends.asp

Association, N. Z. P. F. M. (2020). *New zealand pet statistics*. https://www.petfoodnz.co.nz/index.htm

Bauer, J. E. (2016). The essential nature of dietary omega-3 fatty acids in dogs [Editorial Material]. *Javma-Journal of the American Veterinary Medical Association*, *249*(11), 1267-1272. <Go to ISI>://WOS:000388905800018

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020a). Effect of Diet on the Growth Performance, Feed Conversion, and Nutrient Content of the House Cricket. *Journal of Insect Science*, *20*(2), Article 10. https://doi.org/10.1093/jisesa/ieaa014

Bawa, M., Songsermpong, S., Kaewtapee, C., & Chanput, W. (2020b). Nutritional, sensory, and texture quality of bread and cookie enriched with house cricket (Acheta domesticus) powder. *Journal of Food Processing and Preservation*, *44*(8), Article e14601. https://doi.org/10.1111/jfpp.14601

Bekhit, A. E.-D. A., Shavandi, A., Jodjaja, T., Birch, J., Teh, S., Mohamed Ahmed, I. A., Al-Juhaimi, F. Y., Saeedi, P., & Bekhit, A. A. (2018). Flaxseed: Composition, detoxification, utilization, and opportunities. *Biocatalysis and Agricultural Biotechnology*, *13*, 129-152. https://doi.org/https://doi.org/10.1016/j.bcab.2017.11.017

Beynen, A. (2018). Beynen AC, 2018. Insect-based petfood.

Bhatty, R. S. (1988). Composition and quality of lentil (lens-culinaris medik) - A REVIEW. *Canadian Institute of Food Science and Technology Journal-Journal De L Institut Canadien De Science Et Technologie Alimentaires*, *21*(2), 144-160. https://doi.org/10.1016/s0315-5463(88)70770-1

Biro, B., Sipos, M. A., Kovacs, A., Badak-Kerti, K., Pasztor-Huszar, K., & Gere, A. (2020). Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. *Foods*, *9*(11), Article

1561. https://doi.org/10.3390/foods9111561

Bosch, G., Zhang, S., Oonincx, D., & Hendriks, W. H. (2013). Protein quality of insects as potential ingredients for pet foods. *The WALTHAM International Sciences Symposium 2013: from pet food to pet care - bridging the gap, Portland, Oregon, USA, 1-4 October, 2013. Abstracts*, 67-67. <Go to ISI>://CABI:20153213672

Bosch, G., Zhang, S., Oonincx, D. G. A. B., & Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science*, *3*, e29 [24pp.]-e29 [24pp.]. <Go to ISI>://FSTA:2015-07-Wa0077

Caligiani, A., Marseglia, A., Sorci, A., Bonzanini, F., Lolli, V., Maistrello, L., & Sforza, S. (2019). Influence of the killing method of the black soldier fly on its lipid composition [Article]. *Food Research International*, *116*, 276-282. https://doi.org/10.1016/j.foodres.2018.08.033

Cappelli, A., Oliva, N., Bonaccorsi, G., Lorini, C., & Cini, E. (2020). Assessment of the rheological properties and bread characteristics obtained by innovative protein sources (Cicer arietinum, Acheta domesticus, Tenebrio moitor): Novel food or potential improvers for wheat flour? *Lwt-Food Science and Technology*, *118*, Article 108867. https://doi.org/10.1016/j.lwt.2019.108867

Cargo-Froom, C. L., Fan, M. Z., Pfeuti, G., Pendlebury, C., & Shoveller, A. K. (2019). Apparent and true digestibility of macro and micro nutrients in adult maintenance dog foods containing either a majority of animal or vegetable proteins. *Journal of Animal Science*, *97*(3), 1010-1019. https://doi.org/10.1093/jas/skz001

Carlos, G., dos Santos, F. P., & Froehlich, P. E. (2020). Canine metabolomics advances [Review]. *Metabolomics*, *16*(2), 19, Article 16. https://doi.org/10.1007/s11306-020-1638-7

Case, L. P., Daristotle, L., Hayek, M. G., & Raasch, M. (2010a). *Canine and feline nutrition: a resource for companion animal professionals*. Elsevier Health Sciences.

Clutton-Brock, J. (2012). *Animals as domesticates: a world view through history*. MSU Press.

Cohen, K. M., & Diaz, L. R. (2013). *Dogs: Domestication History, Behavior and Common Health Problems*. Nova Science Publishers.

Combarros, D., Castilla-Castano, E., Lecru, L. A., Pressanti, C., Amalric, N., & Cadiergues, M. C. (2020). A prospective, randomized, double blind, placebo-controlled evaluation of the effects of an n-3 essential fatty acids supplement (Agepi (R) omega 3) on clinical signs, and fatty acid concentrations in the erythrocyte membrane, hair shafts and skin surface of dogs with poor quality coats. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *159*, Article 102140. https://doi.org/10.1016/j.plefa.2020.102140

Council, N. R. (2006). *Nutrient requirements of dogs and cats*. National Academies Press.

Danieli, P. P., Lussiana, C., Gasco, L., Amici, A., & Ronchi, B. (2019). The Effects of Diet Formulation on the Yield, Proximate Composition, and Fatty Acid Profile of the Black Soldier Fly (Hermetia illucens L.) Prepupae Intended for Animal Feed. *Animals*, *9*(4), Article 178. https://doi.org/10.3390/ani9040178

Do, S., Koutsos, E., Utterback, P. L., Parsons, C. M., de Godoy, M. R. C., & Swanson, K. S. (2019). True nutrient and amino acid digestibility of black soldier fly larvae differing in life stage using the precision-fed cecectomized rooster assay. *Journal of Animal Science*, *97*, 64-65. <Go to ISI>://WOS:000507409900118

Do, S., Koutsos, L., Utterback, P. L., Parsons, C. M., de Godoy, M. R. C., & Swanson, K. S. (2020). Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecectomized rooster assay. *Journal of Animal Science*, *98*(1), Article skz363.

https://doi.org/10.1093/jas/skz363

Dossey, A. T., Morales-Ramos, J. A., & Rojas, M. G. (2016). *Insects as sustainable food ingredients: production, processing and food applications*. Academic Press.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., & Lalander, C. (2020). Fatty acid composition of black soldier fly larvae (Hermetia illucens) - Possibilities and limitations for modification through diet. *Waste Management*, *102*, 40-47. https://doi.org/10.1016/j.wasman.2019.10.014

Federation, E. P. F. (2018). *More than 140 Million Cats and Dogs in the EU*. https://fediaf.org/press-releases/2156-more-than-140-million-cats-and-dogs-in-the-eu.html

Figoni, P. I. (2010). *How baking works: exploring the fundamentals of baking science*. John Wiley & Sons.

Finke, M. D. (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *21*(3), 269-285. https://doi.org/10.1002/zoo.10031

Finke, M. D. (2013a). Complete Nutrient Content of Four Species of Feeder Insects. *32*(1), 27-36. https://doi.org/10.1002/zoo.21012

Finke, M. D. (2013b). Complete Nutrient Content of Four Species of Feeder Insects. *Zoo Biology*, *32*(1), 27-36. https://doi.org/10.1002/zoo.21012

Finke, M. D. (2015). Complete Nutrient Content of Four Species of Commercially Available Feeder Insects Fed Enhanced Diets During Growth. *Zoo Biology*, *34*(6), 554-564. https://doi.org/10.1002/zoo.21246

Finke, M. D. J. Z. B. P. i. a. w. t. A. Z., & Association, A. (2007). Estimate of chitin in raw whole insects. *26*(2), 105-115.

Frantz, N. Z., Yamka, R. M., & Friesen, K. G. J. I. J. o. A. R. i. V. M. (2007). The effect of dietary protein on body composition and renal function in geriatric dogs. *5*(2), 57.

Gasco, L., Acuti, G., Bani, P., Zotte, A. D., Danieli, P. P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., & Roncarati, A. (2020a). Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. *Italian Journal of Animal Science*, *19*(1), 360-372. https://doi.org/10.1080/1828051x.2020.1743209

Gasco, L., Biancarosa, I., & Liland, N. S. (2020b). From waste to feed: A review of recent knowledge on insects as producers of protein and fat for animal feeds. *Current Opinion in Green and Sustainable Chemistry*, *23*, 67-79. https://doi.org/10.1016/j.cogsc.2020.03.003

Gasco, L., Biasato, I., Dabbou, S., Schiavone, A., & Gai, F. (2019). Animals Fed Insect-Based Diets: State-of-the-Art on Digestibility, Performance and Product Quality. *Animals*, *9*(4), Article 170. https://doi.org/10.3390/ani9040170

German, A. J., Hall, E. J., & Day, N. (2003). Chronic intestinal inflammation and intestinal disease in dogs. *Journal of Veterinary Internal Medicine*, *17*(1), 8–20. https://doi.org/10.1892/0891-6640(2003)017<0008:CIIAID>2.3.CO;2

Halloran, A., Flore, R., Vantomme, P., & Roos, N. (2018). *Edible insects in sustainable food systems*. Springer.

Hui, Y. H., Corke, H., De Leyn, I., Nip, W.-K., & Cross, N. A. (2008). *Bakery products: science and technology*. John Wiley & Sons.

Huis, A. v., & Tomberlin, J. K. (2017). *Insects as food and feed: from production to consumption/edited by Arnold van Huis, Jeffery K. Tomberlin* (9086862969).

Humbert, B., Bleis, P., Martin, L., Dumon, H., Darmaun, D., & Nguyen, P. (2001). Effects of dietary protein restriction and amino acids deficiency on protein metabolism in dogs. *Journal of Animal Physiology and Animal Nutrition*, *85*(7-8), 255-262. https://doi.org/10.1046/j.1439-0396.2001.00324.x

Hynd, P. (2019). *Animal Nutrition: From Theory to Practice*. CSIRO PUBLISHING.

Kaur, S., Panghal, A., Garg, M. K., Mann, S., Khatkar, S. K., Sharma, P., & Chhikara, N. (2019). Functional and nutraceutical properties of pumpkin - a review. *Nutrition & Food Science*, *50*(2), 384-401. https://doi.org/10.1108/nfs-05-2019-0143

Kilburn, L. R., Allenspach, K., Jergens, A. E., Bourgois-Mochel, A., Mochel, J. P., & Serao, M. C. R. (2020). Apparent total tract digestibility, fecal characteristics, and blood parameters of healthy adult dogs fed high-fat diets. *Journal of Animal Science*, *98*(3), Article skaa043. https://doi.org/10.1093/jas/skaa043

Kilburn, L. R., Carlson, A. T., Lewis, E., & Serao, M. C. R. (2020). Cricket (Gryllodes sigillatus) meal fed to healthy adult dogs does not affect general health and minimally impacts apparent total tract digestibility. *Journal of Animal Science*, *98*(3), Article skaa083. https://doi.org/10.1093/jas/skaa083

Kipkoech, C., Imathiu, S., Roos, N., & Kinyuru, J. N. (2018). Prebiotics potential of chitin derived from farmed crickets: a gateway to improved gut health? *Journal of Insects as Food and Feed*, *4*(Suppl. 1), 87-87. https://doi.org/10.3920/jiff2018.S1

Kowalczewski, P. L., Gumienna, M., Rybicka, I., Gorna, B., Sarbak, P., Dziedzic, K., & Kmiecik, D. (2021). Nutritional Value and Biological Activity of Gluten-Free Bread Enriched with Cricket Powder. *Molecules*, *26*(4), Article 1184. https://doi.org/10.3390/molecules26041184

Kowalczewski, P. L., Walkowiak, K., Masewicz, L., Bartczak, O., Lewandowicz, J., Kubiak, P., & Baranowska, H. M. (2019). Gluten-Free Bread with Cricket Powder-Mechanical Properties and Molecular Water Dynamics in Dough and Ready Product. *Foods*, *8*(7), Article 240. https://doi.org/10.3390/foods8070240

Kröger, S., Heide, C., & Zentek, J. (2020). Evaluation of an extruded diet for adult dogs containing larvae meal from the Black soldier fly (Hermetia illucens). *Animal Feed Science and Technology*, *270*, 114699. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2020.114699

Laflamme, D. P. (2008). Pet food safety: Dietary protein [Review]. *Topics in Companion Animal Medicine*, *23*(3), 154-157. https://doi.org/10.1053/j.tcam.2008.04.009

Lees, GE ; Brown, SA ; Elliott, J ; Grauer, GE ; Vaden, SL (2005). Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). *19*(3), 377-385.

Lei, X. J., Kim, T. H., Park, J. H., & Kim, I. H. (2019). Evaluation of supplementation of defatted black soldier fly (Hermetia Illucens) larvae meal in beagle dogs. *Annals of Animal Science*, *19*(3), 767-777. https://doi.org/10.2478/aoas-2019-0021

Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbo, R., Torstensen, B. E., & Lock, E.-J. (2017). Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media. *Plos One*, *12*(8), Article e0183188. https://doi.org/10.1371/journal.pone.0183188

Lokman, I. H., Ibitoye, E. B., Hezmee, M. N. M., Goh, Y. M., Zuki, A. B. Z., & Jimoh, A. A. (2019).

Effects of chitin and chitosan from cricket and shrimp on growth and carcass performance of broiler chickens. *Tropical Animal Health and Production*, *51*(8), 2219-2225. https://doi.org/10.1007/s11250-019-01936-9

Machado, C. D., & Thys, R. C. S. (2019). Cricket powder (Gryllus assimilis) as a new alternative protein source for gluten-free breads. *Innovative Food Science & Emerging Technologies*, *56*, Article 102180. https://doi.org/10.1016/j.ifset.2019.102180

Martens, P., Su, B., & Deblomme, S. (2019). The Ecological Paw Print of Companion Dogs and Cats. *Bioscience*, *69*(6), 467-474. https://doi.org/10.1093/biosci/biz044

Martin, W. F., Armstrong, L. E., & Rodriguez, N. R. (2005). Dietary protein intake and renal function. *Nutrition & Metabolism*, *2*(25), (20 September 2005)-(2020 September 2005). <Go to ISI>://CABI:20073025905

McCusker, S., Buff, P. R., Yu, Z., & Fascetti, A. J. J. J. o. n. s. (2014). Amino acid content of selected plant, algae and insect species: a search for alternative protein sources for use in pet foods. *3*.

McDonald, P. (2011). *Animal nutrition* (7th ed ed.) [Bibliographies Non-fiction]. Prentice Hall/Pearson.https://ezproxy.massey.ac.nz/login?url=http://ebookcentral.proquest.com/lib/massey/detail.action?docID=5187377

McNamara, J. P. (2006). *Principles of companion animal nutrition*. Pearson/Prentice Hall.

Mehler, S. J., May, L. R., King, C., Harris, W. S., Shah, Z. J. P., Leukotrienes, & Acids, E. F. (2016). A prospective, randomized, double blind, placebo-controlled evaluation of the effects of eicosapentaenoic acid and docosahexaenoic acid on the clinical signs and erythrocyte membrane polyunsaturated fatty acid concentrations in dogs with osteoarthritis. *109*, 1-7.

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (Hermetia illucens) larvae. *Journal of the Science of Food and Agriculture*, *98*(15), 5776-5784. https://doi.org/10.1002/jsfa.9127

Mohamad, L., Dina, F., Hasan, H. A., Sudesh, K., & Baidurah, S. (2020). Effect of feeding strategy on the protein and fatty acid contents of black soldier fly prepupae (Hermetia illucens) for the potential applications as animal feed and promising alternative protein-rich food. In M. A. Kassim & M. S. Mohamed (Eds.), *Third Bioprocessing and Biomanufacturing Symposium 2019* (Vol. 716). https://doi.org/10.1088/1757-899x/716/1/012006

Mohd-Noor, S.-N., Lim, J.-W., Mah-Hussin, M.-L.-A., Ramli, A., Chew, T.-L., Bashir, M. J. K., Tan, W.-N., & Beniers, J. J. A. (2018). Potential of Protein and Lipid Productions from Black Soldier Fly Larvae Fed with Mixture of Waste Coconut Endosperm and Soybean Curd Residue. In F. A. A. Nifa, C. K. Lin, & A. Hussain (Eds.), *Proceedings of the 3rd International Conference on Applied Science and Technology* (Vol. 2016). https://doi.org/10.1063/1.5055500

Mohd-Noor, S.-N., Wong, C.-Y., Lim, J.-W., Mah-Hussin, M.-I.-A., Uemura, Y., Lam, M.-K., Ramli, A., Bashir, M. J. K., & Tham, L. (2017). Optimization of self-fermented period of waste coconut endosperm destined to feed black soldier fly larvae in enhancing the lipid and protein yields. *Renewable Energy*, *111*, 646-654. https://doi.org/10.1016/j.renene.2017.04.067

Moreau, M., Troncy, E., Del Castillo, J., Bedard, C., Gauvin, D., Lussier, B. J. J. o. a. p., & nutrition, a. (2013). Effects of feeding a high omega-3 fatty acids diet in dogs with naturally occurring osteoarthritis. *97*(5), 830-837.

Naqash, F., Gani, A., Gani, A., & Masoodi, F. (2017). Gluten-free baking: Combating the challenges-A review. *Trends in Food Science & Technology*, *66*, 98-107.

Nikoletta, H. (2019). Insects as animal feed. *Magyar Allatorvosok Lapja*, *141*(2), 117-128. <Go to ISI>://WOS:000460087000007

Nissen, L., Samaei, S. P., Babini, E., & Gianotti, A. (2020). Gluten free sourdough bread enriched with cricket flour for protein fortification: Antioxidant improvement and Volatilome characterization. *Food Chemistry*, *333*, Article 127410. https://doi.org/10.1016/j.foodchem.2020.127410

Nyangena, D. N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D., & Fiaboe, K. K. M. (2020). Effects of Traditional Processing Techniques on the Nutritional and Microbiological Quality of Four Edible Insect Species Used for Food and Feed in East Africa [Article]. *Foods*, *9*(5), 16, Article 574. https://doi.org/10.3390/foods9050574

Okin, G. S. (2017). Environmental impacts of food consumption by dogs and cats [Article]. *Plos One*, *12*(8), 14, Article e0181301. https://doi.org/10.1371/journal.pone.0181301

Oomah, B., & Mazza, G. (2000). Functional foods in Wiley Encyclopedia of Science and Technology, Vol. 2. In.

Oonincx, D. G., & De Boer, I. J. J. P. o. (2012). Environmental impact of the production of mealworms as a protein source for humans–a life cycle assessment. *7*(12), e51145.

Oonincx, D. G. A. B., Laurent, S., Veenenbos, M. E., & van Loon, J. J. A. (2020). Dietary enrichment of edible insects with omega 3 fatty acids. *27*(3), 500-509. https://doi.org/10.1111/1744-7917.12669

Oonincx, D. G. A. B., Laurent, S., Veenenbos, M. E., & van Loon, J. J. A. (2020). Dietary enrichment of edible insects with omega 3 fatty acids. *Insect Science*, *27*(3), 500-509. https://doi.org/10.1111/1744-7917.12669

Osimani, A., Milanovic, V., Cardinali, F., Roncolini, A., Garofalo, C., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Raffaelli, N., Zamporlini, F., & Aquilanti, L. (2018). Bread enriched with cricket powder (Acheta domesticus): A technological, microbiological and nutritional evaluation [Article]. *Innovative Food Science & Emerging Technologies*, *48*, 150-163. https://doi.org/10.1016/j.ifset.2018.06.007

Ottoboni, M., Spranghers, T., Pinotti, L., Baldi, A., De Jaeghere, W., & Eeckhout, M. (2018). Inclusion of Hermetia Illucens larvae or prepupae in an experimental extruded feed: process optimisation and impact on in vitro digestibility. *Italian Journal of Animal Science*, *17*(2), 418-427. https://doi.org/10.1080/1828051x.2017.1372698

Parkin, K. L. (2017). *Fennema's food chemistry*. CRC Press.

Pinotti, L., Giromini, C., Ottoboni, M., Tretola, M., & Marchis, D. J. A. (2019). Insects and former foodstuffs for upgrading food waste biomasses/streams to feed ingredients for farm animals. *13*(7), 1365-1375.

Poelaert, C., Francis, F., Alabi, T., Megido, R. C., Crahay, B., Bindelle, J., & Beckers, Y. (2018). Protein value of two insects, subjected to various heat treatments, using growing rats and the protein digestibility-corrected amino acid score. *Journal of Insects as Food and Feed*, *4*(2), 77-87. https://doi.org/10.3920/jiff2017.0003

Pond, W. G., Church, D. C., Pond, K. R., & Schoknecht, P. A. (2005). *Basic animal nutrition and feeding* (5th ed ed.) [Bibliographies

Non-fiction]. Wiley. http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cat00245a&AN=massey.b4553804&site=eds-live&scope=site

Purlis, E. (2010). Browning development in bakery products - A review. *Journal of Food Engineering*, *99*(3), 239-249. https://doi.org/10.1016/j.jfoodeng.2010.03.008

Sadava, D. E., Hillis, D. M., Heller, H. C., & Berenbaum, M. (2009). *Life: the science of biology* (Vol. 2). Macmillan.

Singh, Y., Cullere, M., Kovitvadhi, A., Chundang, P., & Zotte, A. D. (2020). Effect of different killing methods on physicochemical traits, nutritional characteristics, in vitro human digestibility and oxidative stability during storage of the house cricket (Acheta domesticus L.). *Innovative Food Science & Emerging Technologies*, *65*, Article 102444. https://doi.org/10.1016/j.ifset.2020.102444

Sogari, G., Amato, M., Biasato, I., Chiesa, S., & Gasco, L. (2019). The Potential Role of Insects as Feed: A Multi-Perspective Review. *Animals*, *9*(4), Article 119. https://doi.org/10.3390/ani9040119

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., De Smet, S. J. J. o. t. S. o. F., & Agriculture. (2017). Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *97*(8), 2594-2600.

Sterna, V., Zute, S., & Brunava, L. (2016). Oat Grain Composition and its Nutrition Benefice. *Agriculture and Agricultural Science Procedia*, *8*, 252-256. https://doi.org/https://doi.org/10.1016/j.aaspro.2016.02.100

Su, B. T., & Martens, P. (2018). Environmental impacts of food consumption by companion dogs and cats in Japan [Article]. *Ecological Indicators*, *93*, 1043-1049. https://doi.org/10.1016/j.ecolind.2018.06.015

Sykes, N., Beirne, P., Horowitz, A., Jones, I., Kalof, L., Karlsson, E., King, T., Litwak, H., McDonald, R. A., Murphy, L. J., Pemberton, N., Promislow, D., Rowan, A., Stahl, P. W., Tehrani, J., Tourigny, E., Wynne, C. D. L., Strauss, E., & Larson, G. (2020). Humanity's Best Friend: A Dog-Centric Approach to Addressing Global Challenges. *Animals*, *10*(3), Article 502. https://doi.org/10.3390/ani10030502

USDA. (2021). *Food Data Central* https://fdc.nal.usda.gov/index.html

van Huis, A., & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5), Article 43. https://doi.org/10.1007/s13593-017-0452-8

Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2013). *Edible insects: future prospects for food and feed security*. Food and Agriculture Organization of the United Nations.

Varelas, V. (2019). Food Wastes as a Potential New Source for Edible Insect Mass Production for Food and Feed: A review. *Fermentation-Basel*, *5*(3), Article 81. https://doi.org/10.3390/fermentation5030081

Wakshlag, J., Barr, S., & Ordway, G. J. R. V. S. (2002). Effect of dietary protein on lean body wasting.

Wang, S., Nie, S., & Zhu, F. (2016). Chemical constituents and health effects of sweet potato. *Food Research International*, *89*, 90-116. https://doi.org/https://doi.org/10.1016/j.foodres.2016.08.032

Wąsik, M., Mikuła, M., Bartyzel, B. J., Strokowska, N., Sablik, P., Uca, Y. O., & Koczoń, P. J. A. S. P. Z. (2017). Polyunsaturated fatty acids in idiopathic epilepsy treatment in dogs. *15*(2), 3-10.

Wu, G. (2018). *Principles of animal nutrition* [Bibliographies

Online

Non-fiction

Electronic document]. CRC Press, Taylor & Francis Group. http://ezproxy.massey.ac.nz/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=cat00245a&AN=massey.b4653183&site=eds-live&scope=site

http://ezproxy.massey.ac.nz/login?url=https://www.taylorfrancis.com/books/9781315120065

Yavor, K. M., Lehmann, A., & Finkbeiner, M. (2020). Environmental Impacts of a Pet Dog: An LCA Case Study. *Sustainability*, *12*(8), Article 3394. https://doi.org/10.3390/su12083394

Zhen, Y., Chundang, P., Zhang, Y., Wang, M., Vongsangnak, W., Pruksakorn, C., & Kovitvadhi, A. (2020). Impacts of Killing Process on the Nutrient Content, Product Stability and In Vitro Digestibility of Black Soldier Fly (Hermetia illucens) Larvae Meals. *Applied Sciences-Basel*, *10*(17), Article 6099. https://doi.org/10.3390/app10176099

10 Appendix

Table A1. Essential vitamins for animals, functions and clinical signs of deficiency (modified from (Hynd, 2019, pp. 24-27).

Vitamin	Functions	Clinical signs of deficiency
A	• Epithelial maintenance • Keratinisation • Vision (formation of the bleaching pigment rhodopsin) • Antioxidant • Protection against mutagenesis	• Anorexia • Skin lesions • Roughened hair/feathers • Xerophthalmia (eye pressure) • Abnormal bone growth • Poor vision in low light • Incoordination, paresis • Reproductive abnormalities • Congenital deformities
D2 (plants) and D3 (animals)	• Calcium absorption (intestinal) • Calcium resorption (renal)	• Rickets (young) • Osteoporosis (adults) • Lameness, sore joints
E	• Antioxidant (with selenium) • Gene expression • Signal transduction	• Cardiac and skeletal muscle abnormalities • Reproductive failure • Immune dysfunction • Muscular weakness (paresis) • Mad-chick disease (poultry) • leaky capillaries = exudative diathesis (poultry) • Mulberry heart disease in pigs • Liver necrosis
K	• Blood clotting (synthesis of prothrombin)	• Haemorrhage anaemia • Increased clotting time (prothrombin time) • Contusions (bruising) • Growth disorders
B1	• Component of transketolase (pentose phosphate pathway) • Component of decarboxylases (carbohydrate and protein utilisation) • Energy production • Neural function	• Anorexia • Neurological disorders (paraesthesia – tingling) • Polioencephalomalacia (Sykes et al.) • Muscle weakness (paresis)

B2	• Energy production (component of FAD and FMN) • Blood cell synthesis	• Neural dysfunction (curled-toe paralysis in birds) • Anaemia • Poor growth
B3	• Energy production (component of NAD, NADH, NADP and NADPH)	• Diarrhoea • Depression • Dementia • Death • Necrotic enteritis in pigs • Blacktongue • Possibly increased abortions
B5	• Component of CoA (e.g. acetyl CoA) • Fatty acid utilisation • Carbohydrate utilisation • Protein utilisation • Energy production	• Reduced growth rate • Dermatitis in chickens • Haemorrhage • Rough coats • Anorexia • 'Goosestepping' in pigs
B6	• Component of 50 enzymes in carbohydrate and protein metabolism • Skin maintenance • Neural function • Red blood cell formation	• Poor growth • Seizures • Anaemia • Depressed immune system
B8 (vit H)	• Protein and fatty acid metabolism	• Scaly skin, dermatitis • Impaired keratinisation
B9	• Methyl group transfers • Protein metabolism • Gene expression	• Anaemia (macrocytic) • Poor growth • Depressed immunity • Death • Neural tube defects
B12	• Component of methylmalonyl CoA mutase for propionate entry to TCA cycle • Methyl group transfer and folate metabolism • Methionine metabolism • Red blood cell synthesis • DNA synthesis	• Megaloblastic anaemia • Anorexia • Weight loss • Fatty liver • Wool break • Infertility
Choline	• Component of acetylcholine neurotransmitter • Component of phosphatidylcholine • Methyl group transfer	• Liver damage • Fatty liver

Appendix B

Table B1
Ingredient list.

168630	171116	172617	175167	168944	168898
Beef	Chicken	Lamb	Salmon	Wheat Flour	Rice Flour
169739	169741	171400	Poultry Fat	171401	171412
Barley	Oat Meal	Beef Tallow	Poultry Fat	Lard	Coconut Oil
171016	171024	171027	171410	171413	171422
Seasame Oil	Cottonseed Oil	Safflower High Oleic	Peanut Oil	Olive Oil	Palm Kernel Oil
172338	172357	172343	172340	172341	173577
Sunflower High Oleic	Sunflower Oil	Salmon Oil	Herring Oil	Menhaden Oil	Cod Liver Oil
169717	169230	364202	172237	171333	519744
Tapioca	Garlic	Yellow Peas	Distilled Vinegar	Rosemary	Flax meal
170290	172420	168482	171428	172336	171015
Corn Flour	Lentils	Sweet Potato	Babassu	Canola Oil	Palm Oil
748323	1103864	171411	168448	170393	168820
Corn Oil	Safflower Oil	Soybean Oil	Pumpkin	Carrots	Molasses
171320					
Cinnamon					

Appendix C

Raw data of 5.3 Effect of increasing glycerin level on the final Aw value, the sixth trial

Moisture content raw data

Table C1 Raw data of moisture content.

3% Glycerin 15 minutes					
Container Weight	First Weight	Final Weight	Weight Loss	Sample weight	Water Content
34.139	36.254	35.944	0.3100	2.115	0.146572104
35.6513	37.8069	37.4936	0.3133	2.1556	0.145342364
34.1061	36.3275	35.9994	0.3281	2.2214	0.147699649
				Average	**14.65%**
3% Glycerin 16 minutes					
33.5418	35.7779	35.4119	0.366	2.2361	0.163677832
35.5851	37.6188	37.2884	0.3304	2.0337	0.162462507
36.0104	38.168	37.8109	0.3571	2.1576	0.165507972
				Average	**16.39%**
3% Glycerin 17 minutes					
36.0856	38.1785	37.863	0.3155	2.0929	0.150747766
33.5202	35.6439	35.309	0.3349	2.1237	0.157696473
35.9048	37.9394	37.6127	0.3267	2.0346	0.160572103
				Average	**15.63%**
4% Glycerin 15 minutes					
Container Weight	First Weight	Final Weight	Weight Loss	Sample weight	Water Content
36.0762	38.2442	37.8036	0.4406	2.168	0.203228782
34.2522	36.2334	35.8274	0.406	1.9812	0.204926307
35.9028	38.0337	37.6077	0.426	2.1309	0.199915529
				Average	**20.27%**
4% Glycerin 16 minutes					
34.8924	36.8042	36.4498	0.3544	1.9118	0.185375039
33.8781	35.9082	35.5368	0.3714	2.0301	0.182946653
35.9169	38.0234	37.6258	0.3976	2.1065	0.18874911
				Average	**18.57%**
4% Glycerin 17 minutes					
35.066	37.2722	36.921	0.3512	2.2062	0.159187744
35.9633	38.0952	37.7433	0.3519	2.1319	0.165064027
33.5749	35.6197	35.2865	0.3332	2.0448	0.162949922
				Average	**16.24%**
5% Glycerin 15 minutes					
Container Weight	First Weight	Final Weight	Weight Loss	Sample weight	Water Content
36.4228	38.4392	37.998	0.4412	2.0164	0.218805793
35.8509	37.8644	37.4176	0.4468	2.0135	0.22190216
33.6715	35.7535	35.3017	0.4518	2.082	0.217002882
				Average	**21.92%**

5% Glycerin 16 minutes					
34.4189	36.507	36.1313	0.3757	2.0881	0.179924333
35.609	37.6477	37.2924	0.3553	2.0387	0.174277726
33.8241	35.8826	35.5251	0.3575	2.0585	0.173670148
				Average	**17.60%**
5% Glycerin 17 minutes					
35.9618	38.0295	37.7078	0.3217	2.0677	0.155583499
36.1041	38.2705	37.93	0.3405	2.1664	0.157173191
36.9952	38.1913	37.8439	0.3474	1.1961	0.290443943
				Average	**20.11%**

Water Activity raw data

Table.C2 Aw value in the 6th test.

	3%	4%	5%
15 minutes	0.7145	0.7657	0.7637
16 minutes	0.713	0.7429	0.7215
17 minutes	0.7003	0.6472	0.6976

Raw data of 5.4.3 Baking trials at 100℃

Table C3 Aw levels against baking time.

Time	58	61	64	67	70	73	76	79	82
Aw	0.4549 20.07 C	0.4565 20.06 C	0.4201 20.03 C	0.4455 20.03 C	0.4528 20.01 C	0.4299 20.01C	0.3871 20.01 C	0.3986 20.02 C	0.4074 20.02 C
Time	85	88	91	94	97	100	103	106	109
Aw	0.3963 20.02 C	0.3989 20.00 C	0.3993 20.01 C	0.3807 20.01 C	0.3794 20.01 C	0.3791 20.02 C	0.3728 20.02 C	0.3895 20.02 C	0.3797 20.01 C

Raw data of 5.4.3 The sixth test: 100 °C oven temperature test

Table. C4 Aw levels against baking time.

Time	72 min	75 min	78 min	81 min	84 min	87 min	90 min	93 min
Aw	0.3356 20.10C	0.3132 20.08C	0.3116 20.07C	0.3019 19.99C	0.3026 20.02C	0.2928 20.07C	0.289 20.09C	0.2884 20.04C
Time	96 min	99 min	102 min	105 min	108 min	111 min	114 min	117 min
Aw	0.2800 20.04C	0.2839 20.05C	0.2800 20.03C	0.2850 20.02C	0.2828 20.03C	0.2869 20.02C	0.2770 20.04C	0.2796 20.02C
Time	120 min	123 min	126 min	129 min	132 min	135 min	138 min	

Aw	0.2759 20.02C	0.2843 20.02C	0.2780 20.04C	0.2739 20.03C	0.2768 20.04C	0.2741 20.03C	0.2623 20.02C	

Raw data of Chapter 6 Drying test of TF2

Table C5 Raw data of moisture content test.

26 min Sample	24 min Sample	Time	26 min Container Weight	24 min Container Weight	
3.0695	3.0487				
20.0115	20.3867	0 min	3.0695	3.0487	0 min
19.2442	19.3748	1/2 hour	3.0695	3.0487	1/2 hour
18.6906	18.847	1 hour	3.0695	3.0487	1 hour
18.3364	18.475	1.5 hour	3.0695	3.0487	1.5 hour
18.0495	18.178	2 hour	3.0695	3.0487	2 hour
17.8406	17.9649	2.5 hour	3.0695	3.0487	2.5 hour
17.6863	17.804	3 hour	3.0695	3.0487	3 hour
17.719	17.8575	1hour cooling	3.0695	3.0487	1 hour cooling

Raw data of 6.3.3: 100 °C oven temperature test

Table C6 Aw levels against baking time.

Time	58 min	61 min	64	67 min	70 min	73 min	76 min	79 min
Aw	0.4760 20.10C	0.4380 20.09C	0.4199 20.07C	0.3934 20.09C	0.3891 20.08C	0.3646 20.07C	0.3638 20.10C	0.3613 20.09C
Time	82 min	85 min	88 min	91 min	94 min	97 min	100 min	103 min
Aw	0.3685 20.03C	0.3554 20.11C	0.3563 20.07C	0.3624 20.11C	0.3448 20.04C	0.3376 20.03C	0.3313 20.06C	0.3464 20.05C
Time	106 min	109 min	112 min	115 min	118 min	121 min	124 min	127 min
Aw	0.3439 20.05C	0.3439 20.12C	0.3246 20.03C	0.3337 20.04C	0.3231 20.04C	0.3353 20.05C	0.3412 20.05C	0.3327 20.04C

Appendix D Treat formula 3 first test

Test formula

Treat Formula 3 first test.

Ingredient	Target Weight (g)	Actual Weight (g)
Pumpkin Powder	53.0	53.0
Carrots	12.5	12.5
Oats	9.5	9.5
Cricket Flour	13.0	13.0
Canola Oil	4.0	4.0
Glycerin	2.0	2.1
Tapioca Starch	1.0	1.0
Molasses	1.0	1.0
Agar	1.0	1.0
Water	57.3	56.3

Status Log

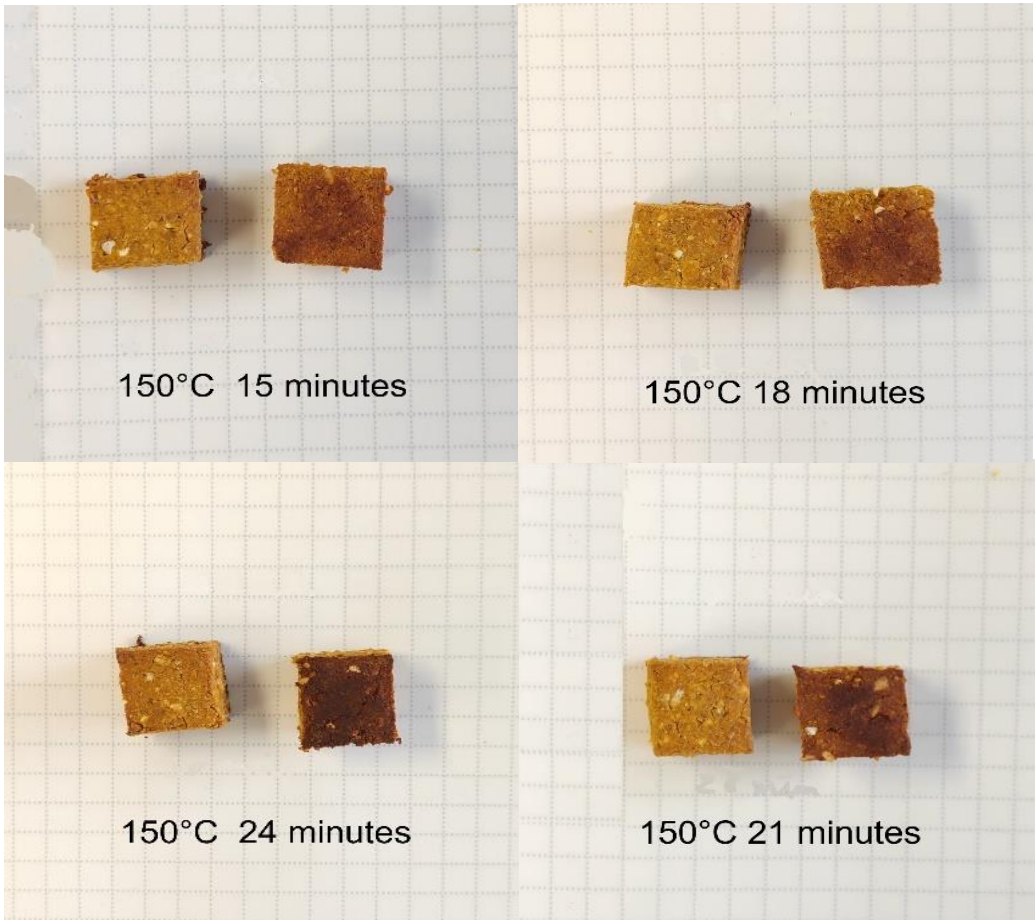
Status Log Formula 3.	
15'00"	Slight color change
18'00"	The bottom side was golden brown
21'00"	The bottom side overcooked
24'00"	Bottom side overcooked, top side showed deep color

* Every time take out and put back use 10 seconds

Test result

1) The best time range was estimated at around 15 ~ 18 minutes continuously baking.
2) Try 17 minutes at 150°C for the next trial (the second batch).

Pictures



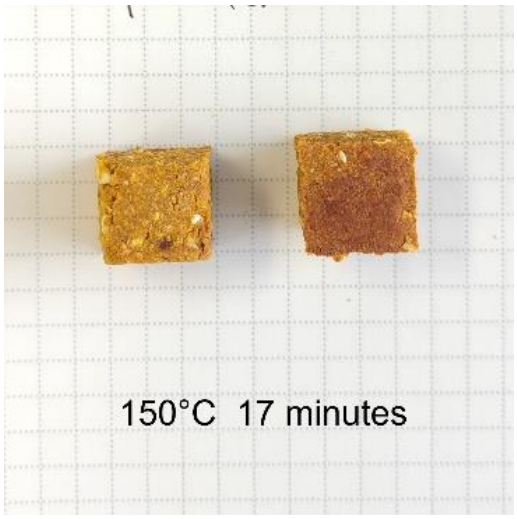
Second batch trial

The processing parameter was 150°C and 17 minutes

Test results.

The formation was good, Aw =0.8030 at 20.54 °C which was too high.

Pictures



Results and conclusion

1) The water activity was too high, need improvement.
2) The bottom and the top side were not heated evenly, need to find a better way to bake.

1.7 **Treat formula 3 second test**

Aim: Test two baking times for each formula to determine a better combination

Test formula

Treat Formula 3.

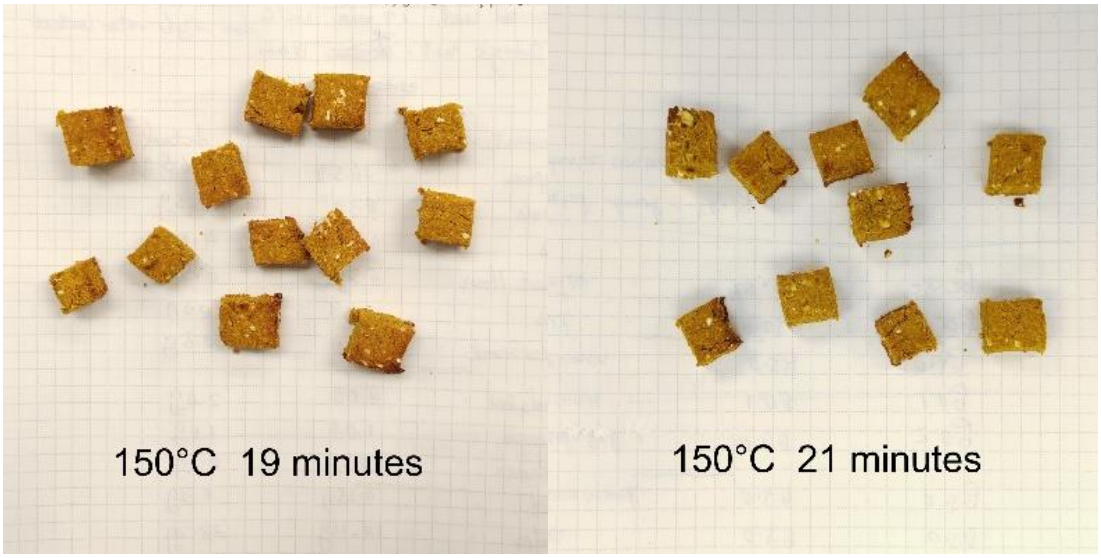
Ingredient	Target Weight (g)	Actual Weight
Pumpkin Powder	26.5	26.5
Carrots	6.3	6.3
Oats	4.8	4.8
Cricket Flour	6.5	6.5
Agar	0.5	0.5
Tapioca Starch	0.5	0.5
Canola Oil	2.0	2.0
Glycerin	1.0	1.0
Molasses	0.5	0.5
Salt	0.3	0.3
Water	28.2	28.2

* At 150 °C 19 min and 21 min

Test result

1) The 19-min sample, the formation was good but Aw=0.7568 was unacceptable.
2) The 21-min sample, a bit over-cooked but Aw=0.7548 which was still too high.
3) Due to carrots may have inferior palatability, will obsolete this formula.

Pictures



Appendix E

1. Baking trials at 150℃

1.1 First Trial

The aim of the trial is 1）To test the basic formality of the complete formula. 2）To determine the range of the optimum baking time. 3) Testing the usage of the modified aluminium oven tray to achieve the same colour on both the top and bottom sides.

1.1.1 Method

1) The cube-shaped samples were split into two batches. 2) The first batch (A batch) was baked and under visual inspection every 3 minutes to observe the color change. 3) The second batch (B batch) was baked (20 minutes) according to the estimation of the time range based on the result of the A batch.

1.1.2 A batch test

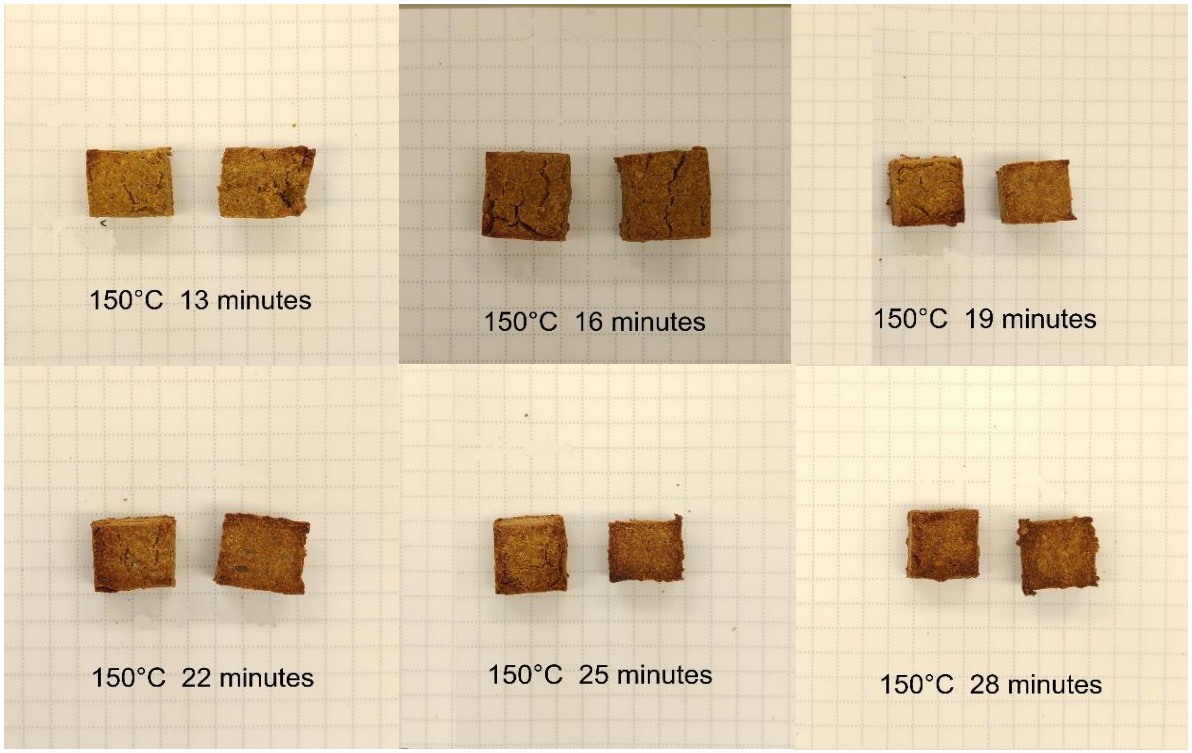
Inspection log

Table 10.1 Status Log Complete Formula 1st test.

13'00"	A bit of color change, not fully cooked
16'00"	Deeper color, need more time
19'00"	No apparent color change, crispy
22'00"	Golden brown, more crispy
25'00"	Over-cooked
28'00"	To much over-cooked

* Every time take out and put back in used about 10 seconds

1.1.3 Pictures



Picture 101 Visual inspection log.

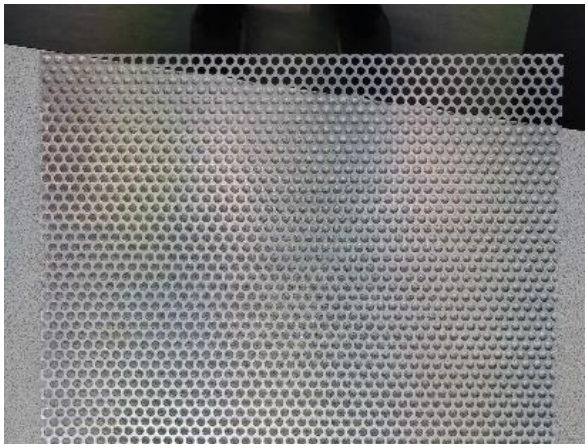
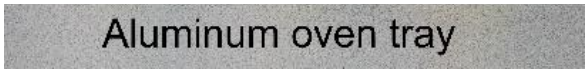


Figure 10-2 Modified aluminium baking tray



Picture 02 Aluminum tray,

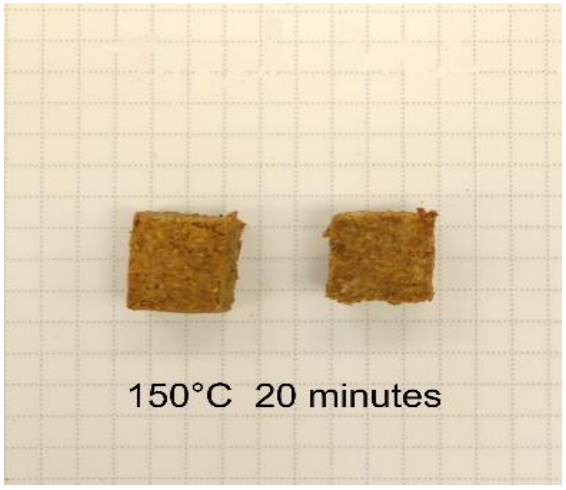
1.1.4 Test results and conclusions

1) The best baking time range was estimated at around 19 ~ 25 minutes.
2) Try 20 minutes at 150°C for the B batch.
3) Water was too much, as the dough was hard to keep formation, hence less water should be tried during the next trial.
4) The aluminium tray worked well, now the top side and the bottom side were similar.

1.1.5 B batch test

The processing parameter was set to 20 minutes at 150°C.

1.1.6 Picture of B batch test



Picture 03 B batch test.

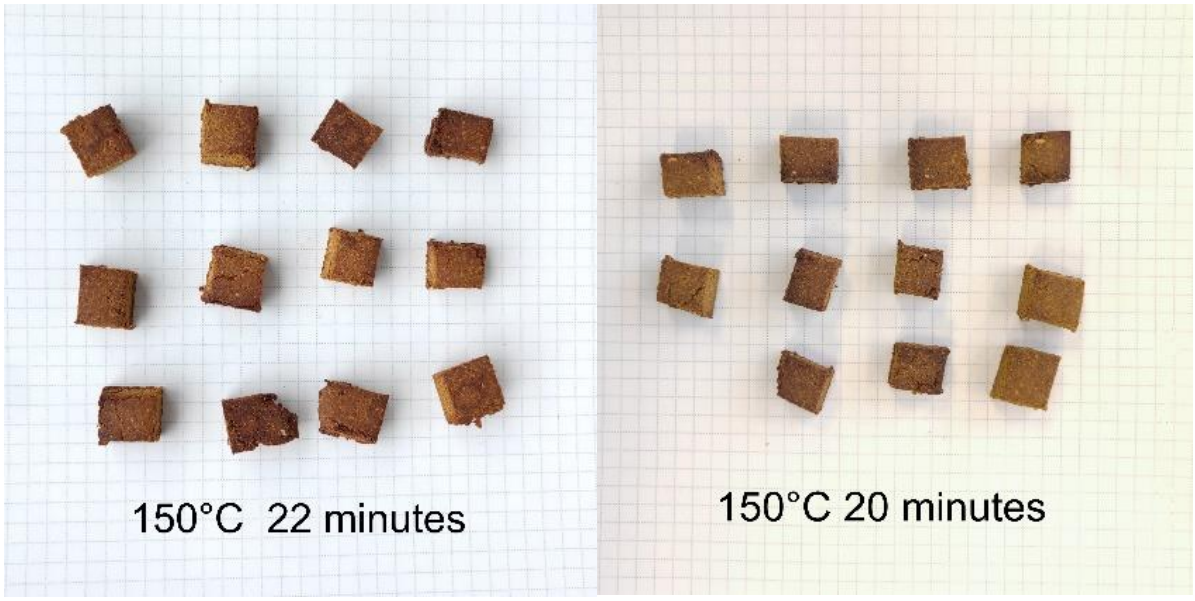
1) The formation was good, Aw $= 0.7469\ at\ 20.11\ °C$.

2) Aw was too high, need lower down the Aw level, hence will increase the baking time and decreased the amount of added water.

1.2 The Second trial

The trial aim is to get a lower water activity (< 0.65) through 1) try longer baking times, 20 and 22 minutes. 2） less added water.

1.2.1 Picture of 2nd trial



Picture 04 Picture of 2nd trial.

1.2.2 Results and conclusions

1) The 22-minute sample had a very deep color hence was a bit over-cooked.
2) The 20-minute sample was in good condition.
3) The water activity of 22 and 20-minute samples were 0.6281 at 20.23 °C and 0.6423 at 20.05 °C.
   Hence the 20 minutes baking time at 150°C was chosen as the baseline for further trials.

1.3 The third trial

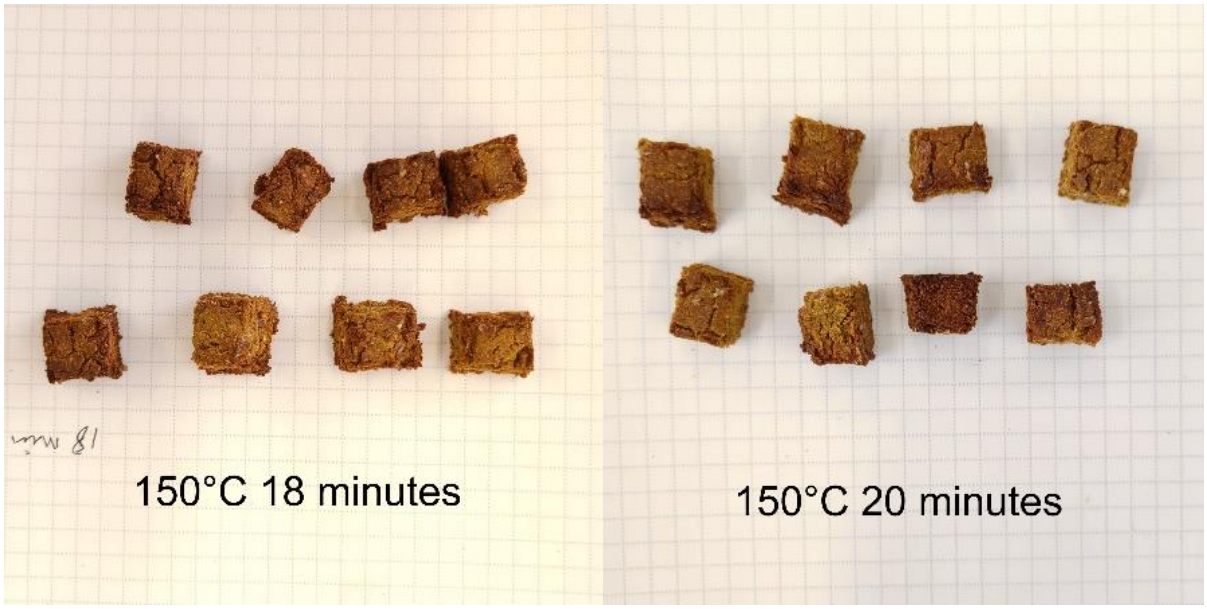
For cost considerations, this test was conducted to test the formula with decreased pumpkin level. The levels of other ingredients were recalculated and adjusted to reach the AAFCO's requirements on the complete dog food formula.

1.3.1 Method

1) Based on the 2nd test, two baking times were chosen to be tested, 20 minutes and 18 minutes.

2) To minimise the variation, the cube-shaped samples from the same dough was split into two batches. One batched was tested for 20 minutes, the other batch was tested for 18 minutes.

1.3.2 Picture of 3rd trial



Picture 05 Results of the 3rd test.

1.3.3 Results and conclusions

1) Difficult dough formation, the dough was easy to be broken into pieces. This could be caused by the adjustment of the ingredient level. The difference in water combination ability and in the strength of interactions between different ingredients each other and water might cause the difficulty of dough formation. Hence further trials with higher water levels should be conducted
2) Both batches were in deep color hence showing over-cooked, shorter baking time should be tested in the next trial.

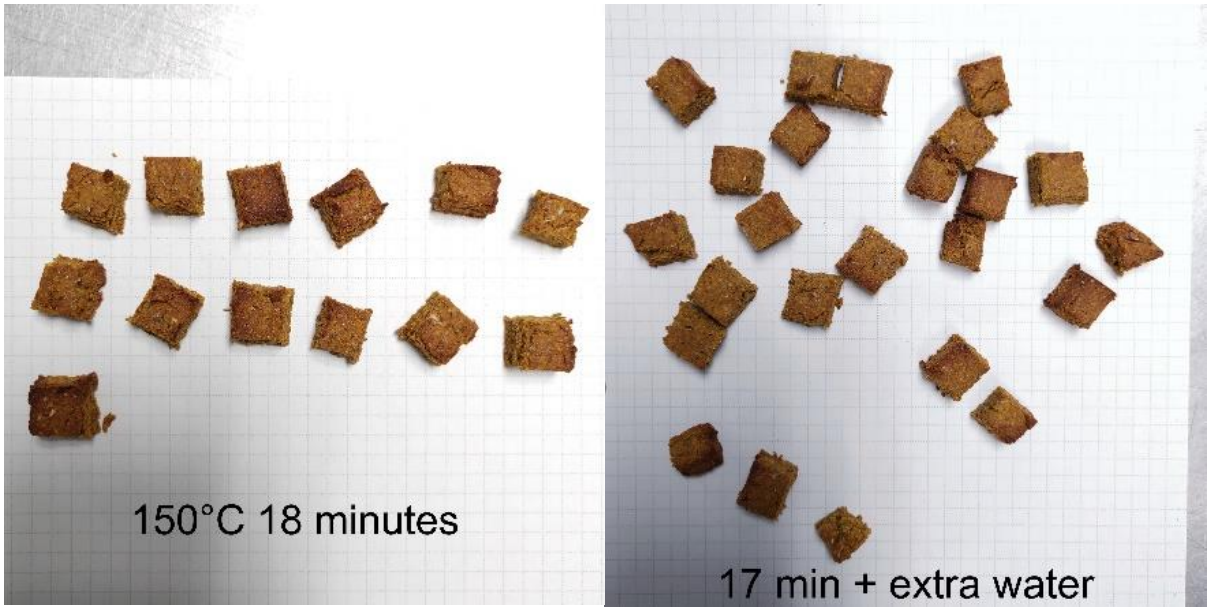
1.4 The fourth trial

Based on the results of the 3rd test, this test was conducted to testify the effect of more water and shorter baking time.

1.4.1 Method

To minimise the variations, the cube-shaped samples from the same dough was split into two batches. One batched was tested for 18 minutes, the other batch was tested for 17 minutes.

1.4.2 Picture of the 4th test



Picture 06 Results of the 4th trial.

1.4.3 Results and conclusions

1) The 18-min sample was a bit over-cooked which was consistent with the 3rd test.
2) The 17-min sample was better in color.
3) The dough formation was improved, as more elastic and tensible.
4) Will run another test to try a shorter time (15 min) and determine Aw.

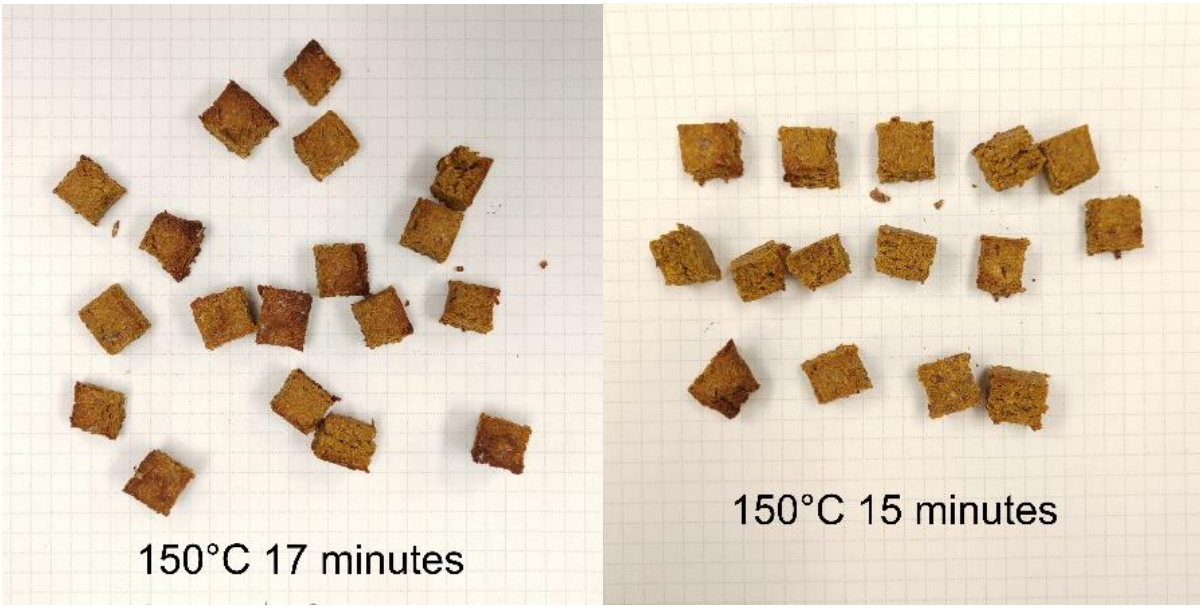
1.5 The fifth test

Based on the test results of the 4th test, this test was conducted to test the possibility of a shorter baking time.

1.5.1 Method

To make a comparison, the cube-shaped samples from the same dough was split into two batches. One batched was baked for 15 minutes, the other batch was baked for 17 minutes.

1.5.2 Pictures of the 5th trial



Picture 07 Results of the 5th test.

1.5.3 Results and conclusions

1) The 17 min sample, Aw = 0.6655 at 20.11°C, water content 14.77%
2) The 15 min sample, Aw = 0.7020 at 20.09°C, water content 16.78%
3) Compared the two samples, the 17-min sample showed a better color formation and with lower Aw, hence was kept as the basis of the future tests.

There was potential to further lower down the Aw value by adjusting the glycerin level. Therefore, the next test would be focused on the effect of glycerin level on the final Aw value.

1.6 Effect of increasing glycerin level on the final Aw value, the sixth trial:

Based on the results of the 5th trial and to further lower the water activity, increasing glycerin level was tested. Here glycerin was used as a humectant to hold water molecules hence maintaining the moisture of the food products. This trial was conducted to demonstrate the effect of gradient glycerin level on the Aw performance of the final products.

1.6.1 Methods

1) Three formulas with 3%, 4%, and 5% percent glycerin were tested.

2) The same amount of flaxseed meal was deducted from the formula when an extra amount of glycerin was added.

3) At each glycerin level, three baking times (15, 16, and 17 minutes) were tested for comparison.

4) Water activity and moisture content was determined after cooling overnight.

1.6.2 Test Formula

Table 02 Complete Formula with gradient glycerin level.

Ingredient	Percentage (As-is)		
	3% Glycerin	4% Glycerin	5% Glycerin
Cricket Flour	34%	34%	34%
Pumpkin Powder	25%	25%	25%
Lentils Powder	13%	13%	13%
Flaxseedf Meal	11%	10%	9%
Peanut Butter	4%	4%	4%
Vegetable Glycerin	**3%**	**4%**	**5%**
Coconut Oil	2%	2%	2%
Flaxseed Oil	2%	2%	2%
Molasses	3%	3%	3%
Salt (1)	0.5%	0.5%	0.5%
Subtotal (2)	98%	98%	98%
Water (3)	57.40%	57.40%	57.40%

(1) In the formulation calculation, the level of salt and supplements (to balance nutrient levels) was assumpted as 1%, and the flavor was assumpted as 2%, hence the rest ingredients was 97%.
(2)The subtotal should be 100% if flavor and salt and supplements were added.
(3)The water amount is calculated based on the assumption that the "Subtotal" is 100%.

1.6.3 Results and conclusion

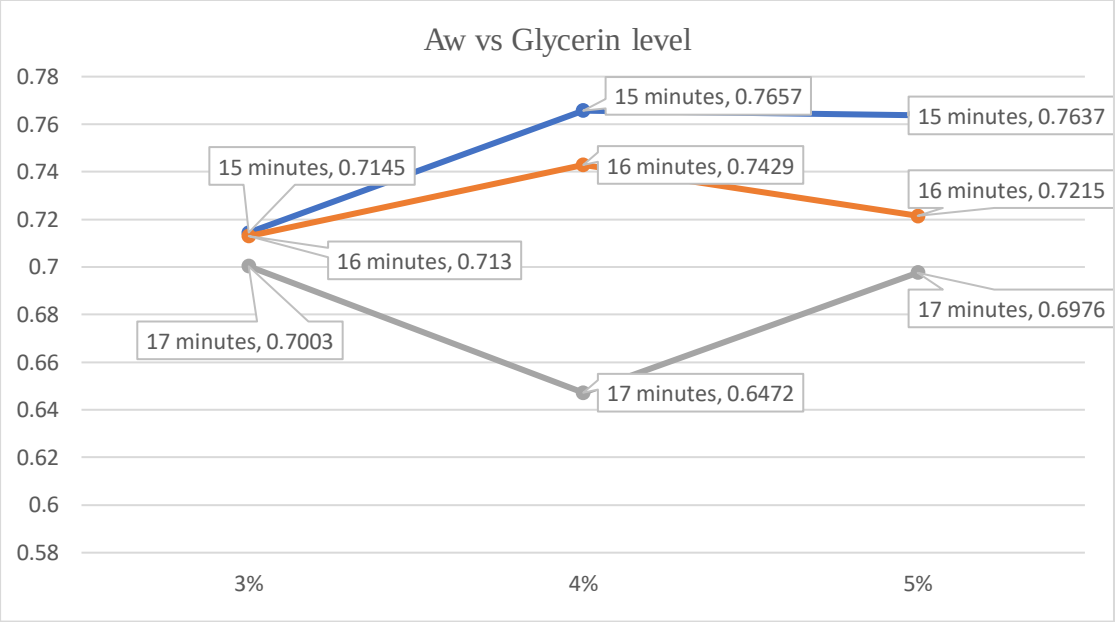


Figure 3.2 Effects of gradient glycerin on Aw value.

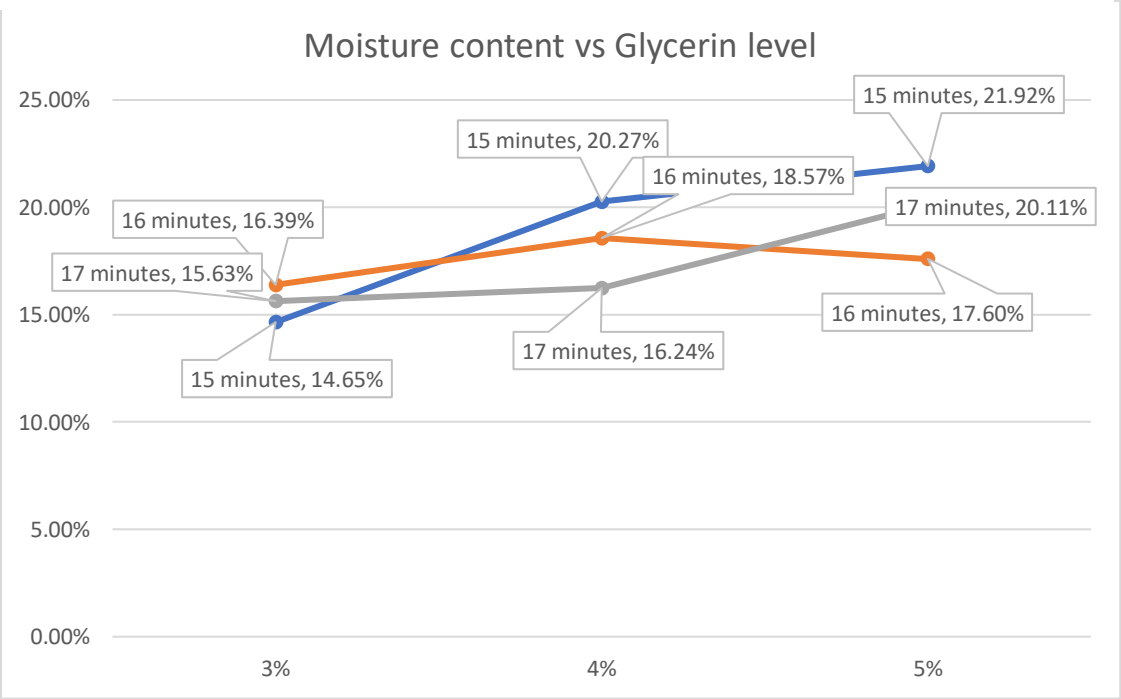


Figure 10-2 Effects of gradient glycerin on moisture conten.t

As shown in above Figure 3.1 and Figure 3.2 (the raw data is included in Appendix 1.1), it was shown that there is no obvious correlation between the water activity and glycerin level in the samples with the same baking time. Meanwhile, the relationship between moisture content and glycerin level was also not clear. In short, the results showed that glycerine is not very effective at reducing water activity.

Baking trials at 120℃ and 100℃

1.7 Baking trials at 120℃ part 1, the 7th trial

This test was aimed to verify the feasibility of baking the samples at 120°C and find out the relationship between the water activity and the baking time, hence it was possible to estimate a time range within which the desired Aw level can be reached.

1.7.1 Methods

1) The cube-shaped samples were split into two batches.

2) The first batch (A batch) was baked and samples (3-4 cubes) were taken out every 3 minutes for the water activity test.

3) The second batch (B batch) was baked according to the estimation of the time range based on the result of the A batch (51 minutes) and was tested against water activity.

1.7.2 Results and conclusion

The formula was the first one in the 6th test (3% glycerin).

1.7.3 Results of A batch

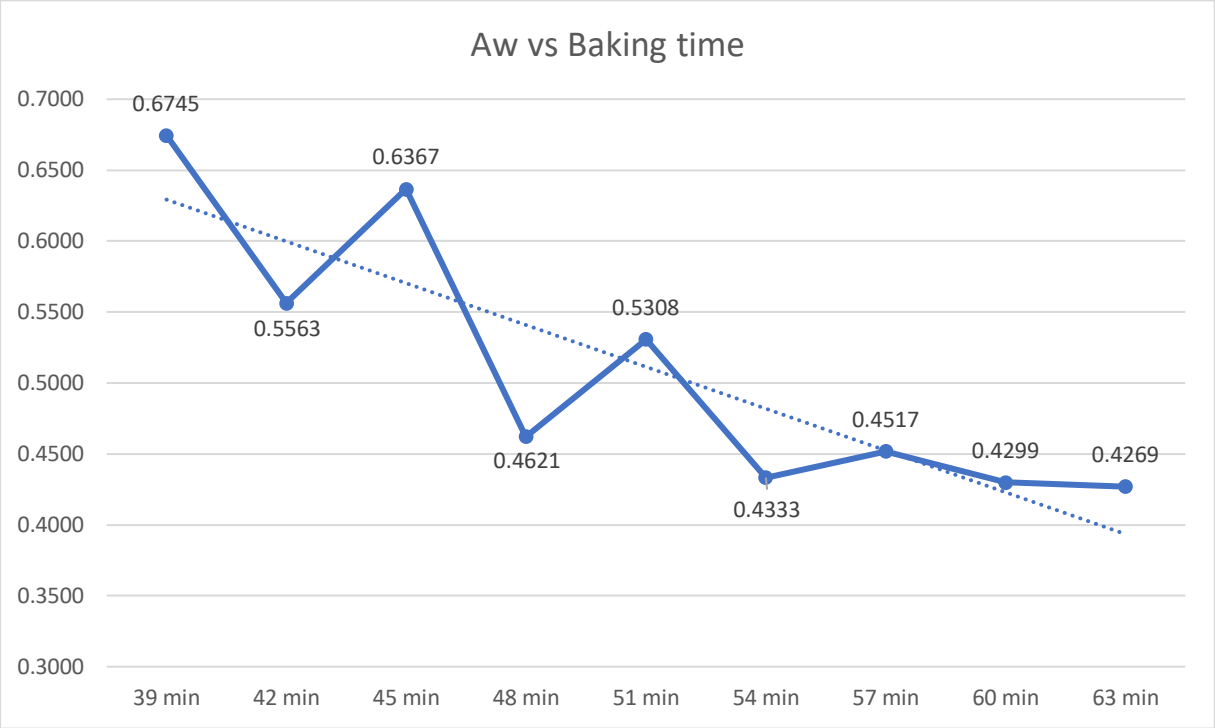


Figure 10-3 Aw levels against baking time (A batch).

Table 10.3 Aw levels against baking time (A batch).

Time	39 min	42 min	45 min	48 min	51 min	54 min	57 min	60 min	63 min
Aw	0.6745	0.5563	0.6367	0.4621	0.5308	0.4333	0.4517	0.4299	0.4269

1.7.4 Result of B batch

The Aw value at 51 minutes was 0.3428 at 20.01°C

1.7.5 Conclusion

1) As the trend line in Figure 3.3 shows that the Aw level was negatively related to the baking time.
2) From Figure 3.3, the optimum range of baking time could be roughly estimated as somewhere between 45~55 minutes.
3) The Aw value of the B batch at 51 minutes was much lower than the A batch sample of the A batch at the same time. This will be discussed in the final discussion part.
4) The next test would be conducted to further narrow down the range of baking time.

1.8 Baking trials at 120℃ part 2, the 8th trial

This test was aimed to further narrow down the range of baking time. The formula was the same as the 7th test.

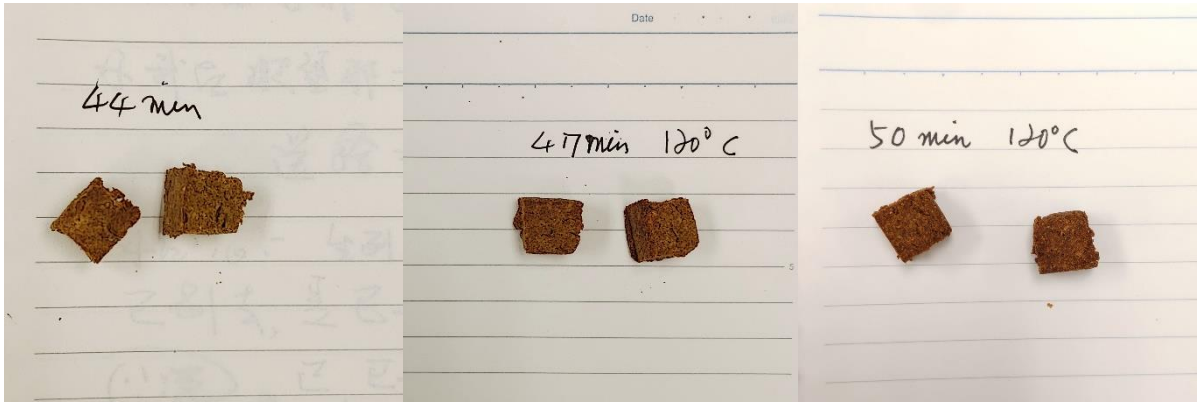
1.8.1 Methods

1) Three baking times (44, 47, and 50 minutes) were tested.

2) The cube-shaped samples made from the same dough was split into three batches, then were baked consecutively.

3) Water activity was determined after cooling overnight.

1.8.2 Pictures of the 8th trial



Picture 08 Results of the 8th test (before grinding).

1.8.3 Comparisons after grinding

As shown in Figure 5-12, the color changes of the samples were more visible after grinding. Over time, the color became deeper.

Picture 09 Comparison after grinding.

1.8.4 Results and conclusions

1) The water activity values of the samples baked at 44, 47, and 50 minutes were 0.6399 at 20.09 °C, 0.4200 at 20.10 °C, and 0.2686 at 20.04 °C respectively.

2) Taking into account both the water activity level and the color change (that the 50-min sample was a bit overcooked) it was estimated that at the 120 °C oven temperature, the optimum baking time was around 47 ± 3 minutes.

1.8.4 Baking trials at 100℃, the ninth trial

This test was aimed to try a lower baking temperature to observe the color and water activity changes when baked at 100 °C oven temperature.

1.8.5 Methods

1.8.6 Methods

1) The cube-shaped samples were split into three batches.

2) The first batch (A batch) was baked and samples (3-4 cubes) were taken out every 3 minutes from the 58 minutes for the water activity test.

3) The second batch (B batch) was baked continuously for 79 minutes and was tested against water activity.

4) The third batch (C batch) was baked continuously for 88 minutes and was tested against water activity.

1.8.6 Aw changes over time of A batch.

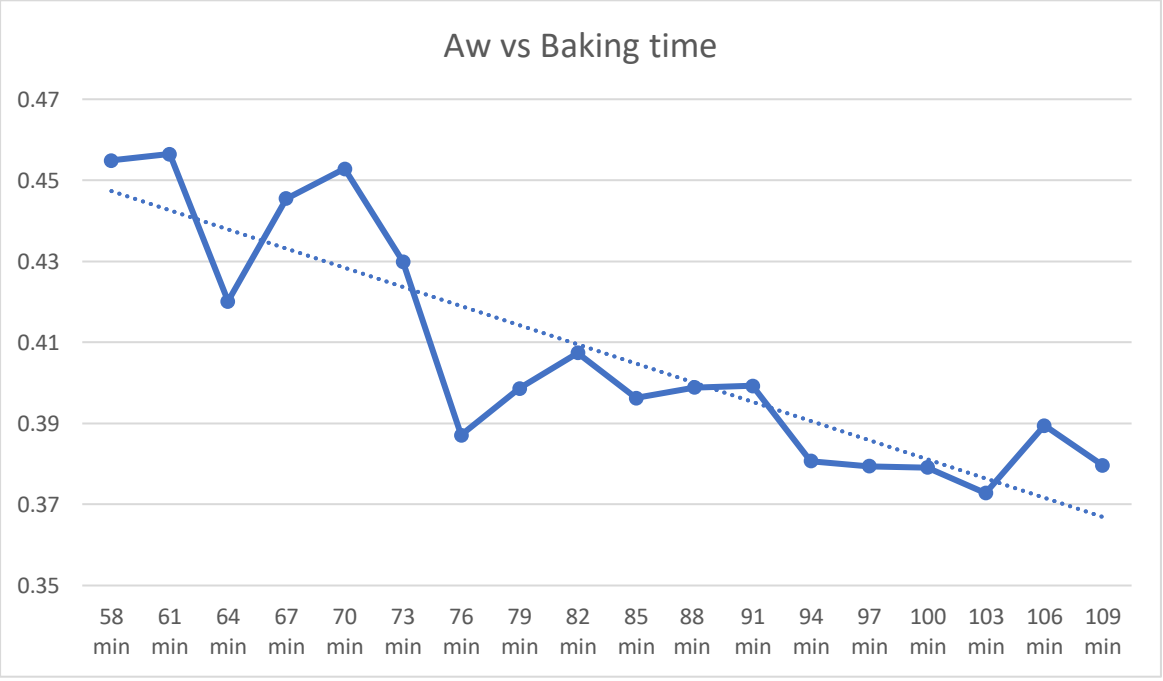
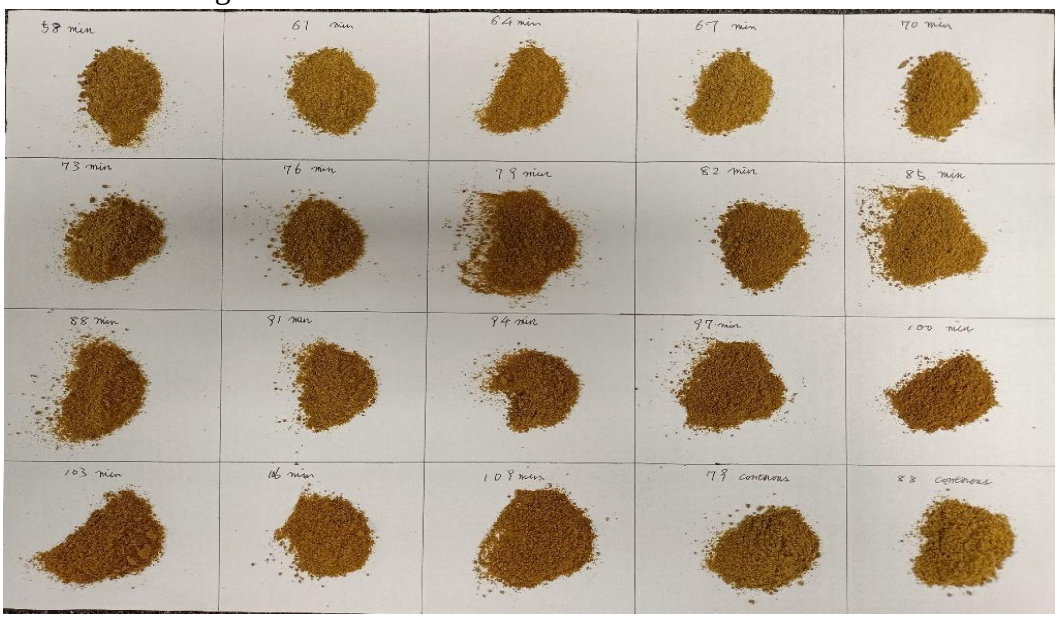


Figure 10-4 Aw values over time of A batch (*raw data is in Appendix C 1.2).

1.8.7 Color changes over time



Picture 10 Color changes over time.

1.8.8 Water activity values of B and C batch

1) The Aw value of the B batch (79 minutes continuous baking) was 0.3533 at 20.02 °C.

2) The Aw value of the C batch (88 minutes continuous baking) was 0.3201 at 20.05 °C.

1.8.9 Results and conclusion

1) The observation of color changes at 100 °C oven temperature was difficult. The reason was that the browning reactions that give color to bakery products, namely Maillard reaction and caramelization, normally happened above 120 °C.

2) The Aw test showed that the Aw value decreased continuously over time which means the required Aw value could be reached given enough time.

3) The Aw values of the B and C batch were lower than the samples which were taken out during the A batch test with close baking time. This is discussed in the final discussion part.

Development of Treats

2.1 Treats dog food formula 1

2.1.1 Test formulas

Table 10.4 Test formulas TF1.

Ingredient	Percentage (DM)			
	Trial Two	Trial Three	Trial Four/Five	Trial Six
Cricket Flour	17%	17%	17%	17%
Sweet Potato	52%	52%	52%	52%
Oat Meal	20%	20%	20%	20%
Tapioca Starch	1%	1%	1%	1%
Canola Oil	3%	3%	3%	3%
Molasses	1%	1.10%	1%	1%
Vegetable Glycerin	2%	2%	2%	2.2%
Agar	1.2%	1%	1%	1%
Salt			0.6%	0.6%
Water	54%	57.30%	57.80%	57.80%

The development of the treats dog food formula 1 (TF1) had been through several adjustments for different purposes. An overall summary is provided in Table 6.2.

Table 10.5 Summary of treat formula 1.

	Temperature	Variation & notes
1st	200 °C	10 minutes test
2nd	200 °C	15 minutes test
3rd	150 °C	10 minutes and re-baking test
4th	150 °C	25 and 27 minutes comparison test
5th	150 °C	29 and 31minutes comparison test
6th	100 °C	Decreased oven temperature to 100 °C

2.2 Baking trials at 200℃

2.2.1 The first trial

2.2.1.1 Aim of the test

1）To test the basic formality of the Treats formula 1 (TF1). 2）To determine the range of the optimum baking time.

2.2.1.2 Method

The cube-shaped samples were baked in the oven at 200 °C for 10 minutes.

2.2.1.3 Pictures

Picture 11 Results of trial 1.

2.2.1.4 Results and conclusions

Undercooked, a longer baking time should be tried in the next trial.

2.2.2 The second trial

2.2.2.1 Aim of the test

This test was conducted based on the results of the first-time test and a longer baking time was adopted.

2.2.2.2 Method

The shaped dice were baked at 200 °C oven temperature for 15 minutes.

2.2.2.3 Pictures

Picture 12 Second trial of TF1.

2.2.2.4 Results and conclusions

1) Overcooked. Five minutes longer baking time made the change from an undercooked sample to an overcooked one. A lower baking time should be tried in the next trial.

2) The dough formation was improved with less added water.

Baking trials at 150℃

2.2.3 The third trial

2.2.3.1 Aim of the test

1）This test was aimed to try the 150 ℃ oven temperature. 2）To optimize methodology.

2.2.3.2 Method

1) Start baking at 150℃ for 10 minutes then observe. 2) Re-bake the sample to estimate the right baking time by continuously taking out the sample from the oven every 3 minutes and observing the color change.

2.2.3.3 Picture of the third trial

Picture 13 TF1 the third trial.

2.2.3.4 Test Result

1) The sample was undercooked.

2) The sample should be used to be re-baked so that a new method could be testified.

2.2.3.5 Trial 3 part 2 extra Re-baking test

Table 10.6 Status log.

4'30"	Good smell
4'50"	Took out, still under-cooked
14'20"	No apparent color change
17'35"	Bottom side golden-brown
20'35"	No apparent color change
22'40"	Both sides golden-brown

2.2.3.6 Picture of the samples after re-baking

Picture 14 Samples after re-baking.

2.2.3.7 Results and conclusion

1) The lower oven temperature led to a longer baking time.
2) Taking into account the first stage 10 min baking and each time taking-out consumed time. The optimized baking time was estimated at around 25 minutes.

3) A more systematically take-out-put-in method can be testified in future tests.

2.2.4 The fourth trial

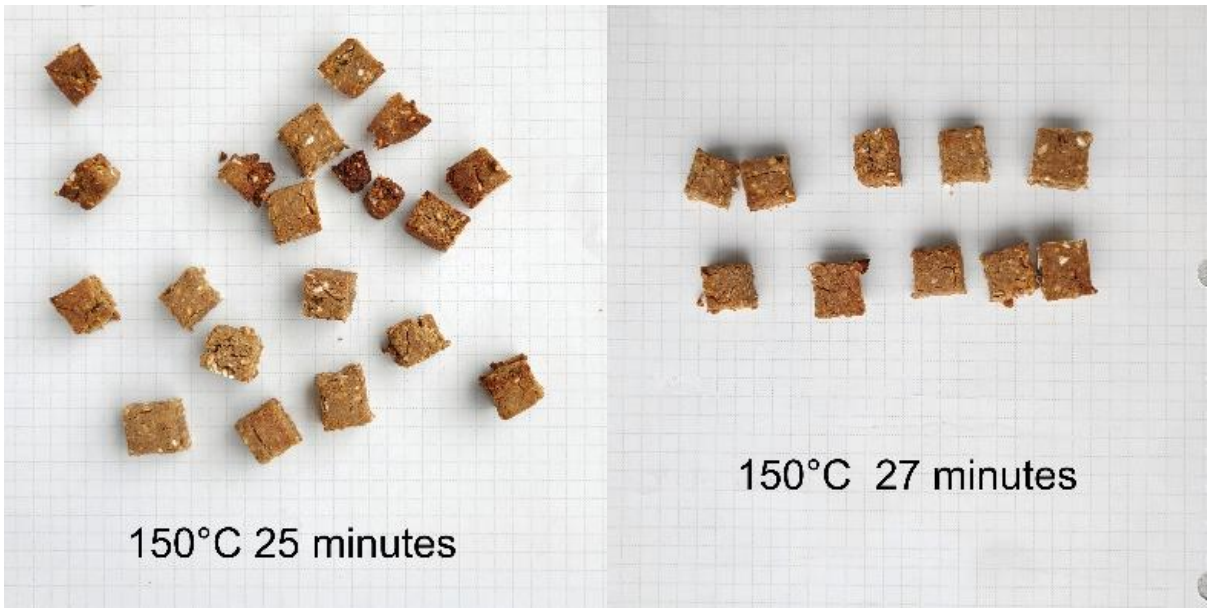
2.2.4.1 Aim of the test

Based on the result from the 3rd test, this test was aimed to try the 25 and 27 minutes baking time at 150 °C oven temperature.

2.2.4.2 Method

The cube-shaped samples from the same dough were split into two batches. One batched was baked for 25 minutes, the other batch was baked for 27 minutes.

2.2.4.3 Pictures



Picture 15 TF1 at 150 °C for 25 and 27 minutes.

2.2.4.4 Results and conclusion

1) Both the 25 and 27-min samples were good at formation, solid, and well-formed.

2) In the 27-min sample, the color was close to the 25-min one.
3) The 25-min sample, Aw=0.7549 20.10°C; the 27-min one, Aw= 0.6807 19.99°C
4) The water activity was too high hence a longer baking time should be tested in the next trial.

2.2.5 The fifth trial

2.2.5.1 Aim of the test

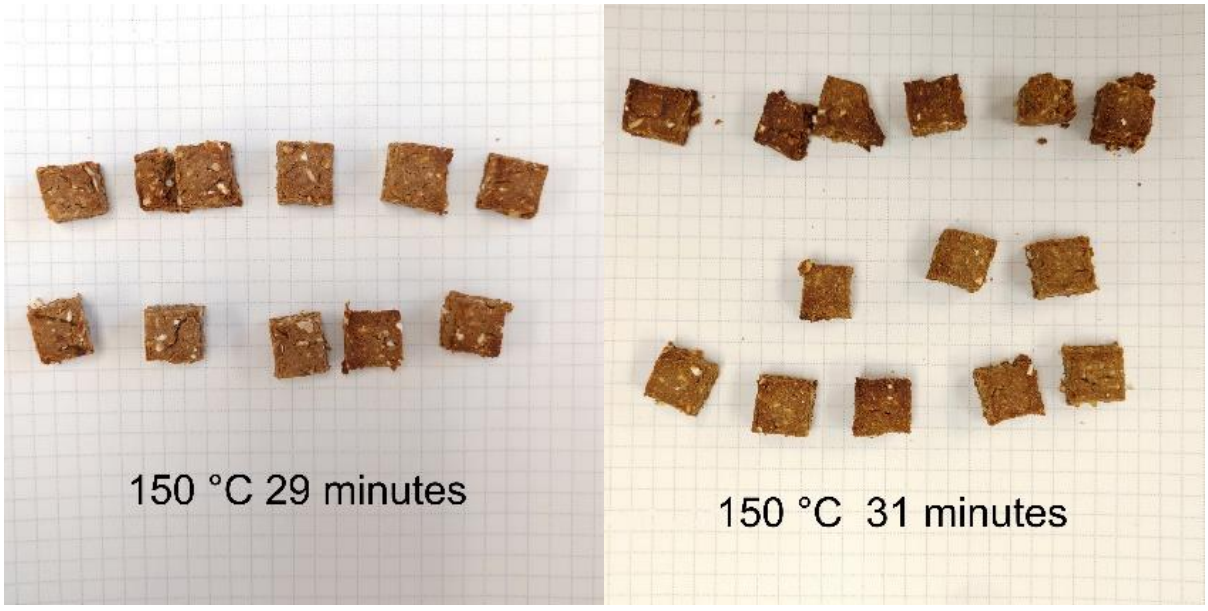
Based on the result from the 3rd test, this test was aimed to try the 29 and 31 minutes baking time at 150 °C oven temperature.

2.2.5.2 Method

1) The cube-shaped samples from the same dough were split into two batches. One batched was baked for 29 minutes, the other batch was baked for 31 minutes.

2) Water content was tested after cooling overnight.

2.2.5.3 Pictures



Picture 16 TF1 at 150 °C for 29 and 31 minutes.

2.2.5.4 Results and conclusion

1) The 29-min sample, Aw = 0.5504 at 20.06 °C, Water Content = 10.14 %

2) The 31-min sample, Aw = 0.4901 at 19.97 ℃, Water Content = 9.15 %

3) Will keep the 150 °C and 29 minutes as finalised combination.

Baking trials at 100℃

2.2.6 The sixth trial

2.2.6.1 Aim of the test

This test was aimed to try a lower baking temperature to observe the color and water activity changes when baked at 100 °C oven temperature.

2.2.6.2 Methods

1) The cube-shaped samples were split into three batches.

2) The first batch (A batch) was baked and samples (3-4 cubes) were taken out every 3 minutes from the 72 minutes for the water activity test.

3) The second batch (B batch) was baked continuously for 96 minutes and was tested against water activity.

4) The third batch (C batch) was baked continuously for 105 minutes and was tested against water activity.

2.2.6.3 Aw changes over time of A batch

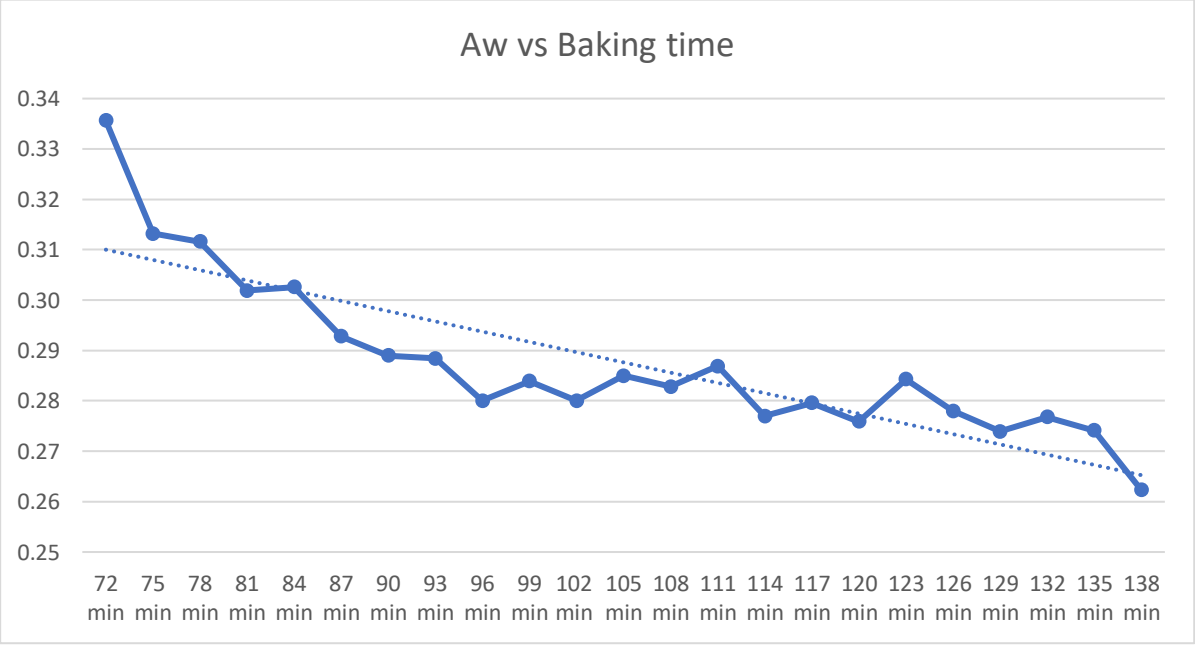
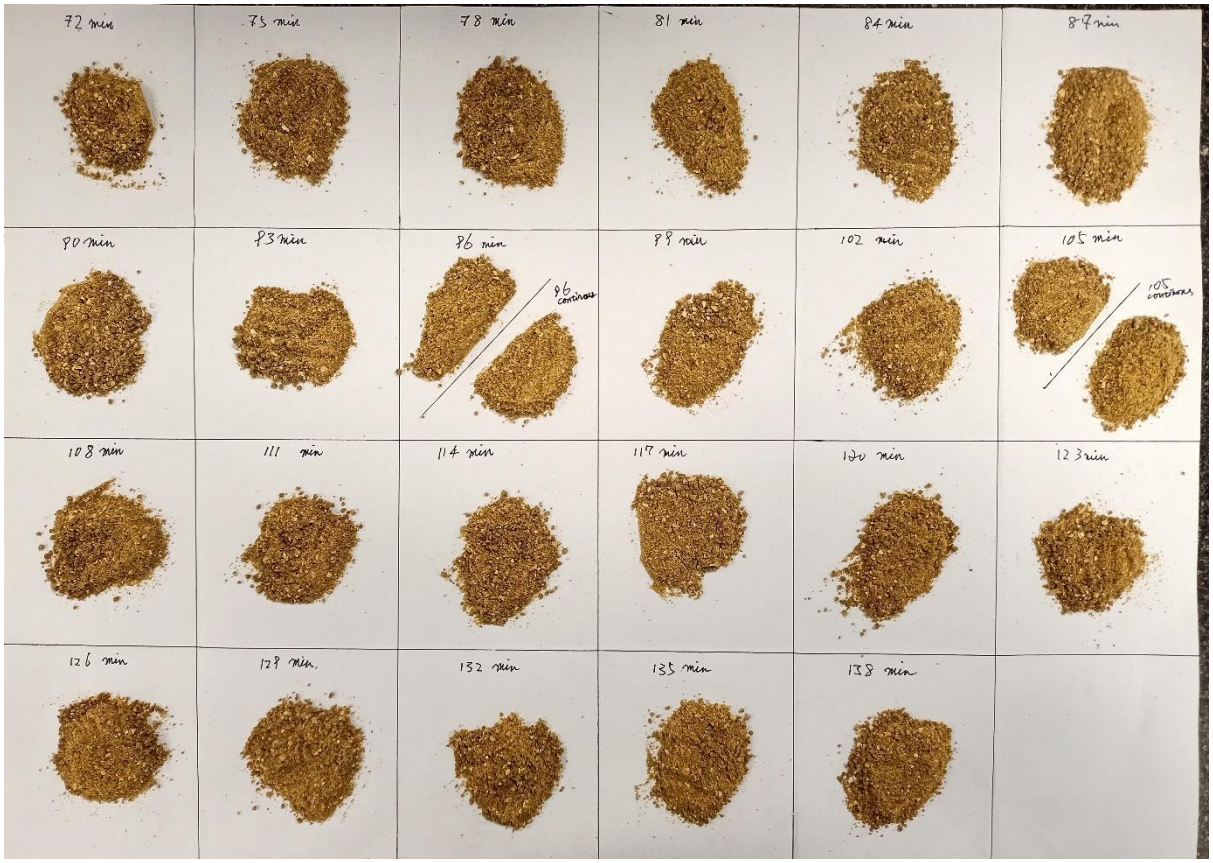


Figure 0-5 Aw values over time of A batch (*raw data is in Appendix C 1.3).

2.2.6.4 Color changes over time



Picture 17 Color changes over time.

2.2.6.5 Water activity values of B and C batch

1) The Aw value of the B batch (96 minutes continuous baking) was 0.2314 at 19.99 °C.

2) The Aw value of the C batch (105 minutes continuous baking) was 0.1843 at 19.97 °C.

2.2.6.6 Results and conclusion

1) The observation of color changes at 100 °C oven temperature was difficult. This was consistent with test 3.2.10 (Complete dog food formula 100 °C test).

2) The Aw test showed that the Aw value decreased continuously over time which means the required Aw value could be reached given enough time.

3) The Aw values of the B and C batch were lower than the samples which were taken out during the A batch test with close baking time. This is discussed in the final discussion part.

2.3 Treats dog food formula 2

Test formulas

Table 10.7 Test formulas of TF2.

Ingredient	Percentage (DM)			
	Trial One	Trial Two	Trial Four	Trial Five
Cricket Flour	25%	25%	25%	25%
Lentils Flour	24%	24%	24%	24%
Pumpkin Powder	26%	26%	26%	26%
Flaxseed meal	13%	13%	13%	13%
Peanut Butter	4%	4%	4%	4%
Molasses	3%	3%	3%	3%
Coconut Oil	2%	2%	2%	2%
Salt		0.6%	0.6%	0.6%
Water	57.3%	57.4%	57.4%	57.3%

*Trial 3 was conducted with samples from trial 2 hence no formula is listed in this table

The development of the treats dog food formula 2 (TF2) had been through several adjustments for different purposes. An overall summary is provided in Table 6.5.

Table 10.8 Summary of the treat dog food formula 2.

	Temperature	Variation & notes
1st	150 ℃	First test
2nd	150 ℃	20 and 22 minutes comparison test
3rd	150 ℃	Three-hour drying test
4th	150 ℃	Tested 24 minutes baking with 40 minutes drying
5th	100 ℃	Decreased oven temperature to 100 ℃

Baking trials at 150℃

2.3.1 The first trial

2.3.1.1 Aim of the test

1）To test the basic formality of the treat dog food formula 2 (TF2). 2）To determine the range of the optimum baking time

2.2.1.1.1 Method

1) The cube-shaped samples were split into two batches. 2) The first batch (A batch) was baked and under visual inspection every 3 minutes to observe the color change. 3) The second batch

(B batch) was baked (22 minutes) according to the estimation of the time range based on the result of the A batch.

2.3.1.2 A batch test

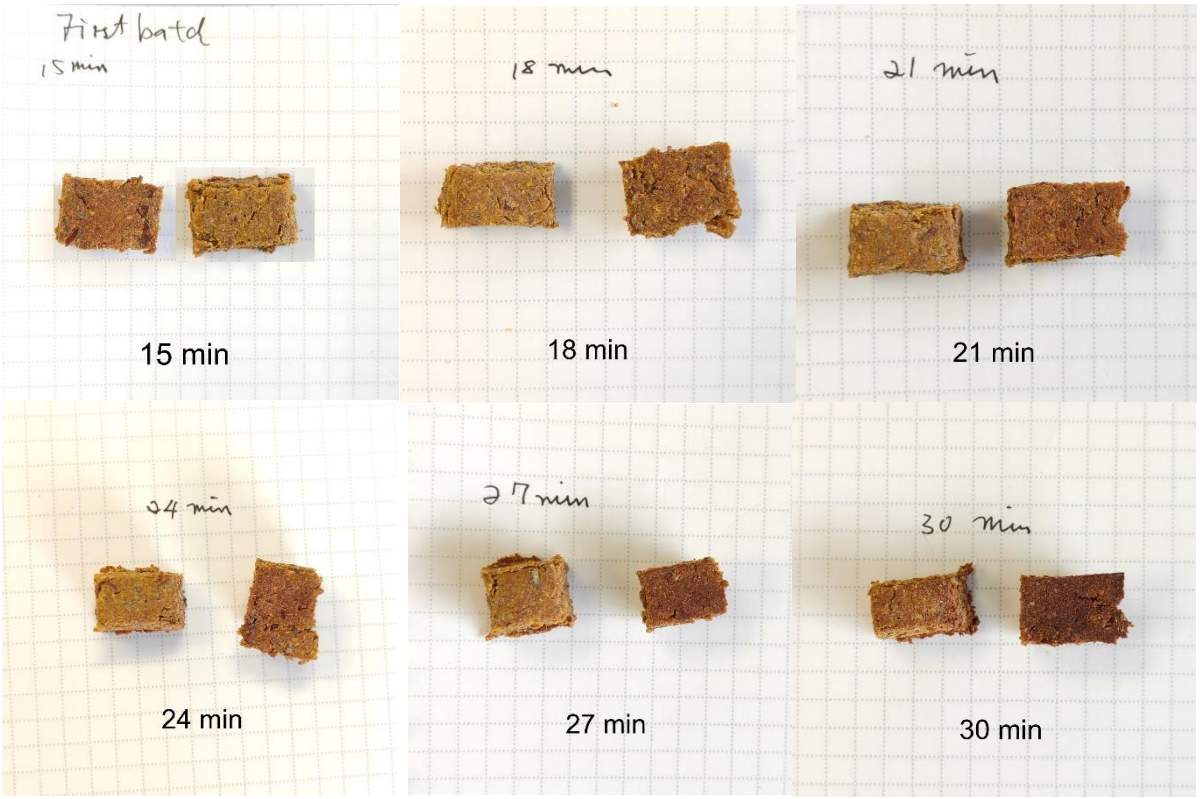
2.3.1.3 Inspection log

Table 10.9 Status log for TF2 A batch test .

13'00"	No obvious color change
15'00"	The top side had a slight color change
18'00"	Obvious color change on the bottom side
21'00"	Golden brown, good color.
24'00"	Tougher crust, but not obviously over-cooked
27'00"	Become crispy with a deep dark color
30'00"	Overcooked; very crispy, deep dark color on both sides.

* Every time take out and put back use around 10 seconds.

2.3.1.4Pictures



Picture 18 Visual inspection log.

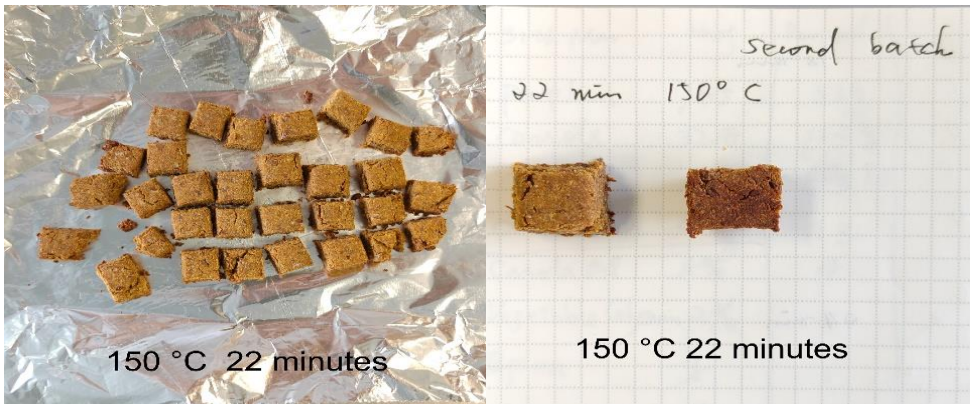
2.3.1.5 Test results and conclusions

1) The optimum time range was estimated around 20 ~ 25 minutes continuously baking.
2) Try 22 minutes baking time at 150°C for the next trial (the second batch).

2.3.1.6 B batch test

The processing parameter was set to 22 minutes at 150°C.

2.3.1.7 Picture of B batch test



Picture 19 B batch test.

2.3.1.8 Results and conclusion of B batch test

1) The formation was good, Aw = 0.7469 at 20.11 °C.

2) Aw was too high, need to lower down the Aw level, hence will increase the baking time.

2.3.2 The second trial

2.3.2.1 Aim of the test

The aim was to test longer baking times, 20 and 22 minutes.

2.3.2.2 Pictures

2.2.1.1.2 Results and conclusion

1) The 24-min sample, formation is good but Aw=0.8202 which is unacceptable.
2) The 26-min sample, a bit over-cooked but Aw=0.7608 which is still too high.
3) The above results showed that there was a limitation when increasing baking time at the constant temperature which could be caused by the limitation of water evaporation speed in the tested environment. Therefore a drying procedure was necessary to reach the desired Aw level.

2.3.3 The Third Trial, Drying test

2.3.1 Aim of the test

Based on the results from the 2nd test, this drying tested was conducted to determine/estimate the drying time for Treat Formula 2. The samples were from the 2nd test.

2.3.2 Method

A modified Air-Oven method was adopted.

1) Accurately weigh aluminium moisture dishes. Place the sample in each dish, and quickly reweigh. 2) Place the dish (with contents) in the oven, the air oven is set at 50 °C. 3) Take out the dishes every 30 minutes and quickly weigh them. 4) Determine the Aw value.

2.2.1.1.3 Drying curve

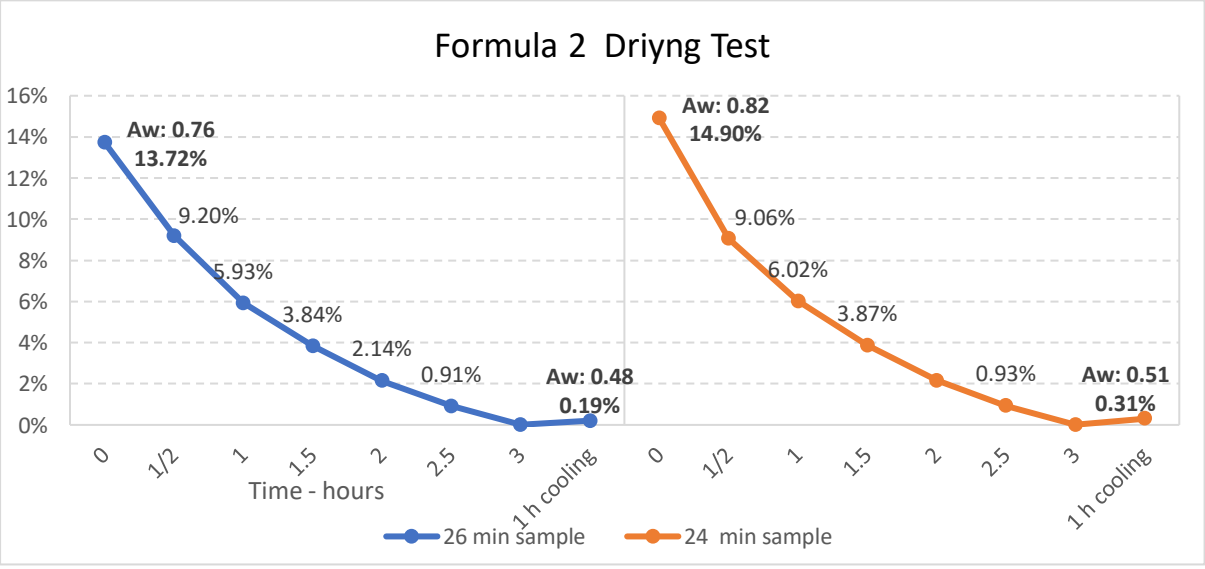


Figure 0-3 Drying curve of the 24-min and 26-min samples (raw data in Appendix C 1.4.

2.3.3 Results and conclusion

1) From figure 6-12, it was roughly estimated the minimum required drying time was between 30~60 minutes.

2) A 40-minute drying test following a baking test with a 24-minute baking time was planned to be conducted.

2.3.4 The fourth trial, the test of baking followed by 40 minutes of drying

2.3.4.1 Aim of the test

This test was conducted to verify the effect of a 40-minute drying after the 24-minute baking.

2.3.4.2 Method

1) The sample was baked at 150 °C for 24 minutes. 2) After baking, the samples were split into two batches. 3) The first batch (A batch) was kept for the Aw and moisture content test. 4) The second batch (B batch) was dried at 50 °C for 40 minutes and be cooled for Aw and moisture content test.

2.3.4.3 Picture

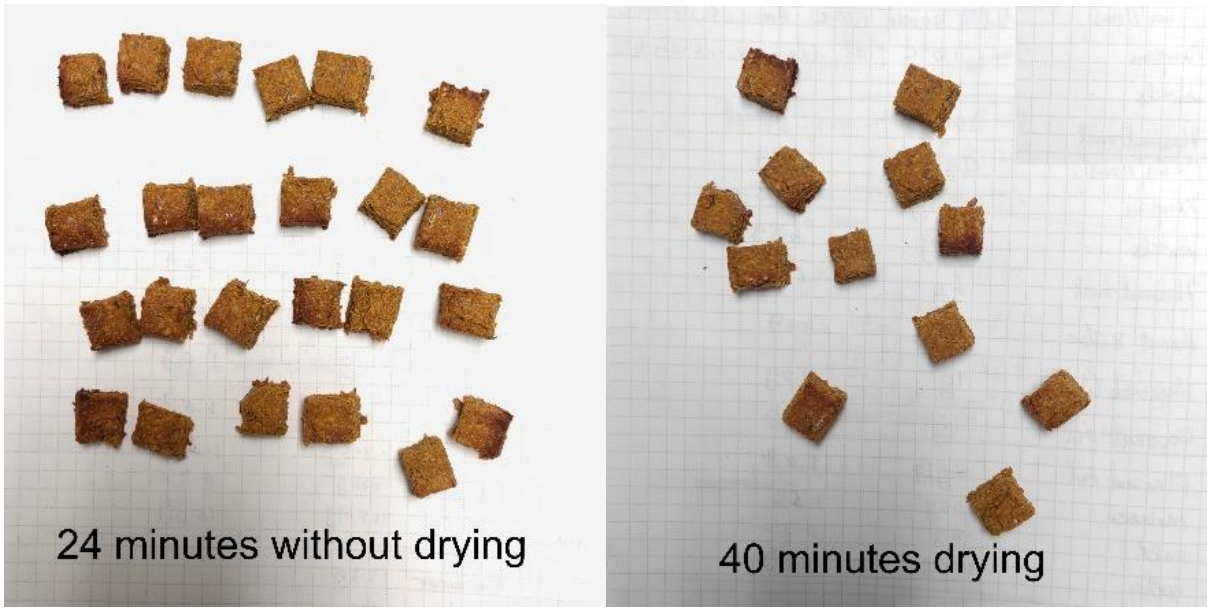


Figure 21 Samples before and after drying.

2.3.4.4 Results and conclusion

1) 24-min sample, Aw=0.7745 at 20.10 °C, water content = 17.06%
2) The sample after 40-min drying, Aw = 0.6580, water content = 13.46%.
3) The result showed that drying after baking can effectively reach the target Aw level.

Baking trials at 100°C

2.3.5 The fifth test

2.3.5.1 Aim of the test

This test was aimed to try a lower baking temperature to observe the color and water activity changes when baked at 100 °C oven temperature.

2.3.5.2 Methods

1) The cube-shaped samples were split into three batches.

2) The first batch (A batch) was baked, and samples (3-4 cubes) were taken out every 3 minutes from the 58 minutes for the water activity test.

3) The second batch (B batch) was baked continuously for 97 minutes and was tested against water activity.

4) The third batch (C batch) was baked continuously for 106 minutes and was tested against water activity.

2.3.5.5 Aw changes over time of A batch

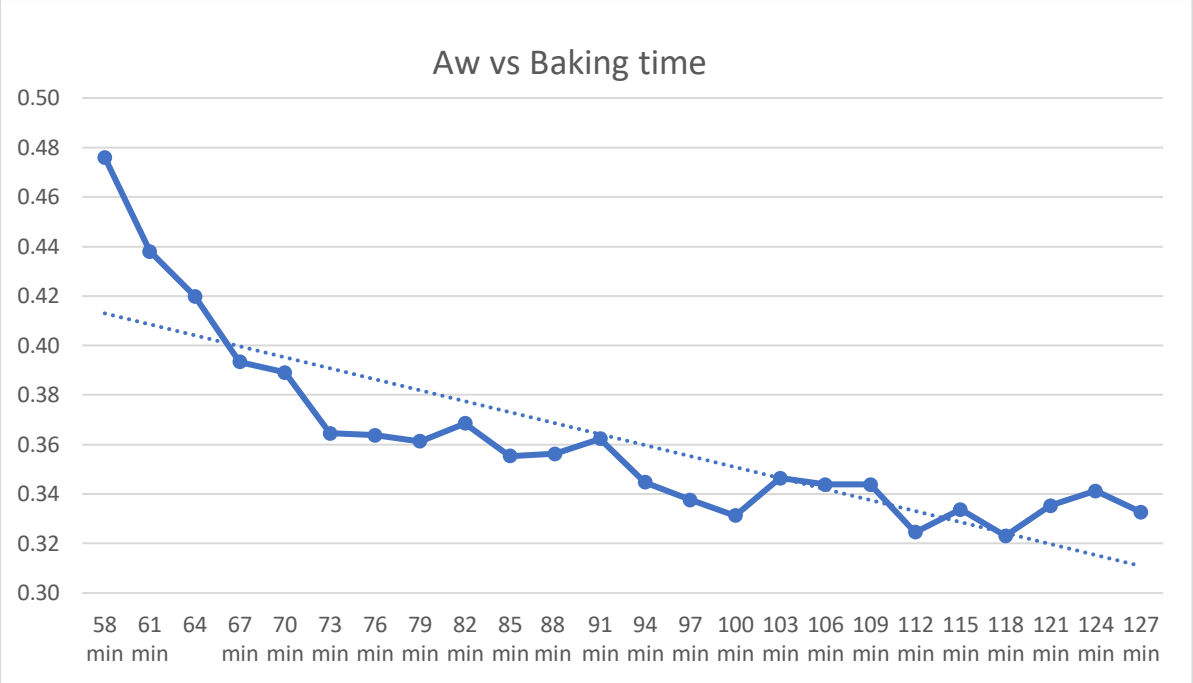
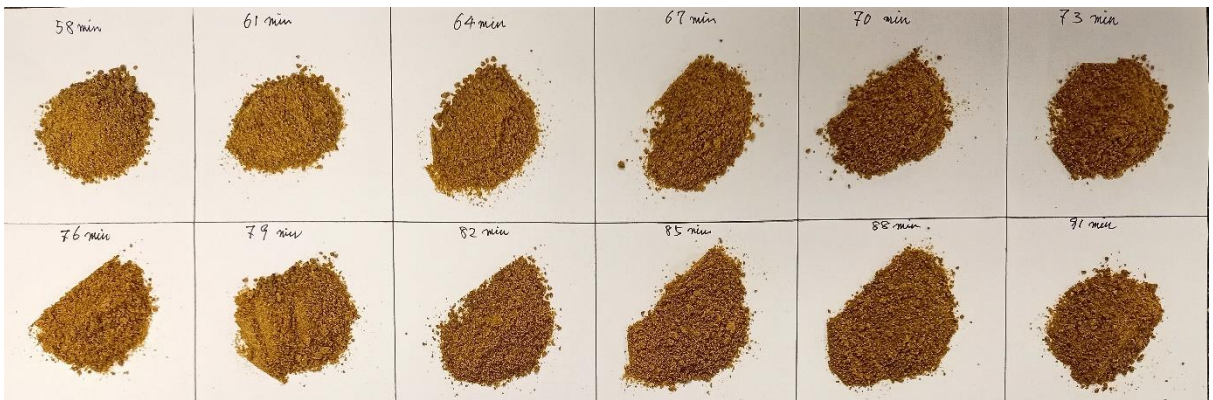


Figure 0-7 Aw values over time of A batch (*raw data is in Appendix C 1.5).

2.3.5.6 Color changes over time



2.3.5.7 Water activity values of B and C batch

1) The Aw value of the B batch (97 minutes continuous baking) was 0.2386 at 19.97 °C.

2) The Aw value of the C batch (106 minutes continuous baking) was 0.2255 at 19.97 °C.

2.3.5.8 Results and conclusion

1) The observation of color changes at 100 °C oven temperature was difficult. This was consistent with tests 3.2.10 and 3.3.6.
2) The Aw test showed that the Aw value decreased continuously over time which means the required Aw value could be reached given enough time.
3) The Aw values of the B and C batch were lower than the samples which were taken out during the A batch test with close baking time. This is discussed in the final discussion part.

2.4 Discussion and conclusion

2.4.1 Treat formula 1

The estimated proper baking time is shown in Figure 6-16. At 150 °C the estimated time range was 27-31 minutes.

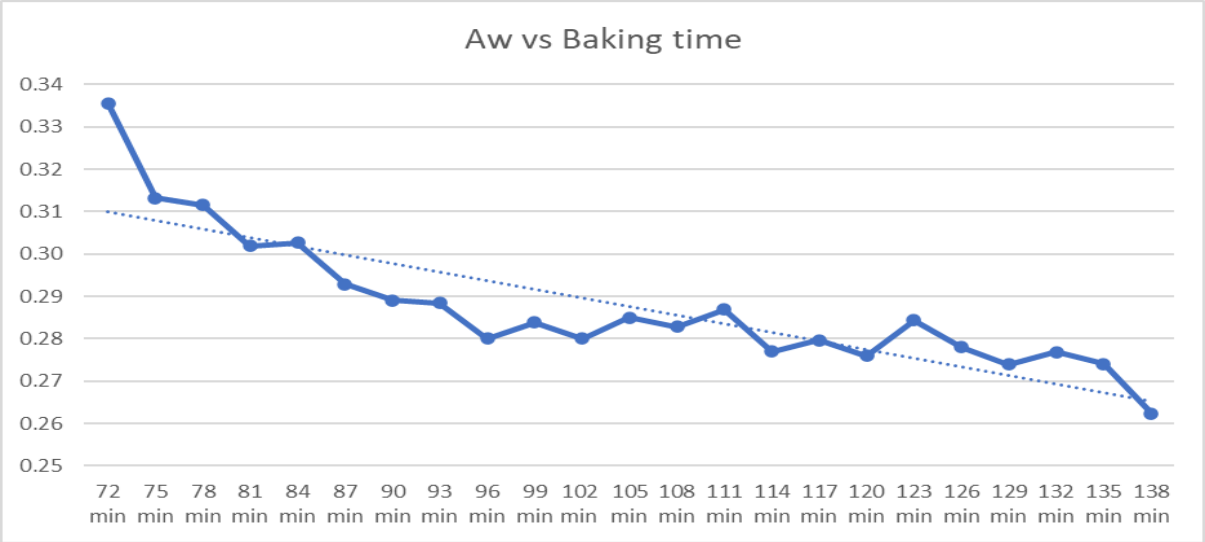


Figure 0-8 Baking time vs oven temperature (TF1).

*The width of the bar indicates the baking time.

In the 3rd trial, the 25- and 27-minute baking times were tested, the resulting Aw value was 0.75 and 0.68 respectively. Given the target-Aw was <0.65 and there were possible fluctuations of the actual temperatures in the oven, the lower limit was estimated as above 27 minutes. In the 4th test, the 29- and 31- minute baking times were tested, the resulting Aw value was 0.55 and 0.49. Taking into account that the 31-minute sample was a bit overcooked, the upper limit was estimated as no more than 31 minutes.

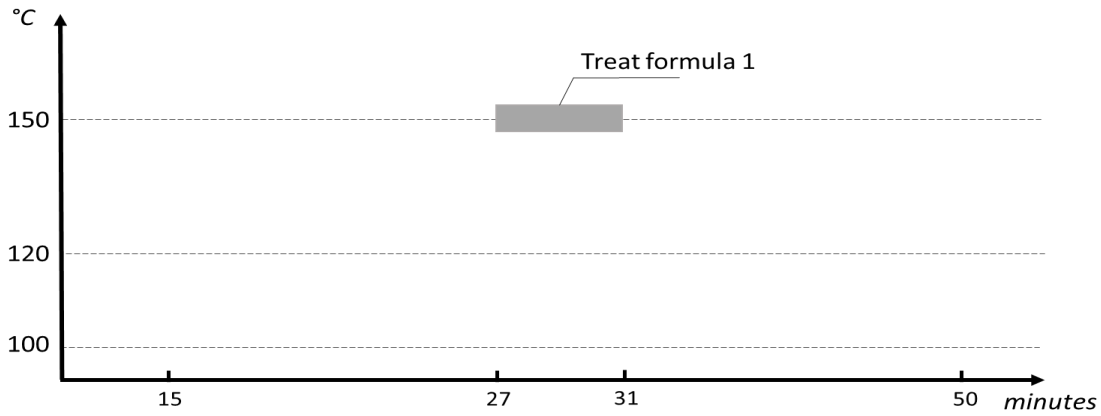


Figure 0-9 Baking time vs oven temperature (TF1).

*The width of the bar indicates the estimated range of baking time.

2.4.2 Treat formula 2

The estimated proper baking time is shown in Figure 0-10. At 150 ℃ the estimated time range was 21-30 minutes baking plus oven drying.

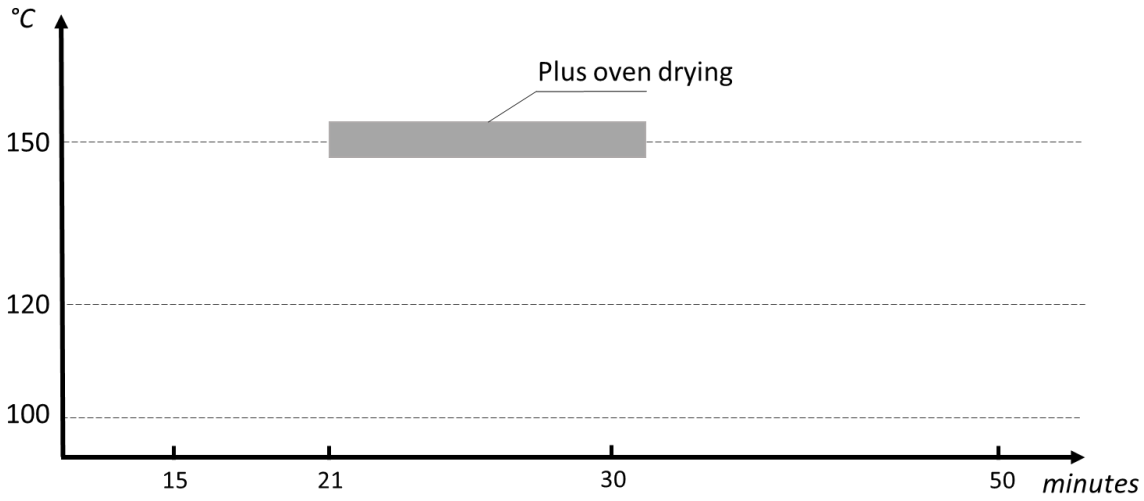


Figure 0-10 Baking time vs oven temperature (TF2).

*The width of the bar indicates the estimated range of baking time.