CHICKPEA AQUAFABA AS AN EMULSIFIER IN FOOD-OIL SYSTEMS

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ABSTRACT

Aquafaba (AQ) is a viscous liquid produced during boiling or pressure-cooking chickpea seed in water. This by-product solution has been gaining popularity since 2014, when a blogger used AQ as an egg replacement in a vegan meringue. Due to its foaming and emulsion properties, AQ is increasingly being used as an egg replacement to add functionality to food products, such as mayonnaise, ice cream, pudding, and baked goods. The objective of this study is to select a chickpea cultivar that produces superior AQ for production of food oil emulsions as well as standardize AQ production. Seed of five chickpea cultivars were selected for AQ production: CDC Leader, CDC Orion, CDC Consul, CDC Luna, and Amit. Emulsion capacity and stability of AQ derived from different chickpea cultivars were determined. Physicochemical properties of different chickpea cultivars, such as hundred seed weight, seed coat incidence, seed dimensions, hydration kinetics, and proximate composition (moisture, ash, protein, fat, carbohydrate, and crude fibre) were also measured to determine possible correlations of these parameters with AQ emulsion properties. The effects of soaking and boiling of chickpea seed on yield and properties of AQ were determined. After selection of optimum conditions for preparing AQ, five drying methods (freeze drying, spray drying, oven drying, rotary evaporation drying, and vacuum drying) were compared. Dried AQ was rehydrated with distilled water, and its emulsion capacity and stability were measured. AQ from CDC Leader had the greatest emulsion capacity (1.10 ± 0.04 m^2 g^{-1}) and stability ($71.9 \pm 0.8\%$) and produced the most stable food oil emulsions. There were no correlations observed between AQ emulsion properties and chickpea seed chemical properties. On the other hand, AQ emulsion properties were negatively correlated with AQ yield and moisture content, indicating that AQ with higher dry-matter content displayed better emulsion properties. Among the treatments tested, soaking chickpea seed in 4 °C water for 16 h followed by cooking for 30 min produced AQ with the greatest emulsification properties. Spray drying achieved a high drying rate and drying efficiency, so it was a superior method for AQ production. In conclusion, AQ can simulate the function of egg in food oil emulsions and can be prepared from all cultivars tested but cooking conditions and genotype must be controlled to produce a consistent product.

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LIST OF ABBREVIATIONS

A	Absorbance
AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AQ	Aquafaba
CDC	Crop Development Centre
cP	Centipoise
CVD	Cardiovascular disease
DL	Detection limit
DLVO	Derjaguin and Landau; Verway and Overbeek theory
EC	Emulsion capacity
ED	Equivalent dimensions
EA	Emulsion activity
EAI	Emulsifying activity index
ES	Emulsion stability
HDL	High-density lipoproteins
HLB	Hydrophile-lipophile balance
HMW	High molecular weight
HSP	Heat shock protein
HSW	Hundred seed weight
LDL	Low-density lipoproteins
LEAP	Late embryogenesis abundant protein
LMW	Low molecular weight
ND	Not detected
OSA	Octenyl succinic anhydride
o/w	Oil in water
PC	Phosphatidylcholine
PLs	Phospholipids
SCI	Seed coat incidence

SD	Standard deviation
SDS	Sodium dodecyl sulfate
SSA	Specific surface area
T	Turbidity
tRNA	Transfer RNA
UV	Ultraviolet
Vis	Visible
w/o	Water in oil
WSA	Seed coat weight per surface area
WSC	Water-soluble carbohydrates

LIST OF SYMBOLS

ΔA	The increase in interfacial area of droplets
ΔG_{form}	The free energy of emulsion formation
$\emptyset$	Oil volume fraction of dispersed phase
p	Calculated probability
r	Pearson correlation coefficient
$T\Delta_{sconfig}$	The configurational entropy of emulsion droplets
γ	The interfacial tension

1. INTRODUCTION

1.1 Overview

Food oil emulsions are significant components of food. Mayonnaise is an edible food emulsion that has grown in popularity since early 1900's. Mayonnaise is a semi-solid oil-in-water (o/w) emulsion made from a mixture of egg yolk, edible vegetable oil, acetic or citric acid, and other ingredients such as salt, spice, natural sweetener, and various natural flavored ingredients (Harrison and Cunningham, 1985; Depree and Savage, 2001). Typically, mayonnaise oil content must be not less than 65% by weight, and the product should contain at least 2.5% acetic or citric acid by weight. The latter might be included in the form of vinegar or lemon juice.

Egg yolk is a typical ingredient in mayonnaise, though occasionally egg white has also been used in making this product as these egg components are efficient natural emulsifiers (Baldwin, 1977; Corran, 1943) for a variety of o/w emulsions. The high emulsifying capacity of egg is related to the phospholipids (PLs) (lecithin), lipoproteins (low-density lipoproteins, LDL, and high-density lipoproteins, HDL), and non-associated proteins (livetin and phosvitin) (Moros et al., 2002; Laca et al., 2010). These proteins have amphiphilic properties and act as surface-active substances (also known as surfactants) in multiphase systems, such as mayonnaise.

Unfortunately, egg is one of the most frequent causes of food allergies, especially in infants and young children (Park et al., 2017). Egg allergens are mainly present in egg white. Ovalbumin, constituting 54% of egg white protein (Park et al., 2017), is one of the major egg allergens (Duan et al., 2017; Abeyrathne et al., 2013). In addition, egg is not suitable for consumers with special dietary restrictions and those that cannot eat egg for religious reasons or personal lifestyle choices (Lin et al., 2017). Moreover, egg yolk contains cholesterol (5.2% of total lipids) which is linked to cardiovascular disease. Although a cholesterol limit is not mentioned in the 2015-2020 Dietary Guideline for Americans, it is still recommended that the elderly and people with previous incidents of heart disease limit dietary cholesterol intake (Lin et al., 2017). Furthermore, a segment of consumers cites environmental concerns related to egg production as a rationale to avoid egg

consumption (Janssen et al., 2016). Therefore, many scientists and food processing companies are developing innovative new egg replacements to cater to a growing demand for egg alternatives.

Aquafaba (AQ) is the viscous liquid resulting from cooking chickpea seed or other legumes in water. AQ has been gaining popularity since 2014, when a novel recipe blogger used the leftover liquid from a chickpea can as an egg replacement in vegan meringue (Révolution végétale, 2014). Due to its desirable foaming and emulsification properties, AQ is now widely used by the vegan community as an egg replacement in many food products such as mayonnaise, meringues, and baked goods. Chickpea AQ components have been identified by Shim et al. (2018). Its applications as a foaming agent have been reported in several studies (Stantiall et al., 2018; Mustafa et al., 2018). However, the substances conferring AQ egg-similar emulsion properties have not been fully elucidated. Meanwhile, AQ qualities differ among diverse cooking conditions and legume genotypes. Therefore, chickpea cultivar selection and AQ process standardization are required to assure the quality of both AQ and AQ-based emulsion.

1.2 Hypothesis

Based on previous studies and AQ functional properties, the central hypothesis of this research is that AQ emulsion properties not only differ among chickpea cultivars, cooking conditions and drying methods, but also have correlations with chickpea seed components and physicochemical properties.

1.3 Objectives

The primary objective of this study is to select AQ made from a desirable chickpea cultivar to produce food oil emulsions and standardize the AQ production process.

The specific goals of this study are as follows:

1) to determine which chickpea cultivar produces AQ with the best emulsion properties;
2) to determine grain composition and physicochemical properties of the different chickpea cultivars used in this study;
3) to determine correlations among AQ emulsion properties, chickpea composition, and chickpea physicochemical properties; and
4) to standardize the conditions for AQ preparation and compare the influences of different commercial drying methods on AQ emulsion properties.

2. LITERATURE REVIEW

2.1 Chickpea

Chickpea (*Cicer arietinum* L.), also called garbanzo bean or Bengal gram, is traditionally cultivated on marginal lands in arid and semi-arid regions of the world (Vineeth et al., 2016; Rao et al., 2002). Chickpea annual global production is 14.0 million tonnes (FAOSTAT, 2017) making it the fourth most important legume crop as a source for protein. India, the largest producer, contributes about 70% of the world's total production and is also the greatest consumer. Canada is the tenth largest producer and produced 12,300 tonnes in 2014 (FAOSTAT, 2017). Based on the seed size and colour, there are two major commercial classes of cultivated chickpea: Desi (Indian origin) and Kabuli (the Mediterranean and Middle Eastern origin). Kabuli cultivars produce white to cream-yellow colour seed that is used widely as cooked whole seed, whereas the Desi cultivar seed can be brown, fawn, orange, or green and is wrinkled at the beak (Chavan et al., 1989). Desi types account for 80-85% of the total chickpea area and are more tolerant to abiotic stress than Kabuli types (Vineeth et al., 2016).

Chickpea is an essential legume crop cultivated worldwide as a rich source of carbohydrate and protein. It is an essential food for growing populations, especially in developing countries. It has also been widely used as green manure and animal fodder (Ahmad et al., 2016). Carbohydrate and protein constitute about 80% of chickpea seed dry mass (Wood, 2010; Geervani, 1991). It has little cholesterol, and is a good source of dietary fibre (DF) (18-22 g/100 g of raw chickpea seed) (Aguilera et al., 2009b; Tosh and Yada, 2010), minerals (Ca, Mg, P and K) and vitamins (riboflavin, niacin, thiamin, folate and ß-carotene; Wood and Grusak, 2007; Jukanti et al., 2012). Chickpea major carbohydrates are presented in Table 2.1. Some monosaccharides have also been reported (Sánchez-Mata et al., 1998), including ribose (0.13 g/100 g), fructose (0.26 g/100 g), glucose (0.065 g/100 g), and galactose (0.05 g/100 g). Maltose (0.60%) and sucrose (1.0-2.0%) are the most abundant free chickpea disaccharides (Wood and Grusak, 2007).

Furthermore, α-galactosides account for about 63% of chickpea total sugar content (Sánchez-

Mata et al., 1998) with ciceritol and stachyose constituting 36-43% and 25%, respectively, of total sugar in chickpea seed (Sánchez-Mata et al., 1998; Aguilera et al., 2009b). Starch is the main chickpea storage polysaccharide (Wood and Grusak, 2007). Its content varies from 41 to 50% of total carbohydrate (Pearson et al., 1981; Dalgetty and Baik, 2003; Özer et al., 2010). About 35% of total starch is resistant starch while the remainder 65% is available starch (Özer et al., 2010; Aguilera et al., 2009a; Aguilera et al., 2009b).

Table 2.1 Different carbohydrate fractions in chickpea seed (Jukanti et al., 2012).

Carbohydrates	Åman (1979)[‡§]	Wang and Daun (2004)[*]		Han and Baik (2006)[†]	Aguilera et al. (2009b)[§‖]
		Kabuli	Desi	Kabuli	
Starch	-	41.1 (38.2-43.9)	36.4 (33.1-40.4)	-	51.9
Sucrose	4.3	3.8 (3.10-4.41)	2.0 (1.56-2.85)	-	15.2
Raffinose	1.00	0.6 (0.48-0.73)	0.5 (0.46-0.77)	50.2	3.2
Stachyose	2.8	2.2 (1.76-2.72)	1.6 (1.25-1.98)	27	17.7
Verbascose	Traces	-	-	ND	-
Ciceritol	-	-	-	67.7	27.6
Fructose	0.1	-	-	-	3.1
Galactose	-	-	-	-	0.1
Galactinol	0.5	-	-	-	-
Glucose	0.1	-	-	-	0.5
Maltose	-	-	-	-	3.3
Manninotriose	3.4	-	-	-	-
Pinitol	0.2	-	-	-	-

- not measured.
ND not detected.
[*]Expressed as g/100 g dry weight. Number in parentheses indicate a range.
[†]Expressed as mg/g.
[‡]Expressed as a percentage of raw seed dry weight.
[§]The type of chickpea is not specified.
[‖]Expressed as g/kg.

Chickpea protein content was reported to be 207 g/kg as an average of eight annual wild

species (Ocampo et al., 1998). Ashur et al. (1973) and Jukanti et al. (2012) demonstrated that chickpea meets individual adult requirements for all essential amino acids except for sulphur-rich amino acids methionine and cystine.

Chickpea oil content varies from 3.8 to 10% (Singh, 1985; Gül et al., 2008). Chickpea oil has linoleic acid, oleic acid, tocopherols, sterols and tocotrienols (Zia-Ul-Haq et al., 2007), with major sterols being sitosterol (73-76% of phytosterols) and campesterol (12-14% of phytosterols), and α-tocopherol (8.2 mg/100 g) being the major tocol (Muhammad Zia-ul-HAQ, 2009).

Two important phenolic compounds found in chickpea seed are the isoflavones biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) and formononetin (7-hydroxy-4'-methoxyisoflavone) (Wood and Grusak, 2007). The concentration of biochanin A in Kabuli-type seed (1420-3080 μg/100 g) is higher than that in Desi-type seed (838 μg/100 g) (Mazur et al., 1998). The amount of formononetin in Kabuli- and Desi-type seed is 215 μg/100 g and 94-126 μg/100 g, respectively (Mazur et al., 1998).

Additionally, due to its content of vitamins, minerals, and bioactive compounds (phytates, phenolic compounds, oligosaccharides, enzyme inhibitors, etc.) chickpea might impart several potential health benefits such as protecting against cardiovascular disease (CVD), type 2 diabetes, digestive disease, and some forms of cancer (Jukanti et al., 2012).

2.2 Emulsification Principle

An emulsion is a two-phase system of immiscible liquids (Lynch and Griffin, 1974). One phase is often in the form of finely divided droplets with diameters generally larger than 0.1 μm (Becher, 1957). This dispersed, internal, or discontinuous phase is suspended in the continuous, or external phase. A high internal-phase-ratio emulsion, such as mayonnaise, is formed when the internal phase volume is greater than that of the external phase. Food emulsion systems are generally divided into two categories: oil-in-water (o/w) emulsions and water-in-oil (w/o) emulsions. Mayonnaise is a typical o/w emulsion which means it consists of oil droplets dispersed throughout a mostly aqueous medium.

Food emulsifiers have an amphiphilic nature so they can absorb at the interface between oil and water phases to form an interfacial film around droplets, thus lower the oil/water interfacial tension and impart emulsion stability (Das and Kinsella, 1990). Two models, based on thermodynamics and interactions, have been developed to describe mechanisms of emulsification.

2.2.1 Thermodynamic Theory

Energy is required for emulsion formation to disperse one phase into the other. During dispersion, the total droplet interfacial area increases, and energy creates a new interfacial area. The free energy of emulsion formation (ΔG_{form}) is represented by Equation 2.1 (Tadros and Vincent, 1983; Chattoraj and Bridi, 1984):

Equation 2.1 The free energy of emulsion formation

$$\Delta G_{form} = \gamma \Delta A - T\Delta S_{config}$$

in which, γ is the interfacial tension, ΔA is the increase in interfacial area of droplets, and $T\Delta S_{config}$ is the configurational entropy of emulsion droplets.

For an o/w emulsion, droplet interfacial area is much larger than initial surface area of unemulsified oil, i.e., ΔA is very large, γΔA becomes larger than $T\Delta S_{config}$, and ΔG_{form} is positive. Emulsion systems are thermodynamically unstable due to this excess energy. Therefore, the systems tend to reduce energy by decreasing total surface area to a minimum, usually by one or more mechanisms. Due to the large fat content in mayonnaise, coalescence is a primary problem for emulsion stability (Jaynes, 1985).

2.2.2 Interaction Theory

Generally, attractive forces tend to destabilize emulsions whereas repulsive forces impart stability. Derjaguin and Landau (1941) and Venway and Overbeek (1948) independently developed the DLVO (Derjaguin Landau Venway Overbeek) theory to explain the basic theory of emulsion stability. Several interactions cooperate within emulsion systems to determine both thermodynamic and kinetic stability: 1) van der Waals interaction (attractive); 2) electrostatic interaction (repulsive); 3) steric interaction (repulsive); 4) hydration force (repulsive); and 5) miscellaneous interactions.

A schematic representation of the potential DLVO theory is shown in Figure 2.1. It can be observed that the emulsified system needs to pass through an energy barrier to reach the minimum energy state described in thermodynamic theory. The higher the energy barrier, the slower the loss of interfacial surface area, and the better the emulsion stability.

Although emulsions are thermodynamically unstable systems, it is necessary to have reasonable stability (weeks to months) for food use. Food emulsion stability declines with time. In

order to predict food stability in the development of food products, experimentation is necessary to practically assess emulsion stability. Stability requirements vary among different food emulsions. Mayonnaise emulsions, for example, are expected to remain stable for over a year. Long-term stability is usually provided by employing macromolecules, such as proteins and polysaccharides (Dickinson et al., 1988; Charalambous and Doxastakis, 1989).

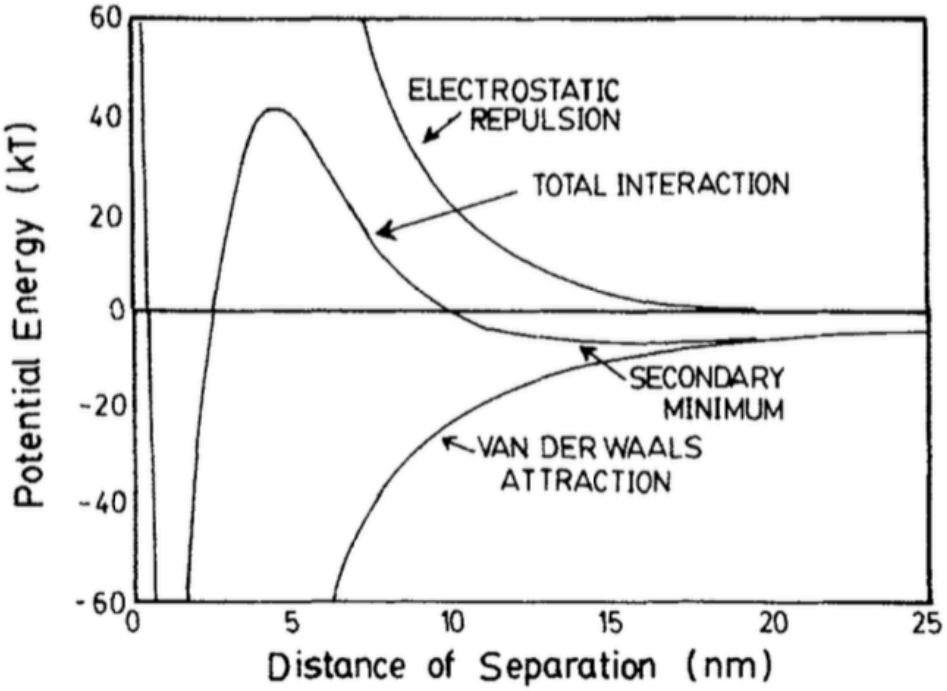


Figure 2.1 Potential energy of interaction versus distance of separation for van der Waals attraction, electrostatic repulsion, and total interaction according to DLVO theory (Parker and Krog, 1987 with permission).

Emulsifiers and stabilizers can be added to increase emulsion stability through several mechanisms (Nawar, 1985). The main mechanism of increased emulsion stability is the reduction of interfacial tension by emulsifiers. The emulsifier joins the oily and aqueous phases of an emulsion into a homogeneous and stable preparation (Waginaire, 1997). Addition of an emulsifier reduces surface tension at an oil-water interface and increases emulsion stability. An emulsifier contains two portions: the lipophilic (oil-loving) portion and a hydrophilic (water-loving) portion. These two portions orient themselves with the emulsion oil or water phase and form a shell around dispersed phase droplets. In this way, emulsifiers prevent dispersed droplets from coalescing and separating, thus increasing emulsion stability (Lynch and Griffin, 1974). The other mechanisms

imparted by emulsifiers and stabilizers are as followed: 1) the repulsive force between droplets with similar surface electrical charge; 2) the formation of mesophases or liquid-crystalline phases which provide the most stable configuration for a specified set of conditions; 3) the inclusion of macromolecules or particulate materials, such as gum and protein, can interact with emulsion components to substantially increase its viscosity and stability.

Conditions present when an emulsion is formed influence emulsion stability. For example, emulsion constituents, emulsifier concentration, emulsion temperature, and emulsifier physical state (crystalline versus fluid) are all critical in determining its stability. The order of addition of constituents can also affect emulsion stability (Friberg and Mandell, 1970). Additionally, the nature of internal and continuous phases is also a crucial factor. For example, it has been found that emulsions prepared with unsaturated fatty acid-based emulsifiers and unsaturated triglyceride oil, or with saturated emulsifiers and saturated triglyceride oil, are more stable than those prepared with emulsifiers and oil with intermediate or mixed saturation (Garti and Remon, 1984).

2.3 Egg Composition and Emulsification Properties

Egg is a natural and efficient o/w emulsifier for a variety of emulsions (Baldwin, 1977; Corran, 1943). Egg is regarded as the most critical ingredient in mayonnaise due to its high emulsifying capacity and stability (Nikzade et al., 2012). Its performance is related to phospholipids (lecithin), lipoproteins (low-density lipoproteins (LDL) and high-density lipoproteins (HDL)), and non-associated proteins (livetin and phosvitin) (Moros et al., 2002; Laca et al., 2010).

The major lipid classes in yolk are triglycerides (62%), phospholipids (33%), and cholesterol (less than 5%). “Lecithin” is class of amphiphilic phospholipid substances that contains phosphatidylcholine (PC) as its major component when obtained from egg yolk (Wabel, 1998). In commercial practice, the term “lecithin” refers to a mixture of phospholipids, including PC and other entrained substances such as triglycerides and minor co-extracted lipids. Lecithin is widely used as an emulsifier in the food industry because of its ability to act as a surfactant in multiphase systems, such as mayonnaise. Phospholipids are amphiphilic molecules built on a glycerol structure which contain one hydrophilic head group substituent (a phosphoric acid) and one or two hydrophobic ester substituents (typically fatty acids). The phosphorous can be attached to other moieties such as choline, serine, ethanolamine, and inositol. These molecules can diffuse to interfaces between immiscible phases, orienting the hydrophobic tail to the non-polar lipid phase

and the hydrophilic head to the aqueous polar phase. In the presence of phospholipids, the interfacial tension decreases at oil-water interfaces thereby developing stable emulsions even though mechanical energy is applied to the system after emulsification (Huopalahti et al., 2013).

The interactions between lipid and protein can result in the formation of lipoprotein structures, which are the main constituents of egg yolk (McCully et al., 1962). The composition of egg yolk as a portion of dry matter is shown in Table 2.2 (Powrie and Nakaï, 1986).

Table 2.2 Composition of hen egg yolk (Powrie and Nakai, 1986).

	Yolk dry matter (%)	Yolk lipids (%)	Yolk proteins (%)	Lipids (%)	Proteins (%)
Yolk	100	100	100	64	32
Plasma	78	93	53	73	25
LDL	66	61	22	88	10
Livetins	10	-	30	-	96
Others	2	-	1	-	90
Granules	22	7	47	31	64
HDL	16	6	35	25	75
Phosvitin	4	-	11	-	95
LDLg	2	1	1	88	10

LDLg - LDL in granules.

Research has shown that LDL fraction can play a primary role in the formation and stabilization of yolk-based emulsions. The LDL fraction is comprised of large spherical particles (~35 nm diameter) with a core of triglyceride, cholesterol, and cholesteryl ester surrounded by a layer of apolipoprotein and phospholipid (Martin et al., 1964). Apolipoprotein is a major molecular class among LDL components and can adsorb at the oil-water interface (Kiosseoglou and Sherman, 1983a; Mizutani and Nakamura, 1984). Anton et al. (2003) extracted LDL from egg yolk and concluded that apolipoprotein showed a high proportion of amphipathic α-helix chains, explaining its capacity to absorb at the oil-water interface in emulsion due to the amphipathic character and flexibility.

Proteins, which constitute most of the interfacial film, have two primary functions in emulsion formation. First, protein could adsorb at the oil-water interface, thereby causing a substantial

decrease in interfacial tension. Secondly, they could form a mechanical barrier due to protein film viscoelastic properties, which protects against disruption. With these properties, proteins can control the colloidal interactions between coated oil droplets, thus having the potential to regulate aggregation and flocculation (Anton et al., 2003).

Molecular flexibility, solubility, and hydrophobicity are critical factors influencing protein impact on emulsions (Graham and Phillips, 1979; Kinsella, 1979; Nakai, 1983). Sell et al. (1935) also concluded that emulsification properties of egg yolk are not just due to one compound such as lecithin, but also determined by an unstable complex of lecithin and protein termed "lecitho-protein".

2.4 Egg Replacement Composition and Emulsification Properties

Many food emulsions, such as mayonnaise and salad dressing, have long used egg as an emulsifier. Nevertheless, due to egg allergies, specific dietary choices (e.g., vegan and vegetarian lifestyles), health considerations (e.g., limitation of dietary cholesterol) and environmental concerns consumers are searching for egg replacements. The egg replacement market, which includes products like pea protein, is estimated to reach 34.8 million USD by 2020 (Research and Markets, 2015). Many studies have identified effective egg replacements in different food products. Here we only discuss egg replacements as emulsifiers in food emulsions.

2.4.1 Emulsifier Hydrophile-lipophile Balance

Emulsifier performance is determined by the chemical composition and the ionization extent. As there are several important aspects of emulsifier performance, it is possible to select superior emulsifiers for specific applications based on the hydrophile-lipophile balance (HLB) value. These values range from 1 to 20 where w/o emulsions are formed by emulsifiers with lipophilic properties whose HLB number is below 9. Conversely, o/w emulsions are best formed with more hydrophilic emulsifiers with HLB numbers from 11 to 20. An intermediate HLB value, from 9 to 11, is classified as intermediate (Lynch and Griffin, 1974). The chemistry of the triglyceride oil used in forming emulsions interacts with the emulsifier and, therefore, the selection of HLB value is dependent on both the oil and emulsifier. For example, emulsifiers with HLB numbers from 7 to 12 are chosen to form o/w emulsions with corn or soybean oils, while an emulsifier with an HLB number of 5 will form an o/w emulsion with cottonseed oil (Powrie and Tung, 1976).

2.4.2 Egg Replacers

The emulsification properties of whey protein, lactoglobulins, and casein have been extensively investigated (McClements et al., 1993; Dickinson and Yamamoto, 1996). Moreover, some vegetable based materials stabilize food emulsions (McWatters and Holmes, 1979). Ghazaei et al. (2015) evaluated a reduced-fat mayonnaise formulation in which egg yolk was partially replaced with octenyl succinic anhydride (OSA)-modified potato starch. Such hybrid products had lower cholesterol content than egg yolk, but 100% substitution of egg yolk produced products with poor consistency and sensory scores compared to control mayonnaise products. Substitution of egg in mayonnaise with up to 50% soy milk produced products with similar viscosity, stability, and acceptability as a control sample containing only whole egg as an emulsifier (Kobra, 2014). Ghoush et al. (2008) employed iota-carrageenan and wheat protein as an emulsifier alternative to egg yolk in mayonnaise. This emulsifier alternative could replace 75% egg without significant changes on mayonnaise physicochemical properties including viscosity and stability. Nikzade et al. (2012) completely replace egg yolk and produced mayonnaise products with reduced cholesterol and fat by exploiting the interaction of gums (guar and xanthan) with soy milk and mono- and diglycerides. The product had similar characteristics when compared to full-fat mayonnaise prepared with egg yolk. In another approach, Raymundo et al. (2002) produced stable lupin protein-stabilized emulsions with xanthan gum. This emulsion had comparable properties to commercial brand mayonnaise. This literature indicates that replacing egg in food emulsions requires other thickening agents such as xanthan gum, guar gum, and glycerides.

2.5 Aquafaba

Aquafaba (AQ) is the viscous liquid remaining that can be drained from cooked chickpea or other legumes. In normal cooking this liquid is discarded. It is often suggested that AQ is therefore an inexpensive liquid resource that is readily obtained from canned or pressure-cooked chickpea. In the first report of AQ (Révolution végétale, 2014), it was described as a foaming agent that could replace egg white. AQ is predominately water (90-95%), which contains carbohydrate, protein and small molecules dissolved while chickpea seed is cooked. Chickpea polysaccharide, protein and small molecules found in AQ afford solutions with many functional properties that can produce stable foams, emulsions, gels, and thickened solutions. There are many reports of AQ in family recipes to replace egg and make fluffy food products, such as mayonnaise, meringue, mousse,

pudding, vegan cakes and whipped cream. More and more people recognize AQ as an effective egg replacer. AQ is not only an inexpensive and accessible resources that can be used as a cholesterol-free egg replacement, it also provides significant utility to the vegan community because it increases food options for vegans, such as eggless vegan cake, vegan mayonnaise, and vegan cream. Moreover, people who have an egg allergy might also benefit from AQ, as it affords more food product options, and at the same time avoids the risk of egg allergy.

2.5.1 AQ History

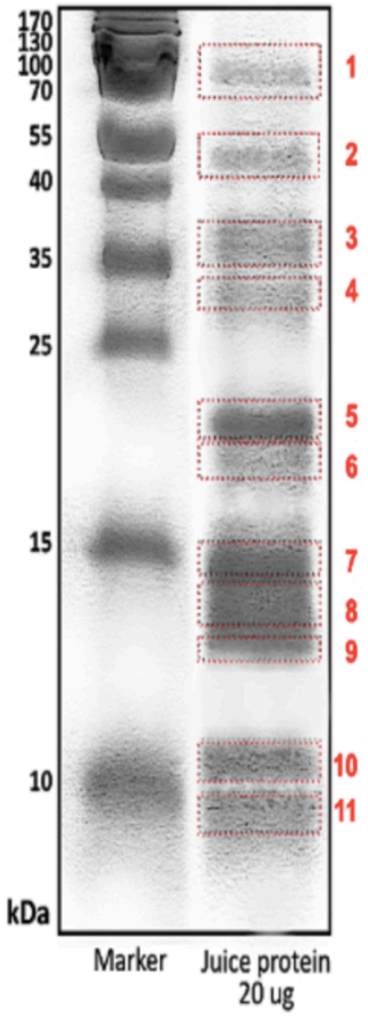
Pulses are usually steeped in water to hydrate the seed, then cooked by either boiling seed in water or cooking in a pressure cooker to soften the seed and improve digestibility. This process generates large volumes of liquid waste that requires treatment before it can be discarded. The viscous liquid waste separated from canned or pressured-cooked chickpea or other legumes such as pea and lentil has found new utility as the product now called aquafaba. AQ was initially described by Joël Roessel as a foaming agent to replace egg white in meringue (Révolution végétale, 2014). He produced AQ with red kidney bean. After Joël's discovery, Goose Wohlt, a software engineer in the US, optimized a vegan meringue recipe prepared with AQ, and posted his recipe to a vegan Facebook® page, What Fat Vegans Eat in February 2015 (Aquafaba, 2016). Since then, people's enthusiasm for AQ was ignited. Many AQ fans posted their recipes through social media such as YouTube® and Facebook®. For example, a new Facebook group was created titled: "Vegan Meringues – Hits and Misses!" especially for sharing AQ recipes and food products. AQ food pictures and recipes are now available on worldwide via the internet.

2.5.2 AQ Composition

AQ compositions consist of water (94%), carbohydrate (4%), protein (1.3%) and ash (0.6%). Shim et al. (2018) investigated AQ compositions recovered from 10 commercial canned chickpea products. AQ moisture content ranged from 93.0% to 95.1%. AQ carbon and protein content as a portion of dry matter ranged from 31.3% to 39.2% and 22.8% to 26.8%, respectively. The authors also identified proteins present in AQ (Figure 2.2). Stantiall et al. (2018) and Serventi et al. (2018) analyzed chemical compositions of AQ from haricot bean, garbanzo chickpea, whole green lentil, split yellow pea, and soybean (Table 2.3). AQ dry matter ranged from 3.28 g/100 g to 5.59 g/100 g. Similar concentrations of water-soluble carbohydrate (WSC) were measured (average 1 g/100 g

AQ) except for whole green lentil (0.61 g/100 g AQ), and yellow soybean (1.66 g/100 g AQ). The majority of WSC was low molecular weight (LMW) carbohydrate such as sugars. Based on the pulse origin of these materials, LMW carbohydrate should consist of sucrose, raffinose, and stachyose (Mokni et al., 2015; Bach, 1997). High molecular weight (HMW) carbohydrate present in AQ was largely regarded to be soluble fibre. Insoluble fibre can constitute a significant portion of the dry matter of AQ made from pulses (0.93 g/100 g haricot bean AQ to 2.46 g/100 g yellow soybean AQ). Based on previous studies, insoluble fibre is likely to be mostly hemicellulose (Önning et al., 1994; Vasishtha and Srivastava, 2013). Starch was not detected by the iodine test in any AQ preparations.

A



B

Gel	Protein name	MW(kDa)	Accession No.
1a	tRNA (Cytosine-5-)-methyltransferase	92.9	A0A163M953
1b	O-acyltransferase	92.1	A0A163IUM5
2a	Provicilin	51.5	Q304D4
2b	Legumin	56.7	Q9SMJ4
3a	Uncharacterized protein	36.0	A0A163HIE9
3b	Oxidoreductase	36.3	A0A163HIE9
4	Dehydrin 1	20.4	Q8GUS4
5	LEAP 2	16.7	O49817
6	LEAP 2	16.7	O49817
7a	LEAP 2	16.7	O49817
7b	LEAP 4	15.7	E7BSD7
7c	Superoxide dismutase [Cu-Zn]	15.3	Q9ZNQ4
7d	LEAP	13.2	A0A076KXB6
8a	LEAP	13.2	A0A076KXB6
8b	Superoxide dismutase [Cu-Zn]	15.3	Q9ZNQ4
8c	LEAP 4	15.7	E7BSD7
8d	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
8e	Histone H3	15.4	A0A163J7K4
8f	LEAP 2	16.7	O49817
8g	Histone H2B	14.9	A0A163DU27
8h	Histone H2AX	14.6	O65759
9a	LEAP	13.2	A0A076KXB6
9b	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
9c	Putative Pi starvation-induced protein	13.9	O65757
10a	18.5 kDa class I HSP (Fragment)	10.1	A0A0B5Z4W5
10b	Non-specific lipid-transfer protein	13.8	A0A076KXC0
11	Defensin	8.8	Q2I2W0

Figure 2.2 (A) SDS-PAGE separation of chickpea AQ protein; (B) Potential protein identification per band. LEAP = late embryogenesis abundant protein, HSP = heat shock protein (Shim et al., 2018 with permission).

Table 2.3 Proximate composition and saponin content of AQ (Serventi et al., 2018; Stantiall et al., 2018).

Nutritional information	Haricot bean	Garbanzo chickpea	Whole green lentil	Split yellow pea	Yellow soybean
Dry matter (g/100 g)	3.28 ± 0.5^{d}	5.13 ± 0.02^{a}	4.69 ± 0.02^{b}	4.41 ± 0.18^{c}	5.59 ± 0.10^{a}
LMW (g/100 g)	0.73 ± 0.03^{c}	1.20 ± 0.02^{a}	0.54 ± 0.03^{d}	1.02 ± 0.03^{b}	1.66 ± 0.03
HMW (g/100 g)	0.16 ± 0.20^{a}	0.04 ± 0.00^{c}	0.07 ± 0.00^{b}	0.09 ± 0.00^{b}	
Insoluble fibre (g/100 g)	0.93 ± 0.05^{d}	2.37 ± 0.02^{a}	2.09 ± 0.02^{b}	1.63 ± 0.18^{c}	2.46 ± 0.10^{a}
Protein (g/100 g)	0.70 ± 0.00^{d}	0.95 ± 0.01^{c}	1.51 ± 0.01^{a}	1.27 ± 0.02^{b}	0.68 ± 0.00^{d}
Fat (g/100 g)	< DL	< DL	< DL	< DL	< DL
Ash (g/100 g)	0.75 ± 0.03^{a}	0.57 ± 0.01^{b}	0.48 ± 0.00^{c}	0.40 ± 0.00^{d}	0.78 ± 0.02^{a}
Saponins (mg/g)	5.9 ± 0.5^{b}	4.5 ± 0.6^{b}	12 ± 1^{a}	4.7 ± 0.4^{b}	6.4 ± 0.6^{b}

The acronyms LMW and HMW refer to water-soluble carbohydrates and refer to low molecular weight and high molecular weight, respectively. Only total water-soluble carbohydrates were measured for yellow soybean. The symbol < DL means below the detection limit. Different letters in the same row show a significant difference ($P < 0.05$) between measurements in the same row.

The protein content of AQ preparations ranged from 0.68 g/100 g for AQ prepared from yellow soybean to 1.51 g/100 g for AQ from whole green lentil. Fat was not detected in any AQ preparations. Total ash content ranged from 0.40 g/100 g (split yellow pea AQ) to 0.78 g/100 g (yellow soybean AQ; Stantiall et al. 2018). Previous studies of mineral loss from soybean after boiling support these findings (Tarafdar and Yadav, 2008). This study identified significant losses of calcium (from 2800 to 2400 mg/kg), magnesium (from 1300 to 1100 mg/kg) and iron (from 0.34 to 0.31 mg/kg) in soybean. Damian et al. (2018) determined AQ mineral composition (Table 2.4). Calcium was present in all AQ preparations (3.1-7.5 mg/100 g AQ). Potassium concentration ranged from 105 mg/100 g for whole green lentil AQ to 210 mg/100 g for haricot bean AQ. Garbanzo chickpea AQ had considerable concentrations of magnesium (24 mg/100 g AQ) and

phosphorous (37 mg/100 g AQ). Whole green lentil AQ contained a 30 mg/100 g sulfur. In addition, other minerals detected in AQ at lower concentrations included copper, iron, manganese, sodium, and zinc. For example, yellow soybean AQ contained 9.6 mg/100 g of calcium, 319 mg/100 g of potassium, 38 mg/100 g of magnesium, and 1.3 mg/100 g of iron.

Table 2.4 Minerals in AQ (Damian et al., 2018; Serventi et al., 2018).

Mineral content (mg/100 g AQ)	Haricot bean	Garbanzo chickpea	Whole green lentil	Split yellow pea
Ca	7.5 ± 0.1^{a}	6.2 ± 0.0^{b}	4.3 ± 0.1^{c}	3.1 ± 0.0^{d}
Cr	< DL	< DL	< DL	< DL
Cu	0.06 ± 0.00^{d}	0.10 ± 0.00^{b}	0.17 ± 0.00^{a}	0.09 ± 0.00^{c}
Fe	0.73 ± 0.00^{a}	0.60 ± 0.01^{b}	0.72 ± 0.03^{a}	0.51 ± 0.01^{c}
K	210 ± 1^{a}	193 ± 2^{b}	169 ± 2^{c}	105 ± 2^{d}
Mg	24 ± 0^{a}	18 ± 0^{b}	14 ± 0^{c}	10 ± 0^{d}
Mn	0.06 ± 0.00^{b}	0.11 ± 0.00^{a}	0.06 ± 0.00^{c}	0.04 ± 0.00^{d}
Mo	0.01 ± 0.00^{c}	0.04 ± 0.00^{a}	0.01 ± 0.00^{b}	0.00 ± 0.00^{d}
Na	0.72 ± 0.00^{c}	4.6 ± 0.03^{a}	0.75 ± 0.01^{c}	0.96 ± 0.01^{b}
P	37 ± 0^{a}	33 ± 0^{c}	36 ± 0^{b}	25 ± 0^{d}
S	18 ± 0^{c}	21 ± 0^{b}	30 ± 0^{a}	18 ± 0^{c}
Zn	0.13 ± 0.00^{d}	0.19 ± 0.00^{c}	0.28 ± 0.00^{a}	0.22 ± 0.00^{b}

< DL - below the detection limit.

The concentration of AQ phenolic compounds was 0.3-0.7 mg/mL (Damian et al., 2018), which is comparable to a previous study with similar cooking treatments: boiling time 60 min for lentil (0.84 mg/g) and 90 min for the other pulses (0.51 mg/g, 0.56 mg/g, and 0.70 mg/g for green pea, yellow pea, and chickpea, respectively) (Xu and Chang, 2008).

Previous research has shown that chickpea saponin is reduced 44-52% by boiling (El-Adawy, 2002), while in lentils, the saponin content only decreased by 6-14% (Ruiz et al., 1996). In Stantiall et al.'s (2018) study, the average AQ saponin content was 5 mg/ g except for whole green lentils (12 mg/g). Later, Damian et al. (2018) identified the accuracy with a different analytical procedure and updated AQ saponins content to 8-14 mg/mL (equal to 0.8-1.4%, w/w). Serventi et al. (2018) determined that yellow soybeans AQ saponin content was 6.4 mg/g. High saponin content in food can lead to toxicity and hemolytic activity. Conversely, these phytochemicals at lower levels can have health-promoting properties, such as reducing blood cholesterol level, blood lipid level, and

improving blood glucose responses (Barakat et al., 2015; Singh et al., 2017). They have antioxidant properties and can decrease the risk of some cancers (Barakat et al., 2015).

Legumes often contain anti-nutritional factors, such as trypsin and chymotrypsin inhibitors, oligosaccharides, lectin and tannin (Lyimo et al., 1992; Muzquiz et al., 1999), which are compounds that can interfere with nutrient absorption. During AQ preparation, presoaking dry legumes in water can reduce the concentration of components that are partly or entirely solubilized in the soaking solution. Soluble molecules including sugars (sucrose, raffinose), α-galactosides, minerals, phytic acid, and proteolytic enzyme inhibitors (Frias et al., 2000a; Frias et al., 2000b) are partially removed. Cooking could destroy or inactivate some heat-sensitive anti-nutritional factors, including trypsin inhibitors, and decrease phytic acid and α-galactoside content (Iyer et al., 1989; Chau et al., 1997; Frias et al., 2000a; Frias et al., 2000b).

2.5.3 AQ Physical Characteristics

AQ physical properties, including pH, density, and viscosity, were analyzed by Serventi et al. (2018) and Stantiall et al. (2018) (Table 2.5). Overall, AQ was slightly acidic with pH ranging from 6.07 to 6.47 whereas its density ranged from 1.02 g/mL for haricot bean to 1.25 g/mL for yellow soybean. The viscosity of AQ from different legumes varied significantly amongst the samples prepared. For instance, the viscosity of garbanzo chickpea AQ was 47 ± 1 mPa*s, whereas haricot bean AQ was only 4.5 ± 2 mPa*s. Insoluble fibre content was positively correlated with AQ viscosity as this insoluble fibre (cellulose, pectin) can increase solution viscosity (Hüttner and Arendt, 2010). Density and viscosity of AQ from commercially canned chickpea ranged from 1009 kg/m^3 to 1180 kg/m^3, and 5.7 cP to 114.2 cP, respectively (Shim et al., 2018). Additionally, yellow soybean AQ water- and oil-absorption capacities were also determined (Serventi et al., 2018). High oil absorption capacity (2.68 ± 0.04 g oil/g sample) and moderate water absorption capacity (1.54 ± 0.32 g water/g sample) were observed, showing potential for this AQ to be used as thickening agent in a high fat matrix.

Table 2.5 Physicochemical properties of AQ (Serventi et al., 2018; Stantiall et al., 2018).

Physicochemical properties	Haricot bean	Garbanzo chickpea	Whole green lentil	Split yellow pea	Yellow soybean	Egg white
pH	6.07 ± 0.01^{d}	6.26 ± 0.06^{c}	6.47 ± 0.03^{b}	6.39 ± 0.05^{bc}	6.07 ± 0.01^{d}	9.20 ± 0.09^{a}

Density (g/mL)	1.017±0.002[b]	1.020±0.002[c]	1.025±0.003[c]	1.021±0.005[c]	1.25±0.00[a]	1.040±0.004[b]
Viscosity (mPa*s)	4.5±2.0[d]	47±1[a]	25±2[b]	8.7±1.2[d]	45±9[a]	15±2[c]
Foaming ability (%)	39±2[b]	58±7[b]	97±6[b]	93±12[b]	65±2[b]	400±49[a]

[a-d] Significant differences in data ($P < 0.05$) in each row are indicated by superscripted letters.

2.5.4 AQ Functionality and Applications

Systems that contain AQ are foamable, gelable, and emulsible. This functionality makes AQ a potential candidate to replace egg in many food recipes, such as meringue, mayonnaise, vegan cakes, and mousses.

2.5.4.1 Foamability

Dry AQ is mainly carbohydrate (sugars, soluble and insoluble fibre) and protein (Stantiall et al., 2018). Soaking and cooking chickpea seed extracts sugars, and the sugar concentration subsequently decreases with cooking (El-Adawy, 2002). The extracted soluble sugars include reducing sugars, sucrose, raffinose, stachyose, and verbascose (Mokni et al., 2015). The water-soluble carbohydrates from chickpea seed can form stable foams (Alajaji and El-Adawy, 2006). Protein can also diffuse from chickpea seed during soaking and cooking (Cherian et al., 2012). Protein has an amphiphilic structure that can contribute to foamability. Proteins can have many hydrophilic amino acids that interact with water, whereas the hydrophobic amino acids can stabilize interactions with the gaseous phase (Mariotti et al., 2013). AQ foaming capacity has a strong positive correlation with protein content ($r^2 = 0.95$) (Stantiall et al., 2018). AQ has saponins that can also produce foam due to their amphipathic nature and stabilize the incorporation of gas into aqueous solutions (Klamczynska et al., 2001; Güçlü-Üstündağ and Mazza, 2007).

Foaming agents have been widely used in sponge cake, meringue, and mousse to afford food with a fluffy texture (Liu et al., 2009). A foam forms when the gas phase is dispersed within a liquid phase by energy from whipping, shaking, or other means of agitation (Wilde and Clarke, 1996). Mustafa et al. (2018) produced eggless sponge cake using AQ as an egg white replacement (Figure 2.3). The colour and texture of AQ sponge cake were similar to those of sponge cake made with egg white. The AQ sponge cake was less springy, and less cohesive than egg white cake. Stantiall et al. (2018) investigated the foaming properties of AQ of haricot bean, garbanzo chickpea,

whole green lentil, and split yellow pea, and their application to replace egg white in meringue (Figure 2.4). In their study, AQ foaming ability was expressed as a percent increase (%) in volume and calculated by foaming ability = (total foam volume – initial AQ volume)/initial AQ volume × 100. AQ foaming ability ranged from 39% to 97%, though still much lower than egg white (400%). Sensory analysis of AQ meringues made from garbanzo chickpea and split yellow pea revealed good palatability and overall quality that was comparable to egg white meringue. Conversely, due to their unappealing taste, meringues prepared from haricot bean and whole green lentil AQ were deemed undesirable.

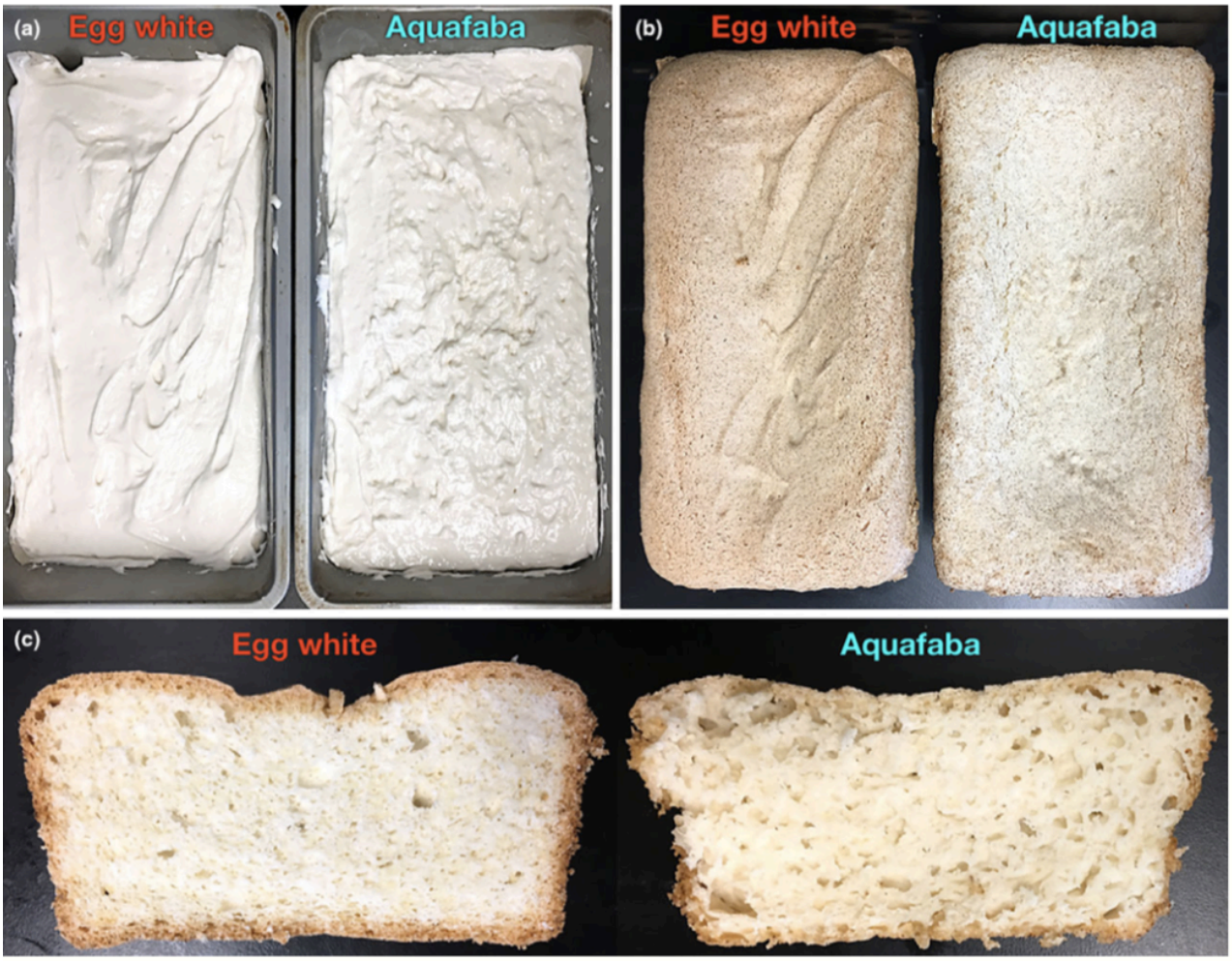


Figure 2.3 Sponge cake prepared with egg white or AQ (A) before baking and (B) after baking. (C) Cross section of baked sponge cake using egg white or AQ (Mustafa et al. 2018 with permission).

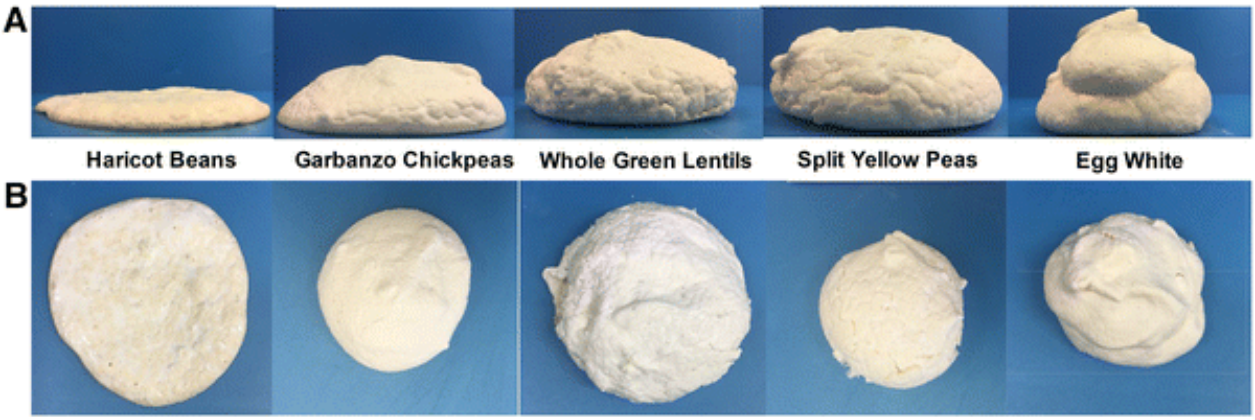


Figure 2.4 Pictures of the meringues: (A) front view and (B) top view (Stantiall et al., 2018 with permission).

Serventi et al. (2018) used the same method above and expressed 65% foaming ability for yellow soybean AQ. This AQ was incorporated into gluten-free crackers and to produce a product with improved water absorption. The AQ increased softness and the crackers did not harden with storage. A similar principle was applied by Bird et al. (2017), who used AQ in gluten-free bread to reduce crumb hardness and enhance crumb homogeneity and gas retention.

2.5.4.2 Emulsibility

Protein has been identified as the principal component that determines emulsibility induced by pulse fractions (Du et al., 2014; Karaca et al., 2011), especially when it was combined with carbohydrates (Sila et al., 2014). In previous studies, phenolic content decreased 30-40% in common bean after cooking (Bressani et al., 1980). The loss of 83-97% of tannin was induced by processing (Rao and Deosthale, 1982). Xu and Chang (2008) determined that after soaking and cooking, 40-68% of legume phenolics diffused into cooking water, with the amount dependent on legume type. It has been reported that the flavonoids rutin and tiliroside could also emulsify oil-water solutions (Luo et al., 2011). Moreover, food physical properties, including emulsibility, solubility, stability, and foamability, can be improved by manipulating the interaction between flavonoids and protein (Vega and Grover, 2011). Legume's saponins are surfactants that can also act as emulsifiers due to their amphiphilic structure (Güçlü-Üstündağ and Mazza, 2007). On the other hand, Chung et al. (2017) presumed that saponins could pack tightly together at the interface, and, therefore, effectively avoid unfavourable molecular interactions between the oil and water phases. This could lowered the interfacial tension to generate smaller droplets during homogenization and lead to higher emulsibility (Chung et al., 2017).

AQ has been proven to produce stable emulsions, and is especially suitable for high-fat matrices (Damian et al., 2018). According to literature, coalescence caused by oil droplet convergence is the main reason for mayonnaise instability (Jaynes, 1985). The most effective way to limit coalescence is prevent oil droplets from getting too close to each other; in other words, generating strong repulsive forces between droplets (Nikzade et al., 2012). It is assumed that AQ can resist droplet coalescence because emulsion samples which contained AQ showed remarkable stability. Damian et al. (2018) studied AQ emulsion properties and produced cream mousse stabilized by AQ. AQ emulsion activity (EA, %) was measured after centrifugation, and calculated by EA = (height of emulsified layer/total height of mixture) × 100. The AQ produced from haricot bean, garbanzo chickpea, whole green lentil, and split yellow pea had high emulsifying activities (46%-54%), which were comparable to the yellow soybean AQ (49.3%) described by Serventi et al. (2018). Sensory analysis revealed that garbanzo chickpea and split yellow pea AQ were highly rated for palatability and had acceptable function to replace egg in this raw confectionery product.

2.5.4.3 Gelation

Soluble fibre components in AQ, such as hemicellulose and pectin, are associated with gelling ability (Hüttner and Arendt, 2010; Li and Nie, 2016). Gelling agents could form a gel by dissolving in a liquid phase as a hydrocolloid mixture and develop weak cohesive internal structures (Hüttner and Arendt, 2010). Calcium was reported to modulate hydrocolloids functionality in food gels (Nussinovitch et al., 1990) and it may act in this manner with AQ. Garbanzo chickpea AQ has good gelling ability (Stantiall et al., 2018). Therefore, AQ has the potential to be applied as a gelling agent in some food products, such as puddings. Moreover, AQ gelling ability is negatively correlated with AQ insoluble fibre content (Stantiall et al., 2018).

2.5.5 Challenges in AQ Application

AQ has been verified as a potential eco-friendly egg replacement product with various nutrients and functional properties. Unfortunately, AQ functional properties vary among legume's genotypes and with cooking conditions (e.g., temperature, pressure, cooking time, and water-chickpea ratio) (Shim et al. 2018). Therefore, although many AQ food recipes are posted on the internet, internet comments note that individuals have failed to reproduce some recipes. Therefore, optimization and standardization of AQ preparation is necessary to generate consistent and reproducible AQ performance in terms of functional properties and food product quality.

2.6 Summary

Chickpea nutrients and its significance as an essential legume crop growing worldwide was described. Meanwhile, the mechanisms of emulsification and the properties of egg in food emulsions was introduced. Egg replacements were discussed as components of food emulsions as consumers make choices to avoid eggs. Avoidance of eggs can occur for a number of reasons including egg allergies, dietary choices, health considerations, and environmental concerns. Therefore, egg replacements as a trend in food emulsion preparation was discussed. AQ, which has excellent emulsification properties, was described as an egg replacement in a wide range of food emulsions. It is a potential eco-friendly by-product with various nutrients, and it has functional properties that resemble egg white. Unfortunately, there is no literature regarding differences in functional properties of AQ prepared from different chickpea genotypes and standard methods for preparing AQ with the best properties. Therefore, more evidence is needed to demonstrate AQ quality differences among chickpea genotypes, and processing conditions for AQ preparation should be standardized so that AQ quality and functional properties are predictable for the user.

The following studies address this shortfall of knowledge. The impact of genotype on AQ properties was determined by an investigation of five representative chickpea cultivars that were chosen to prepare AQ. AQ emulsion properties were measured to identify differences of AQ quality among chickpea genotypes. Moreover, AQ was prepared from the selected chickpea genotypes by different cooking and drying conditions to determine standard processing conditions.

3. MATERIALS AND METHODS

3.1 Materials

Four Kabuli chickpea cultivars (CDC Leader, CDC Orion, CDC Luna, and Amit (B-90)) and one Desi chickpea cultivar (CDC Consul) were generously provided by Dr. Bunyamin Ta'ran from the University of Saskatchewan, Crop Development Centre (CDC) (SK, Canada) (Figure 3.1). The seed was randomly selected and manually cleaned and freed of broken seed, dust, and other foreign materials. Canola oil (purity 100%; ACH Food Companies, Inc., IL, USA) and baking soda ($NaHCO_3$; ARM & HAMMER by Church & Dwight Co., Inc., ON, Canada) were purchased from a local supermarket (Walmart, Saskatoon, SK, Canada). Sodium dodecyl sulfate (SDS) was purchased from GE Healthcare (Mississauga, ON, Canada). Anhydrous ether was obtained from Fisher Scientific Co. (Ottawa, ON, Canada). Sodium hydroxide (NaOH) and sodium chloride (NaCl) was purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Concentrated sulphuric acid (H_2SO_4, ≥96%, w/w) and methanol were acquired from EMD Millipore Corporation (Burlington, MA, USA).

Figure 3.1 Chickpea seed from five cultivars (from left to right: CDC Leader, CDC Orion, CDC Consul, CDC Luna, and Amit).

3.2 Fresh AQ Preparation

Chickpea seed (approx. 100 g) was washed and soaked in distilled water at a ratio of 1:4 (w/w), covered, and kept at 4 ºC for 16 h (Stantiall et al., 2018). Soaking water was then drained and discarded. Soaked chickpea seed (100 g) was rinsed with distilled water and mixed with 100 mL distilled water in a 250 mL sealed glass jar and cooked in a pressure cooker (Instant Pot® 7 in

1 multi-use programmable pressure cooker, IP-DUO60 V2, 6 quart/liters) at a range of 70–80 kPa, 30 min, and 115–118 ºC (Figure 3.2A). Subsequently, the jar was cooled at room temperature for 24 h. Cooled AQ was drained from cooked chickpea seed using a stainless-steel strainer (Figure 3.2B) and stored in a freezer (–18 ºC). Each chickpea cultivar was used to produce AQ in this way four times. Prior to analysis, AQ was thawed at 4 ºC overnight then held at room temperature for 2 h. AQ moisture content was determined by oven drying at 105 ºC overnight according to AACC method 44-15.02.

Figure 3.2 (A) Programmable pressure cooker; (B) AQ drained from cooked chickpea seed; and (C) hand mixer.

3.3 AQ from Five Chickpea Cultivars

3.3.1 AQ Oil Emulsion Preparation

AQ (6 g) produced from each cultivar was mixed with 14 g canola oil using a kitchen hand mixer (Everyday Essentials™, Figure 3.2C). The mixer was set at its maximum speed for 2 min. Canola oil was added dropwise to the AQ to produce oil-in-water emulsions.

3.3.2 Emulsion Capacity

Each AQ oil emulsion was diluted 100-fold with 0.1% SDS (w/v), and emulsion turbidity (500 nm) was calculated immediately after dilution. A Genesys™ 10S UV-vis spectrophotometer (Thermo Scientific™) was used to measure transmittance at 500 nm. Emulsion turbidity value (*T*) was calculated using Equation 3.1:

Equation 3.1 Emulsion turbidity value

$$\boldsymbol{T = \frac{2.303 \times A \times V}{I}}$$

in which *T* is the emulsion turbidity (m^{-1}), *A* is the emulsion "absorbance" measurement at 500 nm (1/transmittance), *V* is the dilution factor, and *I* is the path length (0.01 m).

AQ emulsion capacity (EC) was determined according to Liu et al. (2016). An emulsifying activity index (EAI) was used as an indicator and defined by Wang et al. (2010) and Pearce and Kinsella (1978) using Equations 3.2 and 3.3:

Equation 3.2 Emulsifying activity index (EAI)

$$\boldsymbol{EAI = \frac{2T}{\emptyset \times C}}$$

in which ∅ is the oil volume fraction of dispersed phase and C is the emulsifier concentration (the weight of AQ per unit volume of the aqueous phase before the emulsion is formed) (Pearce and Kinsella, 1978).

Equation 3.3 The oil volume fraction of dispersed phase for EAI

$$\emptyset = \frac{C - A_1 - E(B-C)}{C - A_1 + \frac{(B-C)\{\{1+E)D_0 - E\}}{D_S}}$$

In Equation 3.3, A_1 denotes mass of beaker; B is mass of beaker plus emulsion; C is the mass of beaker plus dry matter; D_0 is the density of oil; D_s is the density of protein solution, and E is the

concentration of solutes (mass per unit mass of solvent) (Pearce and Kinsella, 1978).

All measurements were conducted in triplicate, and results were expressed as mean ± SD.

3.3.3 Emulsion Stability

Emulsion stability (ES) value was determined at room temperature. Each emulsion was transferred into 15 mL centrifuge tube, which was tightly sealed with a plastic cap and centrifuged at 1,860 *g* for 15 min. The weight of the original emulsion before centrifugation (F_0) and the emulsified layer (F_1) after centrifugation were measured (Figure 3.3). The emulsion stability at room temperature was determined by Equation 3.4 (Nikzade et al., 2012):

Equation 3.4 Emulsion stability

$$\boldsymbol{ES = \frac{F_1}{F_0} \times 100\%}$$

All measurements were conducted in triplicate, and results were expressed as mean ± SD.

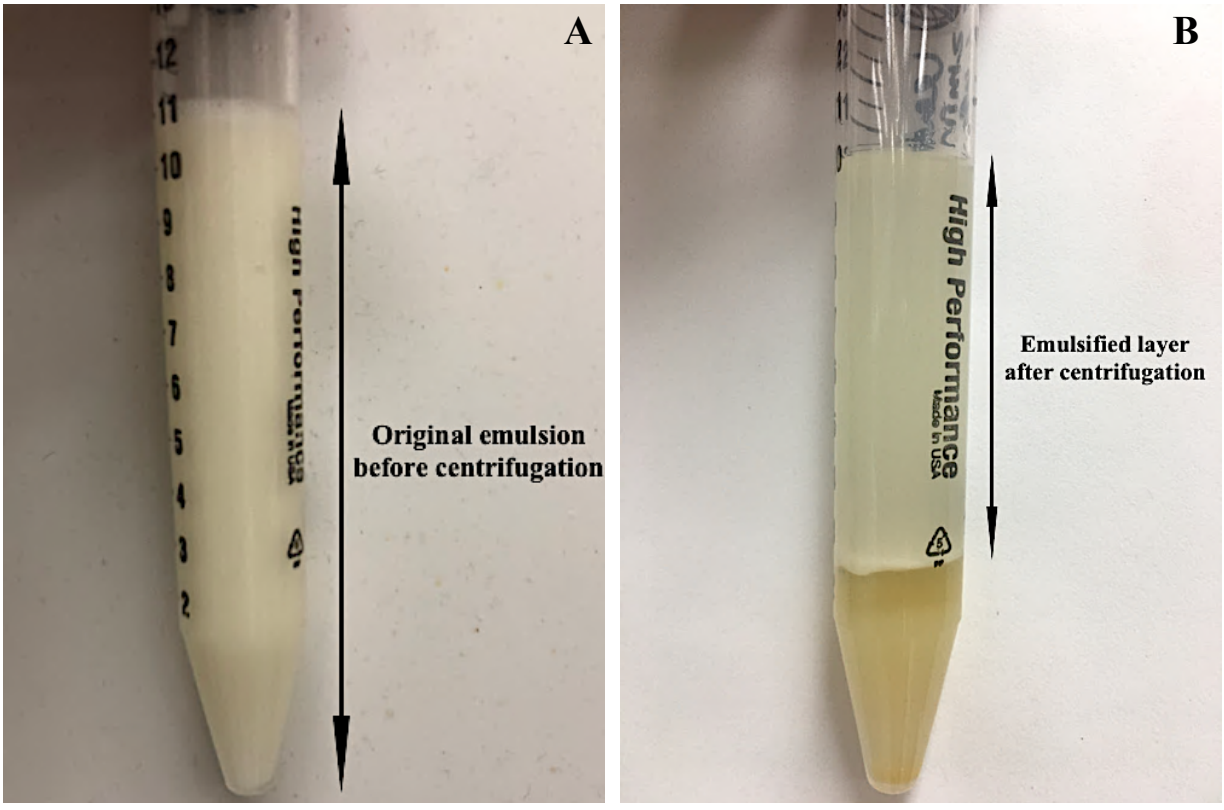


Figure 3.3 AQ-canola oil emulsion (A) before and (B) after centrifugation.

3.4 Chickpea Physical Properties

Hundred seed weight (HSW, g) was determined by randomly selecting and weighing 100 grains selected from five chickpea cultivars. Seed coat incidence (SCI, %) was determined by the method of Avola and Patanè (2010) with minor modification. The seed coat of ten chickpea seed was removed after soaking seed in distilled water at 4 °C for 12 h. Then the seed coat and cotyledons were dried separately at 65 °C for 4 h, then weighed each hour until to a constant weight.

Seed dimensions were determined by randomly selecting ten chickpea seeds and then using a micrometer to record the seed dimensions in three perpendicular directions. Equation 3.5 calculated the geometric average of the diameter of an equivalent dimension (ED, mm):

Equation 3.5 Equivalent dimension

$$\boldsymbol{ED = (L \times W \times T)^{1/3}}$$

in which L, W and T were the major, minor and intermediate axes (mm), respectively (Mohsenin, 1970).

The surface area per unit mass of seed (or, specific surface area, SSA, $mm^2\ mg^{-1}$) and seed coat weight per surface area (namely seed coat thickness, WSA, $mg\ cm^{-2}$) of a single seed were calculated based on the ED and HSW value by the following Equations 3.6 and 3.7, respectively.

Equation 3.6 Specific surface area

$$\boldsymbol{SSA = \frac{\pi \times ED^2 \times 100}{HSW}}$$

Equation 3.7 Seed coat weight per surface

$$\boldsymbol{WSA = \frac{HSW}{100 \times \pi \times ED^2}}$$

3.5 Chickpea Hydration Kinetics

Hydration kinetic tests were performed by the method of Avola and Patanè (2010) with minor modification. Chickpea seed was soaked at room temperature (20–22°C) and weighed periodically to determine water uptake kinetics. Ten seeds were transferred to a 200 mL beaker, which contained 150 mL deionized water or aqueous solutions of 0.5% (w/v) NaCl or $NaHCO_3$. Beakers were held at a constant temperature of 22 °C. Each hour up to the 8th hour, then at 24 h

after initial imbibition, the seed was drained, and free water was absorbed by a Kimwipes® Low-Lint wiper, then weighed. A clean wiper was used for each weighing to avoid contamination with solutes or water.

A two-parameter asymptotic equation (Equation 3.8) was used to model water uptake kinetics (SigmaPlot 9.0; Systat Software, Inc., San Jose, CA, USA):

Equation 3.8 Hydration weight after soaking for time

$$\boldsymbol{H_t = H_{max} \times (1 - e^{-kx})}$$

in which H_t is hydration weight (g seed^{-1}) after soaking for time t (h), H_{max} is the asymptote of the curve (to estimate seed weight at full hydration), k is a curve parameter that is related to the initial hydration rate (estimating H_{rate}).

All measurements were conducted in triplicate, and results were expressed as mean ± SD.

3.6 Chickpea Chemical Properties

The moisture content of whole chickpea seed was measured by the ASAE S352.2 air oven method (103°C, 72 h, 15 g) (ASAE Standards, 1988). Selected whole chickpea seed was ground with a disc mill before proximate composition analysis. Analyses of crude protein, crude fat, ash, and crude fibre were performed using Association of Official Analytical Chemists (AOAC, 2016) methods. In brief, nitrogen content was analyzed by combustion (AOAC Method 990.03) using a LECO (Saint Joseph, MI, USA) nitrogen analyzer. Protein content was calculated as nitrogen content multiplied by a conversion factor 6.25. Fat was extracted from ground samples according to AOAC method 920.39 using anhydrous ether in a Soxhlet apparatus (Extraction system B-811, BÜCHI Labortechnik AG., Switzerland). Ground chickpea samples were weighed (2 g) onto filter paper which was then placed in a cellulose Soxhlet extraction thimble and washed five times with 20 mL distilled H_2O each time. After drying in an oven at 102 °C for 2 h, oil was extracted over 5 h in a Soxhlet apparatus with anhydrous ether. Chickpea ash content was determined by AOAC method 942.05. Samples were weighed (2 g) in separate pre-weighed porcelain crucibles and placed in a preheated furnace (600 °C) for 2 h. Crucibles were then transferred to a desiccator, cooled and reweighed. Sample weight remaining after ignition of a 2 g sample was regarded as ash content. Carbohydrate content was determined by subtracting the total percentage of protein, fat, fibre and ash components from 100 percent. Crude fibre content was determined by AOAC method 962.09 with minor modification. Samples were digested with 1.25% (w/v) boiling H_2SO_4 (30 min)

followed by 1.25% (w/v) boiling NaOH (30 min) and washed with methanol. Samples were then dried to constant weight and the residue burned. Weight loss on ignition of the dried residue was regarded as crude fibre content. All measurements were conducted in triplicate, and results were expressed as mean ± SD.

3.7 AQ Standardization

3.7.1 Cooking Conditions

Chickpea seed (approx. 100 g) from selected cultivars was washed and soaked in distilled water at a ratio of 1:4 (w/w) over various intervals (1 or 16 h) at different temperatures (4 or 85 ºC). After soaking water was drained and discarded. Soaked chickpea seed (100 g) was rinsed with distilled water and mixed with 100 mL distilled water in a 250 mL sealed glass jar and cooked in a pressure cooker (70–80 kPa, and 115–118 ºC) for different periods (20, 30 or 60 min). After cooking jars were cooled at room temperature for 24 h. Cooled AQ was drained from cooked chickpea seed using a stainless-steel strainer and stored in a freezer (–18 ºC). For each analysis of AQ properties, a sample was thawed at 4 ºC overnight and then warmed to 22 C for 2 h. AQ preparation conditions are provided in Table 3.1. AQ emulsion properties were measured to determine the most desirable cooking conditions. All measurements were conducted in triplicate, and results expressed as mean ± SD.

Table 3.1 AQ preparation conditions.

Condition No.	A	B	C	D	E
Soaking time (h)	16	1	1	16	16
Soaking temperature (ºC)	4	85	85	4	4
Soaking water additives (w/w)	NA	0.2% $NaHCO_3$	NA	NA	NA
Cooking time (min)	30	20	30	60	20

NA - no additives.

3.7.2 Comparison of Drying Methods

The effect of five drying methods on AQ properties was determined on AQ prepared from selected chickpea cultivars. In addition, dried AQ emulsion capacity and stability were measured to determine if the drying process influenced AQ properties and emulsion quality. AQ (750 g) was

weighed and randomly separated to 5 equal parts (150 g) and marked as sample No.1 to No.5. Sample No.1 was frozen to –20 °C then dried in a freeze dryer (Figure 3.4A, FreeZone 12 Liter Console Freeze Dryer with Stoppering Tray Dryer, Labconco Corporation, Kansas City, USA) until the sample temperature rose to –5 °C, indicating the sample had been thoroughly dried. Sample No.2 was spray dried (Figure 3.4B, Mini Spray Dryer B-290, BUCHI Labortechnik AG, Switzerland) and was dried at 150 °C immediately. Sample No.3 was dried in an oven (Figure 3.4C, VWR® Signature™ Forced Air Safety Oven, Radnor, Pennsylvania, USA) at 80 °C to a consistent weight. Sample No.4 was dried in a round-bottom flask connected with a rotary evaporator (Figure 3.4D, Büchi® Model R-200 BUCHI Labortechnik AG, Switzerland) at 50°C under vacuum pressure. Sample No.5 was dried in a vacuum pressure oven (Figure 3.4E, Fisherbrand™ Isotemp™ Model 281A vacuum oven, Fisher Scientific International, Ottawa, ON, Canada) at 60 °C for 12 h.

Figure 3.4 (A) Freeze dryer with stoppering tray dryer; (B) spray dryer; (C) oven; (D) rotary evaporator; and (E) vacuum oven.

Dried AQ was rehydrated by adding distilled water to replace water lost during drying. Moisture loss could be determined by dried AQ yield. Rehydrated AQ was mixed with oil to make emulsion following the method above (3.3.1).

3.8 Statistical Analysis

Three replications were used to obtain the average and standard deviation values for all tests. Data are presented as mean ± standard deviation (SD) (n = 3). The analyses are processed with Microsoft® Excel® 2018 and a completely randomized design and the Statistical Package for the Social Science (SPSS) version 25.0 (IBM Corp., AR, NY, USA). The analysis of variance (ANOVA) and Tukey's tests were used to evaluate the statistical significance of differences in properties and composition. Statistical significance was accepted at $P < 0.05$. The mathematical model parameters used in chickpea seed hydration kinetics measurement were estimated using a nonlinear regression procedure performed by SigmaPlot software (Systat Software Inc. SJ, CA, USA). Model suitability was evaluated using the coefficient of determination (R^2), which indicates the model predictive quality (the higher the value for R^2, the better the goodness of fit, and up to a value of 1 meaning exact fit). The hydration kinetics parameters given by the nonlinear regressions were used to compare chickpea cultivars and soaking treatments including soaking time and soaking solutions. A t-test was used referenced from Douglas (2012). Pearson correlation coefficients (r) for the relationships between all characteristics were calculated.

4. RESULTS AND DISCUSSIONS

4.1 Aquafaba Produced from Different Chickpea Cultivars

Chickpea seed was washed and soaked in distilled water and kept at 4 ºC for 16 h. Next, soaked chickpea seed (100 g) was separated from soaking water and mixed with 100 mL distilled water in a 250 mL sealed glass jar, and cooked in a pressure cooker for 30 min. The liquid remaining after chickpea seed was cooked is AQ. Aquafaba (AQ) prepared from different chickpea cultivars showed significant different yields and moisture contents (Figures 4.1 and 4.2). Fresh AQ yields ranged from 70.90 g/100 g seed to 107.44 g/100 g seed with the highest yield produced by CDC Luna and the lowest one by CDC Leader. AQ moisture content ranged from 92.4% to 94.2% with the highest moisture content in AQ produced by CDC Luna and the lowest by CDC Leader. Commercially, high yield AQ with low moisture content (high dry matter content) would be of better economic value.

Colour and turbidity of AQ varied with chickpea cultivar (Figures 4.3 and 4.4). AQ produced from CDC Leader and CDC Orion had similar colour and high turbidity. The AQ samples were pale yellow and cloudy liquid. Whereas, AQ produced from Amit was bright yellow and cloudy. Interestingly, AQ produced by CDC Luna had the lowest turbidity and was nearly translucent and bright yellow. More remarkable, AQ produced by CDC Consul was a dark brown colour and had high turbidity. This dark brown colour might arise from tannins in the chickpea seed (Lyimo et al., 1992; Muzquiz et al., 1999). In general, AQ colour might arise from chickpea seed pigments, vitamins, and other plant secondary metabolites content and varieties. Chickpea contains pigments mainly falling into the carotenoids class (β-carotene, cryptoxanthin, lutein, and zeaxanthin) as well as small amounts of chlorophyll (Abbo et al., 2005). Moreover, there are also some water-soluble vitamins in chickpea, such as thiamin, riboflavin, niacin, vitamin B_6, folate, and ascorbic acid (USDA, 2018). Additionally, some flavonoid compounds, including anthocyanin, flavonols, isoflavones, flavonol glucosides, phlobaphenes, proanthocyanidin, leucoanthocyanidin, and proanthocyanidin in the seed coat also contributed to AQ colour (Furukawa et al., 2006).

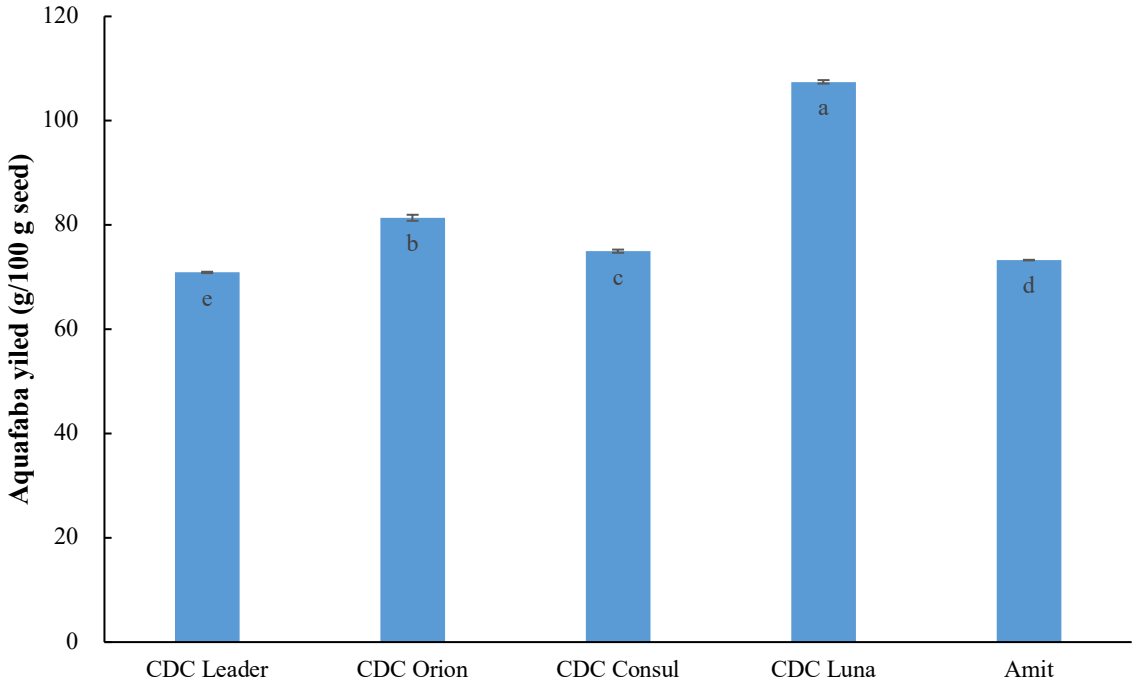


Figure 4.1 Fresh AQ yield (g/100 g seed) prepared from different chickpea cultivars.

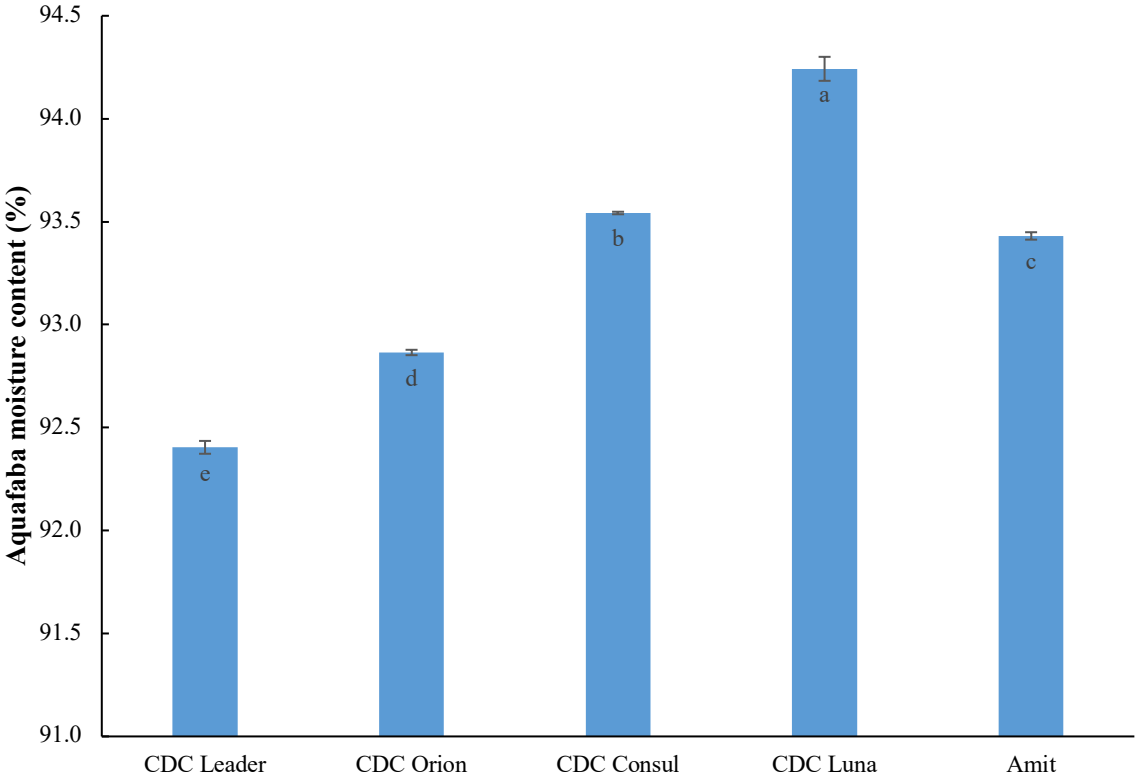


Figure 4.2 Moisture content (%) of fresh AQ prepared from different chickpea cultivars.

Hemicellulose (Hromádková and Ebringerová, 2003) and cellulose (Sun et al., 2004b) were disrupted by high temperature (70–80 kPa), high pressure (115–118 ºC) cooking for 30 min, leading to destruction of the chickpea cell wall and breakage of the links between lignin and hemicelluloses (Sun et al., 2004a). In the current study, CDC Leader, CDC Orion, CDC Luna, and Amit are Kabuli class chickpea cultivars which normally have a white to cream-yellow colour, while CDC Consul is Desi class with brown to fawn colours. This pigment difference could explain why CDC Consul has a unique dark brown colour among these chickpea cultivars. AQ turbidity is also a result of the disruption of chickpea seed microstructure during cooking, leaching of organic compounds, pigments, proteins, sugars, starch, and vitamins into the cooking water (Yildirim and Öner, 2015).

Figure 4.3 AQ and chickpeas from different chickpea cultivars prepared in jars. From left to right: CDC Consul, CDC Luna, CDC Orion, CDC Leader, and Amit.

Figure 4.4 AQ separated from chickpea seed. From left to right: Amit, CDC Orion, CDC Leader, CDC Luna, CDC Consul.

4.2 AQ Selection from Different Chickpea Cultivars

4.2.1 AQ Emulsion Capacity

Today, healthier and nutritious foods are demanded by conscious consumers. Food oil emulsion, such as mayonnaise and salad dressing, is often mentioned due to its high fat and cholesterol content. Plant-based proteins, including soybean and wheat proteins, are accessible in replacing egg as emulsifiers in mayonnaise emulsion systems (Ghoush et al., 2008; Puppo et al., 2000). Nikzade et al. (2012) developed a combination of soy milk, gums, and other stabilizers to replace egg in a low cholesterol-low fat mayonnaise formula. The application and development of different ingredients in reduced fat/cholesterol salad dressing and mayonnaise have been summarized by Ma and Boye (2013).

As a novel emulsifier, AQ can potentially stabilize oil-in-water emulsions. EAI values of AQ prepared from each of five chickpea cultivars were measured (Figure 4.5). They ranged from 1.10 ± 0.04 to 1.30 ± 0.05 m^2g^{-1} with the highest EAI observed from AQ prepared from CDC Leader, while the lowest EAI occurred with CDC Orion. There were no significant differences among EAIs of CDC Consul (1.21 ± 0.02 m^2g^{-1}), CDC Luna (1.17 ± 0.07 m^2g^{-1}), and Amit (1.20 ± 0.05 m^2g^{-1}).

In this study, AQ prepared by CDC Leader induced higher EAI (1.30 ± 0.05 m^2g^{-1}) than AQ prepared by other chickpea cultivars, indicating better emulsification activity of CDC Leader AQ. Emulsification activity could be partly ascribed to proteins present which could help to decrease oil-water interface surface tension and provide electrostatic repulsion on oil droplet surfaces thereby emulsifying and stabilizing emulsions (Nakamura et al., 2004; Jitngarmkusol et al., 2008). Importantly, the contribution of hydrophobic polysaccharides and amphiphilic phytochemicals to emulsification activity cannot be neglected. Improved rheological properties of hydrophilic polysaccharides induced steric and mechanical stabilization effects, which slowed or even prevented emulsion droplet aggregation by forming thick charged layers (Randall et al., 1988).

4.2.2 AQ Emulsion Stability

Emulsion stability of AQ from cooked whole seed of five chickpea cultivars was also investigated in this study (Figure 4.5). AQ emulsion stability ranged from 71.9 ± 0.8% to 77.1 ± 0.5% with the highest emulsion stability also observed from AQ prepared by CDC Leader and Amit, while the lowest emulsion stability for AQ prepared by CDC Luna. There was no significant

difference between the emulsion stability of AQ from CDC Orion (75.6%) and CDC Consul (74.7%). Nikzade et al. (2012) measured emulsion stability of mayonnaise samples made by soymilk and different compositions of xanthan gum, guar gum, and mono/diglyceride emulsifiers. In their comparison the ES values for emulsions from samples ranged from 65.67% to 97.78%. In comparison, this study shows that AQ has better emulsification effects than some existing commercial egg replacements when used as emulsifiers in mayonnaise. Thus, these results suggested that AQ might be used as a stabilizing agent in manufacturing egg-free food oil emulsions, and AQ produced from CDC Leader would be the superior choice.

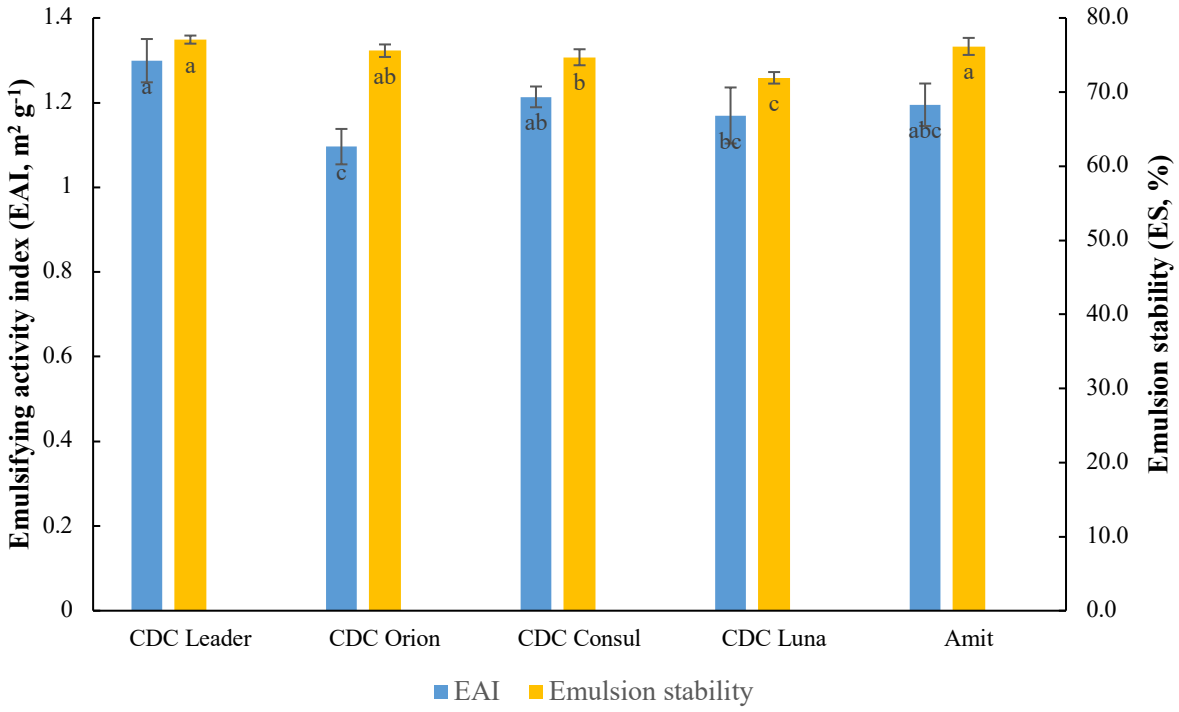


Figure 4.5 Emulsion capacity and stability of AQ prepared from different chickpea cultivars.

4.3 Chickpea Physical Properties

Physical characteristics of seed from different chickpea cultivars are shown in Table 4.1. Highly significant differences on the HSW and WSA among chickpea cultivars were observed from ANOVA results. HSW and ED ranged from 22.43 ± 0.08 g to 42.9 ± 0.3 g and 6.89 ± 0.5 mm to 8.49 ± 0.2 mm, respectively. CDC Leader exhibited heavier (42.90 ± 0.3 g/100 seed) and larger (8.49 ± 0.2 mm) seed compared with the other cultivars. CDC Orion was not significantly ($P >$ 0.05) different from CDC Luna, except for WSA (10 ± 1 mg cm^{-2}). CDC Consul exhibited the greatest SCI (11.2 ± 2%) and WSA (15 ± 1 mg cm^{-1}). Amit showed the highest SSA (0.668 ± 0.09

$mm^2\ mg^{-1}$). These observed values are comparable to results reported previously (Avola and Patanè 2010), where three Sicilian chickpea cultivars (Calia, Etna and Principe) were evaluated for their HSW, ED, SCI, SSA, and WSA. In their study, chickpea HSW value ranged from 31.3 g/100 seed to 48.8g/100 seed. Three Sicilian chickpea cultivars had similar ED and SCI with an average of 7.8 mm and 5.78%, respectively. The SSA value differed among chickpea cultivars and ranged from 0.45 $mm^2\ mg^{-1}$ to 0.6 $mm^2\ mg^{-1}$. The WSA value showed a wide range from 8.3 to 11.9 mg cm^{-2}.

4.4 Hydration Kinetics

Chickpea seed was soaked with water to hydrate, soften its texture and reduce the cooking time before boiling, making it easier to cook. The relationship between soaking time and cumulative values of water uptake (Figure 4.6) was described by a nonlinear iterative regression method with an exponential equation (Equation 3.8). The applied model fitted the experimental data with an R^2 that was greater than 0.94 for all cultivars. Therefore, a single curve for water uptake was used in further analysis with all data combined. Soaking processes achieve rapid water uptake (H_{rate} = 0.38 g H_2O g min^{-1}), and after soaking for 6 h, water absorbed reached 90% of seed dry weight. Subsequently, water absorption rate declined until the hydrated seed weight was approximately 2.06-fold greater than before hydration, where total hydration reached saturation at 1.06 g H_2O g dw^{-1} (H_{max}). Similar trends for chickpea seed hydration were described by several authors (Avola and Patanè, 2010; Clemente et al., 1998; Turhan et al., 2002; Ibarz et al., 2004; Patanè et al., 2004; Wood and Harden, 2006; Gowen et al., 2007). However, in Avola and Patanè (2010), Ibarz et al. (2004) and Patanè's (2004) studies, water content of chickpea seed exceeded 90% of total water imbibition after 4 h.

Statistic analysis revealed no significant differences among H_{max}, except Amit seed which absorbed more water than the other chickpea cultivars (1.20 g H_2O g dw^{-1}) in all soaking solutions (Tables 4.1 and 4.2). The modeled hydration rate (H_{rate}) of these five chickpea cultivars were mostly similar and ranged from 0.328 to 0.417 g H_2O g dw^{-1}. Interestingly, the H_{max} and H_{rate} of chickpea seed soaking in NaCl solution was slightly lower compared with that obtained in deionized water. However, seed of CDC Leader, exhibited similar H_{rate} in both deionized water and NaCl solution. Conversely, soaking seed in $NaHCO_3$ solution increased H_{max} for CDC Luna and H_{rate} for CDC Consul. These results are partially in agreement with previous reports that the presence of salt in soaking solution results in a slower seed hydration (Pinto and Esin, 2004), but in contrast with the

Table 4.1 Physical and chemical characteristics of seed from five chickpea cultivars.

Characteristics	Unit	CDC Leader	CDC Orion	CDC Consul	CDC Luna	Amit
Physical						
Hundred seed weight, HSW	G	42.90 ± 0.3^{a}	41.03 ± 0.6^{b}	33.34 ± 0.4^{d}	40.37 ± 0.6^{bc}	22.43 ± 0.08^{e}
Diameter Equivalent, ED	Mm	8.49 ± 0.2^{a}	8.45 ± 0.3^{a}	8.21 ± 0.2^{a}	8.24 ± 0.1^{a}	6.89 ± 0.5^{b}
Seed Coat Incidence, SCI	%	3.89 ± 0.3^{c}	6.63 ± 2^{b}	11.2 ± 2^{a}	5.39 ± 0.6^{bc}	4.65 ± 0.9^{c}
Specific Surface Area, SSA	mm^2mg^{-1}	0.528 ± 0.03^{b}	0.547 ± 0.04^{b}	0.621 ± 0.03^{ab}	0.529 ± 0.02^{b}	0.668 ± 0.09^{a}
Seed Coat weight per surface area, WSA	mg cm^{-2}	6.9 ± 0.4^{d}	10 ± 1^{c}	15 ± 1^{a}	5.7 ± 0.2^{d}	12 ± 2^{b}
Technological						
Hydration capacity (t= ∞), H_{max}	g (H_2O g dw^{-1})	1.036 ± 0.02^{b}	1.073 ± 0.01^{b}	0.9870 ± 0.03^{b}	1.025 ± 0.1^{b}	1.198 ± 0.03^{a}
Hydration rate, H_{rate}	g (H_2O g min^{-1})	0.3537 ± 0.01^{ab}	0.3914 ± 0.04^{ab}	0.3412 ± 0.09^{b}	0.4542 ± 0.03^{a}	0.4112 ± 0.04^{ab}
Chemical						
Moisture	%	8.86 ± 0.07^{c}	9.24 ± 0.08^{b}	10.7 ± 0.1^{a}	5.48 ± 0.02^{d}	5.29 ± 0.01^{e}
Carbohydrate	g (100 g dw^{-1})	65.4 ± 2^{ab}	61.8 ± 2^{b}	65.2 ± 2^{a}	67.4 ± 0.7^{a}	66.8 ± 1^{ab}
Protein	g (100 g dw^{-1})	20.9 ± 0.1^{b}	23.6 ± 0.08^{a}	18.7 ± 1^{c}	18.3 ± 0.3^{c}	20.2 ± 0.1^{b}
Fat	g (100 g dw^{-1})	6.49 ± 0.5^{ab}	5.96 ± 0.5^{b}	4.64 ± 0.5^{c}	7.24 ± 0.5^{a}	4.10 ± 0.5^{cd}
Ash	g (100 g dw^{-1})	3.0 ± 0.1^{c}	3.2 ± 0.0^{b}	2.9 ± 0.1^{d}	2.5 ± 0.0^{e}	3.4 ± 0.1^{a}
Fibre	g (100 g dw^{-1})	4.32 ± 1^{b}	5.36 ± 2^{ab}	8.59 ± 0.6^{a}	4.63 ± 1^{b}	5.55 ± 0.8^{ab}

Data are expressed as mean ± standard deviation (n = 3). Value within rows followed by the same letter (e.g., a, b, c, d.) indicates no significant difference ($P > 0.05$) between varieties by Tukey's test.

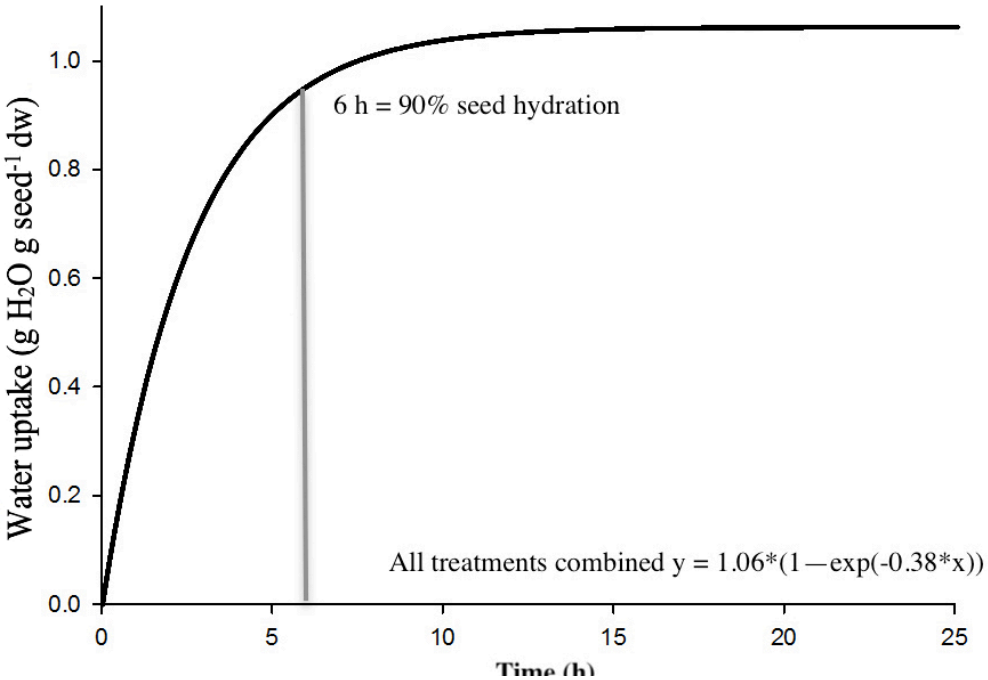


Figure 4.6 Chickpea seed water absorption kinetics. A common curve fitted all data (different chickpea cultivars and hydration solutions).

Table 4.2 Kinetic constants of the nonlinear regression analysis for chickpea seed hydration.

Hydration solution	H_{max} g H_2O g dw^{-1}	H_{rate} g min^{-1}	R^2
CDC Leader			
H_2O	1.058	0.356	0.996
NaCl	1.014	0.359	0.996
$NaHCO_3$	1.035	0.347	0.996
CDC Orion			
H_2O	1.078	0.431	0.992
NaCl	1.060	0.364	0.993
$NaHCO_3$	1.080	0.379	0.992
CDC Consul			
H_2O	1.023	0.313	0.974
NaCl	0.973	0.268	0.941
$NaHCO_3$	0.965	0.442	0.968
CDC Luna			
H_2O	0.993	0.479	0.986
NaCl	0.948	0.425	0.993
$NaHCO_3$	1.133	0.459	0.943
Amit			
H_2O	1.192	0.456	0.988
NaCl	1.172	0.384	0.998
$NaHCO_3$	1.230	0.393	0.988
All data combined	1.062	0.380	0.929

findings of Avola and Patanè (2010) and Clemente et al. (1998), where no effect was observed of soaking chickpea seed in salt solution. There were two possible explanations for increased H_{max} results in $NaHCO_3$ solution: 1) the osmotic pressure gradient across membranes of cotyledon cells was decreased (Woodstock, 1988); 2) there was an interaction of carbonate ions with biopolymers in cotyledon cells which might produce molecular unfolding with a possible exposure of new sites for water binding (Leopold, 1983).

4.5 Chickpea Chemical Properties

The main chemical constituents (moisture, carbohydrate, protein, fat, ash and fibre content) of different cultivars chickpea are summarized in Table 4.1. The moisture content of raw chickpea seed showed significant difference among chickpea cultivars (5.29 ± 0.01% to 10.7 ± 0.1%), with the highest moisture content for CDC Consul and the lowest for Amit. Carbohydrate was the main component in all of the samples, while protein was the second major component. CDC Luna had the highest carbohydrate content (67.4 ± 0.7 g 100 g dw^{-1}), followed by Amit (66.8 ± 1 g 100 g dw^{-1}) and CDC Leader (65.4 ± 2 g 100 g dw^{-1}). Protein content ranged from 18.3 ± 0.3 (CDC Luna) to 23.6 ± 0.08 g 100 g dw^{-1} (CDC Orion). The former also contained more fat (7.24 g ± 0.5 g 100 g dw^{-1}) than other chickpea cultivars, while Amit had the lowest fat content (4.10 ± 0.5 g 100 g dw^{-1}). Chickpea ash content did not differ with genotype. The mean value of ash content was 3.0 g 100 g dw^{-1}. Crude fibre content ranged from 4.32 ± 1 g 100 g dw^{-1} to 8.59 ± 0.6 g 100 g dw^{-1}. These observations are in agreement with previous studies by Xu et al. (2014), Sotelo et al. (1987), Jood et al. (1998), Özer et al. (2010) and de Almeida Costa et al. (2006) for chemical composition of raw chickpea seed from different chickpea cultivars. In addition, Khattak et al. (2006) analyzed protein and ash content of seven Kabuli chickpea cultivars which ranged from 18.08 to 19.22% and 2.45% to 2.94%, and thus was similar to the values in this study.

4.6 Correlation Analysis

The overall interrelationships among chickpea physical, chemical, hydration characteristics, and AQ yield, emulsion capacity, and stability are shown in Table 4.3. AQ emulsion properties have often been linked to AQ carbohydrate content, protein content (Du et al., 2014; Karaca et al., 2011; Sila et al., 2014), and some phytochemicals such as phenolics (Luo et al., 2011) and saponins (Vega and Grover, 2011; Güçlü-Üstündağ and Mazza, 2007; Chung et al., 2017). In this study, we

only determined chickpea seed proximate composition (protein, fat, carbohydrate, fibre, and ash), and did not observe any possible correlation between these parameters and AQ emulsion properties (emulsion capacity and stability). This could be due to different physical characteristics exhibited in the seed of different chickpea cultivars. For example, seed coat thickness and seed hardness may influence the dispersal of chemical substances into AQ, even under identical cooking conditions. Therefore, a weak correlation between chickpea seed proximate composition (carbohydrate, protein, etc.) and AQ emulsion properties did not suggest that AQ chemical components were not correlated to AQ emulsion properties, and vice versa.

AQ yield was inversely correlated to both ES ($r = –0.94^{*}$) and ash content ($r = –0.92^{*}$). Emulsion stability was also negatively correlated to AQ moisture content ($r = –0.91^{*}$), suggesting that AQ with higher dry matter content have better emulsion properties. Seed coat incidence, SSA and WSA were not related to seed dimension, but SSA was found to have the highest negative correlation with seed weight ($r = –0.97^{**}$). Hundred seed weight (HSW) was also positively correlated to ED ($r = 0.97^{**}$), indicating that heavier and larger chickpea seeds develop smaller surface area per unit mass. Fibre was closely correlated to seed coat incidence (SCI: $r = 0.95^{*}$) and seed coat thickness (WSA: $r = 0.90^{*}$). Therefore, a chickpea cultivar with high SCI and WSA will have high fibre content in the seed coat (Sreerama et al., 2010). Finally, fat content was negatively correlated with SSA ($r = –0.96^{*}$) and WSA ($r = –0.90^{*}$), complementary to Gil et al. (1996) who observed a similar relationship in Desi and Kabuli chickpea. In their study, fat content was also positively and significantly correlated to HSW for both chickpea classes. However, in this study, correlation between chickpea fat content and HSW ($r = 0.87^{ns}$) was insignificant. This study supported observations made by Khattak et al. (2006), who revealed a strong positive correlation between seed size and seed weight. Moreover, they found that seed size was positively correlated with chickpea hydration capacity and protein content, as well as moisture content. However, no similar correlation was observed in this study. The weaker correlation observed in this study might be attributed to genetic/environmental variability in chickpea genotypes used in these different experiments.

4.7 AQ Standardization

The chickpea cultivar, CDC Leader, was chosen to standardize AQ production processes and further AQ-oil emulsion studies because it demonstrated the highest EAI and ES.

Table 4.3 Correlation coefficient among the sixteen physical, chemical, and hydration attributes for the five chickpea cultivars and AQ yield, emulsion capacity, and emulsion stability.

	ES	EC	HSW	SCI	ED	SSA	WSA	Carboh.	AQ moisture	Protein	Fibre	Fat	Ash	H_{max}	H_{rate}
AQ yield	-0.94*	-0.28^{ns}	0.47^{ns}	0.09^{ns}	0.42^{ns}	-0.55^{ns}	-0.50^{ns}	-0.29^{ns}	0.71^{ns}	-0.48^{ns}	-0.15^{ns}	0.71^{ns}	-0.92*	-0.54^{ns}	0.32^{ns}
ES		0.40^{ns}	-0.17^{ns}	-0.24^{ns}	-0.16^{ns}	-0.24^{ns}	0.25^{ns}	-0.37^{ns}	-0.91*	-0.58^{ns}	-0.06^{ns}	-0.44^{ns}	0.82^{ns}	0.39^{ns}	-0.37^{ns}
EC			0.02^{ns}	0.20^{ns}	0.01^{ns}	-0.00^{ns}	-0.02^{ns}	0.47^{ns}	-0.34^{ns}	0.39^{ns}	-0.06^{ns}	-0.01^{ns}	-0.08^{ns}	-0.17^{ns}	-0.51^{ns}
HSW				-0.10^{ns}	0.97**	-0.97**	-0.63^{ns}	-0.39^{ns}	-0.28^{ns}	0.24^{ns}	-0.38^{ns}	0.87^{ns}	-0.52^{ns}	-0.71^{ns}	-0.19^{ns}
SCI					0.15^{ns}	0.30^{ns}	0.75^{ns}	0.24^{ns}	0.26^{ns}	-0.26^{ns}	0.95*	-0.39^{ns}	-0.18^{ns}	-0.52^{ns}	-0.62^{ns}
ED						-0.88^{ns}	-0.42^{ns}	-0.48^{ns}	-0.28^{ns}	0.21^{ns}	-0.13^{ns}	0.74^{ns}	-0.51^{ns}	-0.83^{ns}	-0.38^{ns}
SSA							0.80^{ns}	0.25^{ns}	0.19^{ns}	-0.21^{ns}	0.57^{ns}	-0.96*	0.55^{ns}	0.58^{ns}	-0.03^{ns}
WSA								-0.20^{ns}	0.02^{ns}	-0.04^{ns}	0.90*	-0.90*	0.45^{ns}	0.12^{ns}	-0.47^{ns}
Carbohydrate									0.59^{ns}	-0.85^{ns}	-0.11^{ns}	-0.01^{ns}	-0.40^{ns}	-0.14^{ns}	0.27^{ns}
AQ moisture										-0.72^{ns}	0.22^{ns}	0.04^{ns}	-0.59^{ns}	-0.07^{ns}	0.43^{ns}
Protein											-0.31^{ns}	0.01^{ns}	0.65^{ns}	0.30^{ns}	0.09^{ns}
Fibre												-0.64^{ns}	0.02^{ns}	-0.30^{ns}	-0.63^{ns}
Fat													-0.68^{ns}	-0.51^{ns}	0.23^{ns}
Ash														0.74^{ns}	0.01^{ns}
H_{max}															0.60^{ns}

AQ, aquafaba; ES, emulsion stability; EC, emulsion capacity; HSW, 100 seed weight; ED, equivalent dimension; SCI, seed coat incidence; SSA, specific surface area; WSA, weight of seed coat per surface area; H_{max}, max hydration capacity; H_{rate}, initial hydration rate.

*, ** indicate significant for $P < 0.05$, 0.01, respectively.

4.7.1 Optimization of AQ Preparation

Conditions for AQ preparation were investigated. Cooking time is readily controlled and regarded as an essential parameter in the acceptability of chickpea. Long cooking time leads to softer texture and can cause loss of nutrients. On the other hand, short cooking time does not sufficiently soften chickpea seed and can limit seed digestibility. Additionally, cooking chickpea seed with $NaHCO_3$ solution as a soaking medium might provide faster water absorption rate and shorter cooking times (Clemente et al., 1998). Therefore, chickpea soaking/cooking times and soaking solution were determined to optimize AQ cooking conditions.

Chickpea seed of CDC Leader was soaked and cooked for different intervals (conditions A to E). After cooking and separation, the AQ emulsion properties were measured (Figure 4.7). Overall, AQ prepared by soaking chickpea seed in 4°C water for 16 h and cooking for 30 min (condition A) had the highest EAI (1.30 ± 0.05 m^2 g^{-1}) compared with AQ made using other conditions. Interestingly, AQ EAI showed lower values when the cooking time was either increased to 60 min (0.944 ± 0.07 m^2g^{-1}) (condition D) or decreased to 20 min (0.699 ± 0.09 m^2 g^{-1}) (condition E) indicating that when chickpea was cooked for 20 min to 30 min, EAI increased; however, when chickpea was cooked for 30 min to 60 min, EAI decreased. Soaking seed in a 0.2% (w/w) $NaHCO_3$ solution at 85 °C for 1 h and cooking for 20 min (condition B) decreased AQ EAI to 1.17 ± 0.06 m^2 g^{-1}. Lower EAI (0.843 ± 0.1 m^2 g^{-1}) was observed when AQ was made by soaking seed in 85 °C water for 1 h and cooking for 30 min (condition C). This verified that soaking chickpea in a 0.2% (w/w) $NaHCO_3$ solution promoted AQ EAI by increasing chickpea water absorption rate and accelerating chickpea microstructure disruption, thereby leaching more substances with emulsion forming properties.

AQ prepared by cooking condition A demonstrated high ES (77.1 ± 0.5%). Shortening soaking time to 1 h at 85 °C water did not increase AQ stability, regardless of the addition of 0.2% w/w $NaHCO_3$ (76.1 ± 0.3%) (condition B) or not (74.0 ± 1%) (condition C). There was no significant difference when prolonging cooking time to 60 min (77.5 ± 1%) (condition D). However, decreasing cooking time to 20 min (condition E) decreased AQ emulsion stability to 72.0 ± 2%.

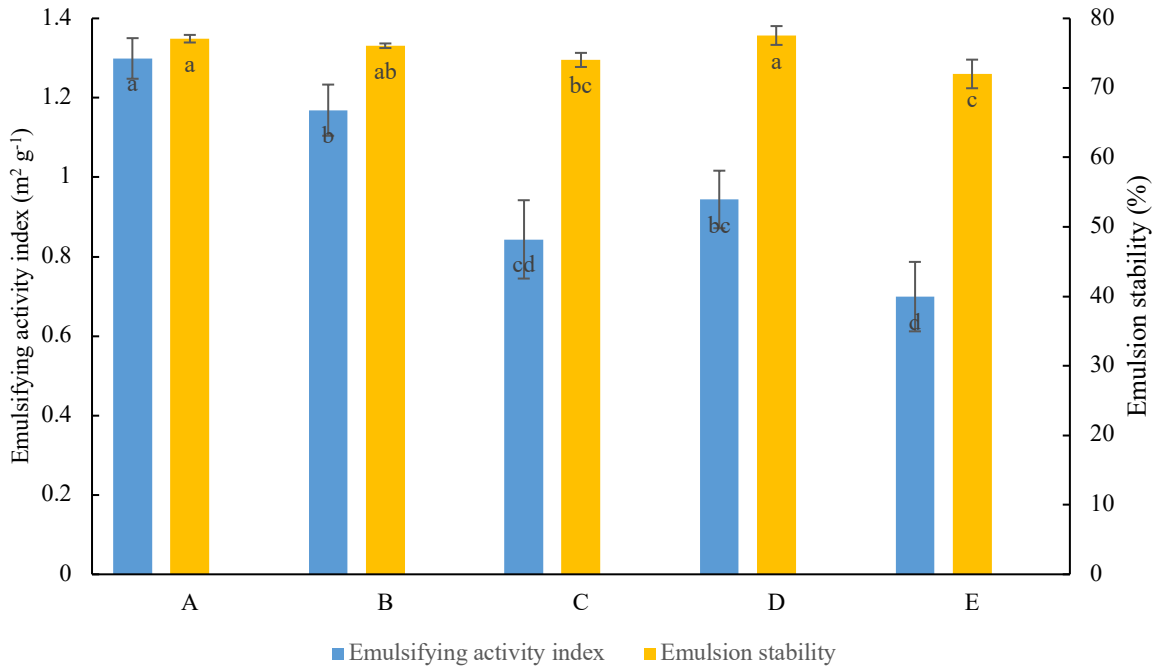


Figure 4.7 Emulsion capacity and stability of AQ prepared with CDC Leader using different cooking conditions (A: Soaking seed in 4 °C water for 16 h then cooking for 30 min; B: Soaking seed in 85 °C water with 0.2% (w/w) $NaHCO_3$ for 1 h and cooking for 20 min; C: Soaking seed in 85 °C water for 1 h and cooking for 30 min; D: Soaking seed in 4 °C water for 16 h and cooking for 1 h; and E: Soaking seed in 4 °C water for 16 h and cooking for 20 min).

Phenolics have the ability to emulsify oil-water solutions (Luo et al., 2011). More importantly, emulsibility could be improved through interactions between phenolics and protein (Vega and Grover, 2011). Xu and Chang (2008) observed that the amount of phenolic compounds in lentil extract solutions decreased from 1.25 mg/g to 0.84 mg/g when the cooking time was increased from 30 min to 60 min. Therefore, one possible mechanism, by which long cooking times lowered emulsion properties, is through the release of phenolic compounds between 20 min to 60 min of cooking followed by the degradation of these compounds due to their heat sensitivity at boiling temperatures (Siah et al., 2014). Oxidation of hydroxyl groups in phenolics is a possible mechanism of thermal degradation as most phenolic compounds are non-conjugated. Therefore, these functional groups are readily degraded (Vallverdú-Queralt et al., 2014).

4.7.2 Comparison of Drying Methods

The moisture content of fresh AQ is over 90%. Furthermore, the likely application of AQ is as a food ingredient. However, it is highly desirable to prepare AQ as a concentrate to reduce the costs of shipping large amounts of water. More importantly, AQ is rich in carbohydrates and proteins (Stantiall et al., 2018) which are nutrients for microorganisms (Pastor et al., 2014). Therefore, it would be desirable to reduce AQ water activity prior to shipping. Drying AQ to remove water before shipping or storage is an excellent way to transport it efficiently and economically, maintain its quality, and save storage space. However, the effects of drying on AQ functional properties is unknown. Moreover, there are many approaches to dry liquids and gels, and it is important to determine which method best preserves AQ functionality.

AQ (100 g) prepared from CDC Leader was dried using several approaches, and the emulsion forming properties were compared to determine which approach provides the most desirable AQ product (Figures 4.8 and 4.9).

Images of dried AQ prepared by different drying methods are provided in Figure 4.8. Both freeze dried AQ (Figure 4.8A) and spray dried AQ (Figure 4.8B) were powdery. On the contrary, oven drying, AQ samples (Figure 4.8C) changed from pale yellow to dark brown, and its structure became brittle. Meanwhile, AQ sample prepared by drying at 40 °C using a rotary evaporation (Figure 4.8D) resulted in a thick rubbery gel that adhered to the evaporator flask and had to be removed using a spatula. Finally, AQ sample dried via vacuum evaporation in an oven was slow and had higher moisture content when compared with other dried AQ samples. The texture of the vacuum dried sample (Figure 4.8E) was like a rubber sheet.

All drying methods were able to remove a significant portion of water present in the AQ. The amount of water removed (g) and drying time (h) of different drying methods were calculated and are reported in Table 4.4. Spray drying could remove the largest amount of water (95.00±0.03 g) in the shortest time (0.29±0.00 h). Freeze drying, oven drying, rotary evaporation drying, and vacuum drying took a longer time to remove water from AQ. Powders are preferable to pastes for home and industrial applications as they are more readily dispensed. Both freeze drying and spray drying methods produced powder, but it is important that the functionality of AQ after rehydration, must be maintained. Freeze drying methods are not typically preferred in industry due to the high capital cost of equipment and the requirement for large amounts of energy.

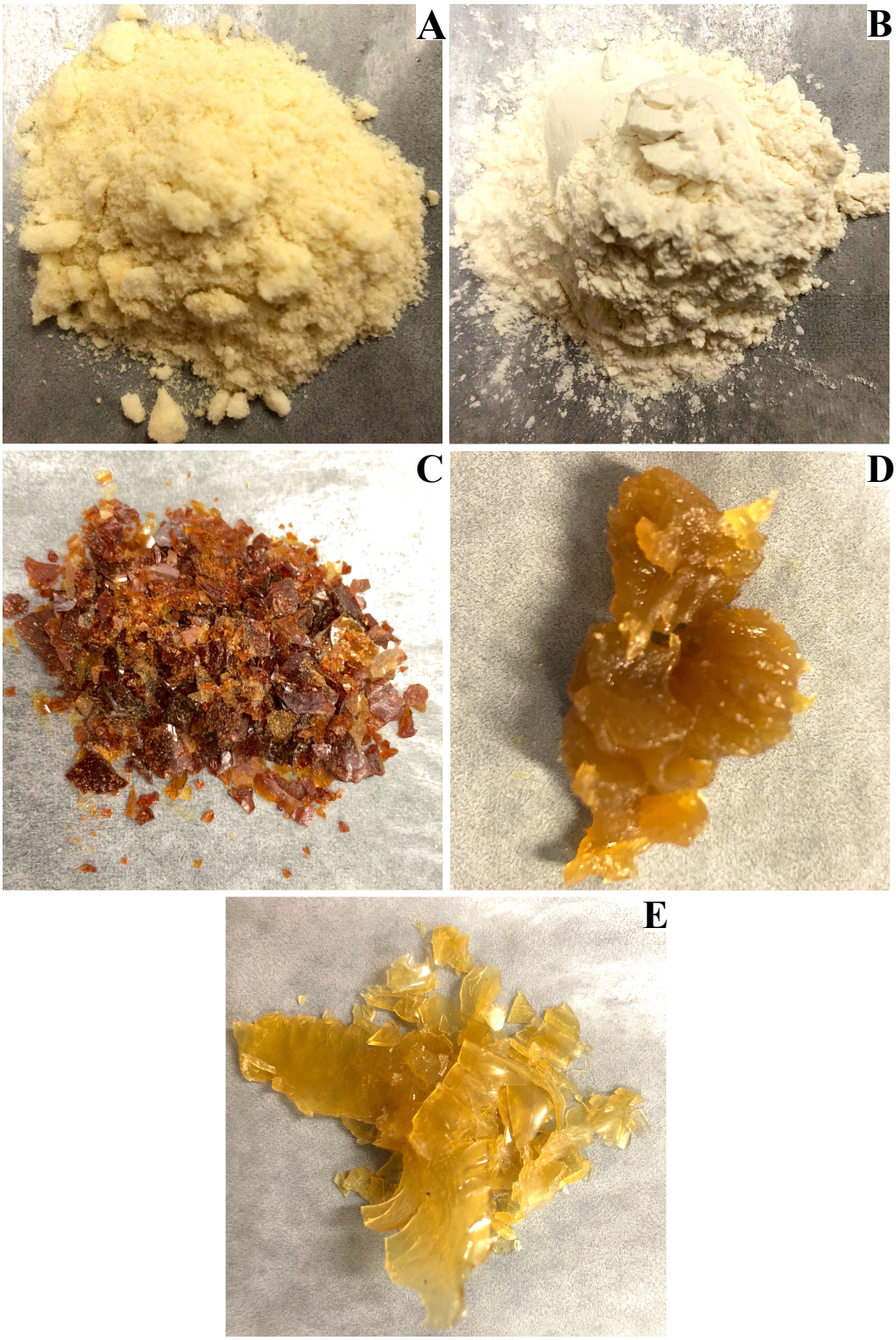


Figure 4.8 AQ samples prepared by different drying methods (A) freeze drying; (B) spray drying; (C) oven drying; (D) rotary evaporation drying; and (E) vacuum drying.

Table 4.4 Amount of water removed, drying time, and dried AQ yield of different drying methods for 100 g fresh AQ and water mass added to rehydrate AQ.

Drying methods	Amount of water removed (g)	Drying time (h)	Dried AQ yield (g/100 g fresh AQ)	Water added (g/10 g dried AQ)
Spray drying	95.0 ± 0.0^{a}	0.3 ± 0.0^{d}	5.01 ± 0.03^{e}	190
Freeze drying	92.9 ± 0.0^{b}	129 ± 5^{a}	7.06 ± 0.04^{d}	132
Oven drying	92.8 ± 0.1^{c}	29.0 ± 2^{c}	7.22 ± 0.06^{c}	129
Rotary evaporation drying	91.2 ± 0.1^{e}	3.22 ± 0.08^{d}	8.78 ± 0.09^{a}	104
Vacuum drying	92.6 ± 0.0^{d}	45.6 ± 2^{b}	7.37 ± 0.01^{b}	126

Data are expressed as mean ± standard deviation (n = 3). Value within rows followed by the same letter indicate no significant difference ($P > 0.05$) between varieties by Tukey's test.

Dried AQ (10 g) was rehydrated by the addition of distilled water until the moisture content, before drying, was attained. Moisture loss was determined from dried AQ yield (Table 4.4), with the highest AQ yield (8.78 ± 0.09%) occurring from rotary evaporation. The AQ product of rotary evaporation still had a higher moisture content than other products and this led to its gel-like properties. AQ dried by freeze drying, oven drying, and vacuum drying had similar yields (7.06–7.37%). Spray dried AQ had a much lower yield (5.01 ± 0.03%), due to losses in the lab spray dryer where product recovery is difficult. However, such losses would become negligible in a commercial spray dryer.

Rehydrated AQ samples were mixed with canola oil, and emulsion properties of the mixtures were measured (Figure 4.9). The spray dried sample showed the highest EAI (1.26 $m^2 g^{-1}$), which was comparable with AQ (1.30 $m^2 g^{-1}$) that was not previously dried. There were no significant differences among EAI of freeze dried (1.10 $m^2 g^{-1}$), oven dried (1.08 $m^2 g^{-1}$) and vacuum pressure dried samples (1.10 $m^2 g^{-1}$). AQ samples dried in a rotary evaporator achieved the lowest EAI (0.94 $m^2 g^{-1}$).

Oven dried AQ sample produced emulsions with the highest ES (78.8%) and demonstrated superior stability than fresh AQ emulsion (77.1%). Oxidation and thermal reactions of AQ components (such as a polysaccharide and protein) during drying for more than 12 h at 80 °C, might have contributed to a higher ES.

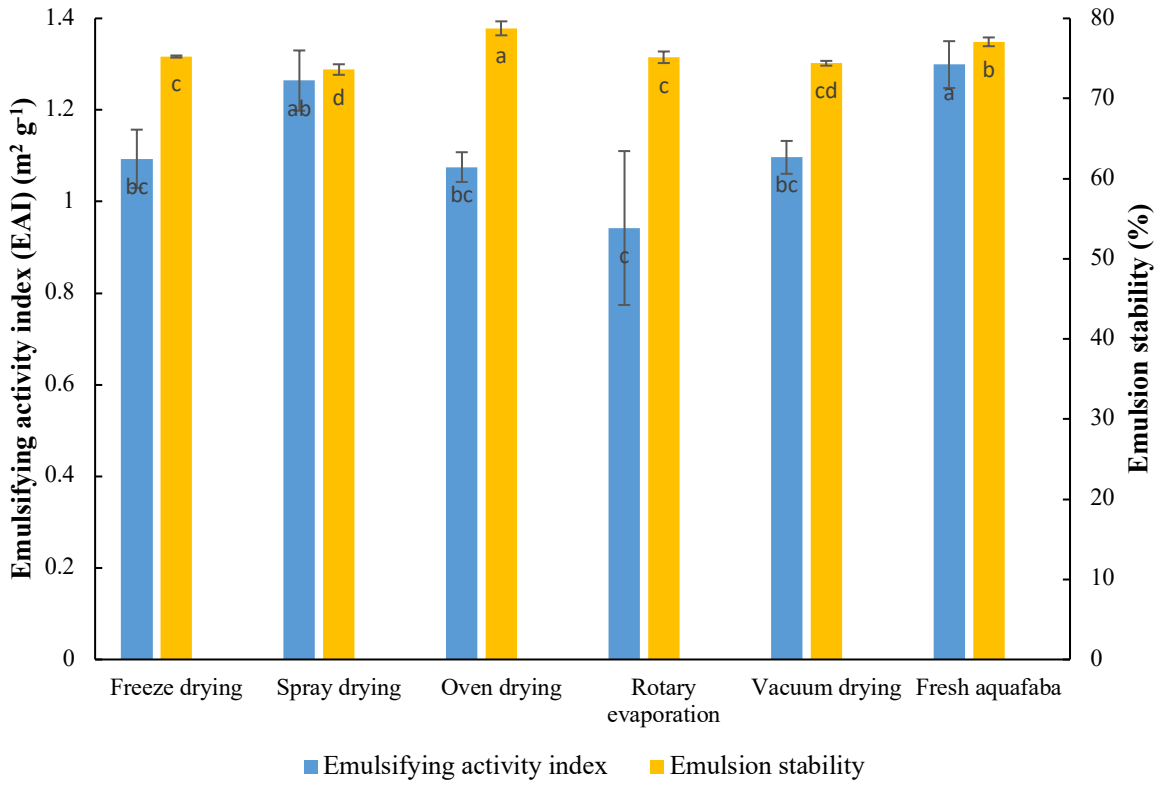


Figure 4.9 Emulsion capacity index and stability of AQ prepared with CDC Leader and dried by different drying methods.

However, there was evidence of browning in the oven dried samples. Emulsion stability of freeze dried (75.2%), rotary evaporation dried (75.2%) and vacuum pressure dried (74.4%) samples did not differ significantly from each other. Spray dried samples had relatively lower ES (73.6%) compared with other dried samples. Spray dried AQ yield value was also lower than the other samples, which might have led to an AQ concentration of constituents during rehydration. AQ concentration has been verified having a negative correlation with ES, previously discussed above in section 4.6. Therefore, the lower ES of rehydrated spray dried AQ might be due to this factor.

In summary, spray drying can be regarded as a favoured method for AQ drying as it lowers AQ water content with high efficiency and produces powder that is readily reconstituted without significant negative influences on its emulsion properties. Therefore, spray drying could lead to an AQ product suitable for long-term storage. Additionally, dried AQ products would be suitable emulsifiers for use in a wide range of food products.

5. SUMMARY AND CONCLUSIONS

The central hypothesis of this research was that AQ emulsion properties not only differed among chickpea cultivars, cooking conditions and drying methods, but also correlated with chickpea seed compositions, physical properties, and hydration characteristics. To validate our hypothesis, AQ was produced from chickpea seed from five different cultivars then the emulsion capacity and stability were measured using absorbance and centrifugation methodologies, respectively. Next, physicochemical properties and hydration kinetics of chickpea seed from different cultivars were evaluated. From this, the overall interrelationship among AQ emulsion properties and chickpea physical, chemical, hydration characteristics, and AQ yield were identified. Finally, the impacts of different soaking and boiling processes, and subsequent drying methods, on AQ emulsion capacity and stability were also investigated.

As described in this research, AQ produced from different chickpea cultivars illustrated significant differences in emulsion properties. Aquafaba (AQ) emulsion capacity and stability, among the five chickpea cultivars, ranged from 1.10 to 1.30 $m^2 g^{-1}$ and 71.9 to 77.1%, respectively. Furthermore, AQ obtained from CDC Leader produced emulsions demonstrating superior emulsion capacity and stability. Therefore, AQ produced by CDC Leader was used in further studies to optimize AQ cooking conditions and determine the impact of drying methods on AQ quality and emulsion properties.

Chickpea seed physicochemical properties and hydration characteristics were measured. Chickpea seed physicochemical properties and hydration characteristics did not show any strong correlation with AQ emulsion capacity and stability. This could be due to differences in the dispersal of chemical substances into AQ during cooking. On the other hand, AQ emulsion properties were negatively correlated with its yield and moisture content, suggesting that more dry matter in AQ leads to improved emulsion properties.

Chickpea seed of CDC Leader was soaked and cooked for different times and AQ emulsion properties were measured to identify the best AQ production conditions. Results revealed that AQ prepared by soaking chickpea seed in 4 °C water for 16 h and cooking for 30 min displayed the

highest emulsion capacity (1.30 m^2 g^{-1}) and stability (77.1%). Furthermore, the influence from different methods of drying on AQ emulsion properties, and drying rates, were investigated. It was found that spray dried AQ resulted in a powder possessing superior emulsion properties and drying rate. Therefore, spray drying was regarded as a preferred AQ drying method.

In conclusion, AQ produced by soaking chickpea seed of CDC Leader in 4 °C water for 16 h and cooking for 30 min, was spray dried to obtain a powder suitable for long term storage. Altogether, AQ exhibits excellent emulsification properties and, when dried, produces a powdered material suitable for storage and transportation. Together this suggests the potential to produce AQ as a concentrated emulsifier to replace egg in food oil emulsions. Selection of chickpea genotype and control of AQ processing is required to standardize emulsion properties. The results demonstrated here can help design useful and novel, egg-free, AQ emulsion products such as, egg-free mayonnaise and salad dressing.

6. FURTHER DIRECTIONS

Results from this study demonstrate the possible application of AQ as an emulsifier to replace egg in food oil emulsions. Moreover, results from this study revealed that chickpea seed physicochemical properties and hydration characteristics did not correlate with AQ emulsion properties. However, the current study does not indicate if AQ physicochemical properties are associated with AQ emulsion properties. It is possible that AQ emulsion capacity and stability are closely correlated to AQ physicochemical properties such as pH, viscosity, protein content, and carbohydrate content. Additionally, AQ as an emulsifier ingredient for food product development (e.g. replacement for eggs in complex emulsions such as mayonnaise and salad dressing) were not investigated in this study.

Therefore, further studies could focus on determining the relationship between AQ emulsion properties and physicochemical properties. A composition with AQ as an emulsifier in mayonnaise or salad dressing could be developed. A sensory study could be designed to evaluate the acceptance of AQ and determine the impact of the product on flavor. Furthermore, all available chickpea cultivars could be investigated. Also, additional AQ cooking parameters (cooking pressure, temperature) and drying methods (Artisan Rototherm drying) could be investigated.

In summary, the future directions of this work could range from AQ food product development to the standardization of AQ quality. Those works together could provide compelling evidence on the efficacy of AQ in commercial food oil emulsions.

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APPENDIX A

Dried AQ dissolved in water to refresh AQ

Figure A.1 Dried AQ dissolved in water to refresh AQ. (A) Freeze dried AQ; (B) spray dried AQ; (C) oven dried AQ; (D) rotary evaporation dried AQ; and (E) vacuum oven dried AQ

APPENDIX B

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Fri 2019-03-08 10:49 AM

To: He, Rachel <yuh885@mail.usask.ca>;

Cc: Ali-Rae Clark <ali-rae.clark@jove.com>;

Hi Rachel,

Thank you for the quick response.

Please consider this explicit permission to re-use Figure 6 from JoVE 56305. Please ensure that the original JoVE article is cited in the figure legend as well as the reference.

If you have any further questions, please do not hesitate to contact me.

Best,

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