Purdue University
Purdue e-Pubs

Open Access Dissertations | Theses and Dissertations

8-2018

The effects of mastication and digestion on the bioaccessibility of energy from nuts

Breanna M. McArthur
Purdue University

Follow this and additional works at: https://docs.lib.purdue.edu/open_access_dissertations

Recommended Citation
McArthur, Breanna M., "The effects of mastication and digestion on the bioaccessibility of energy from nuts" (2018). *Open Access Dissertations*. 2020.
https://docs.lib.purdue.edu/open_access_dissertations/2020

This document has been made available through Purdue e-Pubs, a service of the Purdue University Libraries. Please contact epubs@purdue.edu for additional information.

THE EFFECTS OF MASTICATION AND DIGESTION ON THE BIOACCESSIBILIITY OF ENERGY FROM WALNUTS

by

Breanna M. McArthur

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Food Science

West Lafayette, Indiana

August 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Richard D. Mattes, Chair
Department of Nutrition Science
Dr. Kimberly K. Buhman
Department of Nutrition Science
Dr. Mario Ferruzzi
Department of Food Science
Dr. Osvaldo Campanella
Department of Food Science

Approved by:
Dr. Arun Bhunia
Head of the Graduate Program

To my God, Jesus Christ, for giving power, love, and a sound mind.

ACKNOWLEDGMENTS

I would firstly like to express heartfelt thanks to my major professor, Rick Mattes, who is comical, genuine and the true embodiment of a mentor. Thank you for guiding me, often with large doses of patience, in all the time of research and scientific writing. I am really blessed to have had the opportunity to work with you.

I am also deeply thankful to my committee members, Dr. Mario Ferruzzi, Dr. Kim Buhman, and Dr. Oswaldo Campanella for their insightful comments, guidance and support throughout my Ph.D.

Special mention goes to Dr. Martha Verghese and her team at Alabama A&M University, not only for their faith in my ability to succeed in Food Science, but also for giving me so many wonderful opportunities. Thank you all for going far beyond the call of duty.

My sincere thanks goes to the members of the Mattes lab, past and present: Jaapna Dhillan, Cordelia Running, Kelly Higgins, Steph Hunter, Robin Rhine, and Judy Robin who assisted me in many ways throughout the last 4 years.

I would like to thank the members of the Ferruzzi lab who I have worked with over the years; you have all been wonderful colleagues and friends, making my time in the lab enjoyable and entertaining. Many thanks to my girls: Jennifer Allen, Hawi Debalo, and Ingrid Aragon-Gallego, for helping me with the digestion experiments and coping with my endless questions.

Special thanks goes to my family and friends who have been my biggest supporters throughout all of my studies. Your prayers and endless encouragement over the years has not only kept me going, but has kept me happy in the process.

Finally, my deepest praises and thanks goes to God, for His never-ending faithfulness.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: McArthur, Breanna, M. PhD
Institution: Purdue University
Degree Received: August 2018
Title: The Effects of Mastication and Digestion on the Bioaccessibility of Energy from Walnuts.
Committee Chair: Richard D. Mattes

Walnuts consumption provides multiple health benefits, but the high energy and fat content of walnuts raises continued concerns regarding their role in weight management. These concerns persist despite the evidence from epidemiological and clinical studies indicating that nut consumption does not increase the risk of weight gain. The predominate factors contributing to the inverse or neutral relationship between walnut consumption and increased body weight is their high satiety properties and poor bioaccessibility of energy during digestion. Mastication is associated with each of these factors; however, its role in energy balance has not yet been clearly characterized. Moreover, mastication is modulated by factors associated with meal ingestion, such as palatability and prandial fluid intake. Yet, there has been a dearth of information on the mastication of nuts in the context of the diet, where these inputs are present. Consequently, this dissertation focused on mastication and had four primary aims. The first aim was to evaluate the oral processing of walnuts in isolation and under manipulated palatability and fluid intake. The second aim was to evaluate the contribution of mastication to the satiety effects of walnuts. The third aim was to evaluate the contribution of mastication to the low digestion efficiency of walnuts. The forth aim was to contrast the mastication and digestion of walnuts with almonds and pistachios, nuts that present different physical properties.

Fifty health adults (25 males, 25 females; BMI 24.7 ± 3.4 kg/m^2; age: 18–52 years old) were enrolled in a randomized cross-over trial in which nuts (walnuts, almonds, pistachios) with and without water, juice, sweetened yogurt, and plain yogurt were ingested. Chewing forces, pre-swallowing particle size, along with satiety sensation and gut hormones following walnut consumption (whole nut or nut butter) were measured. Results suggest that walnuts with yogurt yielded larger particle sizes than chewing walnuts in isolation. Although particle size was not correlated with either food palatability or sweet flavor, the findings indicate that changing the conditions at swallowing might modify the release of energy from nuts. Further, fullness

sensations were higher after whole walnut than walnut butter consumption though there were no significant changes in glucose, insulin, or GLP-1 concentrations. This indicates that mastication has a direct influence on the satiety effects of walnuts, although the mechanism requires further investigation.

In the second part of this work nuts (walnuts, almonds, and pistachios) were chewed by seven healthy adults (3 males, 4 females; BMI: 25 ± 1.19; age: 28 ± 4 years old) and subjected to simulated gastric and intestinal digestion conditions. Results showed that the mean particle size was significantly smaller for walnuts after mastication than after 120 minutes of the intestinal phase of digestion. Compared to almonds, the mean particle size was larger for walnuts post-mastication. Moreover, post gastric and intestinal digestion, the mean particle size was larger for walnuts compared to almonds and pistachios. However, the masticated and digesta particle sizes were not related to the integrity of cell walls nor lipid release. Mastication caused walnut cells to rupture rather than separate and as walnut tissues passed through the gastrointestinal track, lipids coalesced reducing digestion efficiency. The findings from this study suggest that the net release of energy during the digestion of walnuts is determined by the intactness of cell walls as well as by structural and compositional features of walnuts, such as naturally occurring oil bodies.

CHAPTER 1. INTRODUCTION

1.1 Rational

With the rise in overweight and obesity, individuals have increasingly turned to foods that provide a metabolic advantage in an attempt to lower energy intake and aid with healthy eating and weight loss/management (1). In this context, nuts may be an exemplary food. Extensive research has connected the intake of nuts, including walnuts with better metabolic profiles(2), improvements in select biomarkers for cardiometabolic health (3-6) as well as diet quality (5, 7, 8), and a lower or stable body weight (6, 9-11). It is notable that the lack of effect on body weight occurs despite evidence that nut consumption is associated with higher total daily energy intake (6, 11-14).

Several mechanisms explaining the relationship between nut consumption and a lack of weight gain have been advanced previously. Among these mechanisms, the satiating effects of nuts appears to largely offset the excess energy they provide (13, 15). It has been suggested that the satiating effects of nuts depend on their physical form and perhaps more importantly their high demand for oral processing (16, 17), but the contribution of chewing to the satiety value of nuts has not been widely examined. Walnuts, which are one of the most widely, consumed nuts in the US and uniquely rich in polyunsaturated fatty acids (PUFA) among other nutrients, have been associated with strong satiety responses (18, 19). However, walnuts are lesser studied nuts in regards to their effects body weight, thus they were selected as the focus of this research.

Additionally, inefficiencies in the absorption and utilization of energy from nuts may contribute to a lower risk of weight gain (13). Evidence has established that nuts have indigestible structures that must undergo substantial oral/mechanical processing for optimal release and absorption of energy (17, 20, 21). However, the relationship between the physical structure of nuts and their energy yield is still not fully understood. For example, recent evidence indicates that 21% of the energy from walnuts is not bioaccessible (22). Notably, this low bioaccessibility is analogous to almonds (23) with markedly different physical properties and higher than pistachios (24) with similar characteristics. Further research is now needed to establish the mechanisms responsible for these observations.

Since energy balance is the product of the total diet, the context in which nuts are consumed is relevant (8). National dietary assessments show that nuts are eaten in many contexts (e.g., alone as snacks or with beverages or meals) (8, 25, 26), yet knowledge concerning the oral processing of nuts within the context of the diet is lacking. A better understanding of this may lead to new strategies to derive the greatest health benefit from nuts.

1.2 Study objectives

The main objectives of this dissertation are to:

- Review the literature on the effects of nut intake on body weight as well as the underlying mechanisms involved.
- Evaluate the effects of efficiency of mastication of nuts in isolation and in the context of the diet.
- Evaluate the contribution of mastication to the high satiety property of nuts.
- Contrast the effects of mastication and digestion on the structure and release of lipid from nuts.

1.3 Organization of dissertation

This dissertation is organized into chapters that contain published manuscripts or manuscripts submitted to peer-reviewed journals.

Chapter 2 beings with a general review on the topics related to oral processing, followed by an in-depth discussion of the literature relating the oral processing of nuts to satiety and nutrient bioaccessibility.

Chapter 3 describes an investigation of the efficiency of mastication of nuts in isolation and under conditions of manipulated sweetness and fluid ingestion. Changes in pre-swallowing particle size, bite forces, and palatability were assessed. The contribution of mastication to the satiating properties of nuts was also investigated. Changes in appetite ratings and GLP-1, PYY, and ghrelin were assessed.

Chapter 4 contains findings on the effects of mastication and digestion on energy bioaccessibility as well as changes in particle size and cell wall encapsulation of lipid derived from nuts.

Chapter 5 summarizes the main findings of this dissertation and presents recommendations for future research.

1.4 Study hypotheses

- Walnut particles will be greater when consumed with a liquid or semi-solid, especially if the liquid or semi-solid is sweet.
- Whole nut consumption will yield higher satiety responses than nut butter consumption.
- Mastication of walnuts will lead to less fracturing of cell walls and more separated intact cells compared to almonds and pistachios.
- A greater proportion of lipid-rich cells from walnuts will remain intact during gastro-intestinal digestion compared to pistachios.

1.5 References

1. Mozaffarian D. Dietary and policy priorities for cardiovascular disease, diabetes, and obesity. Circulation. 2016;133(2):187-225.

2. Kendall CW, Josse AR, Esfahani A, Jenkins DJ. Nuts, metabolic syndrome and diabetes. British Journal of Nutrition. 2010;104(4):465-73.

3. Katz DL, Davidhi A, Ma Y, Kavak Y, Bifulco L, Njike VY. Effects of walnuts on endothelial function in overweight adults with visceral obesity: a randomized, controlled, crossover trial. Journal of the American College of Nutrition. 2012;31(6):415-23.

4. Kris-Etherton PM. Walnuts decrease risk of cardiovascular disease: a summary of efficacy and biologic mechanisms. The Journal of nutrition. 2014;144(4):547S-54S.

5. Njike VY, Ayettey R, Petraro P, Treu JA, Katz DL. Walnut ingestion in adults at risk for diabetes: effects on body composition, diet quality, and cardiac risk measures. BMJ Open Diabetes Research & Care. 2015;3(1). doi: 10.1136/bmjdrc-2015-000115.

6. Pan A, Sun Q, Manson JE, Willett WC, Hu FB. Walnut consumption is associated with lower risk of type 2 diabetes in women. The Journal of nutrition. 2013;143(4):512-8.

7. Bitok E, Jaceldo-Siegl K, Rajaram S, Serra-Mir M, Roth I, Feitas-Simoes T, et al. Favourable nutrient intake and displacement with long-term walnut supplementation among elderly: results of a randomised trial. British Journal of Nutrition. 2017;118(3):201-9.

8. Griel AE, Eissenstat B, Juturu V, Hsieh G, Kris-Etherton PM. Improved diet quality with peanut consumption. Journal of the American College of Nutrition. 2004;23(6):660-8.

9. Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition. 2006;96(S2):S52-S60. Epub 2007/04/01. doi: 10.1017/bjn20061864.

10. Jackson CL, Hu FB. Long-term associations of nut consumption with body weight and obesity. The American journal of clinical nutrition. 2014;100(Supplement 1):408S-11S.

11. Sabaté J, Cordero-MacIntyre Z, Siapco G, Torabian S, Haddad E. Does regular walnut consumption lead to weight gain? British Journal of Nutrition. 2005;94(5):859-64.

12. Freisling H, Noh H, Slimani N, Chajès V, May AM, Peeters PH, et al. Nut intake and 5-year changes in body weight and obesity risk in adults: results from the EPIC-PANACEA study. European journal of nutrition.1-10.

13. Fraser GE, Bennett HW, Jaceldo KB, Sabaté J. Effect on body weight of a free 76 kilojoule (320 calorie) daily supplement of almonds for six months. Journal of the American College of Nutrition. 2002;21(3):275-83.

14. Alper C, Mattes R. Effects of chronic peanut consumption on energy balance and hedonics. International journal of obesity. 2002;26(8):1129.

15. Kranz S, Hill A, Fleming J, Hartman T, West S, Kris-Etherton P. Nutrient displacement associated with walnut supplementation in men. Journal of human nutrition and dietetics. 2014;27(s2):247-54.

16. Kirkmeyer S, Mattes R. Effects of food attributes on hunger and food intake. International Journal of Obesity & Related Metabolic Disorders. 2000;24(9).

17. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. The American journal of clinical nutrition. 2009;89(3):794-800. doi: 10.3945/ajcn.2008.26669.

18. Stevenson JL, Paton CM, Cooper JA. Hunger and satiety responses to high-fat meals after a high-polyunsaturated fat diet: A randomized trial. Nutrition. 2017;41:14-23.

19. Alasalvar C, Shahidi F. Tree nuts: composition, phytochemicals, and health effects: CRC Press; 2008.

20. Grundy MML, Grassby T, Mandalari G, Waldron KW, Butterworth PJ, Berry SEE, et al. Effect of mastication on lipid bioaccessibility of almonds in a randomized human study and its implications for digestion kinetics, metabolizable energy, and postprandial lipemia. The American journal of clinical nutrition. 2015;101(1):25-33. doi: 10.3945/ajcn.114.088328. PubMed PMID: PMC4266890.

21. Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, et al. Role of cell walls in the bioaccessibility of lipids in almond seeds. The American journal of clinical nutrition. 2004;80(3):604-13.

22. Baer DJ, Gebauer SK, Novotny JA. Walnuts Consumed by Healthy Adults Provide Less Available Energy than Predicted by the Atwater Factors. The Journal of nutrition. 2015. doi: 10.3945/jn.115.217372.

23. Novotny JA, Gebauer SK, Baer DJ. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. The American journal of clinical nutrition. 2012;96(2):296-301.

24. Baer DJ, Gebauer SK, Novotny JA. Measured energy value of pistachios in the human diet. British Journal of Nutrition. 2012;107(1):120-5.

25. O'Neil CE, Keast DR, Nicklas TA, Fulgoni VL. Out-of-hand nut consumption is associated with improved nutrient intake and health risk markers in US children and adults: National Health and Nutrition Examination Survey 1999-2004. Nutrition research. 2012;32(3):185-94.

26. O'Neil CE, Keast DR, Fulgoni VL, Nicklas TA. Tree nut consumption improves nutrient intake and diet quality in US adults: an analysis of National Health and Nutrition Examination Survey (NHANES) 1999-2004. Asia Pacific journal of clinical nutrition. 2010;19(1):142.

CHAPTER 2. A LITERATURE REVIEW ON THE ENERGETICS OF NUT CONSUMPTION: ORAL PROCESSING, APPETITE, AND ENERGY BALANCE

2.1 Abstract

Tree nuts and peanuts are nutrient rich foods with wide ranging cardiovascular disease and metabolic benefits. Despite these benefits, since nuts are energy dense foods, there is still a fear that their consumption may lead to a greater long-term risk of developing overweight or obesity. However, accumulating evidence indicates that nut consumption is inversely associated with weight gain. This is mainly attributable to the high satiety and low metabolizable energy properties of nuts and perhaps an acute increase in energy expenditure or thermogenesis, resulting in dissipation of another portion of the energy they provide. All of these mechanisms stem from the oral cavity. It is here that appetitive signals, which modulate appetite and perhaps metabolic processes, such as lipid digestion and absorption are generated in response to the chemical and physical properties of nuts. Moreover, the mechanical reduction of nuts into digestible particles through mastication alters their structure with implications for digestive and absorptive efficiency. Further, oral processing stimulates sympathetic nervous system activity that may contribute to energy expenditure.

2.2 Introduction

Several epidemiological and clinical studies indicate that nut consumption is associated with reduced risk for a number of chronic diseases. The mechanisms behind these associations have not been fully characterized. Nevertheless, it is hypothesized that the nutritional composition of nuts induces positive health outcomes, such as in improvements in lipid profiles and glycemic control, as well as reductions in blood pressure, inflammation, and appetite(1, 2). Due to the increasingly demonstrated health benefits of nut consumption on cardiovascular health, the US Food and Drug Administration (FDA) released a health claim acknowledging the these foods may reduce the risk of heart disease (3). Since then, nuts have been included in regulatory guidelines for healthy eating worldwide(4).

Despite the approved health claims for nuts, the healthfulness of nut consumption has been questioned because they are energy dense and rich in fat (albeit mostly unsaturated), which are properties associated with positive energy balance and weight gain. Concerns about body weight remain a barrier to regular nut consumption(5). However, evidence challenging this view has emerged over the past three decades. The available data demonstrate that body weight is lower among nut consumers than non- nut consumers despite the evidence indicating nut consumers have higher energy intake (6). Suggested reasons for this counter-intuitive observation, include underreporting by nut non-consumers, differences in dietary and lifestyle patterns associated with body weight in nut consumers and non-consumers(7), or properties of nuts that affect energy balance(7, 8). The latter is the focus of this review.

Research over the past two decades has revealed three primary factors that collectively account for the limited impact of moderate nut consumption on body weight (7): 1) the energy contained in nuts is not fully bioaccessible resulting in limited absorption efficiency; 2) regular nut consumption may augment resting energy expenditure; 3) nuts have strong satiety effects leading to a high level of dietary compensation. All three of these mechanisms are linked to the oral cavity.

This chapter starts with a brief overview of chewing, followed by a discussion of the factors that influence chewing behavior. The role of nuts in energy balance is then reviewed. In addition, the literature relating the oral processing of nuts to lipid metabolism, appetite, and energy expenditure are discussed.

2.3 Obesity

Globally, the prevalence of obesity has increased and is expected to reach around 20% by 2025 if trends in the mean body mass index (BMI)(9), which characterizes its population distribution continue. Obesity causes various health issues, including increased risk of developing type 2 diabetes, dyslipidemia, hypertension cardiovascular disease, fatty liver disease certain types of cancer dementia obstructive sleep apnea and so on (10). Increases in being overweight and obese reduced life expectancy by 5–13 years increases health care expenditures by 50–200% and dramatically alters quality of life (9-11).

2.3.1 Oral processing and obesity

An association between obesity and oral processing behaviors has been noted (12, 13) . Observational studies suggest there is a positive relationship between fast eating and BMI (14), and researchers have recommended eating slower to protect against excess food intake, by chewing more and/or taking smaller bites (14-16). However, while those studies link oral processing behaviors to a higher BMI and obesity risk, clinical studies suggest that the relationship between oral processing and weight status is less clear. Inverse associations between food hardness and energy intake have been reported from randomized controlled trails, suggesting a positive effect of chewing on energy balance (17-24). In addition, a recent systematic review suggests that there is a positive association between chewing and obesity (25). Due to these discrepancies, associations between oral processing and energy balance demands clarification and was one aim of the present research.

2.3.2 Potential action of oral processing on the health benefits of nuts

Tree nuts, such as walnuts, almonds, pistachios, as well as legume seeds, such as peanuts are particularly aligned with reduced CVD, diabetes, and obesity risk as well as with cholesterol lowering effects due to the unique composition of nuts(26). Many of the health benefits provided by nuts are associated with their favorable fatty acid composition(27). Many investigations have focused specifically on walnuts due to their higher levels of polyunsaturated fatty acids (PUFA), especially ω-3 fatty acids(28). Lipid digestion and absorption influences postprandial lipedema which is related to CHD (29). Moreover, there are other compounds in walnuts and other nuts, including soluble and insoluble fibers, vitamins, minerals, and bioactive compounds, such as carotenoids and antioxidants, with known health benefits.

The majority of the health-promoting nutrients in walnuts and other nuts are enclosed within the food matrix by parenchymal cell walls (30). These cell walls limit the bioaccessibility of nutrients to physical and chemical actions in the gastrointestinal tract (GIT)(30). Failure to disrupt cell walls limits the release (bioaccessibiltiy) of intracellular nutrients to digestive enzymes, leading to losses of health promoting compounds in the stool and also attenuated postprandial lipedema(30, 31). However, mastication ruptures the cell walls making encapsulated nutrients available for digestion and absorption. Thus, mastication efficiency has the potential to influence the health benefits of nuts by altering the integrity of cell walls, which

would affect the accessibility of health-promoting compounds. However, increasing the accessibility of nutrients (e.g., fats) through mechanical disruption of cell walls could also increase blood lipid levels and energy intake. Therefore, further elucidation of the role of chewing on nut ingestion is needed.

2.4 Overview of chewing

As the first part of this dissertation research is related to chewing, a brief review of mastication, assessment of mastication performance, and factors that influence mastication performance and how it influences the microstructure of eating will be discussed here.

Chewing is the action of breaking down solid foods for swallowing and digestion. Food entering the oral cavity is transported from the front of the mouth to the occlusal surfaces of the post-canine teeth, followed by a serious of chewing cycles until a bolus suitable for swallowing is formed(32-34). Additionally, saliva contributes to bolus formation by binding masticated food particles into a coherent bolus that can be easily swallowed (35, 36).

Swallowing is a key step in the beginning of the digestion process. Swallowing transports ingested, partially degraded food from the oral cavity to the stomach for further digestion(37). Although the factors triggering a swallow are under debate, previous studies indicate that the urge to swallow food could be initiated by a threshold level in the food particle size as well as by the degree of lubrication of the food bolus(34). However, these levels depend on the consumer's dental state, the volume of the food ingested, as well as the mechanical properties of the food since foods of low rigidity can be swallowed in larger sizes than harder foods(38). To date most of the evidence suggests that swallowing is primarily determined by the rheological properties of the bolus (e.g., cohesiveness) and these properties vary between individuals (39).

2.4.1 Measuring and monitoring chewing

Mastication performance can be evaluated through recordings of jaw movements and measurements of the particle size of the chewed bolus. Multiple methods for recording jaw movement have been used, such as electromyography (EMG) and video recordings. The EMG method is most commonly used to assess the microstructure of chewing. Unlike video recordings, which provide information about chewing duration, number of chews and chewing rate, EMG methods provide information about bite forces and muscle activities (40).

The bolus particle size can be used as an important indicator of mastication performance, as it influences not only swallowing decisions, but also subsequent digestion processes, such as nutrient extraction and gastric emptying(41). A wide variety of methods have been used to quantify particle sizes, including sieving, colorimetric methods, and optical scanning of chewed particles (40). However, in most studies, particle size is determined by sieving, probably because it is the most feasible as the equipment required is simple and inexpensive compared with other methods. However, variation it the recovered bolus is large because it is difficult to collect every food particle from the oral cavity after expectoration. Therefore, particle size data is typically expressed as the percentage of bolus weight for each particle size range. In some studies the median particle size, which is the theoretical sieve through which 50% of the particle weight can pass is used(42).

2.4.2 Factors influencing chewing performance

2.4.2.1 Intrinsic factors

Several factors can affect mastication performance. Intrinsic factors, such as age(43) and gender(44), as well as dental status and salivary flow rate have a significant impact on mastication performance(36). Several studies have described large intra-individual variation (both within and between foods) in many mastication parameters(38, 42, 45-47), for example the number of chews required for carrots ranged from 9 to 65 and it was 14 to 44 for Brazil nuts(38).

Furthermore, depending on the type of food, there is considerable inter-individual variability in particle size. For example, for soft foods, such as rice, the particle size distribution differs greatly between individuals and correlations between the particle size and type of rice were noted (48). In contrast, for hard foods, such as nuts the inter-individual variability in particle size distribution is smaller (37, 49). One study reported no significant inter-individual variability and showed an obvious difference in the particle size between nuts (peanuts, almonds, and pistachios) (47). Similarly, in another study, no significant inter-individual variability was found in the particle size distribution for nuts (peanut, almond, and pistachios)(42). Therefore, in the case of nuts, extrinsic factors (factors related to the characteristics of food) may contribute more to the variation in mastication performance than inherent consumer traits.

2.4.2.2 Extrinsic factors

Extrinsic factors, such as food structure(50), hardness(51-53), flavor (51, 52, 54-57), as well as food-related lubrication(58-60) are known to influence chewing performance. Among these factors, food hardness has been extensively studied. Harder nuts (e.g., almonds, pistachios, peanuts) require a higher bite force and longer chewing time to form a bolus suitable for swallowing than softer nuts (e.g., peanuts, pistachios, cashews, walnuts) (42, 51, 52). However, in these studies, the particle size in the bolus before swallowing has not differed between hard and soft nuts, despite the large variability in chewing parameters. It is possibly because intra- and inter-individual differences in particle size thresholds for swallowing are fairly small in comparison with the differences in mastication parameters (e.g. number of chews, total EMG activity during a chewing sequence) (37). Thus, individuals with normal dentition may use their masticatory apparatus in different ways to achieve a similar bolus (61).

Large variability in oral processing has been noted for different food types, with the lubrication of a food being a key influential factor (38, 60, 62). Solid foods for example, require extensive processing for safe swallowing(36). In contrast, liquids and semi-solid foods, being already lubricated and without structure, can thus be cleared quickly from the oral cavity without any mastication (36). For example, very moist foods, such as oysters have been shown to be swallowed without any mastication or further lubrication (38). Moreover, previous studies have found that liquid and semi-solid foods are ingested quicker and in larger bites than solid foods (63).

Swallowing studies have shown that drinking while simultaneously eating a cookie or corn beef hash triggers early swallowing of the food (64). Additionally, previous studies demonstrated that adding water to solid foods (peanuts, cakes, toast) reduced jaw muscle activity and the number of chews required until swallowing(60, 62). More recently, Hutchings *et al.* investigated mastication parameters and the final particle size in the food bolus when peanuts were embedded inside two-semi-solid matrices (gelatin gel and chocolate)(45). It was found that the chocolate matrices, containing the peanuts were masticated for a shorter time compared to the gelatin gel matrices. Due to the shorter chewing duration, nuts in the chocolate matrices contained larger bolus particles than nuts in the gelatin matrices. Together, these findings suggest that ingesting liquids with solid foods, including nuts may reduce the bolus preparation time and total muscle activity expended to prepare nuts for swallowing. Given that the current food supply

consists of many fluid foods(65) it is worthwhile to determine whether fluid intake with walnuts significantly alters pre-swallowing particle size and if this has implications for the health benefits of walnuts.

Moreover, palatability has been hypothesized to affect mastication performance. Previously studies indicated that mastication performance may be reduced by increasing (66-68) or decreasing (55) the sensory acceptability of a food. Using different flavored meals Bellisle *et al*., revealed an inverse association between food palatability and both the number of chews and chew time (per unit food)(66, 69). This study also compared the palatability effect between single flavored meals and mixed meals and found that palatability was higher for mixed meals than for any single flavored meal. In addition, the eating rate was increased as compared to single meals (even the most palatable), due to a reduction of chewing activity (e.g., number of chews and chew time). Similar results were reported in another study, which used a similar intervention and found that chewing time was shorter and fewer chews were made when more palatable foods were consumed(70). In contrast, other studies suggested palatability may not influence mastication performance(51, 52). Using a variety of peanuts (raw, roasted salted, roasted unsalted, honey roasted) McKiernan *et al*., reported that palatability does not influence mastication parameters (e.g., number of chews, chewing rate/sec) or the particle size of the swallowed bolus(52). The study by Frecka *et al*., which used different types of almonds (raw, salted, sliced, roasted, honey roasted) also found no relationship between palatability and mastication measures. Several factors may account for these inconsistent results. First, the definition of "chewing rate" was not consistent in these studies and not directly measured. Second, the sample size in these studies was relatively small and characteristics of subjects (e.g. gender, BMI) were different, which may have contributed to the inconsistent results. For example, gender could have influenced the results; it was recently reported that females have smaller bite sizes longer chewing duration and slower eating rates than do males (44). Forth, the experimental procedures were not consistent. The study by Bellisle *et al*. controlled the chewing rate and gave participants free access to drinking water (69), in contrast, the chewing rate used in the latter studies was not controlled and water was not introduced during the testing session (51, 52). Since fluid ingestion may magnify the palatability of a given food and is inversely associated with a meal duration(69), the effects of prandial fluid intake and palatability in association with chewing walnuts will be investigated.

An extrinsic factor that has gained little attention is flavor. A previous study found that sour taste can shorten both the chewing time and increase the rate of ingestion, mainly through effects on salivary flow (71). In addition, the sweetness of food has been shown to increase the speed of eating and reduce the number of bites, which in turn could lead to a high energy intake (56, 71, 72). Frecka *et al*., showed that honey roasted almonds had smaller particle sizes at swallowing than raw almonds because of a faster chewing rate (51). In another study by McKiernan *et al*. found that honey roasted peanuts had larger swallowing particle sizes than raw peanuts, indicating a diminished efficiency in mastication, as reflected by the lower bite forces(52). Due to the differences in particle size and the rate of ingestion, it could be hypothesized that differences in nut flavor reduce subsequent health outcomes, and consequently compromise energy balance. The current research will assess the effects of oral processing of walnuts under conditions of manipulated sweetness.

2.5 Nuts and energy balance

The high energy density of nuts has generated concern that their consumption would promote weight gain; however data collected from over 12,000 participants in the US Department of Agriculture's Continuing Survey of Food Intakes by Individuals revealed that BMI was lower in nut consumers than in those who never ate nuts (23.8 0.1 kg/m2 versus 25.0 0.1 kg/m2) despite their higher energy intakes (2191 kcal/day versus 1997 kcal/day) (73). Moreover, based on the available evidence from prospective studies and interventions, long-term consumption of nuts is associated with lower overweight/obesity risks (2, 7, 74-79). The impact of nuts on body weight has been repeatedly reviewed (2, 7, 8, 80-82) and these reviews support the role of nuts in contributing to the maintenance of healthy body weight.

Furthermore, higher intakes of nuts, walnuts in particular, is associated with a significantly lower risk of type 2 diabetes, and much of this inverse association appears to be mediated by body weight (6, 77). While preliminary, this evidence suggests that walnuts may be differentiated from other energy-dense nuts for their effects on body weight. Walnuts have a unique blend of nutrients (ALA, polyphenols, antioxidants, dietary fiber) combined in a complex food structure that may offer multiple means to affect energy balance, mainly with respect to their impact on satiety and digestion (6).

2.6 Plant cell wall structure and properties

Plant cell walls, composed of polysaccharide networks chiefly composed of cellulose, hemicellulos and pectin constituents are of high importance in regards to extraction of nutrients during digestion (49). These compounds resist degradation in the upper gut and their breakdown within the colon by the gut microbiota is not well characterized (49). Cells that are left intact during digestion act as a physical barrier, entrapping nutrients, and regulating the rate and extent of their release in the gut. Several studies show that the relatively impermeable cell walls limit the release of nutrients as well as the diffusion of enzymes into cells, restricting their bioaccessibility(50, 83-85). This structural integrity and extent of cell wall permeability depends on the inter-cellular adhesion strength and this seems to be of high importance regarding nutrient bioaccessibilty and bioavailability. Mechanical disruption of plant foods through oral processing may impact these properties and therefore alter nutrient release.

2.7 Impact of mastication on raw, whole nuts

Mastication starts the process of digestion (86). Its main role is to mechanically break down solid foods, such as nuts, into smaller particles so that nutrients embedded within cellular compartments of the food matrix can be released (bioaccessible) and potentially available for absorption in the intestine (bioavailable) (49). At the cellular level, mastication results in cell separation, cell rupture, or a combination depending on the structure and composition of the cell wall (49, 87). *Cell separation* occurs when the forces holding the cells together are weaker than the cell walls and is associated with limited release of intracellular nutrients. *Cell rupture* occurs when the forces holding the cells together are stronger than the cell walls and their cellular contents are released under pressure. Generally, the cells of soft plant tissues, such as cooked legumes and ripe fruits separate whereas the cells of crisp/crunchy plant tissues, such as nuts, tend to rupture. For the latter foods, the number of ruptured cells created during mechanical processing affects digestion and absorption of intra-cellular lipids and potentially other nutrients (29). However, even when mastication fails to fully rupture cell walls, fractures and fissures are created that can provide digestive enzymes access to enter cells for digestion (29, 86, 88) and facilitate the release of nutrients (49, 89).

Inadequate mastication may lead to inefficient energy absorption (83). In the case of nuts, lipid bioaccessibility depends on the proportion of ruptured to intact cells after mastication, and this is generally inversely related to the size of particles in the swallowed food bolus (84, 89) as illustrated in Figure 2-1. Whole raw almonds, for example, are chewed into relatively large particles and their particles have a proportion of cells that are not disrupted, thus making them more resistant to lipid release (84, 90). To date, few trials have investigated how chewing affects particle size and lipid release from less firm whole nuts (e.g., walnuts, pine nuts, and cashews). Presumably, the mastication of these nuts may result in smaller bolus particles. Cells may separate rather than rupture and this would limit lipid release/digestion. To verify this hypothesis, the current research will contrast the oral processing of walnut against almond and pistachios.

2.7.1 Impact of mastication on processed nuts

Extrinsic properties of nuts may be augmented by how they are processed which can alter their digestibility. Roasting dehydrates nut tissues causing them to become more brittle. This promotes their degradation during mastication and generally results in greater lipid bioaccessibility and bioavailability (85). Additionally, roasting and nut form (e.g., whole, sliced, butter, oil, and flour, among others) significantly modifies chewing behavior (e.g., bite force, number of masticatory cycles required before swallowing, and final particle size) (52). The implications of processing remain poorly characterized. *In vitro* digestion of almonds demonstrates roasting results in smaller particles when masticated but negligible changes in lipid release (84, 89). Randomized controlled trials (RCT) have yielded different results. In one trial, roasted almonds yielded a greater number of particles with smaller sizes and greater available energy compared to whole raw almonds (50). Conversely, another controlled trial reported particle sizes were significantly larger (> 3.35 mm) after mastication of sliced and roasted almonds compared to other almond varieties (51). The variability in an individual's mastication patterns may, in part, contribute to the discrepancies in lipid availability across studies (34). More research is necessary to understand the significance (or insignificance) of nut processing on nutrient bioavailability and energy extraction.

2.7.2 Impact of mastication on different types of nuts

Overall, the available data demonstrate that insufficient mechanical disruption of nut tissues results in incomplete nutrient release. Though most trials documenting this phenomenon have been conducted with almonds, trials with other nuts have yielded similar findings (91-94). However, energy yields do not conform to projections based on physical properties. Almonds and walnuts differ in hardness yet yield comparable energy (90, 91). Walnuts and pistachios are not markedly different in physical properties (91, 92), but yield discrepant amounts of energy. It is presently not possible to predict energy yields across nut types, therefore, more information is needed to elucidate their contribution to energy balance. Further, nuts are eaten in many ways (e.g., boiled, steamed, or as ingredients) which can greatly affect the bioaccessibility of their contents. Generally, a better understanding of the relationship between nutrient extraction and the digestion of available energy in nuts should provide a basis for processing nuts to achieve different purposes. For individuals in positive energy balance, whole nut consumption may be recommended to lower energy bioaccessibility, whereas for individuals ingesting nuts with the goal of increasing intake of macro- and micronutrients, nut forms with higher nutrient bioavailability may be optimal (e.g., oil and butter).

2.7.3 Fecal Fat Excretion

The accepted energy values of nuts as reported in the United States Department of Agriculture (USDA) National Nutrient Database are based on Atwater factors, a system commonly used to approximate the metabolizable energy (ME) of foods (95). Evidence of the limited energy bioaccessibility from nuts is not reflected in these energy estimates. Recent studies indicate almonds, walnuts, and pistachios provide approximately 24%, 21%, and 5% less ME, respectively than predicted by Atwater factors (90-92). Studies of peanuts (96, 97) and pecans (93) also reveal inefficient energy absorption based on increased fecal fat loss, but the magnitude has not been quantified. Findings from these studies are summarized in Table 2-1. Indeed, nut form also impacts bioaccessibility and bioavailability. Thus, fecal fat excretion varies depending on the physical form of a consumed food. This effect has been documented in trials with various peanut (94, 96) and almond products (e.g., whole, sliced, butter, and oil) (50).

2.8 Impact of mastication on energy expenditure

Another complementary explanation for the inverse or null association between nut consumption and body weight relates to enhanced energy expenditure with chronic nut consumption. Total energy expenditure is primarily comprised of three components: resting energy expenditure (REE) (the energy required to support the body at rest), thermogenic effect of feeding (TEF) (the energy cost of digesting, absorbing and metabolizing food), and the energy expended during physical activity (12). Several trials reveal an increase in thermogenesis with peanut consumption (97-99). One study observed an increase in REE and TEF after providing 320 kcal of high oleic peanuts to men with overweight or obesity in an acute feeding trail. Another trial found that REE was elevated 11% after frequent peanut consumption in healthy adults for 19 weeks; no change in TEF was observed (97). Similarly, another trial noted a 5% increase in REE in participants with overweight compared to normal weight participants following peanut oil ingestion for 8 weeks, and an 11% increase in REE was reported in overweight men only. No differences in TEF were reported in groups that were either lean or overweight (99). Other trials have failed to observe differences in energy expenditure among different types of nuts (e.g., walnuts (100), hazelnuts (101), or almonds (102, 103)). To date, data do not indicate that nut consumption augments physical activity (97, 99, 101-103). Only one study reported an increase in physical activity with regular nut consumption (104). However, the study was not designed to assess changes in energy expenditure.

The mechanisms by which nut consumption may increase energy expenditure are not clear but have been attributed to the combination of mono- and polyunsaturated fatty acids (MUFA and PUFA) and the protein they provide. Protein is the most thermogenic macronutrient (105) and unsaturated fatty acids are oxidized more rapidly than saturated fatty acids (SFA) (106). This would be expected to result in increased TEF which has not been widely observed. Chewing can elevate REE (107-109), but not to the magnitude reported for nut intake. Taken together, a rise in energy expenditure associated with nut consumption has been reported but not consistently. Verification or rejection of this proposed mechanism for energy dissipation would be worthwhile.

Collectively, these data provide a plausible mechanism to explain findings of higher daily energy intake coupled with neutral effects on body weight among nut consumers. Increased fecal

energy loss and elevated energy expenditure would offset the greater energy consumption as measured by bomb calorimetry or calculations based on proximate analyses of nuts.

2.9 Impact of mastication appetite and satiety

Besides having a major role in nut ingestion and digestion, the mastication of nuts has the potential to influence satiety and satiation through several routes (110, 111). First, mastication disrupts the cell walls of nuts, releasing the lipids and proteins from the cells (112), which, in turn, prompt the release of gut-derived hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide (GIP), peptide YY (PYY), and leptin that reportedly enhance satiation and satiety. However, the evidence is mixed regarding whether these hormones alter appetite or if they simply aid the digestive process of food components that promote satiety (113, 114). Studies that measured endocrine responses from nuts have yielded inconsistent results: one trial showed significantly increased PYY after peanut consumption compared to no peanuts (115), whereas other trials reported no significant difference in PYY concentration after consumption of walnuts (100), or pine nut oil (116) compared to no nuts. Similar discrepant results were observed for GLP-1. A significant increase in GLP-1 concentration has been noted after consumption of pine nut oil compared to no nuts (116), while others reported trends but no significant differences with nut consumption compared to no nuts (115). Additional studies noted no significant difference in GLP-1 concentration after whole nut consumption compared to no nuts (100, 117). Other measured hormones (e.g., CCK, ghrelin, leptin, and GIP) also showed inconsistent findings after consumption of nuts compared to no nuts (100, 115-117). Therefore, the role of gut hormone responses in nut-induced satiety are not clear.

Second, the satiety effect of nuts may be partially attributed to their physical form and increased need for mastication. Studies comparing nut forms have isolated the relative importance of oral processing on appetite. One study reported when consumed as a preload, both peanuts and peanut butter led to suppression in hunger ratings, but the decline was less in peanut butter (118). In another study that compared whole almonds, almond butter, almond flour, and almond oil, daylong fullness ratings were significantly higher after consuming whole almonds compared to almond flour and almond oil, and higher fullness ratings were reported for almond butter compared to almond flour (117). These results suggest that whole nuts have stronger

satiation and hunger suppressing effects than forms that have been mechanically reduced. There may be a higher expected satiation with whole nuts that becomes self-fulfilling (119). Thus, it appears there is a stronger contribution of mechanical processing than nutrient signaling to appetitive sensations(83), although a cognitive effect is also plausible (120). Nevertheless, few studies have been performed focusing on the contribution of mastication to appetite and satiety(83). Moreover, these studies have been limited to almonds. However, whole walnut seeds are one of the most widely consumed nut in the United States (1) and have specific properties, such as high alpha-linoleic acid (ALA) content which are potent stimulators of gut-derived satiety peptides(121), such as GLP-1 and PYY as well as behavior outcomes (e.g.,. feelings of fullness) (100). Because of this popularity and the fact that their physical and chemical properties vary markedly from almonds, determining whether the findings with almonds generalize to walnuts is warranted and one of the aims of this work.

2.9.1 Non-oral effects on appetite

Over the past two decades, nuts have become a model food for appetite control: they have been shown to increase satiation (117) and satiety (100), and to decrease hunger (118, 122) and desire to eat (122) ratings. The timing of nut consumption may alter their effects on appetite (122). Several studies indicate a strong appetitive effect when nuts are consumed in the morning. Consumption of whole almonds in cereal at breakfast significantly increased daylong fullness ratings compared to cereal without almonds in adults who were overweight (117). Similarly, walnut consumption as part of a shake at breakfast was associated with higher satiety and fullness ratings before consumption of lunch compared to when an energy, carbohydrate, and fat-matched placebo shake was consumed at breakfast (100). Reported satiety remained significantly increased after 3 and 4 days of consuming the walnut shakes at breakfast (100). When almonds were consumed with a meal at lunch, there was less hunger suppression than when almonds were consumed at breakfast (122). Likewise, there were no significant differences in appetite ratings when peanuts were consumed at lunch compared to an iso-energetic meal (123). This suggests that if consumed with a meal, nuts paired with breakfast elicits optimal suppression of hunger and increased satiety ratings throughout the morning and day, although additional verification is required.

Snack consumption promotes excess energy intake and has been implicated in the obesity epidemic (124, 125). However, nuts consumed as a snack can exert marked suppressive effects on hunger and desire to eat ratings (122). A 4-week randomized controlled, parallel-arm study with participants at risk for type 2 diabetes contrasted almond consumption at breakfast, lunch, or as a morning or afternoon snack compared to no almond consumption. Participants who consumed almonds reported lower hunger and desire to eat ratings before the following meals. However, participants that consumed almonds alone as a morning or afternoon snack reported significantly lower levels of hunger and desire to eat ratings 60 minutes post snack, compared to when almonds were consumed with meals. In that trial, there were no significant differences in fullness ratings (122). In another study, normal weight women reported dose-dependent greater fullness and lower hunger after consumption of 0, 28, or 42 g of raw almonds as a mid-morning snack. Energy intake at lunch was also lower in a step-wise pattern. However, the appetite ratings were not suppressed throughout the day, as no significant group differences in appetitive ratings between lunch and dinner were observed (126). Conversely, another study examining the effects of peanuts consumed with a meal or as an afternoon snack in healthy participants observed average hunger and fullness ratings did not differ between snack groups with and without peanuts or with timing of consumption. However, there was greater energy compensation after consuming the peanut-only load and the snack mix with the peanut load compared to the energy matched control snack (123).

Though not fully consistent, the preponderance of evidence indicates nuts consumed as a snack suppress hunger, augment fullness, and promote energy compensation at a subsequent eating event. If verified through further work, this would support a role for nuts in individuals who choose to snack while attempting to maintain or lose body weight.

2.9.2 Properties of nuts that affect appetite

While no single property of nuts has been shown to account for noted appetitive effects, there are multiple reasons for the decreased ratings in hunger, desire to eat, and increased ratings of fullness and satiety reported across studies. First, nuts provide 0.9-3.5 g of dietary fiber per one-ounce portion (127). Fiber contributes to gastric distension and slows gastric emptying, transit time, and absorption of nutrients from the GI tract. These actions possibly increase feelings of fullness, but do not explain the entire phenomenon. Secondly, nuts provide

unsaturated fatty acids, including 2.5-16.7 g MUFA and 0.4-13.4 g MUFA per one ounce serving (127). Unsaturated fatty acids are more readily oxidized than SFA (128). It has been hypothesized that fatty acid oxidation maintains satiety between meals and delays the onset of feelings of hunger in mice (128). Therefore, the high unsaturated fatty acid content of nuts could contribute to satiety and longer intervals between eating events (128). However, several trials have failed to report differential appetitive effects following nut loads varying in fatty acid composition (129, 130). Nuts are also rich in protein, with a content ranging from 2.2-6.0 g per one ounce (127). Protein is reportedly the most satiating macronutrient (131) and consumption is associated with decreased energy intake (8, 132, 133). Protein consumption leads to the secretion of satiety hormones such as GIP, GLP-1, CCK, PYY, and inhibition of ghrelin, all of which may promote satiety. Although, as noted above, nut consumption does not have a robust effect on gut peptide secretion. Nevertheless, the composition of nuts (e.g., fiber, unsaturated fat, and protein) likely contributes to their effects on appetite.

In addition to their chemical composition, there are physical attributes of nuts that could aid in controlling appetite. For example, consuming in-shell nuts may lower energy intake. A randomized, cross-over, controlled-feeding trial in university students, revealed consumption of in-shell pistachios led to lower energy intake than shelled pistachio kernels (134). It was hypothesized that not only does consumer manual shelling slow consumption time, but the empty shells provide a visual clue as to how many nuts were eaten, which may affect appetite (135). However, no significant differences in fullness or satisfaction ratings were reported in this study (134).

Overall, nuts have been shown to increase satiation and satiety and to decrease hunger and desire to eat ratings, especially when eaten at breakfast or as snacks. Multiple nutrient, cognitive, and physical properties of nuts likely act synergistically to impart these sensations and isolation of these components does not yield the same effects.

2.10 Conclusion

Although all nuts are energy dense and nutrient rich, they vary on multiple dimensions such as macro- and micro-nutrient content, phytochemical content, structural and sensory properties, consumption practices, and food forms. Evidence to date indicates no nut yields 100% of its calculated energy content, though there is some variability between nut types and

forms (90-92). Despite these differences, all nuts have the same effect on energy balance. Since nuts are grouped into one category in most epidemiological trials, the ability to determine whether there are differences between nuts is limited. Epidemiological studies investigating the difference between peanuts and tree nuts on obesity reported greater weight maintenance benefits with tree nut consumption than peanut consumption (136, 137). However, this has not been evaluated in a RCT and remains uncertain (137). Nuts are rarely compared between each other in RCTs. If multiple nuts are consumed, they are typically delivered as a mixture of nuts rather than separately to different groups. Overall, there is currently not enough evidence to determine if the various nuts have different effects on appetite or lipid metabolism. The prevailing view is that nuts are more similar than different in their effect on body weight (113).

2.11 References

1. Ros E. Health benefits of nut consumption. Nutrients. 2010;2(7):652-82.

2. Ros E, Tapsell LC, Sabaté J. Nuts and berries for heart health. Current atherosclerosis reports. 2010;12(6):397-406.

3. Food U, Administration D. Qualified health claims: letter of enforcement discretion–nuts and coronary heart disease. Rockville, MD: US Food and Drug Administration. 2003:1-4.

4. Cardoso BR, Duarte GBS, Reis BZ, Cozzolino SMF. Brazil nuts: Nutritional composition, health benefits and safety aspects. Food Research International. 2017;100:9-18. doi: https://doi.org/10.1016/j.foodres.2017.08.036.

5. Brown RC, Yong LC, Gray AR, Tey SL, Chisholm A, Leong SL. Perceptions and Knowledge of Nuts amongst Health Professionals in New Zealand. Nutrients. 2017;9(3):220. doi: 10.3390/nu9030220. PubMed PMID: PMC5372883.

6. Jackson CL, Hu FB. Long-term associations of nut consumption with body weight and obesity. The American journal of clinical nutrition. 2014;100(Supplement 1):408S-11S.

7. Sabaté J. Nut consumption and body weight. The American journal of clinical nutrition. 2003;78(3):647S-50S.

8. Mattes RD, Kris-Etherton PM, Foster GD. Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. The Journal of nutrition. 2008;138(9):1741S-5S.

9. Collaboration NRF. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19· 2 million participants. The Lancet. 2016;387(10026):1377-96.

10. Alston JM, Okrent AM. Obesity in America. The Effects of Farm and Food Policy on Obesity in the United States: Springer; 2017. p. 13-53.

11. Andreyeva T, Sturm R, Ringel JS. Moderate and severe obesity have large differences in health care costs. Obesity. 2004;12(12):1936-43.

12. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. The American journal of clinical nutrition. 2012;95(4):989-94.

13. Romieu I, Dossus L, Barquera S, Blottière HM, Franks PW, Gunter M, et al. Energy balance and obesity: what are the main drivers? Cancer Causes & Control. 2017;28(3):247-58.

14. Okubo H, Miyake Y, Sasaki S, Tanaka K, Hirota Y. Rate of eating in early life is positively associated with current and later body mass index among young Japanese children: the Osaka Maternal and Child Health Study. Nutrition research. 2017;37:20-8.

15. Mattfeld RS, Muth ER, Hoover A. A comparison of bite size and BMI in a cafeteria setting. Physiology & Behavior. 2017;181:38-42. doi: https://doi.org/10.1016/j.physbeh.2017.09.002.

16. Zijlstra N, de Wijk R, Mars M, Stafleu A, de Graaf C. Effect of bite size and oral processing time of a semisolid food on satiation. The American journal of clinical nutrition. 2009;90(2):269-75. doi: 10.3945/ajcn.2009.27694.

17. Stull AJ, Apolzan JW, Thalacker-Mercer AE, Iglay HB, Campbell WW. Liquid and Solid Meal Replacement Products Differentially Affect Postprandial Appetite and Food Intake in Older Adults. Journal of the American Dietetic Association. 2008;108(7):1226-30. doi: 10.1016/j.jada.2008.04.014. PubMed PMID: PMC2556245.

18. Mattes R. Soup and satiety. Physiology & Behavior. 2005;83(5):739-47.

19. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. International Journal of Obesity & Related Metabolic Disorders. 2000;24(6).

20. Lasschuijt MP, Mars M, Stieger M, Miquel-Kergoat S, de Graaf C, Smeets P. Comparison of oro-sensory exposure duration and intensity manipulations on satiation. Physiology & Behavior. 2017;176:76-83.

21. Houchins JA, Tan S-Y, Campbell WW, Mattes RD. Effects of fruit and vegetable, consumed in solid vs. beverage forms on acute and chronic appetitive responses in lean and obese adults. International journal of obesity (2005). 2013;37(8):1109.

22. Kirkmeyer S, Mattes R. Effects of food attributes on hunger and food intake. International Journal of Obesity & Related Metabolic Disorders. 2000;24(9).

23. Cecil J, Francis J, Read N. Relative contributions of intestinal, gastric, oro-sensory influences and information to changes in appetite induced by the same liquid meal. Appetite. 1998;31(3):377-90.

24. Mourao D, Bressan J, Campbell W, Mattes R. Effects of food form on appetite and energy intake in lean and obese young adults. International journal of obesity. 2007;31(11):1688-

25. Tada A, Miura H. Association of mastication and factors affecting masticatory function with obesity in adults: a systematic review. BMC oral health. 2018;18(1):76.

26. Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition. 2006;96(S2):S52-S60. Epub 2007/04/01. doi: 10.1017/bjn20061864.

27. Ros E. Nuts and CVD. British Journal of Nutrition. 2015;113(S2):S111-S20.

28. Feldman EB. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. The Journal of nutrition. 2002;132(5):1062S-101S.

29. Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, et al. Release of protein, lipid, and vitamin E from almond seeds during digestion. Journal of Agricultural and Food Chemistry. 2008;56(9):3409-16.

30. Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, et al. Role of cell walls in the bioaccessibility of lipids in almond seeds. The American journal of clinical nutrition. 2004;80(3):604-13.

31. Berry SE, Tydeman EA, Lewis HB, Phalora R, Rosborough J, Picout DR, et al. Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects. The American journal of clinical nutrition. 2008;88(4):922-9.

32. Lucas P, Prinz J, Agrawal K, Bruce I. Food physics and oral physiology. Food quality and preference. 2002;13(4):203-13.

33. Salles C, Chagnon M-C, Feron G, Guichard E, Laboure H, Morzel M, et al. In-mouth mechanisms leading to flavor release and perception. Critical reviews in food science and nutrition. 2010;51(1):67-90.

34. van der Bilt A, Engelen L, Pereira LJ, van der Glas HW, Abbink JH. Oral physiology and mastication. Physiology & Behavior. 2006;89(1):22-7. doi: https://doi.org/10.1016/j.physbeh.2006.01.025.

35. Pedersen A, Bardow A, Jensen SB, Nauntofte B. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral diseases. 2002;8(3):117-29.

36. Chen J. Food oral processing—A review. Food Hydrocolloids. 2009;23(1):1-25.

37. Bornhorst GM, Singh RP. Bolus formation and disintegration during digestion of food carbohydrates. Comprehensive Reviews in Food Science and Food Safety. 2012;11(2):101-18.

38. Prinz JF, Lucas PW. Swallow thresholds in human mastication. Archives of Oral Biology. 1995;40(5):401-3. doi: https://doi.org/10.1016/0003-9969(94)00185-E.

39. Loret C, Walter M, Pineau N, Peyron M, Hartmann C, Martin N. Physical and related sensory properties of a swallowable bolus. Physiology & Behavior. 2011;104(5):855-64.

40. Van der Bilt A. Assessment of mastication with implications for oral rehabilitation: a review. Journal of Oral Rehabilitation. 2011;38(10):754-80.

41. Grundy MM-L, Edwards CH, Mackie AR, Gidley MJ, Butterworth PJ, Ellis PR. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. British Journal of Nutrition. 2016;116(5):816-33.

42. Jalabert-Malbos M-L, Mishellany-Dutour A, Woda A, Peyron M-A. Particle size distribution in the food bolus after mastication of natural foods. Food quality and preference. 2007;18(5):803-12.

43. Zhu Y, Hollis JH. Differences in chewing behaviors between healthy fully dentate young and older adults assessed by electromyographic recordings. International journal of food sciences and nutrition. 2015;66(4):452-7.

44. Park S, Shin W-S. Differences in eating behaviors and masticatory performances by gender and obesity status. Physiology & Behavior. 2015;138:69-74.

45. Hutchings SC, Foster KD, Bronlund JE, Lentle RG, Jones JR, Morgenstern MP. Mastication of heterogeneous foods: Peanuts inside two different food matrices. Food quality and preference. 2011;22(4):332-9.

46. Hoebler C, Karinthi A, Devaux M-F, Guillon F, Gallant D, Bouchet B, et al. Physical and chemical transformations of cereal food during oral digestion in human subjects. British Journal of Nutrition. 1998;80(5):429-36.

47. Mishellany A, Woda A, Labas R, Peyron M-A. The challenge of mastication: preparing a bolus suitable for deglutition. Dysphagia. 2006;21(2):87-94.

48. Ranawana V, Henry CJK, Pratt M. Degree of habitual mastication seems to contribute to interindividual variations in the glycemic response to rice but not to spaghetti. Nutrition research. 2010;30(6):382-91.

49. Grundy MM, Edwards CH, Mackie AR, Gidley MJ, Butterworth PJ, Ellis PR. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. The British journal of nutrition. 2016;116(5):816-33.

50. Gebauer SK, Novotny JA, Bornhorst GM, Baer DJ. Food processing and structure impact the metabolizable energy of almonds. Food & function. 2016;7(10):4231-8.

51. Frecka JM, Hollis JH, Mattes RD. Effects of appetite, BMI, food form and flavor on mastication: almonds as a test food. Eur J Clin Nutr. 2007;62(10):1231-8.

52. McKiernan F, Mattes RD. Effects of peanut processing on masticatory performance during variable appetitive states. Journal of nutrition and metabolism. 2009;2010.

53. Mioche L, Bourdiol P, Monier S. Chewing behaviour and bolus formation during mastication of meat with different textures. Archives of Oral Biology. 2003;48(3):193-200.

54. Beridot-Therond M, Arts I, Fantino M, De La Gueronniere V. Short-term effects of the flavour of drinks on ingestive behaviours in man. Appetite. 1998;31(1):67-81.

55. Neyraud E, Peyron M, Vieira C, Dransfield E. Influence of bitter taste on mastication pattern. Journal of Dental Research. 2005;84(3):250-4.

56. Forde C, Leong C, Chia-Ming E, McCrickerd K. Fast or slow-foods? Describing natural variations in oral processing characteristics across a wide range of Asian foods. Food & function. 2017;8(2):595-606.

57. Rolls BJ, Kim S, Fedoroff IC. Effects of drinks sweetened with sucrose or aspartame on hunger, thirst and food intake in men. Physiology & Behavior. 1990;48(1):19-26.

58. Foster KD, Grigor J, Cheong JN, Yoo MJ, Bronlund JE, Morgenstern MP. The role of oral processing in dynamic sensory perception. Journal of Food Science. 2011;76(2).

59. Kim EHJ, Jakobsen VB, Wilson AJ, Waters IR, Motoi L, Hedderley DI, et al. Oral processing of mixtures of food particles. Journal of Texture Studies. 2015;46(6):487-98.

60. Engelen L, Fontijn-Tekamp A, van der Bilt A. The influence of product and oral characteristics on swallowing. Archives of Oral Biology. 2005;50(8):739-46.

61. Horio T, Kawamura Y. Effects of texture of food on chewing patterns in the human subject. Journal of Oral Rehabilitation. 1989;16(2):177-83.

62. Liu D, Deng Y, Sha L, Hashem MA, Gai S. Impact of oral processing on texture attributes and taste perception. Journal of food science and technology. 2017;54(8):2585-93.

63. Brudevold F, Kashket S, Kent Jr R. The effect of sucrose and fat in cookies on salivation and oral retention in humans. Journal of Dental Research. 1990;69(6):1278-82.

64. Bellisle F, Guy-Grand B, Le Magnen J. Chewing and swallowing as indices of the stimulation to eat during meals in humans: effects revealed by the edogram method and video recordings. Neuroscience & Biobehavioral Reviews. 2000;24(2):223-8. doi: https://doi.org/10.1016/S0149-7634(99)00075-5.

65. Bellisle F, Le Magnen J. The analysis of human feeding patterns: the edogram. Appetite. 1980;1(2):141-50.

66. Guy-Grand B, Lehnert V, Doassans M, Bellisle F. Type of Test-meal Affects Palatability and Eating Style in Humans. Appetite. 1994;22(2):125-34. doi: https://doi.org/10.1006/appe.1994.1012.

67. Bellisle F, Le Magnen J. The structure of meals in humans: Eating and drinking patterns in lean and obese subjects. Physiology & Behavior. 1981;27(4):649-58. doi: https://doi.org/10.1016/0031-9384(81)90237-7.

68. Bellisle F, Lucas F, Amrani R, Le Magnen J. Deprivation, paiatability and the micro-structure of meals in human subjects. Appetite. 1984;5(2):85-94.

69. Van Der Bilt A, Engelen L, Abbink J, Pereira LJ. Effects of adding fluids to solid foods on muscle activity and number of chewing cycles. European Journal of Oral Sciences. 2007;115(3):198-205.

70. De Wijk R, Zijlstra N, Mars M, De Graaf C, Prinz J. The effects of food viscosity on bite size, bite effort and food intake. Physiology & Behavior. 2008;95(3):527-32.

71. Saitoh E, Shibata S, Matsuo K, Baba M, Fujii W, Palmer JB. Chewing and food consistency: effects on bolus transport and swallow initiation. Dysphagia. 2007;22(2):100-7.

72. Viskaal-van Dongen M, Kok FJ, de Graaf C. Eating rate of commonly consumed foods promotes food and energy intake. Appetite. 2011;56(1):25-31.

73. Coates AM, Howe PR. Edible nuts and metabolic health. Current opinion in lipidology. 2007;18(1):25-30.

74. Sabaté J, Ang Y. Nuts and health outcomes: new epidemiologic evidence–. The American journal of clinical nutrition. 2009;89(5):1643S-8S.

75. Freisling H, Noh H, Slimani N, Chajès V, May AM, Peeters PH, et al. Nut intake and 5-year changes in body weight and obesity risk in adults: results from the EPIC-PANACEA study. European journal of nutrition. 2017:1-10.

76. Bitok E, Jaceldo-Siegl K, Rajaram S, Serra-Mir M, Roth I, Feitas-Simoes T, et al. Favourable nutrient intake and displacement with long-term walnut supplementation among elderly: results of a randomised trial. British Journal of Nutrition. 2017;118(3):201-9.

77. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease: the Adventist Health Study. Archives of Internal medicine. 1992;152(7):1416-24.

78. Sabaté J, Cordero-MacIntyre Z, Siapco G, Torabian S, Haddad E. Does regular walnut consumption lead to weight gain? British Journal of Nutrition. 2005;94(5):859-64.

79. de Souza RGM, Schincaglia RM, Pimentel GD, Mota JF. Nuts and Human Health Outcomes: A Systematic Review. Nutrients. 2017;9(12):1311.

80. Lutz M, Luna L. Nuts and body weight: An overview. Journal of Nutrition and Health Sciences. 2016;3:104.

81. Bes-Rastrollo M, Sabaté J, Gómez-Gracia E, Alonso A, Martínez JA, Martínez-González MA. Nut consumption and weight gain in a Mediterranean cohort: The SUN study. Obesity. 2007;15(1):107-.

82. Tan SY, Dhillon J, Mattes RD. A review of the effects of nuts on appetite, food intake, metabolism, and body weight–. The American journal of clinical nutrition. 2014;100(suppl_1):412S-22S.

83. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. The American journal of clinical nutrition. 2009;89(3):794-800. doi: 10.3945/ajcn.2008.26669.

84. Grundy MML, Grassby T, Mandalari G, Waldron KW, Butterworth PJ, Berry SEE, et al. Effect of mastication on lipid bioaccessibility of almonds in a randomized human study and its implications for digestion kinetics, metabolizable energy, and postprandial lipemia. The American journal of clinical nutrition. 2015;101(1):25-33. doi: 10.3945/ajcn.114.088328. PubMed PMID: PMC4266890.

85. Grundy MML, Lapsley K, Ellis PR. A review of the impact of processing on nutrient bioaccessibility and digestion of almonds. International journal of food science & technology. 2016.

86. Kong F, Singh R. Disintegration of solid foods in human stomach. Journal of Food Science. 2008;73(5).

87. Waldron KW, Parker M, Smith AC. Plant cell walls and food quality. Comprehensive Reviews in Food Science and Food Safety. 2003;2(4):128-46.

88. Grassby T, Picout DR, Mandalari G, Faulks RM, Kendall CW, Rich GT, et al. Modelling of nutrient bioaccessibility in almond seeds based on the fracture properties of their cell walls. Food & function. 2014;5(12):3096-106.

89. Mandalari G, Grundy MM-L, Grassby T, Parker ML, Cross KL, Chessa S, et al. The effects of processing and mastication on almond lipid bioaccessibility using novel methods of in vitro digestion modelling and micro-structural analysis. British Journal of Nutrition. 2014;112(9):1521-

90. Novotny JA, Gebauer SK, Baer DJ. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. The American journal of clinical nutrition. 2012;96(2):296-301.

91. Baer DJ, Gebauer SK, Novotny JA. Walnuts Consumed by Healthy Adults Provide Less Available Energy than Predicted by the Atwater Factors. The Journal of nutrition. 2015. doi: 10.3945/jn.115.217372.

92. Baer DJ, Gebauer SK, Novotny JA. Measured energy value of pistachios in the human diet. British Journal of Nutrition. 2012;107(1):120-5.

93. Haddad E, Sabate J, editors. Effect of pecan consumption on stool fat. FASEB JOurnal; 2000: Federation Amer Soc Exp Bio 9650 Rockvillew Pike, Bethesda, MD 20814-3998 USA.

94. Levine AS, Silvis SE. Absorption of whole peanuts, peanut oil, and peanut butter. New England Journal of Medicine. 1980;303(16):917-8.

95. Zou ML, Moughan PJ, Awati A, Livesey G. Accuracy of the Atwater factors and related food energy conversion factors with low-fat, high-fiber diets when energy intake is reduced spontaneously. The American journal of clinical nutrition. 2007;86(6):1649-56.

96. Traoret C, Lokko P, Cruz A, Oliveira C, Costa N, Bressan J, et al. Peanut digestion and energy balance. International journal of obesity. 2008;32(2):322.

97. Alper C, Mattes R. Effects of chronic peanut consumption on energy balance and hedonics. International journal of obesity. 2002;26(8):1129.

98. Alves RDM, Moreira APB, Macedo VS, Costa NMB, Alfenas RdCG, Bressan J. High-oleic peanuts increase diet-induced thermogenesis in overweight and obese men. Nutrición Hospitalaria. 2014;29(5):1024-32.

99. Coelho SB, de Sales RL, Iyer SS, Bressan J, Costa NMB, Lokko P, et al. Effects of peanut oil load on energy expenditure, body composition, lipid profile, and appetite in lean and overweight adults. Nutrition. 2006;22(6):585-92.

100. Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut Consumption Increases Satiation but Has No Effect on Insulin Resistance or the Metabolic Profile Over a 4-day Period. Obesity. 2010;18(6):1176-82.

101. Tey SL, Brown R, Gray A, Chisholm A, Delahunty C. Nuts improve diet quality compared to other energy-dense snacks while maintaining body weight. J Nutr Metab. 2011;2011. doi: 10.1155/2011/357350.

102. Fraser GE, Bennett HW, Jaceldo KB, Sabaté J. Effect on body weight of a free 76 kilojoule (320 calorie) daily supplement of almonds for six months. Journal of the American College of Nutrition. 2002;21(3):275-83.

103. Hollis J, Mattes R. Effect of chronic consumption of almonds on body weight in healthy humans. Br J Nutr. 2007;98(3):651-6. Epub 2007/04/21. doi: 10.1017/s0007114507734608. PubMed PMID: 17445351.

104. Agebratt C, Ström E, Romu T, Dahlqvist-Leinhard O, Borga M, Leandersson P, et al. A randomized study of the effects of additional fruit and nuts consumption on hepatic fat content, cardiovascular risk factors and basal metabolic rate. PloS one. 2016;11(1):e0147149.

105. Westerterp K, Wilson S, Rolland V. Diet induced thermogenesis measured over 24h in a respiration chamber: effect of diet composition. International Journal of Obesity & Related Metabolic Disorders. 1999;23(3).

106. Jones PJ, Schoeller DA. Polyunsaturated: saturated ratio of diet fat influences energy substrate utilization in the human. Metabolism. 1988;37(2):145-51.

107. Kresge DL, Melanson K. Chewing gum increases energy expenditure before and after controlled breakfasts. Applied Physiology, Nutrition, and Metabolism. 2014;40(4):401-6.

108. Komai N, Motokubota N, Suzuki M, Hayashi I, Moritani T, Nagai N. Thorough Mastication Prior to Swallowing Increases Postprandial Satiety and the Thermic Effect of a Meal in Young Women. Journal of nutritional science and vitaminology. 2016;62(5):288-94.

109. Paphangkorakit J, Leelayuwat N, Boonyawat N, Parniangtong A, Sripratoom J. Effect of chewing speed on energy expenditure in healthy subjects. Acta Odontologica Scandinavica. 2014;72(6):424-7.

110. Jordan HA. Voluntary intragastric feeding: oral and gastric contributions to food intake and hunger in man. J Comp Physiol Psychol. 1969;68(4):498-506. Epub 1969/08/01. PubMed PMID: 5344500.

111. Lavin JH, French SJ, Read NW. Comparison of oral and gastric administration of sucrose and maltose on gastric emptying rate and appetite. Int J Obes Relat Metab Disord. 2002;26(1):80-6. Epub 2002/01/16. doi: 10.1038/sj.ijo.0801828. PubMed PMID: 11791150.

112. Mattes RD, Dreher ML. Nuts and healthy body weight maintenance mechanisms. Asia Pac J Clin Nutr. 2010;19(1):137-41. Epub 2010/03/05. PubMed PMID: 20199999.

113. Tan SY, Dhillon J, Mattes RD. A review of the effects of nuts on appetite, food intake, metabolism, and body weight. Am J Clin Nutr. 2014;100 Suppl 1:412s-22s. Epub 2014/06/13. doi: 10.3945/ajcn.113.071456. PubMed PMID: 24920033.

114. Mars M, Stafleu A, de Graaf C. Use of satiety peptides in assessing the satiating capacity of foods. Physiol Behav. 2012;105(2):483-8. Epub 2011/09/13. doi: 10.1016/j.physbeh.2011.08.033. PubMed PMID: 21907727.

115. Reis CE, Ribeiro DN, Costa NM, Bressan J, Alfenas RC, Mattes RD. Acute and second-meal effects of peanuts on glycaemic response and appetite in obese women with high type 2 diabetes risk: a randomised cross-over clinical trial. Br J Nutr. 2013;109(11):2015-23. Epub 2012/11/06. doi: 10.1017/s0007114512004217. PubMed PMID: 23122211.

116. Pasman WJ, Heimerikx J, Rubingh CM, van den Berg R, O'Shea M, Gambelli L, et al. The effect of Korean pine nut oil on in vitro CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. Lipids Health Dis. 2008;7:10. Epub 2008/03/22. doi: 10.1186/1476-511x-7-10. PubMed PMID: 18355411; PubMed Central PMCID: PMCPMC2322999.

117. Mori AM, Considine RV, Mattes RD. Acute and second-meal effects of almond form in impaired glucose tolerant adults: a randomized crossover trial. Nutr Metab (Lond). 2011;8(1):6. Epub 2011/02/01. doi: 10.1186/1743-7075-8-6. PubMed PMID: 21276226; PubMed Central PMCID: PMCPMC3042001.

118. Kirkmeyer SV, Mattes RD. Effects of food attributes on hunger and food intake. Int J Obes Relat Metab Disord. 2000;24(9):1167-75. Epub 2000/10/18. PubMed PMID: 11033986.

119. Brunstrom JM, Burn JF, Sell NR, Collingwood JM, Rogers PJ, Wilkinson LL, et al. Episodic memory and appetite regulation in humans. PLoS One. 2012;7(12):e50707. Epub

2012/12/12. doi: 10.1371/journal.pone.0050707. PubMed PMID: 23227200; PubMed Central PMCID: PMCPMC3515570.

120. Farr OM, Tuccinardi D, Upadhyay J, Oussaada SM, Mantzoros CS. Walnut consumption increases activation of the insula to highly desirable food cues: A randomized, double-blind, placebo-controlled, cross-over fMRI study. Diabetes, Obesity and Metabolism. 2017.

121. Stevenson JL, Paton CM, Cooper JA. Hunger and satiety responses to high-fat meals after a high-polyunsaturated fat diet: A randomized trial. Nutrition. 2017;41:14-23.

122. Tan SY, Mattes RD. Appetitive, dietary and health effects of almonds consumed with meals or as snacks: a randomized, controlled trial. Eur J Clin Nutr. 2013;67(11):1205-14. Epub 2013/10/03. doi: 10.1038/ejcn.2013.184. PubMed PMID: 24084509; PubMed Central PMCID: PMCPMC3898316.

123. Devitt AA, Kuevi A, Coelho SB, Lartey A, Lokko P, Costa N, et al. Appetitive and Dietary Effects of Consuming an Energy-Dense Food (Peanuts) with or between Meals by Snackers and Nonsnackers. J Nutr Metab. 2011;2011:928352. Epub 2011/08/03. doi: 10.1155/2011/928352. PubMed PMID: 21808728; PubMed Central PMCID: PMCPMC3144712.

124. Piernas C, Popkin BM. Snacking increased among U.S. adults between 1977 and 2006. J Nutr. 2010;140(2):325-32. Epub 2009/12/04. doi: 10.3945/jn.109.112763. PubMed PMID: 19955403; PubMed Central PMCID: PMCPMC2806886.

125. Berteus Forslund H, Torgerson JS, Sjostrom L, Lindroos AK. Snacking frequency in relation to energy intake and food choices in obese men and women compared to a reference population. Int J Obes Relat Metab Disord. 2005;29(6):711-9.

126. Hull S, Re R, Chambers L, Echaniz A, Wickham MS. A mid-morning snack of almonds generates satiety and appropriate adjustment of subsequent food intake in healthy women. Eur J Nutr. 2015;54. doi: 10.1007/s00394-014-0759-z.

127. USDA National Nutrient Database for Standard Reference [Internet]. 2010. Available from: http://www.ars.usda.gov/ba/bhnrc/ndl.

128. Langhans W, Leitner C, Arnold M. Dietary fat sensing via fatty acid oxidation in enterocytes: possible role in the control of eating. Am J Physiol Regul Integr Comp Physiol. 2011;300(3):R554-65. Epub 2010/12/15. doi: 10.1152/ajpregu.00610.2010. PubMed PMID: 21148477.

129. Alfenas RC, Mattes RD. Effect of fat sources on satiety. Obes Res. 2003;11(2):183-7. Epub 2003/02/13. doi: 10.1038/oby.2003.29. PubMed PMID: 12582212.

130. Strik CM, Lithander FE, McGill A-T, MacGibbon AK, McArdle BH, Poppitt SD. No evidence of differential effects of SFA, MUFA or PUFA on post-ingestive satiety and energy intake: a randomised trial of fatty acid saturation. Nutrition journal. 2010;9(1):24.

131. Pesta DH, Samuel VT. A high-protein diet for reducing body fat: mechanisms and possible caveats. Nutr Metab (Lond). 2014;11(1):53. Epub 2014/12/10. doi: 10.1186/1743-7075-11-53. PubMed PMID: 25489333; PubMed Central PMCID: PMCPMC4258944.

132. Jaceldo-Siegl K, Sabaté J, Rajaram S, Fraser GE. Long-term almond supplementation without advice on food replacement induces favourable nutrient modifications to the habitual diets of free-living individuals. British Journal of Nutrition. 2004;92(03):533-40.

133. Bitok E, Jaceldo-Siegl K, Rajaram S, Serra-Mir M, Roth I, Feitas-Simoes T, et al. Favourable nutrient intake and displacement with long-term walnut supplementation among elderly: results of a randomised trial. Br J Nutr. 2017;118(3):201-9. Epub 2017/08/24. doi: 10.1017/s0007114517001957. PubMed PMID: 28831957.

134. Honselman CS, Painter JE, Kennedy-Hagan KJ, Halvorson A, Rhodes K, Brooks TL, et al. In-shell pistachio nuts reduce caloric intake compared to shelled nuts. Appetite. 2011;57(2):414-7. Epub 2011/06/08. doi: 10.1016/j.appet.2011.02.022. PubMed PMID: 21645565.

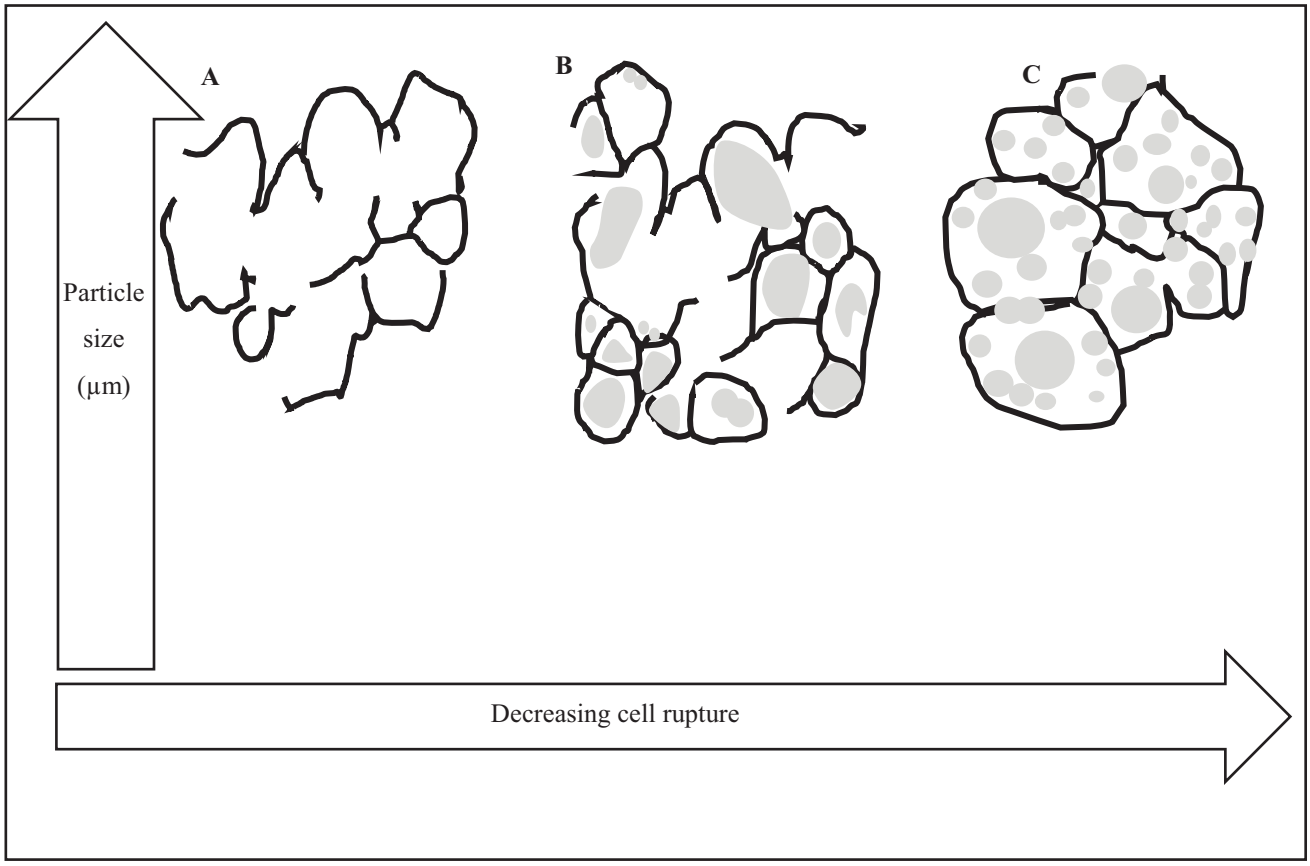
135. Bullo M, Juanola-Falgarona M, Hernandez-Alonso P, Salas-Salvado J. Nutrition attributes and health effects of pistachio nuts. Br J Nutr. 2015;113 Suppl 2:S79-93. Epub 2015/07/08. doi: 10.1017/s0007114514003250. PubMed PMID: 26148925.

136. Bes-Rastrollo M, Wedick NM, Martinez-Gonzalez MA, Li TY, Sampson L, Hu FB. Prospective study of nut consumption, long-term weight change, and obesity risk in women. The American journal of clinical nutrition. 2009;89(6):1913-9.

137. Jaceldo-Siegl K, Haddad E, Oda K, Fraser GE, Sabaté J. Tree Nuts Are Inversely Associated with Metabolic Syndrome and Obesity: The Adventist Health Study-2. PLOS ONE. 2014;9(1):e85133. doi: 10.1371/journal.pone.0085133.

138. Zemaitis J, Sabaté J, editors. Effect of almond consumption on stool weight and stool fat. FASEB Journal; 2001: Federation Amer Soc Exp Bio 9650 Rockville Pike, Bethesda, MD 20814-3998 USA.

Figure 2-1: Structural changes in the microstructure of masticated nut particles of increasing size



Note: cell wall structure is shown in black and intracellular lipids is in grey. (A) Shows cells within smaller bolus particles are ruptured and most of the cellular lipid has been extracted; (B, C) illustrates limited cell wall rupturing within particles of larger sizes and cells largely still filled with lipid.

Table 2-1: Human Feeding Studies with Nuts Reporting Data on Fecal and Energy Loss

Note: CHO, carbohydrate; EI, energy intake; HF, high fiber; LF, low fiber; nm, not measured; P, whole peanuts; PB, peanut butter; PF, peanut flour; PO, peanut oil; R, raw; RO, roasted

Nut type	Intervention	Time (week)	Total fecal energy loss	Fecal fat energy loss	Comments	Reference
Almonds	0 g/day *versus* 43 g/day *versus* 86 g/day + controlled diet	4	nm	2-Fold ↑ with 43 g/d and 86 g/d *versus* 0 g/day	Total energy of the diet not specified	(138)
Almonds	0 g/day *versus* 42 g/d *versus* 84 g/d + controlled diet	2	↑ With 42 g/d and 84 g/d *versus* 0 g/d	4-Fold ↑ with 42 g/d 6-Fold ↑ with 84 g/d	↓ Absorption of total CHO with 42 g/d *versus* 0 g/d. ↓ Absorption of CHO, fiber, and protein with 84 g/d *versus* 0 g/d. Dose response on change in fecal fat. Dose response on change in energy loss.	(90)
Almonds	42/g whole (R and RO) *versus* 42/g slice (R and RO) *versus* 42/g nut butter + controlled diet	3	↑ Whole (R and RO) and sliced R ↔ Nut butter	nm	70%, 75%, 78%, and 98% of energy from whole R, whole RO, sliced R, and butter, was absorbed, respectively.	(50)
Almonds	55 g/day with different levels of controlled chewing (10 *versus* 25 *versus* 40 chews)	3	↑ After 10 chews *versus* 40 chews	↑ After 10 chews *versus* 25 and 40 chews	With lower levels of chewing total energy and fat loss was greater than with higher levels of chewing. No control group	(83)

Table 2-1 continued

Nut type	Intervention	Time (week)	Total fecal energy loss	Fecal fat energy loss	Comments	Reference
Peanuts	Control period *versus* 70 g/d P *versus* PB *versus* PO *versus* PF + controlled non-vegetarian diet	2	↑ With P, PB, and PF ↔ With PF	↑ With P *versus* control ↓ With PB and PF ↑ With P *versus* control ↓ With PB and PF	EI ↑ with P, PB, and PF.	(96)
Peanuts	156 g/d P *versus* PB *versus* PO + 20 g/d HF *versus* 10 g/d LF vegetarian diet	6	nm	↑ With P, PB, and PO on HF/LF	Fiber plus the large quantity of nuts likely overestimate the effects of nut form on stool fat excretion.	(94)
Pecans	0 g/d *versus* 71g/d	4	↑ With 71 g/d *versus* 0 g/d	nm	Total caloric content of the diet not specified. More energy from fat provided by pecan diet (43%) *versus* control (30%) that may introduce confounding.	(93)
Pistachio	0 g/d *versus* 42 g/d *versus* 84 g/d + controlled diet	2	3-Fold ↑ with 42 g/d *versus* 0 g/d 4-Fold ↑ with 84 g/d *versus* 0 g/d	2-Fold ↑ with 84 g/d	↑ EI with 84 g/d *versus* 42 g/d and 0 g/d. No dose response on change in fecal fat. Dose response on change in energy loss. 5% Energy malabsorbed.	(92)
Walnuts	0 g/d *versus* 42 g/d + controlled diet	3	4-Fold ↑	2-Fold ↑	21% Energy malabsorbed.	(91)

CHAPTER 3. MASTICATION OF NUTS UNDER REALISTIC EATING CONDITIONS

Breanna M. McArthur[1] Robert V. Considine[2] and Richard D. Mattes[3] Mastication of nuts under realistic eating conditions: implications for energy balance. *Nutrients* (accepted on May 30 2018) The manuscript has been accepted by *Nutrients* and has been formatted according to the journals requirements

3.1 Abstract

The low digestibility and high satiety effects of nuts have been partly attributed to mastication. This work examines chewing forces and the bolus particle size of nuts (walnuts, almonds, pistachios) varying in physical properties under different conditions (with and without water, juice, sweetened yogurt and plain yogurt) along with satiety sensations and gut hormone concentrations following walnut consumption (whole or butter). In a randomized, cross-over design with 50 adults (25 males, 25 females; Body Mass Index (BMI) 24.7 ± 3.4 kg/m2; age: 18–52 years old (y/o), the chewing forces and particle size distribution of chewed nuts were measured under different chewing conditions. Appetite sensations were measured at regular intervals for 3 h after nut intake, and plasma samples were collected for the measurement of glucose, insulin and Glucagon-like peptide-1 (GLP-1). The three nuts displayed different particle sizes at swallowing though no differences in chewing forces were observed. Walnuts with yogurt yielded larger particle sizes than the other treatments. Particle size was not correlated with either food palatability or flavor. Fullness sensations were higher after whole nut than nut butter consumption though there were no significant changes in glucose, insulin, or GLP-1 concentrations under any condition. Changing the conditions at swallowing might influence the release of energy from nuts.

3.2 Keywords

nuts; physical properties; mastication; bolus formation; swallowing; satiety

3.3 Introduction

Nuts are high-fat, energy-dense foods that, historically, have been associated with adiposity. However, mounting evidence suggests that in the context of a healthy diet, the inclusion of nuts does not promote weight gain (6, 77, 78, 97, 102, 136, 139-142). This has been attributed to their potential to increase energy expenditure (97-99), high satiety value (83, 100, 143-148), and limited energy bioaccessibility (release) (93, 94, 96, 138). Mastication contributes to each of these mechanisms, but in different ways. A better understanding of oral processing may therefore yield insights for manipulating nut consumption to manage energy balance.

Several studies on gum-chewing have documented that mastication elevates energy expenditure (EE) due to the muscular activity involved in chewing (107, 109, 149). Chewing reportedly increases energy expenditure by 11 kcal/h (149), although more recent studies reveal a smaller increment in thermogenesis (107, 108). Other work noted a significantly larger increase in diet-induced thermogenesis (DIT) after consumption of a solid meal compared to the same meal in puree form (108). Although mastication was not measured, multiple studies document acute effects of peanut consumption on energy expenditure (97-99). Supportive findings in trials with other nuts are not robust. One study reported a 14% increment in EE after almond consumption (150), although in another report, no thermogenic response was noted (102). Studies with walnuts (100) and hazelnuts (151) have also revealed no variation in thermogenesis. Consequently, the evidence to date is not conclusive on this mechanism. If the act of chewing does influence thermogenesis, the effect is likely small in magnitude (108).

Investigations on solid and liquid versions of high-carbohydrate, high-protein, and high-fat foods indicate that ratings of hunger and total energy intake are higher following consumption of the liquid versions of each of these foods, regardless of the energy source tested (17-24). These findings suggest that oral processing effort/time may contribute to satiety. However, it is unclear whether this effect is direct or indirect. Some work indicates the act of chewing can enhance satiety by neural and/or endocrine mechanisms. Animal studies show that chewing directly activates satiety centers in the hypothalamus and suppresses food intake (152, 153). Additionally, oral stimulation prompts cephalic phase responses that, in turn, influence the secretion of hormones (e.g., CCK, PYY, GLP-1, insulin) that purportedly mediate appetite and metabolism (154, 155). Alternatively, food components (e.g., protein, fat, fiber) rendered bioavailable through mastication have been correlated with increases in satiety and reductions in energy

intake. Recently, the satiating properties of walnuts have been ascribed to their fatty acid profile, which is especially rich in polyunsaturated fatty acids (e.g., alpha-linolenic acid) (121). However, very few study designs isolate the independent effect of mastication on appetitive responses, so clear conclusions cannot be drawn. One aim of this study was to explore the role of mastication of walnuts on appetitive sensations and selected "satiety hormone" concentrations.

Chewing has a major role in food digestion and nutrient bioaccessibility. Chewing mechanically ruptures the cell walls of plant foods thereby freeing nutrients that may not have been accessible to the body. Randomized controlled trials exploring the relationship between mastication and energy bioaccessiblity from nuts reveal increased energy losses in the stools of subjects on diets rich in walnuts (91), pecans (93), pistachios (92), almonds (90), or peanuts [3,24]. This low bioacessibility is attributed to the resistance of nut parenchymal cell walls to degradation in the gut and inadequate mastication (30). However, one study observed greater lipid absorption (e.g., less fecal fat excretion) after almonds were chewed 40 times versus 10 times (83), calling into question the role of increased chewing as a strategy for weight loss/maintenance. Whereas prolonged oral stimulation may enhance the signals generated for appetite control (156-158), the greater nutrient availability derived through chewing could increase energy absorption. Thus, from this perspective, questions remain as to whether chewing is an aid or hindrance to energy balance.

While there are similarities in nutrient composition and energy density between nuts that support viewing all types of nuts similarly, there are structural and compositional differences that challenge this view. First, nuts differ in their physical properties (e.g., hardness). Almonds for example, require a higher breaking force than peanuts (51, 52). Dissimilarities in hardness between nuts can modify masticatory behavior such as chewing duration and/or bite strength, which determine particle size, and energy bioaccessibility. Second, the context in which nuts are consumed can vary (e.g., nuts alone or in combination with foods and beverages) (159) which can impact their oral processing and availability of nutrients (160). For example, fluid (e.g., water, clear beverages) and semi-solid (e.g., gels, yogurt) foods ingested with nuts shorten the rate/duration of chewing and trigger early swallowing of the mixture (38, 45, 60). Furthermore, prandial fluid intake has been suggested to increase the palatability of a meal which can also lead to fewer chews, larger bite sizes, and an accelerated eating rate (66, 68, 161, 162), notably when the fluid is sweetened. Thus, taking nuts with fluid products, especially sweet ones, may increase

particle sizes within the ingested bolus, affecting the ultimate release of energy from the nuts. However, data concerning this topic are scarce (38, 45), thus this issue was examined in this trial.

Recent evidence indicates 21% of the energy in walnuts is not bioaccessible, which is similar to the energy value for almonds (20% lower than predicted based on Atwater values or bomb calorimetry) though they have dissimilar physical properties (90, 91). Moreover, walnuts reportedly have energy yields that are lower than pistachios (5% less than predicted) with close physical properties (92). Although the mechanisms are unclear, it may be hypothesized that nuts evoke different amounts of fragmentation and cellular disruption in the oral cavity due to differences in their physical properties (163, 164), a postulate that was also examined in this study.

Overall, the goals of this study were two-fold: First, it was of particular interest to contrast the masticatory efficiency of nuts (walnuts, almonds pistachios) varying in physical properties as this may result in changes in pre-swallowing particle size and, consequently, their digestion. Additionally, we investigated the influence of adding high water foods and beverages of varied flavors (sweet vs. plain) to walnuts on masticatory performance and pre-swallowing particle size. We hypothesized that coupling walnuts with a sweet flavor would increase palatability and reduce masticatory efficiency, resulting in larger particle sizes in the swallowed bolus. Additionally, mixing walnuts with fluid foods (liquids and semi-solids) was expected to facilitate swallowing of larger particles. Secondly, we investigated the effects of walnut consumption as whole nuts or butter on appetitive sensations and gut hormone secretion. We hypothesized that the whole nuts would elicit a higher satiety value compared to the nut butter. Differences from other tree nuts in chemical (e.g., fatty acid and antioxidant profile) and physical characteristics make walnuts an intriguing target for the study of the contribution of oral processing to their satiety and low energy yield. Moreover, given that walnuts uniquely provide nutrients associated with various health benefits (165), evaluating how these nuts are orally processed alone and under realistic eating conditions is worthwhile, especially since their health impact may be altered by the efficiency of mastication.

3.4 Materials and Methods

3.4.1.1 Participants

Fifty healthy adults (25 M, 25 F; BMI 24.7 ± 3.4 kg/m2 (range: 19.7–33.7 kg/m2); 25 ± 8 y/o (range: 18–52 years) were recruited through public advertisements. Eligibility criteria included healthy dentition and no nut allergies. All participants were non-smokers and were not taking medications known to affect the study outcomes. Each participant signed an informed consent form approved by the Purdue University Institutional Review Board and received monetary compensation for participation.

3.4.2 Experimental Design

The study followed a within-subject experimental design. Two experiments were carried out in three separate testing sessions. In experiment 1, each participant participated in one session of masticatory performance (Figure 1). Participants reported to the laboratory after having refrained from eating and using oral care products for at least 2 h. Participants were presented 5 g portions of nuts (walnuts, almonds, pistachios) with and without water, apple juice, plain yogurt or sweet yogurt in a randomized order. They were instructed to chew each sample, one at a time, at a constant rate (timed to a metronome at a rate of 1 chew/s for 15 s. Each sample was then expectorated into a pre-weighed plastic container and rated for palatability on a scale of 1 to 9 with 1 = “dislike extremely” and 9 = “like extremely (166).” A separate chewing condition was applied where participants were instructed to chew the walnuts for a predetermined number of chewing cycles (15 s) or until ready to swallow followed by expectoration into pre-weighed containers. For all treatments, electromyography (EMG) activity was recorded throughout the chewing sequences and particle size was determined by wet sieving.

Experiment 2 was divided into two sessions separated by approximately 1 week (Figure 1). Participants reported to the laboratory in the morning after an 8 h fast. They rated their appetitive sensations upon arrival using a visual analogue scale (VAS) presented on a personal digital tablet. Standard appetite questions were used as described previously by Hill et al. (167). After completing the VAS, a catheter was inserted in a vein in the antecubital space of the arm. Following a 10 min acclimatization period, a second appetite questionnaire was completed and a baseline blood sample (time point = 0) was drawn. Participants were then presented with 28 g of whole walnuts (raw) or walnut butter (whole raw walnuts ground to smooth butter consistency

by a standard food processor) in a counterbalanced order. Immediately following walnut consumption, blood samples were drawn at 15, 30, 45, 60, 120, and 180 min. Blood samples were collected in EDTA-coated tubes containing DPP-IV inhibitor on ice, and centrifuged to separate the plasma. Plasma was aliquoted and initially frozen at −20°C prior to storage at −80 °C Plasma GLP-1active was measured in duplicate using a commercially available ELISA kit (Millipore). All samples for an individual were run on the same ELISA plate. Glucose and insulin concentrations were determined using a Roche Cobas Integra Analyzer.

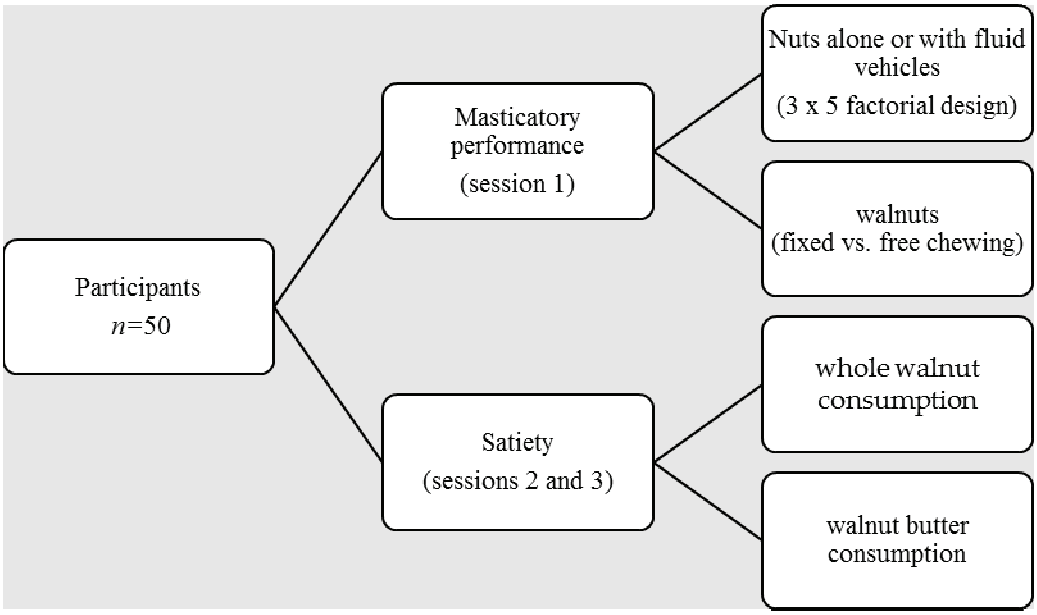


Figure 3-1 Study flow diagram

3.4.3 Test Foods

Three whole nuts were evaluated: Walnuts (raw unsalted, Sacramento, CA, USA), almonds (roasted salted, Sacramento, CA, USA), and pistachios (dry roasted, Kraft Heinz Foods Company, Chicago, IL, USA). Each type of nut was drawn from a single batch and was stored in sealed containers in a refrigerator at 4°C until the day of testing. Each nut sample weighed ~5.0 g. Five eating conditions were assessed: Nuts alone, nuts with water (deionized), nuts with apple juice (Mott's 100% Apple Juice, Mott's LLP, Plano, TX, USA), nuts with sweet yogurt (Greek Gods Greek Yogurt Honey Vanilla, Hain Celestial Group, Inc. Lake Success, NY, USA) and nuts with plain yogurt (Greek Gods Greek Yogurt Traditional Plain, Hain Celestial Group, Inc. Lake Success, NY, USA).

3.4.4 Breaking Force

Texture analyses for each of the three whole nuts was conducted using a TA XT2 Texture Analyzer (Stable Micro Systems, Godalming, UK) fitted with a knife probe and set to penetrate the samples to a depth of 4 mm (almonds, pistachios) and 8 mm (walnuts) at a speed of 1 mm/s. Two different penetration depths were applied since samples differed in shape and dimension (e.g., thickness). Twenty replicates were performed for each nut type and a mean value was calculated.

3.4.5 Masticatory Performance

The microstructure of chewing was characterized by electromyographic (EMG) recording (BioPac Systems, Inc., Goleta, CA, USA). The temporalis and masseter muscles on the dominant chewing side of each participant were identified by palpation and bipolar surface electrodes were placed approximately 3 cm apart along each muscle. A ground electrode was placed on the inside of the participants' opposite wrist. Four parameters were quantified: Maximum bite force (volts); mean bite force (volts); total muscle work (area of the EMG signal); and total number of chews. The raw EMG output was rectified due to the bipolar nature of the signal.

3.4.6 Proportional Particle Size Distribution

A total of 800 boluses were collected (50 subjects × 16 samples) and particle size was determined by sieving the expectorated boluses through a stack of pre-weighted sieves. The mesh sizes were: >3.35 mm, 3.35–2.0 mm, 2.0–1.0 mm, 1–0.50 mm, 0.50–0.25 mm, 0.25–0.125 mm and <0.125 mm (WS Tyler, Mentor, OH, USA). The sieves were arranged in descending order of mesh size. Because wet bolus particles tended to stick together, a 0.1% sodium chloride solution was poured over the expectorated samples and allowed to drain completely through the stack of sieves. The sieves and expectorated samples were then dried for 17 h at 74 °C in an air-dry oven to eliminate all the water. This time/temperature was selected using previously described methods (45, 168), with the noted modifications.

3.5 Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY) The Kolmogorov-Smirnov test was used to verify data distribution normality. Non-parametric

tests were used when the assumptions of parametric tests were not met. A non-parametric Wilcoxon signed-rank test was applied to compare the parameters describing mastication. Repeated measures analysis of variance (ANOVA), with nut type and condition as within subject factors, were applied to test if significant differences exist between nuts and between conditions on palatability ratings, masticatory performance, and proportional particle sizes. Repeated measures mixed models were used to test the overall condition effect (i.e., liquids vs. solid vs. semi-solids, sweet vs. plain) on the particle size distribution. When significant interactions were observed, main effects were tested using paired *t*-tests. Spearman's correlation analysis was performed to examine the relationship between masticatory performance, particle size, and palatability. Repeated measures ANOVA and mixed models were also used to test the effects of nut form (whole vs. nut butter) on appetite and post-prandial responses. When appropriate, *post hoc* comparisons were made with Bonferroni adjustments. Statistical tests were performed at a significance level of $p < 0.05$.

3.6 Results

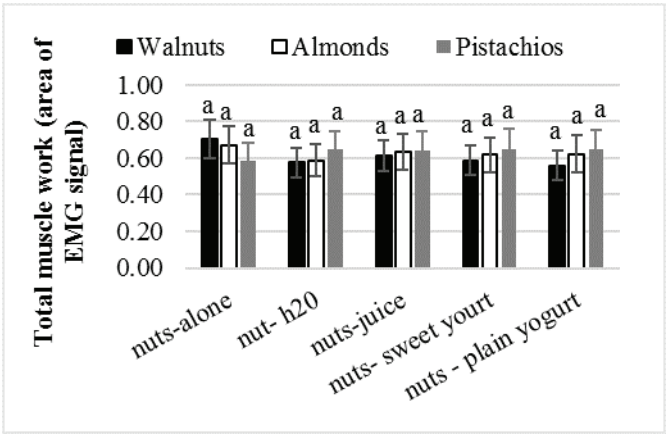
3.6.1 Nut Breaking Force

The instrumental breaking force was the lowest for walnuts (1088 ± 177 g), followed by pistachios (1833 ± 169 g) and almonds (3395 ± 149 g)

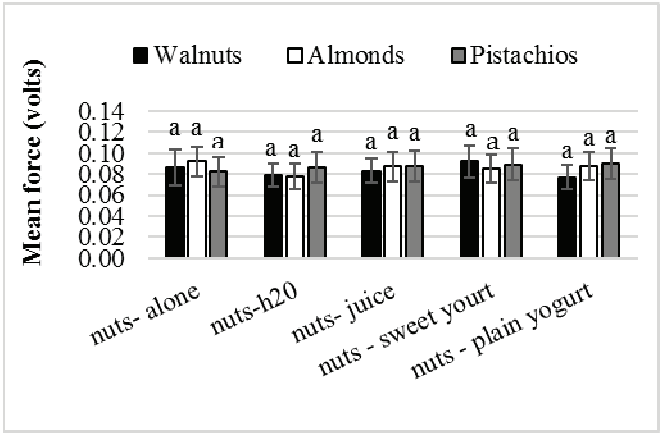
3.6.2 Mastication Parameters

There were no significant differences in chewing outcomes: Mean force, maximum force, and total muscle work (AREA) between eating conditions and nut types (Figure 3-2A–C). Similarly, for walnuts in the free and fixed chewing conditions, the total muscle work ($z = -1.371$, $p = 0.170$), mean ($z = -0.475$, $p = 0.635$) and maximum bite force ($z = -0.005$, $p = 0.996$) were comparable (data not shown). The Wilcoxon signed-rank test revealed that chewing time (second) increased when walnuts were chewed and expectorated at the time participants felt the need to swallow (20 s) compared to when walnuts were chewed for a fixed time (15 s), ($z = -4.583$, $p < 0.0005$).

(A)



(B)



(C)

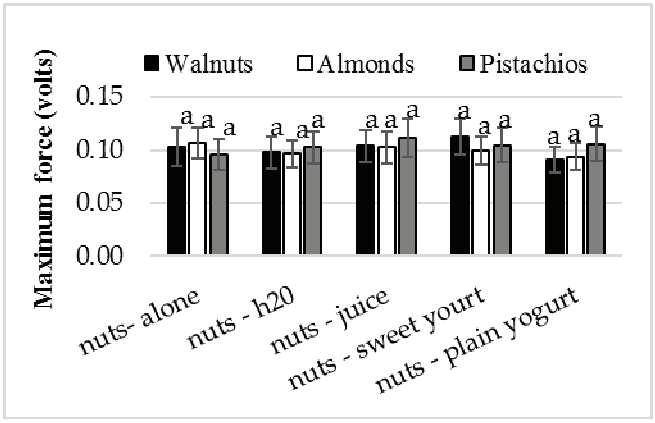
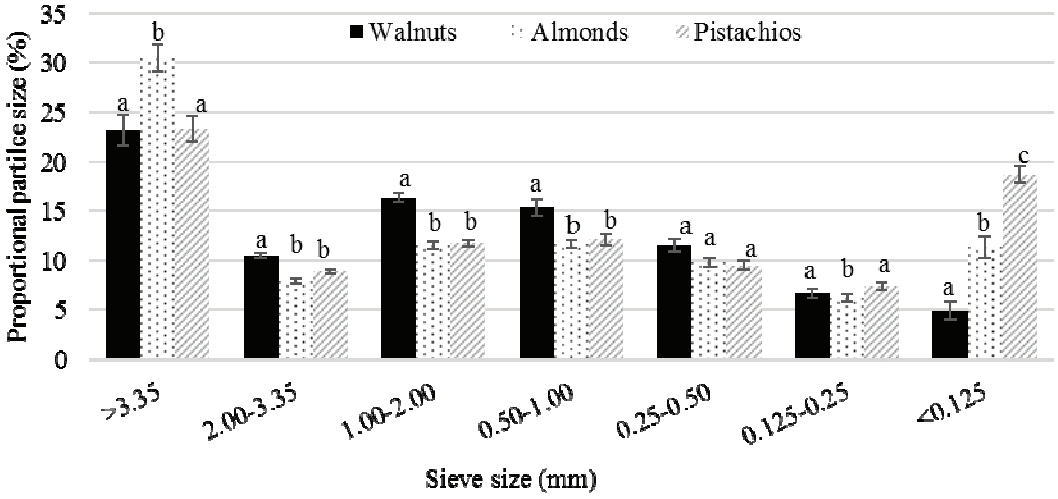


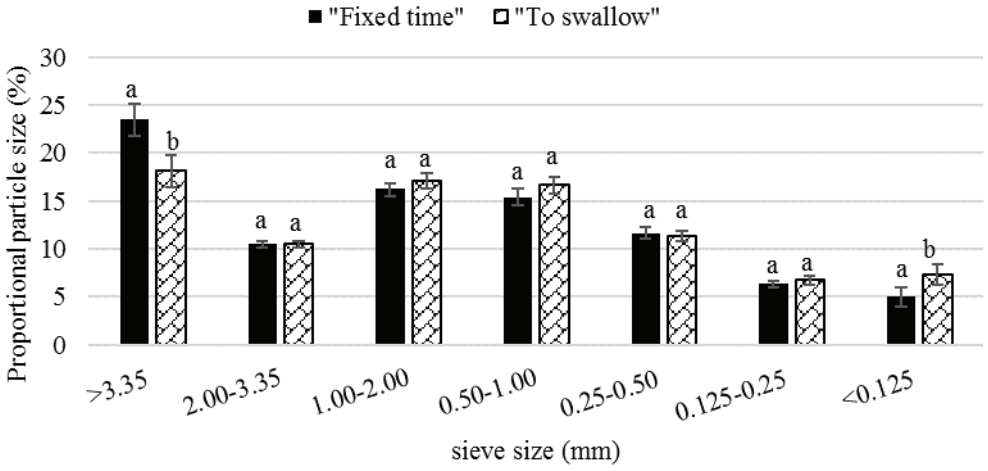
Figure 3-2: Mean± S.E.M (**A**) total muscle work (AREA), (**B**) mean bite force, and (**C**) maximum bite force obtained from EMG recordings.
Conditions with the same lower case letters (a) represent no significant difference between conditions (P>0.05)

3.6.3 Particle Size

There was a moderate, but significant effect of nut (F (2, 80) = 16.096, $p < 0.0001$) and condition (F (3, 139) = 6.906, $p < 0.0001$) on the recovered food mass collected after mastication. The percent recovery was slightly, but significantly lower for the walnuts (88.4 ± 1.1%) compared to the almonds (91.0 ± 0.94%) and pistachios (92.8 ± 0.87%) ($p < 0.01$). The proportion of particles larger than 3.35 mm were significantly greater in almond boluses compared with walnuts and pistachio boluses ($p < 0.01$). The share of particles larger than 3.35 mm was comparable between the pistachios and the walnuts ($p > 0.01$). In contrast, pistachios had a higher proportion of particles less than 0.125 mm compared with the other nuts ($p < 0.01$) (Figure 3-3A). The proportion of particles less than 0.125 mm was significantly larger for walnuts chewed until the point of swallowing than the walnuts chewed for a fixed time ($p = 0.003$) (Figure 3-3B).



(**A**)



(**B**)

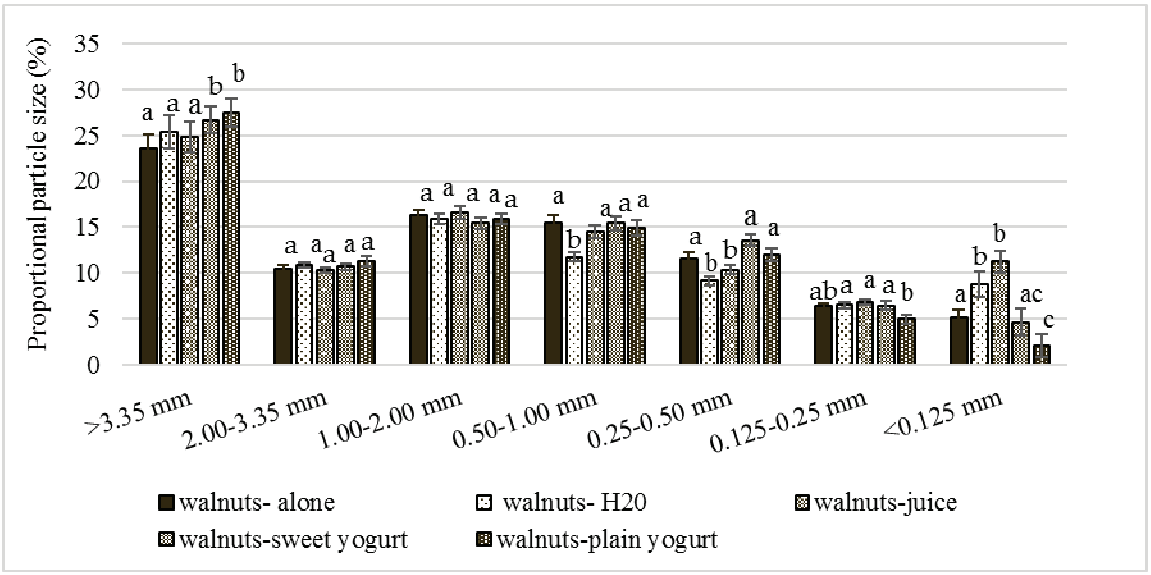
Figure 3-3: (**A**) Mean ± S.E.M particle size distribution of nuts chewed in isolation. Comparisons are based on two-way repeated measures ANOVA with *post hoc* Bonferroni multiple comparison test. Different lower case letters denote significant differences between nuts ($p < 0.05$); (**B**) Mean ± S.E.M particle size distribution by size of walnuts chewed for a fixed time and to the point of swallowing. Letters that are different denote significant differences between mastication protocols ($p < 0.05$).

In an analysis that examined the main effect of condition type (e.g., nut alone, water, juice, sweet yogurt, plain yogurt) at each sieve size level, particles were larger (>3.35 mm) with sweet and plain yogurt compared with the nut alone ($p < 0.01$).

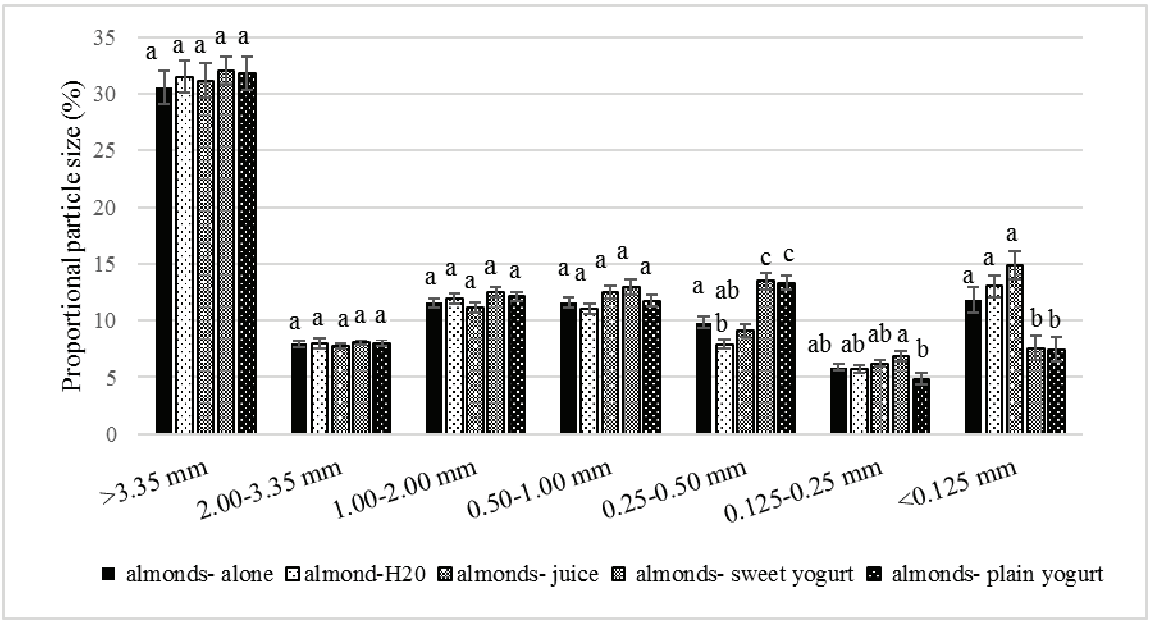
The ANOVA revealed a main effect of condition on particle size (F (3140) = 8.358, $p < 0.0001$). A larger proportion of particles less than 0.125 mm were detected in boluses with water than corresponding boluses without water ($p < 0.01$). Boluses with juice contained more particles less than 0.125 mm than boluses with plain yogurt ($p < 0.01$). In a second analysis that examined the overall condition effect (e.g., solid nuts alone vs. with liquid beverages vs. with semi-solid yogurts) on particle size, a high proportion of bolus particles greater than 3.35 mm was found with the yogurts and the beverages than with the nuts alone ($p < 0.01$).

Significant condition-by-sieve (F (10, 444) = 18.039, $p < 0.0001$) and nut-by-sieve (F (4202) = 104, $p < 0.0001$) interactions were noted ($p < 0.01$). *Post hoc* analyses indicated that walnut particles were significantly larger (> 3.35 mm) with the sweet and plain yogurt compared to the walnuts alone ($p = 0.005$ and $p = 0.002$, respectively) (Figure 3-4A). Similarly, there was a significantly greater proportion of pistachio particles > 3.35 mm with plain yogurt and water compared to alone ($p = 0.009$ and $p < 0.001$, respectively) (Figure 3-4C). Significantly more pistachio particles were < 0.125 mm with juice compared with sweet or plain yogurt (both $p <$

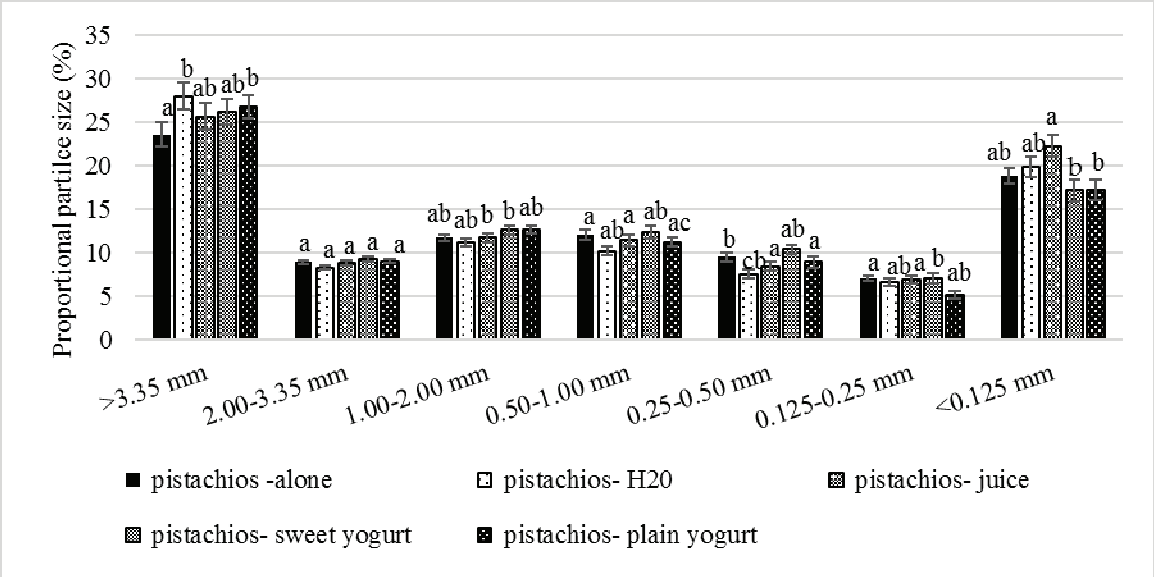
0.01). Almonds with both sweet and plain yogurt resulted in a higher proportion of particles 0.25–0.50 mm compared to all other almond treatments ($p < 0.01$) (Figure 3-4B).



(**A**)



(**B**)



(C)

Figure 3-4: Mean ± S.E.M particle size distribution by condition for nuts: (**A**) walnuts, (**B**) almonds, and (**C**) pistachios.
Comparisons are based on two-way repeated measures ANOVA with *post hoc* Bonferroni multiple comparison test. Different lower case letters denote significant differences between conditions ($p < 0.05$).

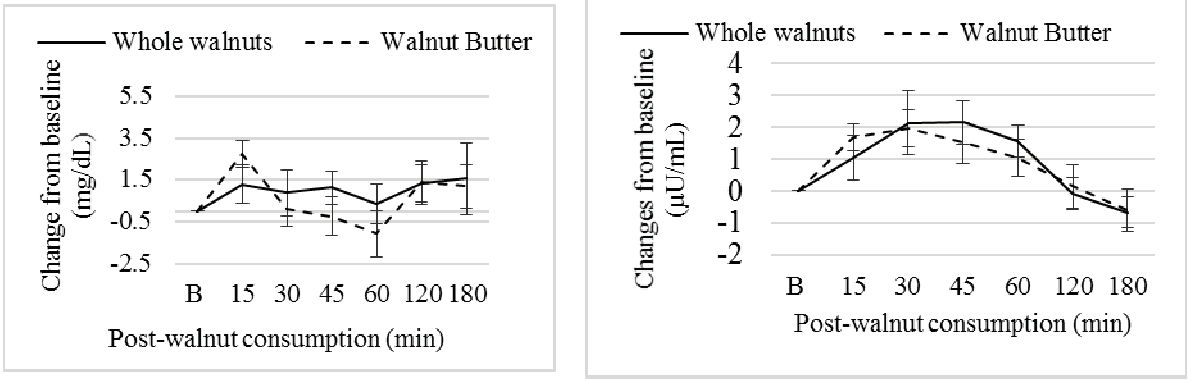
Across all nuts, overall sweetness had no effect on large particle sizes ($p > 0.01$). There was a significant effect of sweet flavor on particles less than 0.50 mm ($p < 0.01$). The proportions of particles in the three smallest sieves were higher in the sweet compared to the plain conditions ($p < 0.001$). This effect was independent of the fluid form (e.g., liquid beverage or semisolid yogurt).

3.6.4 Palatability Ratings

Mean preference scores were 6 ± 0.31, 6 ± 0.30, 6 ± 0.44, 7 ± 0.31, 5 ± 0.39 for nuts alone, nuts with water, nuts with juice, nuts with sweet yogurt, and nut with plain yogurt, respectively. There was no main effect of nut type on preference scores, although there was a significant condition effect (F (3, 149) = 13.5, $p < 0.0005$). Nuts ingested with sweet yogurt were rated as more palatable than nuts with plain yogurt, water, and juice ($p < 0.0005$). Palatability ratings did not correlate with masticatory performance or particle size.

3.6.5 Effect of Walnut Consumption on Metabolic Measure

Baseline glucose and insulin concentrations were not different between sessions. There were no significant effects of nut form or time on plasma glucose or insulin concentrations following ingestion ($p > 0.05$) (Figure 3-5)



(**A**) (**B**)

Figure 3-5: Mean ± S.E.M changes in (**A**) insulin and (**B**) glucose concentration subsequent walnut consumption.

3.6.5.1 Appetitive Sensations

Appetitive sensations at baseline did not differ between testing sessions. There were significant differences in fullness and hunger ratings between time points and treatment groups. Mean fullness was higher and hunger was lower with the whole walnut treatment compared to walnut butter ($p < 0.05$). Ratings of fullness were higher than baseline after 15 and 30 min ($p = 0.014$ and $p = 0.019$, respectfully) and hunger ratings were suppressed below baseline 15 min after whole walnut intake ($p = 0.011$). Additionally, preoccupation with food and thirst was significantly lower with the whole walnuts than with the walnut butter ($p = 0.006$). Desire for something sweet was lower with the walnut butter than the whole walnuts ($p < 0.0005$) (Figure 3-6*)*.

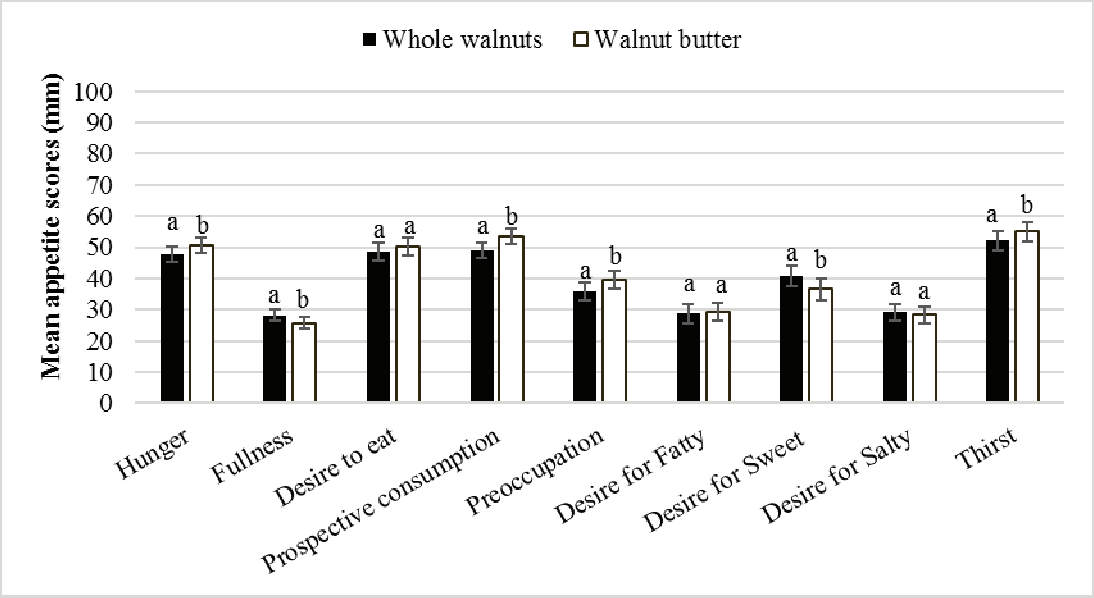


Figure 3-6: Mean ± S.E.M appetite indices subsequent walnut consumption.
Letters that are different denote significant differences between nut form ($p < 0.05$).

3.6.5.2 Gut Hormones

Baseline GLP-1 concentrations were not different between sessions. There were no significant treatment effects on GLP-1 concentrations (Figure 3-7).

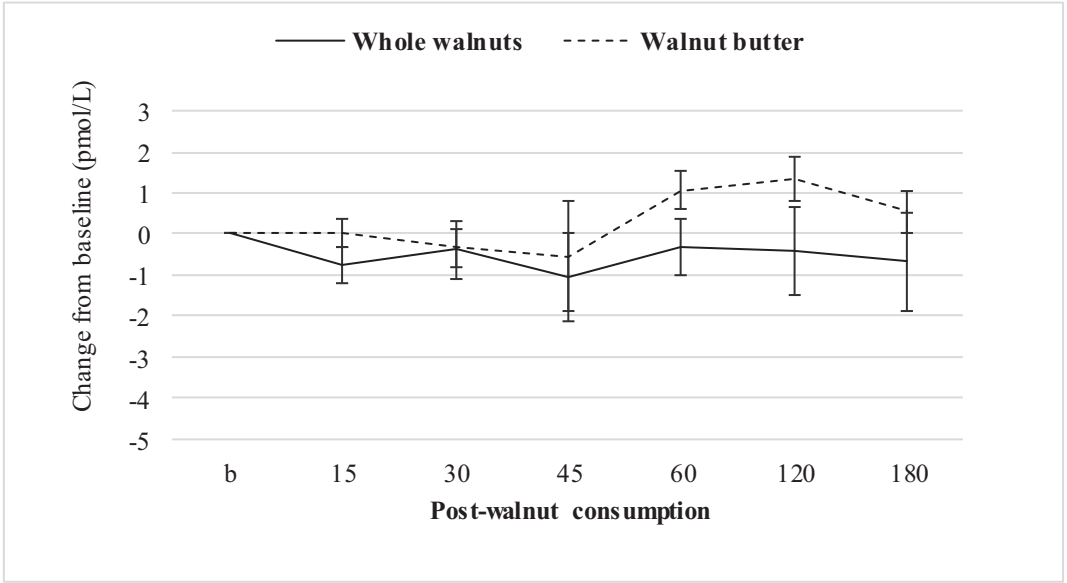


Figure 3-7: Mean ± S.E.M changes in GLP-1 concentrations after walnut consumption.

3.7 Discussion

The primary purpose of mastication is to ensure solid food particles are reduced to a size that can be effectively incorporated into a bolus and safely swallowed. Secondarily, there is evidence that particle size is a determinant of energy and nutrient bioacessibility (84, 85). The optimal size is a function of an individual's anatomy, the nature of the food, and the conditions under which it is ingested (36, 37). Though nuts are viewed as a single class of foods with physical properties that are more similar than dissimilar, the clinical evidence suggests they are not processed equivalently under a given set of conditions or across varying conditions. This is substantiated by the present findings.

The first part of this work aimed to study the efficiency of mastication during the chewing of different types of nuts. Variations in muscle activity have been reported for different types of nuts (51, 52), and other foods such as meat (53), and rice (169). Those observations generally indicate that harder samples require greater initial and mean bite forces. Additionally, hard foods tend to elicit a higher number of chewing cycles relative to soft foods (36, 170). Mastication indices were measured here to explain possible contributions to changes in particle size across three types of nuts. Based on physical properties determined instrumentally, we predicted a rank ordering of almonds > pistachios > walnuts (largest to smallest particle size). However, in the fixed chewing condition, the observed ordering was almonds > walnuts = pistachios. Our observation is not necessarily an outlier as other work has revealed an inverse association between food hardness and mean bolus particle size (42). Additional food properties, such as food/particle shape (e.g., elongated vs. spherical particles), structure, cohesiveness, and elasticity also have an influence on mastication (58). It is likely these additional properties attenuated the effects of nut hardness on particle size. Thus, in this study, measured masticatory intensity (initial and mean bite force) was not predictably related to the hardness of the nuts or ultimate particle size.

This study also assessed whether dietary context influences the oral processing of nuts, since nuts are commonly consumed in conjunction with other foods and beverages. A liquid and semi-solid were chosen as test vehicles mainly because they have been shown to decrease chewing and accelerate swallowing (38, 47, 60, 171, 172). For this reason, it was anticipated that adding a fluid or semi-solid to nuts would result in reduced muscle activity, leading to reduced chewing efficiency and larger particle sizes at swallowing. However, there were no differences

in measured chewing indices between any of the conditions tested. This finding differs from some earlier studies (45, 62, 173), but it agrees with studies by Derks et al. (2015), who did not find differences in chewing behavior between different types of liquid and semi-solid stimuli (174). Other studies have shown that the addition of fluids lowers the chewing forces applied to solid foods, however the decrease in muscle activity was accentuated for soft solids (cakes and toast) rather than hard solids (peanuts) (62). With hard solids, the fluid stimuli had a larger influence on the number of chewing cycles than on muscle activity (62). Additionally, the findings might be explained by the different ratios of nut to semi-solid/liquid used in the different studies. In a chewing study where brazil nuts were suspended in yogurt in varying concentrations, both the number of chews made before swallowing and the time needed to swallow increased significantly with concentration (38).

In terms of particle size, analyses showed significant effects of the addition of liquid and semi-solid vehicles. This is consistent with a number of previous studies (45, 62, 173). With liquid water, bolus particles were smallest (<0.125 mm) compared to all other conditions, possibly due to its low viscosity, which increases sensations of roughness by moistening and separating particles (175). Another possible explanation for this may be that chewing in the presence of water elevates suprahyoid muscle activity, which coordinates tongue movements, including compression between the tongue-palate (174) . Boluses contained a higher proportion of large particles (>3.35 mm) with the semi-solid yogurts compared to masticating the nuts alone. The lubricating effects of yogurt may have masked the perceived size and roughness of the larger particles thereby lowering masticatory efficiency (63). Additionally, embedded in a semi-solid matrix, soft- and round-shaped particles are perceived to be smaller in the mouth than harder particles of the same size range (176), possibly resulting in less chewing. As expected, the walnuts with yogurt gave rise to larger particle sizes than chewing the nuts separately. Similarly, pistachios with yogurt resulted in large particle size, whereas the almonds with yogurt yielded particles of smaller sizes. These results could be explained by the oral viscosity and flow properties of yogurt (177), which may preferentially select larger particles for chewing (oral selection) during the early stage of mastication, thus increasing their chance for fragmentation and hindering their swallowing (59). Additionally, the lubricating properties of a semi-solid reportedly deteriorate with inclusion of hard particles, leading to increased mastication. This could explain the smaller observed particle sizes in the almond-yogurt mixture. Therefore**,** the

present study confirms that properties of liquids and semi-solids influence swallowing decisions between nuts.

Sweet flavor is an important oral sensory property proposed to decrease oral residence time, and accelerate swallowing (39, 161). Therefore, the sweet foods were expected to decrease the chewing efficiency of walnuts, resulting in larger particle sizes in the swallowed bolus. While no differences were observed on the proportion of large particle sizes between the sweet and plain complementary foods, there was a greater proportion of small particles (less than 1 mm) with sweet vehicles than plain vehicles. Because oral movements increase in response to a sweet flavor (178), this may have led to an improved efficiency in the breakdown of the particles. This finding does not support our initial hypothesis on sweet flavor and it is different from previous studies (68, 179). There are multiple possible explanations for this. First, part of the effect noted here may stem from the fact that the sweet stimuli were fluids and semi-solids and the latter property may have dominated. Fluids increase the intensity of shear/squeezing between oral contact surfaces, (e.g., teeth-teeth, teeth-tongue, and tongue-palate) leading to more food fracturing (71). Second, the sweet as well as acid content (citric acid) in juice may have enhanced salivary flow rates, more than yogurt, which may have resulted in an improved masticatory efficiency (e.g., smaller particle sizes compared with yogurt) (33). Thus, the present findings provide suggestive evidence that sweetness results in a bolus with smaller, rather than larger particles.

Previously it was reported that changing the palatability of a meal has marked effects on masticatory function (e.g., chewing rhythm, eating rate, overall intake) (66-68), therefore we considered the effect of palatability on the mastication of walnuts. The prediction was that enhancing the palatability of nuts would result in a reduction in chewing behavior, leading to larger particle sizes at swallowing. However, we did not observe an independent effect of palatability on chewing indices or particle size, possibly because the observed differences in palatability between conditions was limited and all were rated positively. Similar findings have been reported in studies with peanuts (52) and almonds (51). Earlier studies on the microstructure of eating, documented an effect of palatability on masticatory behavior (66-68), but the effects appeared to be food-specific and the stages/duration of mastication were not examined. So the independent effect of palatability on oral processing remains uncertain.

The second part of this work was aimed at studying the role of mastication on the high satiety capacity of walnuts. Regular intake of walnuts generally (100), but not uniformly (180, 181), promotes strong fullness sensations. The reason for the discrepancy in published findings is not apparent, but may relate to methodological variations. For example, the study reporting high fullness assessed sensations after three days of chopped walnut intake relative to no nut intake in a controlled environment with participants that had metabolic syndrome. Whereas the studies failing to observe strong fullness ratings assessed satiety in people with overweight and obesity following a reduced-energy diet with whole walnuts and a reduced-energy diet without walnuts over 6 months in a free-living environment. In the present study, intake of whole walnuts elicited greater fullness compared to the butter form among individuals who are lean. This would suggest that chewing has an influence on appetite, possibly dependent on weight status (182).

The change in appetite with walnut consumption could not be ascribed to the release of GLP-1 or insulin, two reported satiety hormones. No correlation was observed between masticatory indices and concentrations of these hormones in the present study. The lack of effect of endocrine signals on appetite has been reported previously in other nut studies (100, 121, 183). Walnuts and other nuts, generally, have not been effective stimuli for gut peptide secretion(83, 100, 146, 147, 181)], but impart strong satiation/satiety effects. The present study supports the hypothesis that the observed differences in appetite may be attributable to mastication, which may exert its effect directly through neural rather than endocrine mechanisms (120).

A strength of the current study is the cross-over design. Other work has documented marked inter-individual variability in mastication and this hampers identification of treatment effects. Another strength is that the results facilitate understanding of the oral processing of walnuts and other nuts under ecologically valid conditions. In line with previous results (45), we found that changing the eating conditions of nuts, including walnuts, affected pre-swallowing particle size. One limitation of this trial is that we standardized the number of chews for each treatment during the assessment of masticatory performance mainly because of the subject-to-subject variation in habitual chewing rates and times(184). By controlling the chew time/rate the bolus may not faithfully reflect its state at the point of swallowing under natural conditions. Evaluating the oral processing of all treatments under a fixed and experimental controlled condition would have been useful. Another limitation is that we only measured short-term satiety, which may not be predictive of long-term ingestive behavior. A previous study observed

increased fullness following a walnut breakfast only after the third and fourth day of the intervention (100). The authors concluded that the mechanisms responsible for the satiety effects of walnuts may not manifest over a short time period.

3.8 Conclusions

In summary, the breakdown pattern for nuts differed and was not explained by a sole physical property (hardness). Breakdown is likely determined by multiple food properties (elasticity, plasticity, shape, etc.), consumer variations (e.g., dentition, swallowing threshold) and the conditions under which the nuts are consumed (e.g., complementary food viscosity, taste quality). In contrast to some prior findings with other foods [61], sweet flavor was associated with a greater preponderance of small particle sizes. Further study will be required to determine if this is a specific effect with nuts.

Fullness increased after the mastication of whole walnuts compared to walnut butter, though gut peptide concentrations remained unchanged. The present findings raise the question of whether the differences in oral processing translate into altered digestive processes (185). Additional studies are warranted to fully understand the significance of these results on both the bioaccessibility and bioavailability of energy from walnuts.

3.9 Acknowledgments

R.D.M and B.M.M designed the research project; B.M.M conducted the research. R.V.C analyzed the satiety hormones. R.D.M and B.M.M wrote the manuscript and R.V.C. provided feedback and edits to the manuscript. All authors take responsibility for the final content of the manuscript.

R.D.M received a research grant from the California Walnut Commission to conduct the study. B.M.M. was supported by a National Institute of Health (NIH)-training grant during her doctoral training.

The authors declare no conflict of interest. Funding sources had no role in the design of the study, or in the collection, analyses, or interpretation of data.

3.10 References

1. Jackson CL, Hu FB. Long-term associations of nut consumption with body weight and obesity. The American journal of clinical nutrition. 2014;100(Supplement 1):408S-11S.

2. Fraser GE, Bennett HW, Jaceldo KB, Sabaté J. Effect on body weight of a free 76 kilojoule (320 calorie) daily supplement of almonds for six months. Journal of the American College of Nutrition. 2002;21(3):275-83.

3. Alper C, Mattes R. Effects of chronic peanut consumption on energy balance and hedonics. International journal of obesity. 2002;26(8):1129.

4. Sabaté J, Cordero-MacIntyre Z, Siapco G, Torabian S, Haddad E. Does regular walnut consumption lead to weight gain? British Journal of Nutrition. 2005;94(5):859-64.

5. Bes-Rastrollo M, Wedick NM, Martinez-Gonzalez MA, Li TY, Sampson L, Hu FB. Prospective study of nut consumption, long-term weight change, and obesity risk in women. The American journal of clinical nutrition. 2009;89(6):1913-9.

6. Almario RU, Vonghavaravat V, Wong R, Kasim-Karakas SE. Effects of walnut consumption on plasma fatty acids and lipoproteins in combined hyperlipidemia. The American journal of clinical nutrition. 2001;74(1):72-9.

7. Fraser GE, Sabate J, Beeson WL, Strahan TM. A possible protective effect of nut consumption on risk of coronary heart disease: the Adventist Health Study. Archives of Internal medicine. 1992;152(7):1416-24.

8. Albert CM, Gaziano JM, Willett WC, Manson JE. Nut consumption and decreased risk of sudden cardiac death in the Physicians' Health Study. Archives of Internal medicine. 2002;162(12):1382-7.

9. Morgan WA, Clayshulte BJ. Pecans lower low density lipoprotein cholesterol in people with normal lipid levels. Journal of the American Dietetic Association. 2000;100(3):312-8.

10. Jenkins DJ, Hu FB, Tapsell LC, Josse AR, Kendall CW. Possible benefit of nuts in type 2 diabetes. The Journal of nutrition. 2008;138(9):1752S-6S.

11. Coelho SB, de Sales RL, Iyer SS, Bressan J, Costa NMB, Lokko P, et al. Effects of peanut oil load on energy expenditure, body composition, lipid profile, and appetite in lean and overweight adults. Nutrition. 2006;22(6):585-92.

12. Alves RDM, Moreira APB, Macedo VS, Costa NMB, Alfenas RdCG, Bressan J. High-oleic peanuts increase diet-induced thermogenesis in overweight and obese men. Nutrición Hospitalaria. 2014;29(5):1024-32.

13. Casas-Agustench P, López-Uriarte P, Bullo M, Ros E, Gómez-Flores A, Salas-Salvadó J. Acute effects of three high-fat meals with different fat saturations on energy expenditure, substrate oxidation and satiety. Clinical nutrition. 2009;28(1):39-45.

14. Pasman WJ, Heimerikx J, Rubingh CM, van den Berg R, O'Shea M, Gambelli L, et al. The effect of Korean pine nut oil on in vitro CCK release, on appetite sensations and on gut hormones in post-menopausal overweight women. Lipids in Health and Disease. 2008;7(1):10.

15. Tan SY, Mattes R. Appetitive, dietary and health effects of almonds consumed with meals or as snacks: a randomized, controlled trial. European journal of clinical nutrition. 2013;67(11):1205.

16. Reis CE, Ribeiro DN, Costa NM, Bressan J, Alfenas RC, Mattes RD. Acute and second-meal effects of peanuts on glycaemic response and appetite in obese women with high type 2 diabetes risk: a randomised cross-over clinical trial. British Journal of Nutrition. 2013;109(11):2015-23.

17. Barbour JA, Howe PR, Buckley JD, Wright GC, Bryan J, Coates AM. Lower energy intake following consumption of Hi-oleic and regular peanuts compared with iso-energetic consumption of potato crisps. Appetite. 2014;82:124-30.

18. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. The American journal of clinical nutrition. 2009;89(3):794-800. doi: 10.3945/ajcn.2008.26669.

19. Brennan AM, Sweeney LL, Liu X, Mantzoros CS. Walnut Consumption Increases Satiation but Has No Effect on Insulin Resistance or the Metabolic Profile Over a 4-day Period. Obesity. 2010;18(6):1176-82.

20. Katz DL, Davidhi A, Ma Y, Kavak Y, Bifulco L, Njike VY. Effects of walnuts on endothelial function in overweight adults with visceral obesity: a randomized, controlled, crossover trial. Journal of the American College of Nutrition. 2012;31(6):415-23.

21. Haddad E, Sabate J, editors. Effect of pecan consumption on stool fat. FASEB JOURNAL; 2000: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.

22. Zemaitis J, Sabaté J, editors. Effect of almond consumption on stool weight and stool fat. FASEB JOURNAL; 2001: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.

23. Levine AS, Silvis SE. Absorption of whole peanuts, peanut oil, and peanut butter. New England Journal of Medicine. 1980;303(16):917-8.

24. Traoret C, Lokko P, Cruz A, Oliveira C, Costa N, Bressan J, et al. Peanut digestion and energy balance. International journal of obesity. 2008;32(2):322.

25. Levine J, Baukol P, Pavlidis I. The energy expended in chewing gum. New England Journal of Medicine. 1999;341(27):2100-.

26. Kresge DL, Melanson K. Chewing gum increases energy expenditure before and after controlled breakfasts. Applied Physiology, Nutrition, and Metabolism. 2014;40(4):401-6.

27. Paphangkorakit J, Leelayuwat N, Boonyawat N, Parniangtong A, Sripratoom J. Effect of chewing speed on energy expenditure in healthy subjects. Acta Odontologica Scandinavica. 2014;72(6):424-7.

28. Komai N, Motokubota N, Suzuki M, Hayashi I, Moritani T, Nagai N. Thorough Mastication Prior to Swallowing Increases Postprandial Satiety and the Thermic Effect of a Meal in Young Women. Journal of nutritional science and vitaminology. 2016;62(5):288-94.

29. Hollis J, Mattes R. Effect of chronic consumption of almonds on body weight in healthy humans. British Journal of Nutrition. 2007;98(3):651-6.

30. Tey SL, Brown R, Gray A, Chisholm A, Delahunty C. Nuts improve diet quality compared to other energy-dense snacks while maintaining body weight. Journal of nutrition and metabolism. 2011;2011.

31. Stull AJ, Apolzan JW, Thalacker-Mercer AE, Iglay HB, Campbell WW. Liquid and Solid Meal Replacement Products Differentially Affect Postprandial Appetite and Food Intake in Older Adults. Journal of the American Dietetic Association. 2008;108(7):1226-30. doi: 10.1016/j.jada.2008.04.014. PubMed PMID: PMC2556245.

32. Mattes R. Soup and satiety. Physiology & Behavior. 2005;83(5):739-47.

33. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. International Journal of Obesity & Related Metabolic Disorders. 2000;24(6).

34. Lasschuijt MP, Mars M, Stieger M, Miquel-Kergoat S, de Graaf C, Smeets P. Comparison of oro-sensory exposure duration and intensity manipulations on satiation. Physiology & Behavior. 2017;176:76-83.

35. Houchins JA, Tan S-Y, Campbell WW, Mattes RD. Effects of fruit and vegetable, consumed in solid vs. beverage forms on acute and chronic appetitive responses in lean and obese adults. International journal of obesity (2005). 2013;37(8):1109.

36. Kirkmeyer S, Mattes R. Effects of food attributes on hunger and food intake. International Journal of Obesity & Related Metabolic Disorders. 2000;24(9).

37. Cecil J, Francis J, Read N. Relative contributions of intestinal, gastric, oro-sensory influences and information to changes in appetite induced by the same liquid meal. Appetite. 1998;31(3):377-90.

38. Mourao D, Bressan J, Campbell W, Mattes R. Effects of food form on appetite and energy intake in lean and obese young adults. International journal of obesity. 2007;31(11):1688-95.

39. Sakata T. Histamine receptor and its regulation of energy metabolism. Obesity. 1995;3(S4).

40. Sakata T, Yoshimatsu H, Masaki T, Tsuda K. Anti-obesity actions of mastication driven by histamine neurons in rats. Experimental Biology and Medicine. 2003;228(10):1106-10.

41. Arosio M, Ronchi CL, Beck-Peccoz P, Gebbia C, Giavoli C, Cappiello V, et al. Effects of modified sham feeding on ghrelin levels in healthy human subjects. The Journal of Clinical Endocrinology & Metabolism. 2004;89(10):5101-4.

42. Zhu Y, Hsu WH, Hollis JH. Modified sham feeding of foods with different macronutrient compositions differentially influences cephalic change of insulin, ghrelin, and NMR-based metabolomic profiles. Physiology & Behavior. 2014;135(Supplement C):135-42. doi: https://doi.org/10.1016/j.physbeh.2014.06.009.

43. Stevenson JL, Paton CM, Cooper JA. Hunger and satiety responses to high-fat meals after a high-polyunsaturated fat diet: A randomized trial. Nutrition. 2017;41:14-23.

44. Baer DJ, Gebauer SK, Novotny JA. Walnuts Consumed by Healthy Adults Provide Less Available Energy than Predicted by the Atwater Factors. The Journal of nutrition. 2015. doi: 10.3945/jn.115.217372.

45. Baer DJ, Gebauer SK, Novotny JA. Measured energy value of pistachios in the human diet. British Journal of Nutrition. 2012;107(1):120-5.

46. Novotny JA, Gebauer SK, Baer DJ. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. The American journal of clinical nutrition. 2012;96(2):296-301.

47. Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, et al. Role of cell walls in the bioaccessibility of lipids in almond seeds. The American journal of clinical nutrition. 2004;80(3):604-13.

48. Miquel-Kergoat S, Azais-Braesco V, Burton-Freeman B, Hetherington MM. Effects of chewing on appetite, food intake and gut hormones: A systematic review and meta-analysis. Physiology & Behavior. 2015;151:88-96.

49. Li J, Zhang N, Hu L, Li Z, Li R, Li C, et al. Improvement in chewing activity reduces energy intake in one meal and modulates plasma gut hormone concentrations in obese and lean young Chinese men. The American journal of clinical nutrition. 2011;94(3):709-16.

50. Zhu Y, Hsu WH, Hollis JH. Increasing the number of masticatory cycles is associated with reduced appetite and altered postprandial plasma concentrations of gut hormones, insulin and glucose. British Journal of Nutrition. 2013;110(2):384-90.

51. Frecka JM, Hollis JH, Mattes RD. Effects of appetite, BMI, food form and flavor on mastication: almonds as a test food. Eur J Clin Nutr. 2007;62(10):1231-8.

52. McKiernan F, Mattes RD. Effects of peanut processing on masticatory performance during variable appetitive states. Journal of nutrition and metabolism. 2009;2010.

53. King JC, Blumberg J, Ingwersen L, Jenab M, Tucker KL. Tree nuts and peanuts as components of a healthy diet. The Journal of nutrition. 2008;138(9):1736S-40S.

54. Mandalari G, Bisignano C, Filocamo A, Chessa S, Sarò M, Torre G, et al. Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion. Nutrition.29(1):338-44. doi: 10.1016/j.nut.2012.08.004.

55. Hutchings SC, Foster KD, Bronlund JE, Lentle RG, Jones JR, Morgenstern MP. Mastication of heterogeneous foods: Peanuts inside two different food matrices. Food quality and preference. 2011;22(4):332-9.

56. Engelen L, Fontijn-Tekamp A, van der Bilt A. The influence of product and oral characteristics on swallowing. Archives of Oral Biology. 2005;50(8):739-46.

57. Prinz JF, Lucas PW. Swallow thresholds in human mastication. Archives of Oral Biology. 1995;40(5):401-3. doi: https://doi.org/10.1016/0003-9969(94)00185-E.

58. Bellisle F, Guy-Grand B, Le Magnen J. Chewing and swallowing as indices of the stimulation to eat during meals in humans: effects revealed by the edogram method and video recordings. Neuroscience & Biobehavioral Reviews. 2000;24(2):223-8. doi: https://doi.org/10.1016/S0149-7634(99)00075-5.

59. Guy-Grand B, Lehnert V, Doassans M, Bellisle F. Type of Test-meal Affects Palatability and Eating Style in Humans. Appetite. 1994;22(2):125-34. doi: https://doi.org/10.1006/appe.1994.1012.

60. Rolls BJ, Wood RJ, Stevens RM. Palatability and body fluid homeostasis. Physiology & Behavior. 1978;20(1):15-9. doi: https://doi.org/10.1016/0031-9384(78)90196-8.

61. Mosca AC, van de Velde F, Bult JHF, van Boekel MAJS, Stieger M. Taste enhancement in food gels: Effect of fracture properties on oral breakdown, bolus formation and sweetness intensity. Food Hydrocolloids. 2015;43:794-802. doi: https://doi.org/10.1016/j.foodhyd.2014.08.009.

62. Pereira LJ, Duarte Gaviao MB, Van Der Bilt A. Influence of oral characteristics and food products on masticatory function. Acta Odontologica Scandinavica. 2006;64(4):193-201.

63. Singh H, Ye A, Ferrua MJ. Aspects of food structures in the digestive tract. Current Opinion in Food Science. 2015;3:85-93.

64. Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (Juglans regia) chemical composition and research in human health. Critical reviews in food science and nutrition. 2016;56(8):1231-41.

65. Yeh L, Kim K, Chompreeda P, Rimkeeree H, Yau N, Lundahl D. Comparison in use of the 9-point hedonic scale between Americans, Chinese, Koreans, and Thai. Food quality and preference. 1998;9(6):413-9.

66. Hill AJ, Blundell JE. Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. Journal of Psychiatric Research. 1982;17(2):203-12. doi: https://doi.org/10.1016/0022-3956(82)90023-1.

67. Ow RKK, Carlsson GE, Karlsson S. Relationship of masticatory mandibular movements to masticatory performance of dentate adults: a method study. Journal of Oral Rehabilitation. 1998;25(11):821-9. doi: 10.1046/j.1365-2842.1998.00325.x.

68. Grundy MML, Lapsley K, Ellis PR. A review of the impact of processing on nutrient bioaccessibility and digestion of almonds. International journal of food science & technology. 2016.

69. Grundy MML, Grassby T, Mandalari G, Waldron KW, Butterworth PJ, Berry SEE, et al. Effect of mastication on lipid bioaccessibility of almonds in a randomized human study and its implications for digestion kinetics, metabolizable energy, and postprandial lipemia. The American journal of clinical nutrition. 2015;101(1):25-33. doi: 10.3945/ajcn.114.088328. PubMed PMID: PMC4266890.

70. Chen J. Food oral processing—A review. Food Hydrocolloids. 2009;23(1):1-25.

71. Bornhorst GM, Singh RP. Bolus formation and disintegration during digestion of food carbohydrates. Comprehensive Reviews in Food Science and Food Safety. 2012;11(2):101-18.

72. Mioche L, Bourdiol P, Monier S. Chewing behaviour and bolus formation during mastication of meat with different textures. Archives of Oral Biology. 2003;48(3):193-200.

73. Moongngarm A, Bronlund JE, Grigg N, Sriwai N. Chewing behavior and bolus properties as affected by different rice types. International Journal of Medical and Biological Sciences. 2012;6(5).

74. de Lavergne MD, van de Velde F, Stieger M. Bolus matters: the influence of food oral breakdown on dynamic texture perception. Food & function. 2017;8(2):464-80.

75. Jalabert-Malbos M-L, Mishellany-Dutour A, Woda A, Peyron M-A. Particle size distribution in the food bolus after mastication of natural foods. Food quality and preference. 2007;18(5):803-12.

76. Foster KD, Grigor J, Cheong JN, Yoo MJ, Bronlund JE, Morgenstern MP. The role of oral processing in dynamic sensory perception. Journal of Food Science. 2011;76(2).

77. Engmann J, Burbidge AS. Fluid mechanics of eating, swallowing and digestion–overview and perspectives. Food & function. 2013;4(3):443-7.

78. Fontijn-Tekamp FA, van der Bilt A, Abbink JH, Bosman F. Swallowing threshold and masticatory performance in dentate adults. Physiology & Behavior. 2004;83(3):431-6. doi: https://doi.org/10.1016/j.physbeh.2004.08.026.

79. Mishellany A, Woda A, Labas R, Peyron M-A. The challenge of mastication: preparing a bolus suitable for deglutition. Dysphagia. 2006;21(2):87-94.

80. Pereira LJ, de Wijk RA, Gavião MBD, van der Bilt A. Effects of added fluids on the perception of solid food. Physiology & Behavior. 2006;88(4):538-44.

81. Van Der Bilt A, Engelen L, Abbink J, Pereira LJ. Effects of adding fluids to solid foods on muscle activity and number of chewing cycles. European Journal of Oral Sciences. 2007;115(3):198-205.

82. Derks J, De Wijk R, De Graaf C, Stieger M. Influence of stimulus properties and sensory task instructions on oral processing behavior of liquid stimuli. Journal of Texture Studies. 2016;47(1):49-57.

83. De Wijk RA, Prinz JF. Mechanisms underlying the role of friction in oral texture. Journal of Texture Studies. 2006;37(4):413-27.

84. De Wijk R, Zijlstra N, Mars M, De Graaf C, Prinz J. The effects of food viscosity on bite size, bite effort and food intake. Physiology & Behavior. 2008;95(3):527-32.

85. Engelen L, de Wijk RA, van der Bilt A, Prinz JF, Janssen AM, Bosman F. Relating particles and texture perception. Physiology & Behavior. 2005;86(1-2):111-7.

86. Lee W, Lucey J. Formation and physical properties of yogurt. Asian-Australasian Journal of Animal Sciences. 2010;23(9):1127-36.

87. Kim EHJ, Jakobsen VB, Wilson AJ, Waters IR, Motoi L, Hedderley DI, et al. Oral processing of mixtures of food particles. Journal of Texture Studies. 2015;46(6):487-98.

88. Loret C, Walter M, Pineau N, Peyron M, Hartmann C, Martin N. Physical and related sensory properties of a swallowable bolus. Physiology & Behavior. 2011;104(5):855-64.

89. de Wijk RA, Janssen AM, Prinz JF. Oral movements and the perception of semi-solid foods. Physiology & Behavior. 2011;104(3):423-8.

90. Bobroff EM, Kissileff HR. Effects of changes in palatability on food intake and the cumulative food intake curve in man. Appetite. 1986;7(1):85-96.

91. Liu D, Deng Y, Sha L, Hashem MA, Gai S. Impact of oral processing on texture attributes and taste perception. Journal of food science and technology. 2017;54(8):2585-93.

92. Salles C, Chagnon M-C, Feron G, Guichard E, Laboure H, Morzel M, et al. In-mouth mechanisms leading to flavor release and perception. Critical reviews in food science and nutrition. 2010;51(1):67-90.

93. Bellisle F, Le Magnen J. The analysis of human feeding patterns: the edogram. Appetite. 1980;1(2):141-50.

94. Rock CL, Flatt SW, Barkai H-S, Pakiz B, Heath DD. A walnut-containing meal had similar effects on early satiety, CCK, and PYY, but attenuated the postprandial GLP-1 and insulin response compared to a nut-free control meal. Appetite. 2017.

95. Rock CL, Flatt SW, Barkai H-S, Pakiz B, Heath DD. Walnut consumption in a weight reduction intervention: effects on body weight, biological measures, blood pressure and satiety. Nutrition Journal. 2017;16:76. doi: 10.1186/s12937-017-0304-z. PubMed PMID: PMC5715655.

96. Mattes RD, Considine RV. Oral processing effort, appetite and acute energy intake in lean and obese adults. Physiology & Behavior. 2013;120:173-81. doi: http://dx.doi.org/10.1016/j.physbeh.2013.08.013.

97. Burton-Freeman B. Sex and cognitive dietary restraint influence cholecystokinin release and satiety in response to preloads varying in fatty acid composition and content. The Journal of nutrition. 2005;135(6):1407-14.

98. Farr OM, Tuccinardi D, Upadhyay J, Oussaada SM, Mantzoros CS. Walnut consumption increases activation of the insula to highly desirable food cues: A randomized, double-blind, placebo-controlled, cross-over fMRI study. Diabetes, Obesity and Metabolism. 2017.

99. Prinz JF, Lucas PW. An optimization model for mastication and swallowing in mammals. Proceedings of the Royal Society B: Biological Sciences. 1997;264(1389):1715-21. PubMed PMID: PMC1688744.

100. Krop EM, Hetherington MM, Nekitsing C, Miquel S, Postelnicu L, Sarkar A. Influence of oral processing on appetite and food intake – A systematic review and meta-analysis. Appetite. 2018;125:253-69. doi: https://doi.org/10.1016/j.appet.2018.01.018.

CHAPTER 4. ENERGY EXTRACTION FROM WALNUTS

McArthur, B.[1], Mattes R.D.[2] Energy Extraction from Walnuts. The manuscript was submitted to *British Journal of Nutrition* and has been formatted according to the journal requirements.

4.1 Abstract

The bioaccessibility of fat has implications for satiety and postprandial lipidemia. The prevailing view holds that the integrity of plant cell wall structure is the primary determinant of energy and nutrient extraction from plant cells as they pass through the GI tract. However, comparisons across nuts (walnuts, almonds, pistachios) with varying physical properties do not support his view. In this study, masticated samples of three nuts from healthy adults were exposed to a static model of gastric digestion followed by simulated intestinal digestion. Primary outcomes were particle size and lipid release at each phase of digestion. Walnuts produced a significantly larger particle size post-mastication compared to almonds. Under gastric and intestinal conditions, the particle size was larger for walnuts compared to pistachios and almonds ($P<0.05$). However, the masticated and digesta particle sizes were not related to the integrity of cell walls nor lipid release. The total lipid release was comparable between nuts after the *in vitro* intestinal phase ($P>0.05$). Microstructural examination showed ruptured and fissured cell walls that would allow digestion of cellular contents and this may be governed by internal cellular properties such as lipid droplet state. Contrary to our hypothesis, the cell walls of walnuts tend to rupture rather separate and as walnut tissue passes through the GI track, lipids tend to coalesce reducing digestion efficiency.

4.2 Key words

Walnuts; Nuts; Mastication; Digestion; Energy extraction; Lipid bioaccessibility

4.3 Introduction

Walnuts have high satiety value, evoke a low postprandial lipemic response and protect against metabolic disorders such as CVD and type II diabetes (1-7). Additionally, the energy

they contain is not efficiently absorbed, accounting for the limited impact they have on energy balance. The low bioaccessibilty of lipid from walnuts, and other nuts, has been attributed primarily to the presence of intact cell walls that hinder access/binding of lipases to oil bodies enclosed within the cells (8). Where cell structures remain intact, nutrients (e.g., lipids, protein, vitamin E) are lost via fecal excretion (9-11). However, mechanical (e.g., chewing, chopping, grinding) or thermal degradation of cellular structures promotes the ingress of digestive enzymes and liberation of intracellular nutrients that are then digested (12-14). When access is not limited, for example as in isolated oil bodies or finely ground nuts, structural features of lipid control the extent of lipolysis (15).

Consistent with these physical properties, randomized controlled trials have shown decreases in postprandial triacylglycerol responses in humans fed muffins with whole nuts compared to milled nuts(6, 16) as well as improved accessibility of nutrients with decreased size of masticated almond particles(12). However, these investigations have mostly concentrated on the effects of altering the form (e.g., whole, milled, homogenized, roasted) more than the type of nut. Indeed, human studies have reported that there are appreciable differences in the digestion and release of lipid from different nut types: pistachios > almonds ≈ walnuts (17-19). These findings do not coincide with predictions based on the physical properties (e.g., hardness) of these nuts. Likely, the effect of nut type on lipid digestibility relates to the way that nuts are degraded during transit through the GI tract, but direct evidence is not available.

Mastication is a primary determinant of the bioaccessibilty of lipid (and other nutrients) and the subsequent postprandial responses. It therefore warrants consideration for its potential role in walnut lipid bioaccessiblity. Previous in vivo studies report that boluses formed from hard, brittle foods, such as almonds, consist of large particles that contain mostly intact cells with low lipid bioaccesssibility (8, 12). These and other studies showed that during mastication, some cell walls rupture and their contents become exposed to digestive fluids. No studies have examined whether chewing has equivalent effects on less brittle nuts, such as walnuts. Plant foods with a soft texture, generally separate rather than fracture under pressure, resulting in small intact particles during mastication . The maintenance of intact cell walls may reduce the release of nutrients in the digestive tract, as has been shown for fruits and vegetables (20, 21). Whether this finding holds for walnuts has not been studied and warrants investigation. Additionally, intensive thermal or mechanical processing conditions result in loss of structural integrity which leads to

more fractured cells during mastication and a higher accessibility/absorption of nutrients as shown for roasted compared with raw nuts (22, 23) . Walnuts are most frequently consumed raw so should be less susceptible to this effect.

The aim of this study was to provide insight into the structural and biochemical degradation of walnuts during mastication as well as simulated gastrointestinal (gastric and intestinal) digestion and its effects on lipid release. Interest in this question was driven by reports that extraction of energy from almonds, walnuts and pistachios is approximately 20%, 21% and 5%, respectively (17-19). These values do not coincide with structural properties. We hypothesized that walnuts would be chewed into smaller particle sizes, but their cells would separate under applied force and therefore elicit a low bioaccessibility value comparable to almonds. Pistachios were predicted to be more structurally degraded during digestive transit and therefore exhibit greater nutrient losses than walnuts and almonds.

4.4 Materials and Methods

4.4.1 Materials

Whole nuts were used in this study. The walnuts were unsalted and provided by the California Walnut Commission (Sacramento, CA, USA). The almonds were roasted and salted and were provided by the Almond Board of California (Sacramento, CA, USA). Pistachios were dry roasted (Kraft Heinz Foods Company, Chicago, IL) and were purchased from a local retailer in West Lafayette, IN, USA. These forms were selected as they are the most commonly consumed forms. The nuts were stored in sealed containers at 4ºC until the day of testing. Digestive enzymes, porcine pepsin (no. P-7125; ≤400 unit per mg powder), porcine pancreatin (no. P-1750; 4 x USP-US Pharmacopeia specification,), lipase from porcine pancreas type II (no. L3126; 100-400 units per mg powder), and bile extract porcine (EC 232-369-0) were purchased from Sigma-Aldrich (St. Louis, MO USA). The same material lots were used for all digestion experiments. All other chemicals and solvents in this study were of analytical grade.

4.4.2 In vivo mastication

Mastication of nuts for the in vitro experiments was conducted by seven healthy volunteers (age: 28 ± 4; BMI: 25 ± 1.19; gender: 3 males, 4 females) according to the procedure

of Grundy et al. (12), with modifications in relation to the starting material . On the day of testing, volunteers were presented 5g of each nut in a random order. Volunteers were asked to chew each nut until they felt the urge to swallow, at which time they expectorated the sample into individual pre-weighted plastic (50 mL) centrifuge tubes. They then rinsed their mouth with 20 mL of water and emptied the rinse into the same tube to create a final volume of 30 mL. All expectorated boluses were used in the static in vitro digestion model, simulating gastric and intestinal digestion. Individual samples (1 mL) were taken immediately after the oral phase, at the end of the in vitro gastric digestion phase and at the end of the in vitro intestinal digestion phase and were stored at 4◦C before particle size determination on the same day and for microscopy analysis. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Purdue University Institute Review Board, USA, approved all procedures involving human subjects. Written informed consent was obtained from all subjects.

4.4.3 *In vitro* GI digestion

In vitro digestions simulating gastric and intestinal digestion were performed as described by Lipkie et al. (24) . Gastric digestion was carried out immediately after the oral phase on the chewed nut samples. Samples (30 mL) were vortexed and acidified with 1.0 N HCI until it reached pH 3.5 ± 0.1. Then, gastric digestion was performed with the addition of 2 mL of pepsin solution (10 mg/mL) and the pH of the mixture was adjusted once more to 2.5 ± 0.1 with 1.0 N HCL. The final volume was adjusted to 40 mL with saline (0.9% NaCl), capped with nitrogen to minimize contact with oxygen, and then incubated at 37 °C in a shaking water bath for 60 min. Thereafter, the pH of the digesta was adjusted to 5.0 ± 0.1 with 1 N NaHCO3. The intestinal digestion was performed with the addition of 2 mL of pancreatin (20 mg/mL)-lipase (10 mg/mL) solution and 3 mL of bile (30 mg/ mL). Further, the pH was adjusted to 6.5 ± 0.1 with 1 N NaHCO3 and the final volume was brought to 50 mL with saline, after which the headspace of the tube was flushed again with nitrogen and incubated in a shaking water bath at 37°C for 120 min. Following the intestinal digestion, the digesta was subjected to 60 min of 10,000 g centrifugation (Allegra X-22 R, Beckman Coulters, USA) to remove the aqueous fraction and isolate the suspended particles. The recovered particles were washed with water and stored at

4ºC for further experiments. All *in vitro* digestions were performed in quadruplicate. Samples and replicates were run in randomized order.

4.4.4 Particle size

The protocol used for the particle size measurements was adapted from previous work (12, 25). Equal samples collected after mastication and simulated gastric and intestinal digestion (walnuts, n=4, almonds, n=4, pistachios n=4) were poured onto a 2000 μm aperture sieve (WS Tyler, Mentor, OH) placed on top of a sieve base (36 μm mesh size) and then washed with 20 mL of deionized (DI) water. Once the water passed through the mesh, retained particles were transferred into a 1000 mL beaker. Particle sizes > 2000 μm and < 36 μm were removed to prevented obstruction in the instrument and interference with the measurements, respectively. Small particles have been reported to correspond only to cell wall fragments and intracellular contents (12). Suspended particles were loaded into a light scattering apparatus (Malvern Mastersizer HU 2000, Malvern Instruments Ltd., Worcestershire, UK). The refractive index of the walnuts, almonds, pistachios and water is 1.47(26), 1.46(27), and 1.46(25) and 1.33, respectively. The speeds of the stirrer and the pump were 700 and 1175 rpm, respectively. Ten consecutive 10- second measurements were taken for each sample, to give the average particle size distribution of the digested nuts. The mean volume diameter (d [4,3]) of the particle was calculated from the intensity profile of the scattered light with the Mie theory by use of the instrument's software.

4.4.5 Total lipid extraction

Pre-digested nuts and digested residues, recovered at the end of the intestinal phase, were analyzed for total lipid using a Soxhlet extraction method (28), with Petroleum Ether as the solvent. The digested residues were centrifuged (2500 x g, 10 min) prior to analyses to remove the residual liquid phase. The residues were then dried and analyzed. The results of lipid content analysis are expressed as a percentage of fresh weight. The relative bioaccessibility of lipid in the nuts was calculated as follows: [the lipid present in the original non-digested sample-the lipid retained in the digested material (non-bioaccessibile fraction)] * 100/ [the lipid present in the original non-digested sample] *100.

4.4.6 Microscopy analysis

Microstructural analysis of pre- and post-digested nut cotyledon tissue was performed using light microscopy (LM) and transmission electron microscopy (TEM). Nut tissues were fixed with 2.25% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and then post-fixed with a buffered 1% osmium tetroxide solution containing 0.8% potassium ferricyanid, (pH 7.4) and left overnight. The specimens were then dehydrated in ethanol serial dilutions: 50%, 70%, 95%, (v/v) ethanol in distilled water for 10 min intervals and then finally for three 10 min intervals in 100% (v/v) ethanol. For LM and TEM, specimens were embedded in Embed 812 resin and placed in molds and polymerized at 70ºC. Semi-thick sections (0.5 μm) for LM were cut on a Reichert-Jung Ultracut E ultramicrotome (Leica Microsystem Ltd, UK), mounted on a glass slide and then stained with 1% (v/w) toluidine blue. Specimens were examined on a Leica microscope (W.Nuhsbaum, Inc., McHenry, IL) with LAS V4.3 software. Thin sections (80 nm) for TEM were cut on the same microtome and stained in 2% uranyl acetate (w/v) and lead citrate. Specimens examined by TEM were viewed on a FEI Tecnai G2T12 transmission electron microscope (FEI Europe) equipped with a tungsten source and operating at 80 kV.

The quantitative measurements of parenchymal cells were made using the images acquired from LM and TEM. Changes in the integrity of cells following mastication and in vitro digestion were estimated using the LM micrographs. Three hundred cells (100 cells/nut; 3 nuts) from the cotyledon tissues in each nut were examined to estimate the proportion of ruptured cells, relative to those that were intact. This number of cells was selected based on a previous microscopy study with nuts (29). Light micrographs were captured (40 X) from randomly selected areas within the cotyledon tissue of each specimen. The number of ruptured cells in the whole area of the micrograph was manually counted; results were expressed as a percentage of the total number of cells. Image analysis (ImageJ software) of TEM micrographs was used to quantify the diameter of cells and oil bodies.

4.5 Statistical analysis

Data were analyzed using SPSS version 22.0 (IBM Corp.) Statistical significance was set at a probability level of 0.05 ($P<0.05$). All data were normally distributed (analyzed by using the Shapiro-Wilk test). Linear mixed models with repeated measures were used to test for

differences in particle size, lipid release, integrity of cell walls, dimensions of cells and oil droplets. Nut type and digestion phase were treated as fixed effects. *Post hoc* analysis using Bonferroni adjustments was applied to examine pairwise differences. Results are expressed as means ± standard error.

4.6 Results

4.6.1 In vivo mastication

The number of chews was statistically different between nuts (P<0.05). More chewing cycles were necessary to reach swallowing for the almonds than the walnuts and pistachios (P=0.01), but no differences were observed between the walnuts and pistachios (P>0.05). The average number of chews per nut was: 35 ± 4 for the almonds (mean values ranged from 20-60), 30 ± 4 for the walnuts (16-53), and 30 ± 4 for the pistachios (14-55).

4.6.2 Particle size distribution

Figure 4-1A presents the mean particle size ($d_{[4,3]}$) of the different nuts after the three phases of digestion. The phase of digestion had a significant effect on the particle size of the walnuts and pistachios (both P<0.005), but the particle size did not differ significantly across phases for the almonds. Walnuts produced particles that were significantly larger post intestinal digestion (395.56 ± 9.7 μm) than oral (338.08 ± 9.7 μm) and gastric digestion (339.97± 10.18 μm). Similarly, pistachio particles were larger after the intestinal phase (347.142 ± 9.5 μm) compared to the oral (317.06± 9.6 μm) (P=0.004) and gastric phases (289.99 ± 9.6 μm) (P<.005). Moreover, there is an effect of nut type on the mean particle size following digestion (Figure 4-1B). The mean particle size was significantly larger for the walnuts than the almonds following oral digestion (P=0.010), but not different than the pistachios (P=0.084). No significant differences between the pistachios and almonds were observed post oral digestion. The average particle size was significantly larger for the walnuts after gastric digestion (339.58±10.16) compared with the almonds (291.65±9.77) and pistachios (290.47±9.77) (both P<0.005). Following intestinal digestion the mean particle size was larger for the walnuts (396.43±9.8 μm) than the almonds (301.31±9.7 μm) and pistachios (347.14±9.7 μm) (both P<0.005). Almonds yielded the smallest particle size after intestinal digestion (P<.0005).

4.6.3 Proportion of ruptured cells

There was no main effect of nut type or digestion phase on cell wall rupturing (P>0.05) (Figure 4-2). However, there was a significant interaction (P=0.007). Pairwise comparisons showed that significantly more walnut cells were ruptured after the intestinal phase compared with pistachio cells (P=0.005).

4.6.4 Lipid bioaccessibility

The mean total lipid content present in the native walnuts, almonds, and pistachios was 66, 50, and 46 % w/w, respectively. These values are similar to those found in the literature. Approximately 77, 76, and 78% of the original lipid in the walnuts, almonds, and pistachios, respectively was released following the intestinal phase of digestion, with no significant differences between these nuts (P>0.05).

4.6.5 Microstructure

The internal structure of cotyledons (e.g., lipid-bearing tissue) was observed in pre- and post-digested nuts using light as well as transmission electron microscopy. Pre-digested (native) cotyledon consist of compactly packed isodiametric parenchymal cells, with an intact middle lamella (the zone defining the boundary between walls form adjacent cells), and intact (undamaged) cell walls. The raw walnuts had thin cell walls compared to the roasted almonds and pistachios (Table 4-1). Within native nuts, nutrients remained encapsulated within the cell (Figure 4-4). As noted in prior reports (30-33), intracellular oil bodies were the most representative storage components. These lipids are protein stabilized oil bodies as their entire surface is covered by protein bodies or oleosins (32, 33). TEM micrographs showed variation in the organization of lipid between nuts. In the raw walnut and roasted almond (Figure 4-4 A1 and B1, respectively), lipid consisted of a single and dense agglomerate, whereas in the roasted pistachio, lipid was organized into smaller dispersed droplets (Figure 4-4 C1). Furthermore, light imaging (40x objective) showed that parenchymal cells from raw walnuts and roasted almonds exhibited tightly packed cells (Figure 4-4 A1 and B1, respectively), whereas from roasted pistachios, cells were more loosely packed; this difference is probably caused by roasting, as reported by other investigators (32). (Figure 4-4 C1).

Figure 4-5 - 4-7 compares the micrographs of walnuts, almonds, and pistachios after mastication and in vitro digestion. Following mastication, the cell walls for each nut appeared fissured. For walnuts, cell distortion and rupturing rather than separation was observed mainly in peripheral cells located beneath the fractured surface, increasing intracellular nutrient accessibility to digestive enzymes (Figure 4-5 A1). Moreover, portions of the lipid droplets were clearly organized into smaller spherical structures when compared against the native nut (Figure 4-5 A1). For almonds, the first layer of cells was largely ruptured, as in the walnuts, and released cellular contents (Figure 4-5 B1). A higher level of cellular integrity was observed in the underlying cells, which is consistent with previous microstructural studies with almonds (8, 12). Extensive cell wall degradation was observed in the pistachios compared to the almonds and walnuts (Figure 4-5 C1).

After gastric digestion, most protein bodies in walnuts were aggregated and disassociated from the surface of the lipid droplet, resulting in their coalescence (Figure 4-6 D1). In almonds, protein bodies remained attached to the oil droplet surfaces (Figure 4-6 E1), while in pistachios, proteins were mostly disrupted and in some cases, remnants of the protein bodies adhering to the lipid granules could be found (Figure 4-6 F1). Post digested pistachios exhibited smaller oil bodies compared to the walnuts and almonds (Table 4-1). Moreover, for pistachios a thickened middle lamella was noted, indicating some cell wall swelling may have occurred under gastric conditions (Figure 4-6 F1). Further erosion was identifiable in the roasted almonds and the intracellular compounds are clearly accessible (Figure 4-6 E1). Some cell separate was seen in the walnuts probably due to the acidic hydrolysis of middle lamella reducing cell-cell adhesion (13, 20) (Figure 4-6 D2). Progressive degradation of lipid and intracellular contents was observed when tissues collected after chewing (Figure 4-5 A1-C1, A2-C2) were compared with tissues after gastric (Figure 4-6 D1-F1, D2-F2) and intestinal digestion (Figure 4-7 G1-I1, G2-I2). Further, undigested lipid and protein bodies were clearly visible following the intestinal phase in all nut samples. No statistically significant main effect of digestion phase (oral, gastric, intestine) was detected on the morphology of cell and oil bodies, although both variables increased during digestive transit (Figure 4-8).

4.7 Discussion

Clinical trials document limited efficiency of energy extraction from almonds (19), pecans (34), peanuts (9) , pistachios (17) and walnuts (18). The most widely proposed mechanism for this effect highlights the structural integrity of cell walls and their encasement of energy-yielding nutrients (especially fat in the case of nuts). However, this mechanism does not account for the published energy losses from walnuts, almonds and pistachios. The former two reportedly yield about 80% of their predicted energy (based on Atwater factors) while the measured yield from the latter was reported as approximately 95%. The physical properties of these three nuts would predict a rank ordering of: almonds > pistachios> walnuts. This suggests additional factors may be involved in the response to digestive processes for these nuts. The present trial explored this hypothesis. In contrast to some previous studies concerning lipid bioaccessibilty of nut tissues (11), the present design ensured that the role of oral processing was included in the analysis. Samples tested in in vitro gastric and intestinal models were chewed by humans under naturalistic conditions and drawn from the participants at the point they chose to swallow.

Not surprisingly, fewer chewing cycles were required to reach the swallowing threshold for walnuts and pistachios compared to almonds. The observed difference in chewing cycles may partially relate to their physical characteristics (e.g., hardness, brittleness). Increased food hardness is associated with a greater number of chews before swallowing (35). Roasting nuts also results in smaller particles after mastication than oral processing of raw nuts (25). The more malleable structure of walnut tissue could facilitate swallowing larger particles (36). Additionally, the thinner cell walls (Table 4-1) and more disrupted parenchyma (Figure 4-5) in the walnuts and pistachios, respectively, may have resulted in structures that were more easily fractured and hydrated by saliva during mastication. However, weak structure is not a likely explanation here as we previously demonstrated that under fixed chewing conditions, walnuts do not degrade to a greater degree than almonds or pistachios. Since there were differences in chewing between the almonds (roasted) and the pistachios (dry-roasted) compared to the walnuts, there may also be an effect of roasting on masticatory behavior. Such an effect has been reported (37). Moreover, different ways of roasting (e.g., hot air vs. oil roasting, variation in heating temperatures and times) lead to alterations in the number of chews mainly by changing the parenchyma structure and properties (20).

The larger particle size (volume mean diameter ([d43]) in the walnuts after simulated gastrointestinal digestion are in line with previous reports (26), indicating that GI conditions destabilize some of the walnut protein bodies (oleosins) that may have led to oil-body (OB) aggregation/coalescence. This aggregation/coalescence can exert pressure on the cell walls and thus the volume of the recovered particles. Walnut proteins are primarily glutelin, which are readily denatured by low pH, as would occur in the stomach (26) . In contrast, almonds showed a continuous decrease in mean particle size during 60 min of gastric and 120 min of intestinal digestion. This can be attributed to their resistant interfacial proteins (amandin and other almond proteins) to hydrolysis by pepsin. This results in higher OB stability and less aggregated bodies (38). Thus, there is greater surface area for digestive enzymes and bile to access. The change in particle size of almonds, is in agreement with that reported for raw, sliced almonds and roasted hazelnut OB preparations (15, 38). For pistachios, the small d43 values after gastric digestion might reflect an enhanced stability against OB aggregation. Hydrophilic components of its protein bodies are hydrolyzed during roasting rendering them more lipophilic and better suited to stabilize the OBs (15). Conversely, roasting of pistachios could have accelerated the disintegration of particles during in vitro gastric digestion, as has been demonstrated with almonds and peanuts (22, 23). However, oil bodies in roasted nut cells tend to coalesce during digestion (12), likely due to the development of more porous or fractured cell walls from the heating process. This allows cellular infiltration of the digestive juices and consequent destabilization of the oil bodies. This could explain the higher d43 values of the pistachios after intestinal digestion. This is in agreement with a previous study for almond extract (free oil bodies), where the natural layer surrounding the almond oil bodies induced a stronger decrease in triglyceride absorption and appearance in the blood postprandially compared with almond oil emulsions (oil emulsified with milk protein) (39).

It has been shown that trituration of almonds by oral or mechanical processing, increases the release of lipid from the cells on the periphery of particles as a result of cell rupture (8). Because of the different physical properties (i.e., soft texture) of walnut seeds, we predicted that chewing would result in cell separation rather than fracture with reduced release of lipid from walnut tissue. Contrary to this expectation, walnut cells ruptured, rather than separated which is probably due to their strong cell-cell adhesion (Figure 4-4 A2). In nuts and seeds, cell separation is caused mainly by weakening the cell-cell adhesions during gastric digestion, as can be seen in

the native walnuts (Figure 4-5 D1). However, we observed that nut cells have the potential to separate as a result of thermal processing, which can be seen in the micrograph of the native pistachios (Figure 4-4 A3). No studies performed so far have shown any evidence of cell separation occurring in raw or even thermally processed nuts that have been chopped, or chewed, except in ingested nuts after gastric digestion in vitro or microbial fermentation in vivo (8, 13). Our findings indicate that tissue fracturing rather than cell separation may be the main mode of tissue failure in walnuts.

Grundy *et al* (2015) recently reported a negative linear relationship (R^2=0.65) between particle size and free fatty acid release for both raw and roasted almonds (12). Based on these data, it was expected that the extent of lipolysis would be greatest for the particles with smallest size. This corresponded to the sample with the largest proportion of ruptured cells on the surfaces of the particles. The lipid released from these fractured cells would be more accessible to intestinal lipase. However, our results show that the amount of lipid released is not a function of the number of ruptured cells on the fractured surface of walnut tissue. These observations are consistent with previous studies that also demonstrated non-linear or non-existent relationships between particle size and nutrient bioaccessiiltity (29, 40). For example, a study with raw and cooked carrots (gently and intensely cooked) showed that the dependency of b-carotene bioaccessibilty on particle size became more pronounced as the thermal process became less intense (41). Previous studies from our group also showed that increasing the intensity of mastication resulted in a higher lipid release from almond tissues, but no specific dependency of the lipid bioaccessibility on particle size was observed (40).

The loss of lipid from particle surface cells suggests that the cell wall becomes a less efficient barrier to digestion with time (42). There is now convincing evidence that the internal structure of nuts (oil droplets, protein bodies) can be retained to a greater or lesser extent during digestion and can variably hinder or augment digestion and absorption. As a result, we suggest that the structural integrity (intact cells) may not be the primary factor in influencing lipid bioaccessibility in walnuts and that the internal structure of the nut content has the potential to greatly influence postprandial lipid metabolism. Digestion of oil bodies has been studied in almonds, walnuts and hazelnuts (15, 26, 38). These studies show that gastric digestion of oleosins allows more rapid access of the lipase to the oil-water interface for efficient lipolysis of the lipid droplet. Interestingly, in vitro intestinal digestion of a walnut oil bodies showed the

spontaneous formation of a multiple emulsion. This was likely driven by the interaction of PUFA as free FFA and 2-MAG, walnut peptides from proteolysis by digestive enzymes, and negatively charged bile salts (26). The oil and water droplets were stabilized by crystals of lipolytic products and/or bile salts and these structures are predicted to play a major role in how lipids are digested and absorbed from walnuts. These results confirm that in addition to intact cells, there are other physiochemical factors, such as the nature of the interfacial layer, that influence the extraction of energy from walnuts.

An additional factor influencing lipid bioaccessibility may be the increase in porosity of the cell wall during digestion as a result of swelling of the cell walls during digestion. This would increase the influx of lipase and subsequent leakage of hydrolyzed lipids. Some evidence of cell wall swelling has been reported for raw and roasted nuts (22, 23). In the present trial, an increase in porosity may have occurred especially for walnuts (Table 4-1) and pistachios (Figure 4-5 F1). However, the swelling of cell walls has been previously shown to occur slowly and over much longer times (i.e., 3- 24 hours) (11, 22, 23).

In our *in vitro* digestion experiment, the quantity of non-digestible lipid was higher (~22%) for pistachios than has been previously reported in human studies (~5%)(17). This may reflect differences in roasting conditions (e.g., temperature, time). Roasting induces microstructural and chemical changes (e.g., partial cell wall rupture, cell wall swelling, protein denaturation) that facilitate lipolysis (43).

The fraction of bound lipid was comparable for walnuts and almonds (i.e., 24%) after the intestinal phase of digestion. These values are markedly lower that those reported previously which indicated as much as 47% of the lipid still remained in the cellular structure of almond tissue at this stage of digestion. Given findings of lipid malabsorption (excretion) in the range of (21% and 24% for walnuts and almonds, respectively from in vivo studies (18, 19), the previous data require that a high proportion of lipid is extracted in the colon. The effects of lipid reaching the colon either undigested or in digested form and its interactions with the gut microbiota are unclear. Emerging research indicates that both the type and form of nuts may differentially alter microbial metabolism in the colon (6, 44). The present values are in line with little lipid loss in the colon. Future studies in this area are required to determine the role of the gut microbiota in lipid metabolism. It is also possible that the discrepancy in the previous and present studies in lipid bioaccessibility estimates only reflects methodological approaches. Different amounts of

shaking to mimic the mixing/force in the gastric phase of digestion, types/concentrations of enzymes introduced, different digestion conditions/times) and/or a difference in the amount of material digested could potentially explain the observed difference (25, 45, 46).

4.8 Conclusion

Nut structure and internal constituent properties may decrease lipid bioaccessibilty during digestion. Understanding the mechanisms that allow nuts to be a highly energy dense food without promoting positive energy balance is of particular intest since nuts are an increasingly consumed food with postive health benefits and new strategies could be developed to optimize nut-based functional ingredients. Our results show that chewing causes a rupture of cell walls but the amount of lipid released does not corespond with the number of ruptured cells on the fracture surface of nut tissue. Moreover, the ratio of ruptured cells to intact cells was not related to particle size. In this work, evidence of additional mechanisms by which the structural features of nuts can reduced lipid bioaccessibility was provided. Examination of nut microstructure indicates that the fissures of cell walls as well as lipid storage properties are also important for energy extraction. These findings indicate walnuts, almonds and pistachios yield similar, but limited amounts of energy (~80%) during digestion, likely through varied mechanisms. For walnuts, the limited bioaccessibility may stem from the ready hydrolysis of their oliosins at low pH allowing for lipid droplet coalescense and resistance to lipolysis.

4.9 Acknowledgements

We gratefully acknowledge the Muti-Scale Imaging Center, Purdue University, particularly Christopher Gilpin, Laurie Mueller, and Robert Seiler for helping with the LM and TEM imaging.

The present study was funded by the California Walnut Commission (CA, USA) grant 208 491. The contribution of each author are as follows: R.D.M and B.M.M designed the research project; B.M.M conducted the research. R.D.M and B.M.M wrote the manuscript. All authors take responsibility for the final content of the manuscript.

The authors declare that there are no conflict of interest.

4.10 References

1. Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. British Journal of Nutrition. 2006;96(S2):S52-S60. Epub 2007/04/01. doi: 10.1017/bjn20061864.

2. de Souza RGM, Schincaglia RM, Pimentel GD, Mota JF. Nuts and Human Health Outcomes: A Systematic Review. Nutrients. 2017;9(12):1311.

3. Hayes D, Angove MJ, Tucci J, Dennis C. Walnuts (Juglans regia) chemical composition and research in human health. Critical reviews in food science and nutrition. 2016;56(8):1231-41.

4. Kris-Etherton PM. Walnuts decrease risk of cardiovascular disease: a summary of efficacy and biologic mechanisms. The Journal of nutrition. 2014;144(4):547S-54S.

5. Zhou D, Yu H, He F, Reilly KH, Zhang J, Li S, et al. Nut consumption in relation to cardiovascular disease risk and type 2 diabetes: a systematic review and meta-analysis of prospective studies–. The American journal of clinical nutrition. 2014;100(1):270-7.

6. Mandalari G, Parker ML, Grundy MM-L, Grassby T, Smeriglio A, Bisignano C, et al. Understanding the Effect of Particle Size and Processing on Almond Lipid Bioaccessibility through Microstructural Analysis: From Mastication to Faecal Collection. Nutrients. 2018;10(2):213.

7. Berry SE, Tydeman EA, Lewis HB, Phalora R, Rosborough J, Picout DR, et al. Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects–. The American journal of clinical nutrition. 2008;88(4):922-9.

8. Ellis PR, Kendall CW, Ren Y, Parker C, Pacy JF, Waldron KW, et al. Role of cell walls in the bioaccessibility of lipids in almond seeds. The American journal of clinical nutrition. 2004;80(3):604-13.

9. Levine AS, Silvis SE. Absorption of whole peanuts, peanut oil, and peanut butter. New England Journal of Medicine. 1980;303(16):917-8.

10. Zemaitis J, Sabaté J, editors. Effect of almond consumption on stool weight and stool fat. FASEB JOURNAL; 2001: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.

11. Mandalari G, Faulks RM, Rich GT, Lo Turco V, Picout DR, Lo Curto RB, et al. Release of protein, lipid, and vitamin E from almond seeds during digestion. Journal of Agricultural and Food Chemistry. 2008;56(9):3409-16.

12. Grundy MML, Grassby T, Mandalari G, Waldron KW, Butterworth PJ, Berry SEE, et al. Effect of mastication on lipid bioaccessibility of almonds in a randomized human study and its implications for digestion kinetics, metabolizable energy, and postprandial lipemia. The American journal of clinical nutrition. 2015;101(1):25-33. doi: 10.3945/ajcn.114.088328. PubMed PMID: PMC4266890.

13. Kong F, Singh RP. Digestion of raw and roasted almonds in simulated gastric environment. Food biophysics. 2009;4(4):365-77.

14. Gebauer SK, Novotny JA, Bornhorst GM, Baer DJ. Food processing and structure impact the metabolizable energy of almonds. Food & function. 2016;7(10):4231-8.

15. Capuano E, Pellegrini N, Ntone E, Nikiforidis CV. In vitro lipid digestion in raw and roasted hazelnut particles and oil bodies. Food & function. 2018.

16. Berry SE, Tydeman EA, Lewis HB, Phalora R, Rosborough J, Picout DR, et al. Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects. The American journal of clinical nutrition. 2008;88(4):922-9.

17. Baer DJ, Gebauer SK, Novotny JA. Measured energy value of pistachios in the human diet. British Journal of Nutrition. 2012;107(1):120-5.

18. Baer DJ, Gebauer SK, Novotny JA. Walnuts Consumed by Healthy Adults Provide Less Available Energy than Predicted by the Atwater Factors. The Journal of nutrition. 2015. doi: 10.3945/jn.115.217372.

19. Novotny JA, Gebauer SK, Baer DJ. Discrepancy between the Atwater factor predicted and empirically measured energy values of almonds in human diets. The American journal of clinical nutrition. 2012;96(2):296-301.

20. Grundy MM, Edwards CH, Mackie AR, Gidley MJ, Butterworth PJ, Ellis PR. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. The British journal of nutrition. 2016;116(5):816-33.

21. Glahn RP, Tako E, Cichy K, Wiesinger J. The cotyledon cell wall and intracellular matrix are factors that limit iron bioavailability of the common bean (Phaseolus vulgaris). Food & function. 2016;7(7):3193-200.

22. Kong F, Oztop MH, Singh RP, McCarthy MJ. Effect of boiling, roasting and frying on disintegration of peanuts in simulated gastric environment. LWT-Food Science and Technology. 2013;50(1):32-8.

23. Kong F, Singh R. Disintegration of solid foods in human stomach. Journal of Food Science. 2008;73(5).

24. Lipkie TE, De Moura FF, Zhao Z-Y, Albertsen MC, Che P, Glassman K, et al. Bioaccessibility of carotenoids from transgenic provitamin A biofortified sorghum. Journal of agricultural and food chemistry. 2013;61(24):5764-71.

25. Mandalari G, Grundy MM-L, Grassby T, Parker ML, Cross KL, Chessa S, et al. The effects of processing and mastication on almond lipid bioaccessibility using novel methods of in vitro digestion modelling and micro-structural analysis. British Journal of Nutrition. 2014;112(9):1521-9.

26. Gallier S, Tate H, Singh H. In vitro gastric and intestinal digestion of a walnut oil body dispersion. Journal of agricultural and food chemistry. 2013;61(2):410-7.

27. Yıldız M, Gürcan ŞT, Özdemir M. Oil composition of pistachio nuts (Pistacia vera L.) from Turkey. Lipid/Fett. 1998;100(3):84-6.

28. Horwitz W, Latimer G. Official Methods of Analysis of AOAC International, Gaithersburg MA, USA. Association of Official Analytical chemist. 2000.

29. Grassby T, Picout DR, Mandalari G, Faulks RM, Kendall CW, Rich GT, et al. Modelling of nutrient bioaccessibility in almond seeds based on the fracture properties of their cell walls. Food & function. 2014;5(12):3096-106.

30. Varela P, Aguilera JM, Fiszman S. Quantification of fracture properties and microstructural features of roasted Marcona almonds by image analysis. LWT - Food Science and Technology. 2008;41(1):10-7. doi: https://doi.org/10.1016/j.lwt.2007.02.013.

31. Young CT, Schadel WE, Pattee HE, Sanders TH. The microstructure of almond (Prunus dulcis (Mill.) DA Webb cv.'Nonpareil') cotyledon. LWT-Food Science and Technology. 2004;37(3):317-22.

32. Saklar S, Ungan S, Katnas S. Microstructural changes in hazelnuts during roasting. Food Research International. 2003;36(1):19-23.

33. Dourado F, Barros A, Mota M, Coimbra MA, Gama FM. Anatomy and cell wall polysaccharides of almond (Prunus dulcis DA Webb) seeds. Journal of agricultural and food chemistry. 2004;52(5):1364-70.

34. Haddad E, Sabate J, editors. Effect of pecan consumption on stool fat. FASEB JOURNAL; 2000: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.

35. Kohyama K, Hatakeyama E, Sasaki T, Dan H, Azuma T, Karita K. Effects of sample hardness on human chewing force: a model study using silicone rubber. Archives of Oral Biology. 2004;49(10):805-16. doi: https://doi.org/10.1016/j.archoralbio.2004.04.006.

36. Prinz JF, Lucas PW. Swallow thresholds in human mastication. Archives of Oral Biology. 1995;40(5):401-3. doi: https://doi.org/10.1016/0003-9969(94)00185-E.

37. McKiernan F, Mattes RD. Effects of peanut processing on masticatory performance during variable appetitive states. Journal of nutrition and metabolism. 2009;2010.

38. Gallier S, Singh H. Behavior of almond oil bodies during in vitro gastric and intestinal digestion. Food & function. 2012;3(5):547-55.

39. Gallier S, Acton D, Garg M, Singh H. Natural and processed milk and oil body emulsions: Bioavailability, bioaccessibility and functionality. Food Structure. 2017;13:13-23.

40. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. The American journal of clinical nutrition. 2009;89(3):794-800. doi: 10.3945/ajcn.2008.26669.

41. Lemmens L, Van Buggenhout S, Van Loey AM, Hendrickx ME. Particle size reduction leading to cell wall rupture is more important for the β-carotene bioaccessibility of raw compared to thermally processed carrots. Journal of agricultural and food chemistry. 2010;58(24):12769-76.

42. Grassby T, Mandalari G, Grundy MM-L, Edwards CH, Bisignano C, Trombetta D, et al. In vitro and in vivo modeling of lipid bioaccessibility and digestion from almond muffins: The importance of the cell-wall barrier mechanism. Journal of functional foods. 2017;37:263-71.

43. Groopman EE, Carmody RN, Wrangham RW. Cooking increases net energy gain from a lipid-rich food. American journal of physical anthropology. 2015;156(1):11-8.

44. Ukhanova M, Wang X, Baer DJ, Novotny JA, Fredborg M, Mai V. Effects of almond and pistachio consumption on gut microbiota composition in a randomised cross-over human feeding study. British Journal of Nutrition. 2014;111(12):2146-52.

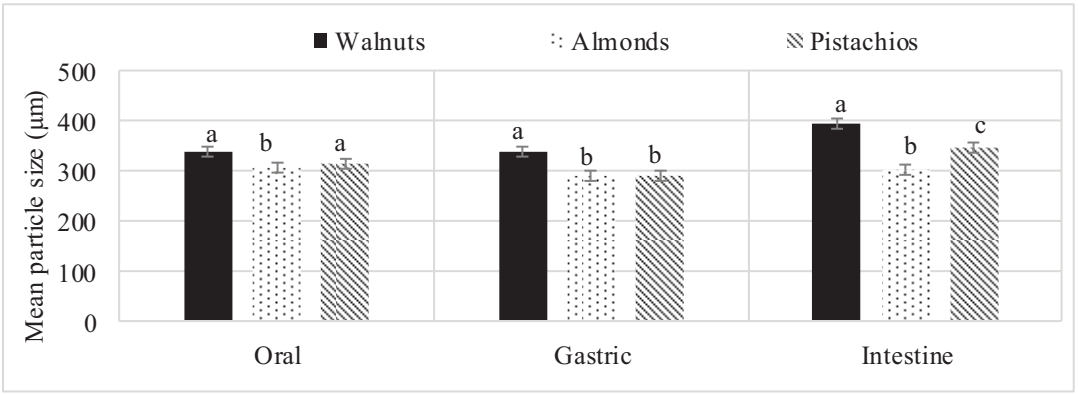
45. Roman MJ, Burri BJ, Singh RP. Release and bioaccessibility of β-carotene from fortified almond butter during in vitro digestion. Journal of agricultural and food chemistry. 2012;60(38):9659-66.

46. Li Y, Hu M, McClements DJ. Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: proposal for a standardised pH-stat method. Food Chemistry. 2011;126(2):498-505.

Table 4-1: Mean diameter of cell oil bodies and walls for pre-digested (native) and post-digested nuts (n=20). Values are means ± S.E.M. Values in a column with the same letter are not significantly different (P<0.05)

	Oil body diameter per cell (µm)		**Cell walls diameter per cell (µm)**	
	Pre-digested	Post-digested	Pre-digested	Post-digested
Walnuts	28.53±2.53[a]	14.77±2.92[ab]	0.862±0.09[a]	1.39 ± 0.14[a]
Almonds	34.11±2.53[a]	21.07±2.93[b]	1.58±0.09[b]	1.48 ±0.14[a]
Pistachios	3.63±2.53[b]	8.80±2.93[a]	1.39±0.09[b]	1.38±0.14[a]

(A)



(B)

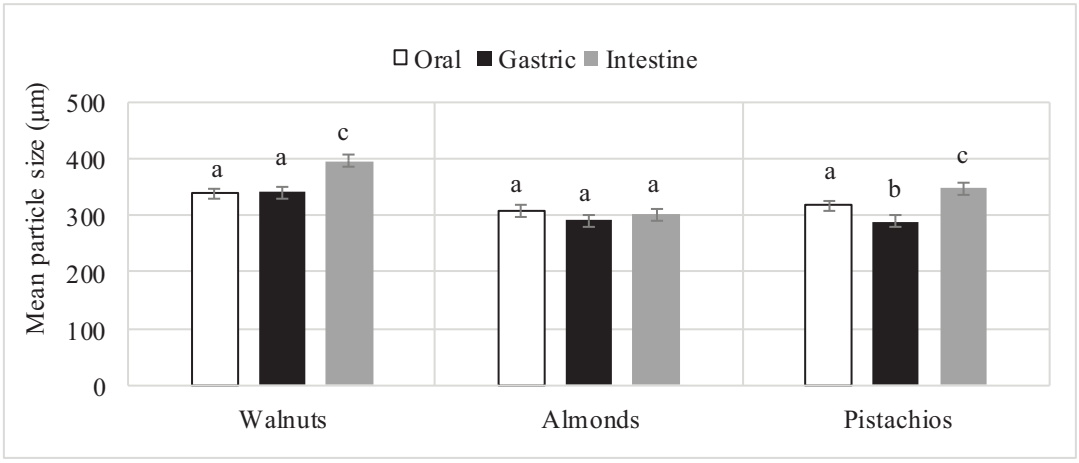


Figure 4-1: (A) Mean particle size comparisons between digestion phases for each nut. (B) Mean particle sizes of nuts after each phase of digestion.
Values are mean ± SEM (n=7). Bar values with different superscript are significantly different (P<0.05)

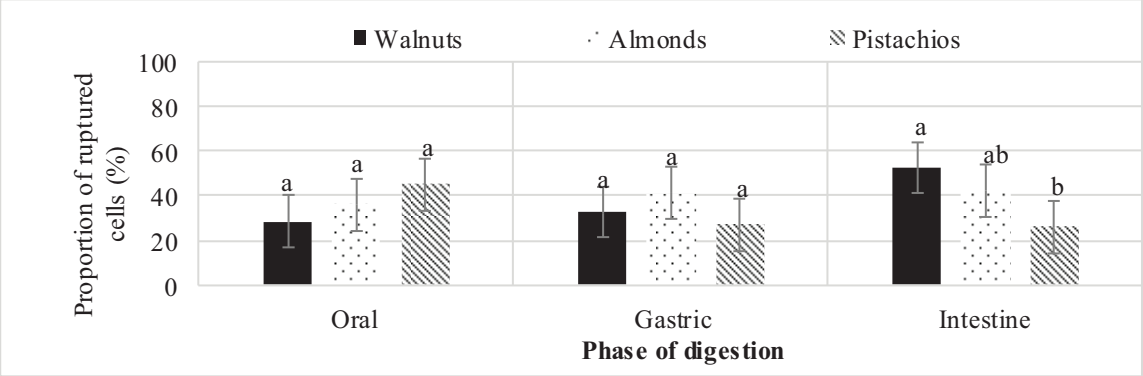


Figure 4-2: Mean proportion of ruptured cells for different nuts after each phase of digestion. Bars sharing the same superscript are not significantly different (P>0.05)

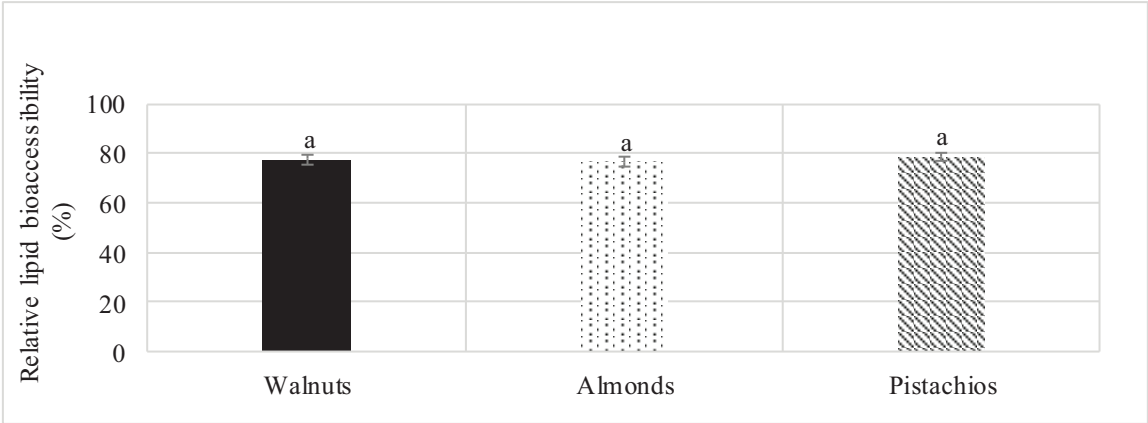
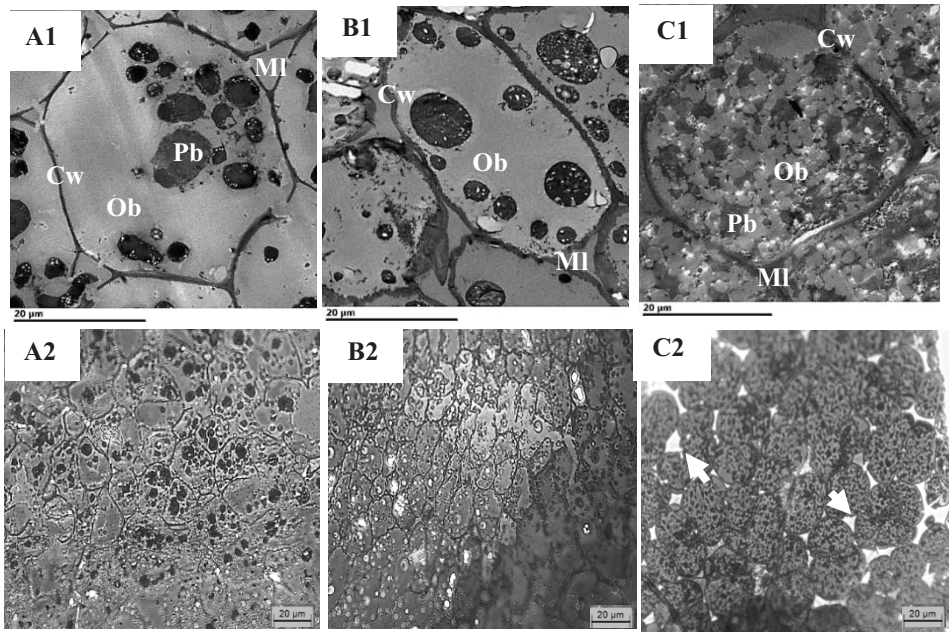


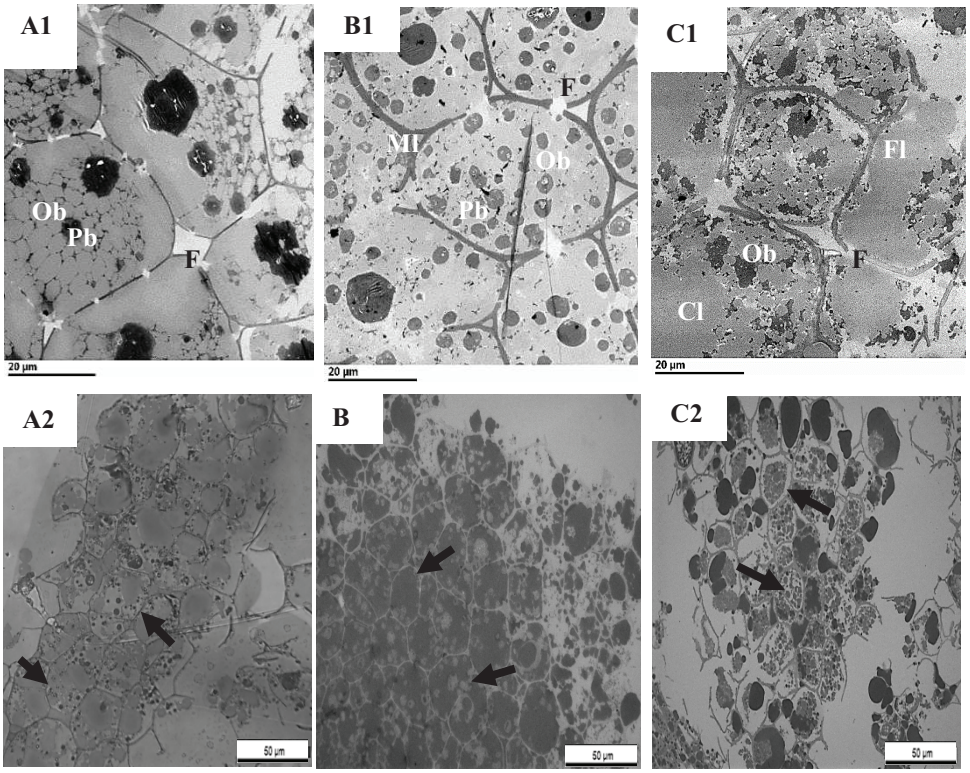
Figure 4-3: Relative lipid biaoccessibility as a percentage of the total lipid.
Values are mean ± SEM. Bars with the same superscripts are not significantly different (P>0.05)

Figure 4-4: TEM (A1-C1) and LM (A2-C2) of pre-digested walnuts (A1, A2), almonds (B1, B2), and pistachios (C1, C2).



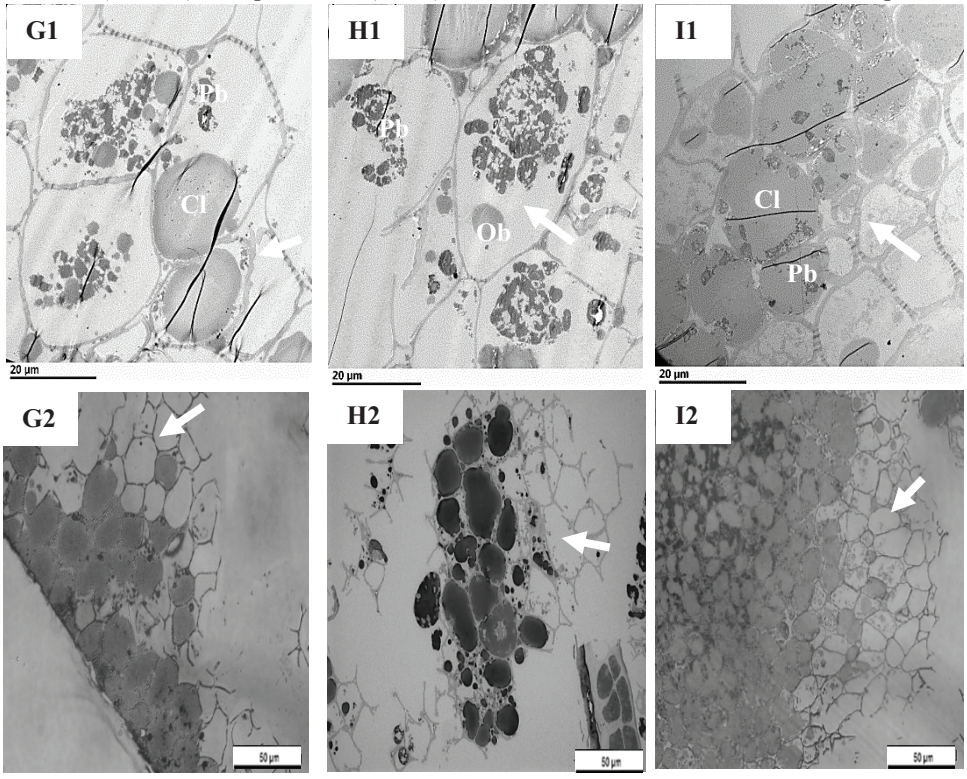
The figure shows intact cells and their contents. Cw, cell wall; Ml, middle lamella; Pb, protein body (black); Ob, oil body (grey); TEM, transmission election microscopy; LM, light microscopy (arrows point to loosely packed parenchyma cells). Scale bars = 20 µm

Figure 4-5: TEM (A1-C1) and LM (A2-C2) micrographs of sections of nut tissues from walnuts (A1, A2), almonds (B1, B2), and pistachios (C1, C2) recovered after mastication.



The figure shows the fractured layer of parenchyma tissue; note coalesced lipid (oil bodies) from fractured cells and free lipid on the peripheral edge of the tissue. F, fissures; Ml, middle lamella; Ob, oil body; Cl; coalesced lipid; Fl; free lipid; TEM, transmission election microscopy; LM, light microscopy (arrows point to intact cells underneath the fractured layer of parenchyma tissue; note coalesced lipid (oil bodies) from fractured cells and free lipid on the peripheral edge of the tissue) Scale bar A1-C2 = 20 µm; A2-C2 = 50 µm.

Figure 4-6: TEM (G1-I1) and LM (G2-I2) micrographs of sections of nut tissues from walnuts (G1, G2), almonds (H1, H2), and pistachios (I1, I2) recovered after 120 min of the intestinal phase.



Cl; coalesced lipid; Ob; oil bodies; Pb; protein body; (arrows show depleted intact cells) Scale bar G1-I2 = 20 µm; G2-I2 = 50 µm.

CHAPTER 5. SUMMARY AND FUTURE RESEARCH DIRECTIONS

5.1 Summary

Nut intake can aid energy balance due to their strong satiating effects, the inefficiency of absorption of their energy, and possibly augmentation of thermogenesis (Chapter 2). Oral processing effects the bioaccessibilty and satiety properties of nuts, but additional data are needed to draw definitive conclusions regarding the role of mastication in their thermogenic effects (Chapter 2). Additionally, the way that nuts are ingested can affect the oral processing applied to them, with implications for digestive and absorptive efficiency (Chapter 2).

This work focused on walnut ingestion with four overall primary aims. The first was to investigate the microstructure of chewing raw, natural walnuts in isolation and under realistic eating conditions as well as under experimentally controlled-chewing and free-chewing conditions. The second aim was to determine the role of oral processing in the high satiety properties of walnuts. The third aim was to investigate the mechanisms responsible for the low bioaccessibilty of energy from walnuts. The fourth aim was to examine how structural differences of whole nuts (walnuts, almonds, and pistachios) affect *in vivo* chewing and *in vitro* lipid digestion. This work also contributes to the research methods for assessing masticatory efficiency by using a free and experimentally controlled chewing protocol to characterize the particle size distribution of walnuts at the point of swallowing.

5.2 Major findings

This work provides new information concerning the oral processing of nuts in the context of the diet. Additionally, new insight are provided into the mechanism contributing to the low digestion efficiency and high satiation potential of walnuts. The major findings are presented below.

5.2.1 The effects of food structure, food properties and food flavor on mastication efficiency of whole nuts

- Mastication of whole nuts (walnuts, almonds, pistachios) resulted in:

- Nut-dependent size distribution of the bolus particles, however, food hardness was not the dominant factor in the variation of the particle size distribution
- Larger particle sizes at swallowing for almonds followed by walnuts and then
- No significant difference in mastication parameters between nuts

- Mastication of nuts with different types of beverages and semi-solid yogurts resulted in;
  - Larger particle sizes at swallowing with sweet and plain yogurts
  - Smaller particle sizes at swallowing with water and juice
  - No significant differences in mastication parameters between fluid forms or types
- Mastication of nuts alone compared to nuts with fluid products resulted in:
  - Larger particle sizes at swallowing for walnuts ingested with yogurts compared to the nut alone
  - Palatability had no significant effect on the particle size nor mastication parameters
  - Sweet flavor had no significant effect on the proportion of large sizes nor mastication parameters

5.2.2 The effects of mastication on the satiety properties of walnuts

- Higher fullness after whole nut intake than nut butter intake; however, there were no changes in GLP-1, glucose, or insulin concentrations
- There was no relationship between the change in fullness and bolus particle size

5.2.3 The effects of mastication and digestion on the extraction of energy from walnuts

- Chewing ruptured rather than separated cotyledon cells
- Walnuts gave larger particles after the intestinal phase compared to the oral and gastric phases

- Approximately 23% of the energy provided by walnuts remained encapsulated in the cells after *in vitro* digestion

- Larger particle sizes for walnuts after the oral, gastric and intestinal phase of digestion compared with almonds and pistachios
- Smaller particle sizes for the pistachios after gastric digestion than after the oral and intestinal phase
- The ratio of intact cells to ruptured cells did not change significantly between nuts nor between digestion phases
- There was no difference in lipid release between nuts
- There was no specific relationship between lipid biaaccessibility and particle size
- Progressive depletion of intracellular nutrients from intact cells was observed in all nut samples collected after the gastric and duodenal phase
- The loss of nutrients from intact cells may be at least in part attributed to fissures created during the oral phase
- Walnut oil bodies with their unique microstructure may also modify lipid digestion

5.3 Future research directions and recommendation

- *Could environmental factors influence oral processing and chewing efficiency?*
    - Oral processing and chewing efficiency are modulated by several factors, such a drinking, food palatability, and environmental aspects (Chapter 2 and 3(161, 179, 186, 187)). Among these factors, environmental aspects, such as TV watching, music, or use of electronic devices, have not been extensively investigated. Although, these factors, may play an important role in chewing efficiency. Recently, it has been suggested that eating while watching TV or using electronic devices may lead to distracted chewing, which presumably cause a decreased in the number of chewing cycles and increased particle size at swallowing(187). If true, this could increase energy intake. Given the current high level of snacking (188), particularly with nuts, it would be interesting to study whether environmental factors have potential to modify pre-swallowing particle size and if this has implications for energy balance.

- *How does the oral processing of walnuts affect appetitive sensations?*
    - We demonstrated that the oral processing of walnuts leads to higher fullness than walnut butter. What is not clear from the appetite effects of mastication is the mechanisms of action. Animal studies suggest mastication can activate satiety centers though histamine neurons (152, 153). With the development of functional neuro-imaging techniques(120, 189), it is now possible to conduct studies to elucidate the neurological mechanism how mastication directly contributes to satiety and satiation. The current literatures provide few published studies on this issue(120, 189), hence it is crucial to design such studies to providing convincing evidence in explain the direct effect of chewing on appetite.

- *Would adding walnuts to yogurts or beverages influence nutrient bioaccessibility and hormonal signals?*
    - Although the chewing study presented in Chapter 4 highlighted intra-individual differences in chewing efficiency and particle sizes in response to changing the oral processing conditions of nuts, the real question is whether these differences result in variability in subsequent digestive processes, such as nutrient bioaccessibilty, gastric emptying, and gut-derived satiety signals. Can difference in the particle size in response to the different oral processing conditions influence appetite, short and long-term satiety, or lipid metabolism? If the degree of intra-individual variability in particle size was large enough it could have potential implications for *in vivo* blood lipids responses and body weight to walnut consumption, potentially through alterations in the availability of lipids and other nutrients. Although this research sets the base for future research, firm conclusions can only be reached upon the completion *in vitro* and *in vivo* studies.

- *Could sweet yogurt increase the 'desire to consume' nuts long-term?*
    a. The present study noted that raw nuts, which are neutral in flavor, resulted in the moderately high liking scores. Similarly, the addition of water, juice, or plain yogurt to nuts did not appear to influence liking. Whereas for nuts with sweet yogurt, liking scores were statically significantly higher than both beverages and

plain yogurt. Given that the acceptance of all nuts with sweet yogurt was high, future studies are needed to determine whether this may enhance compliance with the recommendations to consume nuts daily as a part of heart healthy diet(190).

- *What physical properties of nuts influence their breakdown behavior during mastication?*
    - Physical properties of nuts, particularly hardness, influence the entire digestion process, starting with mastication (49, 52, 86, 191). However, the present findings did not confirm a dominating influence of the nuts' hardness on the particles size at swallowing (Chapter 3). As nuts probably inherently possess and exhibit other physical characteristics, such as brittleness, chewiness, and elasticity it is hypothesized that these properties have a role in the fracture events occurring during chewing. Currently, the exact food properties affecting mastication are unknown and warrant additional investigation. Texture profile analysis (TPA) may highlight the dominate properties (e.g. mechanical, structural, textural) of nuts that influence the particle size distribution of the food bolus as well provide the potential to link the breakdown pathways during mastication to the physical properties of nuts(192).

- *What approaches may be implemented for to the assessment of mastication performance?*
    - Electromyography (EMG) signals were measured as an indicator of the masticatory performance (Chapter 4). While EMG signals are commonly used to study oral physiology parameters during solid food comminution, these data may not reliably indicate absolute forces either generated within muscles or created by muscles elsewhere in the body (193, 194). The use of kinesthesiological methods, which characterize mandibular movements, in combination with EMG methods may result in a better recording of muscle activity. Based on the data presented in Chapter 4 there was indication that the chewing time altered the chewing efficiency of walnuts. While the time spent

chewing in the "fixed time" condition was slightly lower than the time spent chewing in the" free time" condition, there were significant intra-individual differences in particle size of the chewed food between the fixed and free chewing conditions. Based on the intra-individual differences noted in the chewing study in response to walnuts we recommended using a fixed and free chewing protocol to assess mastication efficiency. This will ensure that the studied bolus reflects its natural state at the point of swallowing. Moreover, following the work on particle sizing we recommend using for masticated or digested nuts a laser diffraction technique combined with mechanical sieving if the size range is not covered by the laser instrument. It is important to note that a direct comparison of the two techniques is not easy to achieve as they give particles sizes in two different units (e.g., volume vs weight) (Chapter 3 and Chapter 4). Obtaining a shape factor that allows the two types of data to coincide has been suggested, but this is probably difficult as particle shape has to be constant over the size ranges(195). The process was also compromised by the multimodal pattern of the particle size distribution. Using the latest particle sizer from Malvern (Malvern Mastersizer 3000) could overcome these issues as the instrument has the capacity to measure particles, wet and dry, with sizes ranging from 0.01 to 3500 μm.

- *What is the effect of intact cells within the colon?*
    - This work studied the behavior of walnut after simulated gastric and intestinal digestion (Chapter 4). However, subjecting the digested walnuts to the *in vitro* colonic phase could provide information about their behavior and structure in this compartment, especially the degree of degradation of walnut materials (e.g., particles, oleosins). As there is growing interesting in how plant food material properties could influence the rate of lipid digestion and hence the bioavailability of lipids, more research is required in this area.

- *Do intracellular compounds have potential to hinder lipolysis in walnuts?*

- The present study provides further evidence that intact cell walls act as a barrier that limited the digestibility of lipid from walnuts. However, we observed that intact cells only account for part of the low lipid bioaccessibilty of walnuts. Recent evidence suggests that that structure of walnut oil bodies may reduce even further the digestibility of lipid contained in the cell mainly due to their interfacial layer (e, g. proteins (oleosin), phospholipids, MAG, various kinds of fatty acid esters). Therefore, it would be worthwhile to investigate the potential role of walnut oil bodies on energy extraction. There are a number of questions within this area that needed to investigated such as, how the action of lipase is modified by the structure and composition of the adsorbed layers on the droplets surface. Furthermore, the mechanisms controlling lipase through the cell walls may be different than those governing the binding to the droplet surface(196, 197). CLSM combined with fluorescent techniques instead of LM could provide a more detailed image of the structure and properties of OBs, better insight on enzyme interactions with walnut materials, and useful information about the effects intracellular compounds on nutrient extraction(88, 198).

5.4 References

1. Bobroff EM, Kissileff HR. Effects of changes in palatability on food intake and the cumulative food intake curve in man. Appetite. 1986;7(1):85-96.

2. Rolls BJ, Wood RJ, Stevens RM. Palatability and body fluid homeostasis. Physiology & Behavior. 1978;20(1):15-9. doi: https://doi.org/10.1016/0031-9384(78)90196-8.

3. Yeomans MR, Gray RW, Mitchell CJ, True S. Independent Effects of Palatability and Within-meal Pauses on Intake and Appetite Ratings in Human Volunteers. Appetite. 1997;29(1):61-76. doi: https://doi.org/10.1006/appe.1997.0092.

4. Pereira L, Bilt A. The influence of oral processing, food perception and social aspects on food consumption: a review. Journal of Oral Rehabilitation. 2016;43(8):630-48.

5. Mattes RD. Snacking: A cause for concern. Physiology & Behavior. 2018.

6. Sakata T. Histamine receptor and its regulation of energy metabolism. Obesity. 1995;3(S4).

7. Sakata T, Yoshimatsu H, Masaki T, Tsuda K. Anti-obesity actions of mastication driven by histamine neurons in rats. Experimental Biology and Medicine. 2003;228(10):1106-10.

8. Sayer RD, Dhillon J, Tamer GG, Cornier M-A, Chen N, Wright AJ, et al. Consuming almonds vs. Isoenergetic baked food does not differentially influence postprandial appetite or neural reward responses to visual food stimuli. Nutrients. 2017;9(8):807.

9. Farr OM, Tuccinardi D, Upadhyay J, Oussaada SM, Mantzoros CS. Walnut consumption increases activation of the insula to highly desirable food cues: A randomized, double-blind, placebo-controlled, cross-over fMRI study. Diabetes, Obesity and Metabolism. 2017.

10. Food, Administration D. Qualified Health Claims: Letter of Enforcement Discretion–Nuts and Coronary Heart Disease. Docket No 02P-0505. Food and Drug Administration: Washington, DC. 2003.

11. Grundy MM, Edwards CH, Mackie AR, Gidley MJ, Butterworth PJ, Ellis PR. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. The British journal of nutrition. 2016;116(5):816-33.

12. McKiernan F, Mattes RD. Effects of peanut processing on masticatory performance during variable appetitive states. Journal of nutrition and metabolism. 2009;2010.

13. Kong F, Oztop MH, Singh RP, McCarthy MJ. Effect of boiling, roasting and frying on disintegration of peanuts in simulated gastric environment. LWT-Food Science and Technology. 2013;50(1):32-8.

14. Kong F, Singh R. Disintegration of solid foods in human stomach. Journal of Food Science. 2008;73(5).

15. Ocon A, ANZALDÚA-MORALES A, Quintero A, Gastelum G. Texture of pecans measured by sensory and instrumental means. Journal of Food Science. 1995;60(6):1333-6.

16. Koç H, Vinyard C, Essick G, Foegeding E. Food oral processing: conversion of food structure to textural perception. Annual review of food science and technology. 2013;4:237-66.

17. Vinyard CJ, Fiszman S. Using electromyography as a research tool in food science. Current Opinion in Food Science. 2016;9:50-5.

18. Wang Q, Ellis PR, Ross-Murphy SB. Dissolution kinetics of guar gum powders—III. Effect of particle size. Carbohydrate polymers. 2006;64(2):239-46.

19. Capuano E, Pellegrini N, Ntone E, Nikiforidis CV. In vitro lipid digestion in raw and roasted hazelnut particles and oil bodies. Food & function. 2018.

20. Gallier S, Tate H, Singh H. In vitro gastric and intestinal digestion of a walnut oil body dispersion. Journal of agricultural and food chemistry. 2013;61(2):410-7.

21. Grassby T, Mandalari G, Grundy MM-L, Edwards CH, Bisignano C, Trombetta D, et al. In vitro and in vivo modeling of lipid bioaccessibility and digestion from almond muffins: The importance of the cell-wall barrier mechanism. Journal of functional foods. 2017;37:263-71.

22. Grassby T, Picout DR, Mandalari G, Faulks RM, Kendall CW, Rich GT, et al. Modelling of nutrient bioaccessibility in almond seeds based on the fracture properties of their cell walls. Food & function. 2014;5(12):3096-106.

APPENDIX

Appendix A Institutional Review Board Documents

Revised 10/10 Ref. # _____

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

Purdue University

Institutional Review Board

---

1. Project Title: The effects of mastication and digestion on the bio accessibility of energy from walnuts

2. Full Review ☒ Expedited Review ☐

3. Anticipated Funding Source: California Walnut Commission

4. Principal Investigator *[See Policy on Eligibility to serve as a Principal Investigator for Research Involving Human Subjects]*:

Name and Title	Department, Building, Phone, FAX, E-mail address
Richard D Mattes	Nutrition Science, Stone Hall, Ph: 765-494-0062
(Distinguished Professor)	Fax: 765-494-0674, mattes@purdue.edu.

5. Co-investigators and key personnel *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title	Department, Building, Phone, FAX, E-mail address

6. Consultants *[See Education Policy for Conducting Human Subjects Research]*:

Name and Title	Department, Building, Phone, FAX, E-mail address
Judy George, Lab Manager	Nutrition Science, Stone Hall, Ph: 765-494-6192
	Fax:765-494-494-0606, georgej@purdue.edu
Robin Rhine, Lab Technician	Nutrition Science, Stone Hall, Ph: 765-494-6192
	Fax:765-494-494-0606, rrhine@purdue.edu

7. The principal investigator agrees to carry out the proposed project as stated in the application and to promptly report to the Institutional Review Board any proposed changes and/or unanticipated problems involving risks to subjects or others participating in the approved project in accordance with the HRPP Guideline 207 Researcher Responsibilities, Purdue Research Foundation-Purdue University Statement of Principles and the Confidentiality Statement. The principal investigator has received a copy of the Federal-Wide Assurance (FWA) and has access to copies of 45 CFR 46 and the Belmont Report. The principal investigator agrees to inform the Institutional Review Board and complete all necessary reports should the principal investigator terminate University association.

_____

Principal Investigator Signature Date

8. The Department Head (or authorized agent) has read and approved the application. S/he affirms that the use of human subjects in this project is relevant to answer the research question being asked and has scientific or scholarly merit. Additionally s/he agrees to maintain research records in accordance with the IRB's research records retention requirement should the principal investigator terminate association with the University.

_____

Department Head *(printed)* Department Name

_____

Department Head Signature Date

APPLICATION TO USE HUMAN RESEARCH SUBJECTS

9. This project will be conducted at the following location(s): (please indicate city & state)

- ☒ Purdue West Lafayette Campus
- ☐ Purdue Regional Campus (Specify) _____
- ☐ Other (Specify): _____

10. If this project will involve potentially vulnerable subject populations, please check all that apply.

- ☐ Minors under age 18
- ☐ Pregnant Women
- ☐ Fetus/fetal tissue
- ☐ Prisoners Or Incarcerated Individuals
- ☐ University Students (PSYC Dept. subject pool ___)
- ☐ Elderly Persons
- ☐ Economically/Educationally Disadvantaged Persons
- ☐ Mentally/Emotionally/Developmentally Disabled Persons
- ☐ Minority Groups and/or Non-English Speakers
- ☐ Intervention(s) that include medical or psychological treatment

11. Indicate the anticipated maximum number of subjects to be enrolled in this protocol as justified by the hypothesis and study procedures: 75

12. This project involves the use of an **Investigational New Drug** (IND) or an **Approved Drug For An Unapproved Use**.

☐ YES ☒ NO

Drug name, IND number and company: _____

13. This project involves the use of an **Investigational Medical Device** or an **Approved Medical Device For An Unapproved Use**.

☐ YES ☒ NO

Device name, IDE number and company: _____

14. The project involves the use of **Radiation or Radioisotopes**:

☐ YES ☒ NO

15. Does this project call for: (check-mark all that apply to this study)

- ☐ Use of Voice, Video, Digital, or Image Recordings?

☒ Subject Compensation? Please indicate the maximum payment amount to subjects. $100.00

Purdue's Human Subjects Payment Policy Participant Payment Disclosure Form

☐ VO2 Max Exercise?

☐ More Than Minimal Risk?

☐ Waiver of Informed Consent?

☐ Extra Costs To Subjects?

☒ The Use of Blood? Total Amount of Blood 180ml

Over Time Period (days) 2

☐ The Use of rDNA or Biohazardous materials?

☐ The Use of Human Tissue or Cell Lines?

☐ The Use of Other Fluids that Could Mask the Presence of Blood (Including Urine and Feces)?

☐ The Use of Protected Health Information (Obtained from Healthcare Practitioners or Institutions)?

☐ The Use of academic records?

16. Does investigator or key personnel have a potential financial or other conflict of interest in this study?

☐ YES ☒ NO

APPLICATION NARRATIVE

A. PROPOSED RESEARCH RATIONALE

Background

In light of the high prevalence of overweight and obesity in the nation, health benefits of nuts will be evaluated in large measure by their effect on energy intake and body weight. Despite evidence to the contrary (Tan, et al., 2014), one of the biggest obstacles to increased walnut consumption is concern about their contribution to energy intake and potential to promote weight gain. Thus, it is critical to characterize their role in energy balance and to document the mechanisms by which they may be consumed as part of a healthful diet without posing a risk for overweight/obesity.

The proposed study will focus on oral processing as recent findings document that the efficiency of energy extraction from nuts is less than predicted and this may be attributable to the efficiency of mastication. Recent evidence indicates up to 20% of the energy from walnuts is not absorbed. This low bio accessibility is comparable to almonds with markedly different physical properties and much higher than for pistachios with similar physical characteristics. These counter-intuitive findings require mechanistic explanations so they may be accepted and used to formulate policy and clinical recommendations. It is proposed that the differences in the structure of nuts accounts for the energy yield results. For example, almonds are predicted to fracture while walnuts are predicted to separate under the applied pressure of chewing. The dissimilarity of walnuts and pistachios in terms of energy yield suggests differences in post-swallowing digestion. The whole nut yields superior effects on fullness and this leads to focus on oral processing. It may be that the mechanical act of chewing generates satiety sensations or it may be that the mechanical disruption of the parenchymal call walls releases lipid and protein and their higher concentrations activate the release of gut peptides associated with satiety.

B. SPECIFIC PROCEDURES TO BE FOLLOWED

Screening Procedures

Potential participants meeting preset criteria will be scheduled for a visit at the laboratory having refrained from eating, drinking, chewing gum or using oral care products for at least two hours prior to their visit. Participant's height will be measured with participants in bare feet with a Holtain stadiometer (essentially a ruler on a wall). Participants will be asked to wear light clothing to facilitate the measurement of body weight and composition. Body composition will be assessed via bioelectrical impedance (Body Fat Analyzer Scale, Model TBF 410, Tanita Corporation of America, Inc., Arlington Heights, IL). This just entails standing on a scale like a bathroom scale. Participants will sample all foods and rate them for palatability. Those meeting the eligibility criteria will be asked to read and sign an informed consent document. Electrodes will be placed for electromygraphic recording. Participants will then be presented with each stimulus in a

random order and asked to chew the stimulus 15 times per mouthful at the rate of one chew per second, they then will expectorate the bolus into a set of graded, stacked sieves. They will rinse three times to cleanse the palate of residual material expectorating after each rinse into the same set of sieves. Participants will then be asked to chew the nuts alone (all three types separately) to the point where they would swallow and then spit the bolus into a beaker.

Stimuli

The nuts will include walnuts, almonds and pistachios. The chewing conditions will include: 1) 5g nuts alone, 2) 5g nuts with 5ml water, 3) 5g nuts with 5ml apple juice, 4) 5g nuts with 5g plain yogurt, 5) 5g nuts with 5gsweet yogurt.

	Walnuts	Almonds	Pistachios
Nut alone			
Nut with water			
Nut with apple Juice			
Nut with sweet yogurt			
Nut with plain yogurt			

Test Days 1 and 2

Participants will report to the Laboratory of Sensory and Ingestive Studies after an overnight fast and having refrained from using oral care products for at least two hours prior to their visit. Upon arrival, their blood glucose concentration will be measured by a glucometer (One Touch® Glucometer, LifeScan, Inc.) to confirm that participants are in a fasted state. Participants are then asked to answer a validated appetite questionnaire on a palm pilot (199-201) (in Section L). The appetite questionnaire assesses hunger, fullness, desire to eat, future consumption, preoccupation with food, thirst, and desire to eat something salty, fatty or sweet. Stylus placement on the response line directly translates to percent score.

The test session will continue only if plasma glucose is <110mg/dl and self-reported hunger is rated greater than “strong” on the gLMS scale. If these conditions are not met,

the trial will be rescheduled. If all conditions are met, electrodes will be placed for electromygraphic recording. The participant will then be placed in a semi-supine position and a catheter will be placed in a vein in the antecubital space of one arm. A 9ml baseline blood sample will be collected. Participants will complete a validated appetite questionnaire on a palm pilot right after the baseline blood draw. Participants will then be presented with either 5g walnuts or 5g walnut butter and asked to chew the stimulus 15 times per mouthful at the rate of one chew per second; they then will expectorate the bolus into a beaker. Participants will then be presented with either 5g walnuts or 5g walnut butter and asked to chew until the point where they would normally swallow and then spit the bolus into a beaker. The participants will then consume 28g of walnuts or 28g walnut butter given in random order. Additional blood samples and appetite questionnaire will be taken at 15, 30, 45, 60,120, and 180 minutes. The blood will be stored in a minus 80 freezer to be analyzed at a later date for insulin, glucose, GLP-1, Ghrelin, and PYY.

C. SUBJECTS TO BE INCLUDED

Describe:

50 individuals (male and female) ages 18-60 years with a BMI between 18-35 kg/m2 from any ethnic/racial background will be recruited. Additional eligible criteria include:

- Natural dentition and no oral pathology
- Rate hedonic value of all study foods between 3 and 7 on a 9-point category scale.

D. RECRUITMENT OF SUBJECTS AND OBTAINING INFORMED CONSENT

Participants will be recruited through public advertisements on the Laboratory for Sensory and Ingestive Studies website: www.cfs.purdue.edu/lsis (IRB approval #504002017 and posted flyers (see attached). Advertisement is electronic and paper media may also be used (see attached). Those meeting the preset criteria described above will be contacted via their indicated preferred method (i.e., phone or e-mail) to schedule a screening visit.

E. PROCEDURES FOR PAYMENT OF SUBJECTS

Participants will receive a payment of $125.00 as compensation for any inconvenience caused by participating in this study. A partial payment of $25.00 will be made to participants should they withdraw or be withdrawn from the study for sessions completed.

F. CONFIDENTIALITY

The record of participant progress in the study will be kept in a confidential file in a locked filing cabinet. The confidentiality of any computer record will also be carefully guarded by never including the participant's name on any data file. The information will be stored electronically in a password-protected file. A copy of the written consent form will be retained for three years after termination of the study at which time it will be destroyed. No information by which participants can be identified will be released or published. However, participants will be informed that to process their payments, it will be necessary to provide their name, social security number, and address to the university business office. In addition, participants will be notified that their research records may be reviewed by the National Institutes of Health and by Departments at Purdue University responsible for regulatory and research oversight.

G. POTENTIAL RISKS TO SUBJECTS

The food that participants receive are made of commercially available products and pose no foreseeable risk. All blood will be collected by an experienced technician using sterile techniques, but the procedure may result in a bruise, soreness and infection at the site of collection. The total amount of blood collected over all test sessions will be less than the amount normally given at a blood donation.

H. BENEFITS TO BE GAINED BY THE INDIVIDUAL AND/OR SOCIETY

There are no foreseeable direct benefits to participants. The knowledge gained from this study may provide new insights for the management of obesity, cardiovascular disease, and diabetes – some of the nation's most pressing public health problems.

I. INVESTIGATOR'S EVALUATION OF THE RISK-BENEFIT RATIO

Aside from routine blood draws, participants will be faced with no greater risk than normally encountered on a daily basis. The findings may yield insights for obesity, cardiovascular disease, and diabetes. Thus, the potential benefits outweigh the possible risks.

J. WRITTEN INFORMED CONSENT FORM ***(to be attached to the Application Narrative)***

See attached consent form

K. WAIVER OF INFORMED CONSENT OR SIGNED CONSENT

Not applicable

L. INTERNATIONAL RESEARCH

Not applicable

M. SUPPORTING DOCUMENTS ***(to be attached to the Application Narrative*****)**

Recruitment Flyer

Multi-media advertisement

Walnut Study

PI: Dr. Richard Mattes

Participants needed to study the satiation properties of walnuts

Earn $125.00

<u>Eligibility</u>

Age 18 – 60

No nut allergies

Willingness to eat walnuts

Contact: Breanna McArthur at bmcarth@purdue.edu

Walnut Study
bmcarth@purdue.edu

TV Advertisement and Classified Advertisement

Healthy adults are needed for a study relating to the effects of chewing and digestion on the energy from walnuts that is being conducted by the Purdue University Department of Nutrition Science. Earn $125.00 participating in this study. Contact Robin at rrhine@purdue.edu or Judy at georgej@purdue.edu or visit the Laboratory for Sensory and Ingestive Studies web site at http://www.cfs.purdue.edu/lsis/. (Principal Investigator—Dr. Richard Mattes, Department of Foods and Nutrtition Science/Purdue University)

RESEARCH PARTICIPANT CONSENT FORM

The effects of mastication and digestion on the bio accessibility of energy from walnuts

Principal Investigator: Richard D Mattes MPH, PhD, RD
Purdue University, Department of Nutrition Science
Stone Hall, W. Lafayette, IN 47907
(765) 494-0662

What is the purpose of this study?

This study aims to characterize the mechanisms that account for the low yield of energy during digestion of walnuts.

What will I do if I choose to be in this study?

To confirm your eligibility for participation in this study, we ask that you first read this consent form and ask any questions you may have about your participation. If you choose to sign this form, we will then proceed with additional screening and testing measures.

Screening

- Upon arrival, we will take measurements of your height, weight and body composition (percent of muscle, bone and fat in your body). Your height will be measured in bare feet by asking you to stand near a wall with a ruler attached. Your weight and body composition will be measured in bare feet and light clothing on an electronic scale much like a bathroom scale.

- Surface electrodes will be placed 3 cm apart on your jaw and temple on one side of your head. And another will be placed over the opposite wrist.

- You will be given 15 food samples (in random order) and each will be chewed 15 times at the rate of one chew per second (timed to a metronome). The samples will be expectorated into a series of sieves and you will rinse your mouth with 20ml aliquots of mild salt water, expectorating after each rinse into the same set of sieves.

- You will be asked to rate the palatability of each sample.

- You will then be asked to chew the three nuts alone to the point of swallowing. The samples will be expectorated into a beaker and you will rinse your mouth with three 20ml aliquots of mild salt water, expectorating after each rinse into the same beaker.

Test Visits

- Upon arrival, having refrained from eating or using oral care products for at least 2 hours, you will answer an appetite questionnaire on a palm pilot.
- Surface electrodes will be placed 3 cm apart on your jaw and temple on one side of your head. And another will be placed over the opposite wrist.
- You will then be seated on a reclining chair.
- A trained phlebotomist will then place a catheter (flexible needle) in your arm for blood draws. Prior to each draw, a small amount (1-2 ml) of sterile saline (salt solution) will be washed through the catheter to ensure it is clear.
- You will be given either 5g walnuts or 5g walnut butter asked to chew 25 times at the rate of one chew per second (timed to a metronome). The samples will be expectorated into a beaker and you will rinse your mouth with three 20ml aliquots of mild salt water, expectorating after each rinse into the same beaker.
- You will be given either 5g walnuts or 5g walnut butter asked to chew to the point of swallowing. The samples will be expectorated into a beaker and you will rinse your mouth with three 20ml aliquots of mild salt water, expectorating after each rinse into the same beaker.
- Next, you will consume 28g (about 1 ounce) of walnuts or 28g walnut butter.
- Blood draws will take place once the catheter is set and then at each of the following time points thereafter: 15, 30, 45,60, 120 and 180 minutes.
- Each time your blood is drawn, you will be asked to fill out an appetite questionnaire.

How long will I be in the study?

This study requires a total of 3 visits (1 screening visit and 2 test visits) at Stone Hall room 226 and room 155. The screening visit will take approximately 1 hour and each of the test visits will take approximately 3 hours and 15 minutes. There will be a minimum of 24 hours between each visit.

What are the possible risks or discomforts?

The blood collections may result in pain, bruising and/or infection at the site of collection. Some people become lightheaded during blood collections and may faint. Appropriate techniques and trained personnel will be used to minimize these risks. You will be asked to consume nuts. If you even suspect that you have an allergy or sensitivity to any type of nut, you should not participate in this research study.

Are there any potential benefits?

There are no foreseeable benefits to you from your participation in this study. However, the knowledge gained from this work may provide new insights to manage body weight and obesity.

Will I receive payment or other incentive?

You will receive a payment of $125 as compensation for any inconvenience caused by your participation. A payment of $25/session will be made should you withdraw or be withdrawn from the study for sessions completed and these data will be included in the data analyses at the end of the study unless you indicate you would prefer to have it destroyed before that time.

What happens if I become injured or ill because I took part in this study?

If you feel you have been injured due to participation in this study, please contact the principal investigator, Dr. Richard Mattes, at (765) 494-0662 or mattes@purdue.edu. Purdue University will not provide medical treatment or financial compensation if you are injured or become ill as a result of participating in this research project. This does not waive any of your legal rights nor release any claim you might have based on negligence.

Conflict of Interest Disclosure

The following disclosure(s) is(are) made to give you an opportunity to decide if this(these) relationship(s) will affect your willingness to participate in the research study.

Will information about me and my participation be kept confidential?

All information collected in this study will be stored in a locked filing cabinet in a secure location in close proximity to the principal investigator. A copy of this consent form will be retained for three years after termination of the study at which time it will be destroyed. If any publication results from this research, you will not be identified by name. Your identity will not be released to any party outside the research team with the exception that your name, address, and social security number will be provided to the business office to enable processing of your financial compensation. In addition, the project's research records may be reviewed by the National Institutes of Health, and by Departments at Purdue University responsible for regulatory and research oversight.

What are my rights if I take part in this study?

Your participation in this study is voluntary. You may choose not to participate or, if you agree to participate, you can withdraw your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

Who can I contact if I have questions about the study?

If you have questions, comments or concerns about this research project, you can talk to one of the researchers. Please contact the principal investigator, Dr. Richard Mattes, at (765) 494-0662 or mattes@purdue.edu.

If you have questions about your rights while taking part in the study or have concerns about the treatment of research participants, please call the Human Research Protection Program at (765) 494-5942, email (irb@purdue.edu)or write to:

Human Research Protection Program - Purdue University

Ernest C. Young Hall, Room 1032

155 S. Grant St.,

West Lafayette, IN 47907-2114

Documentation of Informed Consent

I have had the opportunity to read this consent form and have the research study explained. I have had the opportunity to ask questions about the research study, and my questions have been answered. I am prepared to participate in the research study described above. I will be offered a copy of this consent form after I sign it.

_____ _____

Participant's Signature Date

_____

Participant's Name

_____ _____

Researcher's Signature Date

- The participant must sign and date the consent form. The only exception is if the study is granted a waiver of signed consent.
- The researcher's signature, above, refers to the research team member who has obtained the participant's consent. The researcher's signature indicates s/he has explained the research to the participant (or the legally authorized representative when IRB approved) and has answered any of the participant's questions.

VITA

Breanna Marina McArthur
PhD candidate, Interdisciplinary Food Science Program
Department of Food Science
Purdue University

EDUCATION

Purdue University, West Lafayette, IN

Doctor of Philosophy, August 2018

Dissertation: The effect of mastication on the bioaccessibility and digestibility of energy from walnuts

Advisor: Dr. Richard D. Mattes

Alabama A&M University, Huntsville, AL

Bachelor of Science in Food Science & Technology with Honors May 2014

RESEARCH EXPERIENCE

Graduate Research Assistant, Department of Food Science, Purdue University, August 2014-August 2018

Investigating the effects of mastication on energy extraction and the satiety properties of walnuts and its interaction with food physical properties

Undergraduate Research Assistant, Department of Food Science, Ohio State University, Columbus OH, summer 2013

Monitoring and measuring long-term storage of cooking oils using novel technology

Undergraduate Research Assistant, Department of Food Science, Cornell University, Ithaca, NY, summer 2012

Characterizing the mode of action of a natural antimicrobial for food preservation

Undergraduate Research Assistant, Department of Food Science, Alabama A&M University, Huntsville, AL August 2011-May 2013

TEACHING EXPERIENCE

Graduate Teaching Assistant, Department of Food Science, Purdue University, January 2018-May 2018

Food Analysis (FS 469), Purdue University

Graduate Teaching Certificate, Purdue University, April 2018

Student Teaching Assistant, Tutorial Assistance Network (TAN), Alabama A&M University August 2012-2013

Facilitated Calculus I peer led team learning sessions

Student Teaching Assistant, Department of Chemistry, Alabama A&M University, August 2011-May 2012

Facilitated General Chemistry I & II promote group problem solving

Trainings and Certifications:

HONORS AND AWARDS

2018 Certificate **of Excellence in Research Office of Interdisciplinary Graduate Programs**, Purdue University

2017 **Ingestive Behavior Research Center NIH Training Grant,** Purdue University
Certificate **of Excellence in Research Office of Interdisciplinary Graduate Programs**, Purdue University

2016 **Ingestive Behavior Research Center NIH Training Grant,** Purdue University

2014 **Industry Fellowship, Department of Food Science**, Purdue University
Purdue Doctoral Fellowship, Purdue University

PUBLICATIONS

McArthur, B.M., Higgins, K.A., Hunter, S.R., Mattes, R.D. The energetics of nut consumption: oral processing, appetite, and energy balance. *Submitted.*

McArthur, B.M., Mattes, R.D. Energy Extraction from Walnuts. *Submitted.*

McArthur, B.M., Considine R.V., Mattes R.D., Mastication of Nuts under Realistic Eating Conditions: Implications for Energy Balance (2018). *Nutrients.*

Carreiro A.L., Dhillon J., Gordon, S., Jacobs A.G., Higgins, K.A., **McArthur, B.M.**, Redan B.W., Rivera R.L., Schmidt L.R., Mattes, R.D. (2016). The macronutrients, appetite, and energy intake. *Annu Rev Nutr.*