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Effects of Maturity on the Development of Oleic Acid and Linoleic Acid in the Four Peanut Market Types

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Abstract

The commercialization of high oleic peanut varieties with the fatty acids, oleic and linoleic present in a ratio greater than 9 has increased the shelf stability of many products containing peanuts significantly. With no visual traits to determine levels of the fatty acids present, mixing of the high oleic peanut types from the normal oleic types has been a problem in the peanut supply chain. This study investigated the effect of the development of the fatty acids in peanuts over their maturation with respect to the different market types (Runner, Viriginia, Spanish, Valencia) to determine if the maturation stage of the peanut could be responsible for the presence of normal oleic peanuts in lots of high oleic peanuts and thus decreasing the purity of the lots. Peanuts had different levels of the main fatty acids present as the oil content increased with maturation. Due to the presence of a natural desaturase enzyme in peanuts, oleic acid is converted to linoleic as the peanut develops resulting in a ratio of oleic acid to linoleic acid of 3 or lower in normal oleic peanuts. In peanuts from high oleic cultivars, the genes encoding for this enzyme are mutated or slow to develop. As this gene is activated in the later stages of peanut maturity, this study proves immature peanuts of the high oleic type may not have the proper ratios of oleic to linoleic to ensure shelf stability despite being from high oleic cultivars. This study describes how the concentrations of oleic and linoleic acid changed with maturation of the peanut seeds and affects the purity of individual lots of high and normal oleic types of peanuts. This effect of maturity was seen to be greater in the large seeded Virginia cultivars compared to the smaller seeded market types.

Keywords: peanut maturity, shelf-life, lipid stability, seed development

1. Introduction

The four peanut market types are Runner, Virginia, Spanish and Valencia. Different peanut products are produced from each cultivar (National Peanut Board, 2020). Runner peanuts, 38 to 70 seeds per ounce, depending on the grade, are used almost entirely for the manufacture of peanut butter but are often used in certain candies. Virginia type peanuts are usually the largest in physical size, 21 to 42 seeds per ounce, and are most often used for snack peanuts sold as “in-shells” or oil roasted peanuts sold in cans or single serve cellophane bags. The Spanish-type, 60 to 80 seeds per ounce, are sold as “redskin” peanuts in cans and may also be used in confections as they have the highest levels of sweet flavor. Valencia peanuts, 75 to 80 seeds per ounce, represent only about 1% of the USA peanut market and are usually consumed as “in-shell” product although they are sometimes used to produce peanut butter or confections.

Peanut seed development takes place entirely underground so that there is no practical way to monitor that development. Like other legumes, peanuts are the seed of the plant and develop from the stem of the flower which forms a peg and moves underground. In the early stages, starch is accumulated to serve as the energy source but then the production of lipid rapidly overtakes it (Pickett, 1950). Initially, the fatty acids produced by the seed are mostly linoleic and palmitic with slightly lesser amounts of oleic acid. As the seed matures, genes coding for fatty acid desaturases are turned on producing enzymes that begin converting oleic acid to linoleic acid. In 1980’s, a mutation was discovered that resulted in peanuts having large amounts of oleic acid compared

to linoleic acid indicating the desaturases were not being produced or activated (Moore & Knauft, 1989; Norden, Gorbet, Knauft, & Young, 1987). When the mutation is present, the seed continues to produce oleic acid but little or none is converted to linoleic acid and the amount of oleic acid greatly exceeds that of linoleic. Genetic markers have been developed to identify the genotypes aiding in the development of peanut cultivars that contain the high oleic trait (Barkley, Chenault Chamberlin, Wang, & Pittman, 2010). However, peanuts pods achieve their final physical size before the seed inside is at final maturity and the oil composition is at its final state leading to the conclusion that despite the proper genetic makeup, an immature peanut may not necessarily have expressed the high oleic trait.

Peanuts contain approximately 50% lipid, mainly composed of triglycerides (Stalker, 1997). Of the fatty acids present in mature peanuts, 80% are a combination of oleic and linoleic acids (Davis, Dean, Faircloth, & Sanders, 2008). Optimum flavor and shelf life are therefore very dependent on the lipid quality. Peanut cultivars with increased levels of oleic acid have proven to have increased shelf life over conventional cultivars (O'Keefe, Wiley, & Knauft, 1993). High oleic (HO) peanuts are those where the ratio of the oleic acid content to the linoleic acid (O/L ratio) present in their lipids is greater than 9, while those below that ratio are considered normal oleic (NO). In some publications, peanuts with O/L ratios below 9 are referred to as low oleic (LO), but in this study, they will be designated as NO. In this study, peanuts were separated into the different maturity classes before analysis (Rucker, Kvien, Vellidis, Hill, & Sharpe, 1994). Although this method has been used for several decades, it is still the only method the peanut industry has to evaluate maturity in raw peanuts at harvest.

Manufacturers of food products containing peanuts prefer to use HO as they have reduced consumer complaints regarding rancidity. This improvement in peanut oil stability has resulted in the high oleic trait being introduced into all the market types and these cultivars are now commercially available. However, there is no visible indicator as to the O/L ratio of a peanut seed. Mixed lots of normal- and high-oleic peanuts has proven to be a challenge in the peanut supply chain that has seen an increase in the past decade (Klevorn, Hendrix, Sanders, & Dean, 2016). Sources of such mixing have more often been attributed to physical mixing along the supply chain from seed supplier to grower to shelling plant to final processor or to outcrossing in the seed itself (Davis, Price, Dean, Sweigart, Cottonaro, & Sanders, 2016). This is of major concern to processors of peanut products where the flavor of an individual peanut is the major part of the consumer experience. A single rancid peanut can result in a negative association for the consumer and loss of repeat purchases. This study is the first to systematically examine the effect of peanut maturity on the O/L ratio of peanuts of the four commercial market types to evaluate the possibility that a mixture of normal- and high-oleic peanuts is a consequence of the presence of immature peanuts as opposed to human error in the bulk handling of the peanut lots or due to plant outcrossing. Adding the sorting criteria of maturity to improve the purity of HO lots of peanuts will provide an economic advantage is reducing rancidity in processed peanut products and the resulting economic disadvantages of poor consumer response to the products.

2. Materials and Methods

2.1 Sample Preparation

Intact peanut plants were received at the USDA, ARS Market Quality and Handling Research Unit in Raleigh, NC, USA. within 24 hours of field harvest from the cooperators. HO Runner (Cultivar 68-17), NO Runner (Cultivar Tifguard), HO Virginia (Cultivar Spain) and NO Virginia (Cultivar Bailey) plants were harvested by the authors themselves from the North Carolina State University Peanut Belt Research Station in Lewiston, NC, USA. Plants of the HO Spanish market type (Cultivar Olé) and NO Spanish market type (Cultivar Pronto) were shipped overnight in refrigerated containers from the USDA, ARS Wheat, Peanut and Other Field Crops Research Center in Stillwater, OK, USA. Similarly, intact plants of the HO Valencia market type (Cultivar Valencia 308) and NO Valencia market type (Cultivar Valencia 309 Tan) were shipped from the University of New Mexico Department of Plant and Environmental Services (Clovis, NM, USA). Six plants of each cultivar (HO and NO) were harvested. Upon receipt, every seed from each plant was removed from the plant, evaluated for maturity using the "hull scrape" or "pod blast" method (Rucker et al, 1994). After seed separation, the seeds from each plant were loaded into a basket fabricated in house using size 24 mesh metal hardware screen. The seeds were then pressure washed with water using a Greenworks Model 1600 pressure washer (Sunrise Global Marketing, Mooresville, NC, USA). The standard vibrating nozzle was set to 1600 psi. This action resulted in the removal of the exocarp layer of the peanut shells and allowed for the seeds to be sorted by their exterior pod mesocarp color which is the industry standard for peanut maturity determination (Williams & Drexler, 1981). Seeds of the same maturity were packed in Ziploc® plastic bags (S.C. Johnson Corp., Racine, WI, USA) for each plant. The seeds from each plant were stored separately. For analysis, each individual seed was assigned a unique sample number. Each seed was weighed and a representative sample for fatty acid determination was

taken using the hollow needle method described by Zeile and others (1993). If the seed was of insufficient size for sampling, the entire seed was crushed and extracted.

2.2 Fatty Acid Determination

Representative samples obtained as described above were directly methylated as previously described (Klevorn et al, 2016). All chemicals and reagents used were purchased from Thermo Fisher (Fairlawn, NJ, USA) unless otherwise specified. In brief, 20 to 40 mg of each peanut seed sample were heated in 1.0 mL of 0.5 N NaOH in MeOH for 10 min at 85°C in glass screw capped tubes using a water bath. After cooling, a 1.0 mL aliquot of 14 % BF_3 in MeOH solution (Sigma Chemical Corp., St. Louis, Mo., U.S.A.) was added and the tubes were sealed and reheated for 5 min. The tubes were cooled to room temperature and 1.0 mL of water followed by 1.0 mL of hexane was added. The tubes were vortexed for 15 sec to mix and extract the fatty acid methyl esters. The tubes were left to stand at room temperature until layers formed. The top layer (hexane) containing the fatty acid methyl esters was removed using a Pasteur pipet and passed through a bed of Na_2SO_4 (approximately 0.5 g) to remove any water present. The extract was then analyzed by GC.

The extracts were injected onto a Perkin Elmer Model Autosampler XL GC (Perkin Elmer, Shelton, CN, USA) fitted with a BPX-70 capillary column (30m x 0.25 mm i.d., 0.25 μ dry film) (SGE Analytical Science, Austin, TX, USA) and a flame ionization detector (FID). The temperature program was 60°C for 2 min, increased to 180°C at 10°C/min, then increased to 235°C at 4°C/min for a total run time of 27.7 min. The injector was heated to 220°C and the detector to 250°C. The carrier gas was helium at a flow rate of 1.85 ml/min with a split flow rate of 40 mL/min. Retention times were established using the Kel-Fim FAME-5 standard mixture (Matreya LLC, Pleasant Gap, PA, USA) and the GLC-21A standard mixture (Nu-Check Prep, Waterville, MN, USA). The fatty acid content of the seeds was calculated by normalization according to AOCS method Ce 1-62 (Firestone, 2003).

3. Results

3.1 Maturity Determination

Due to the indeterminate flowering character of the peanut plant, when peanuts are harvested, every peanut present on a single plant will not necessarily be mature. Although not readily apparent when examining the intact peanut pod, the maturation stage of peanuts can be determined by removal of the top layer of the mesocarp of the shell (Williams & Drexler, 1981). When dampened, the actual color of this underlying level of the shell can be seen. As the peanut matures, the color of this layer changes progressively from white (most immature) to yellow to orange to brown to black (most mature). The shell also become thicker and develops a ridged texture. Determination of pod maturity in this study was done through use of this hull-scrape method (Williams & Drexler, 1981). Investigation of the four commercially produced market types in the United States, runner, virginia, spanish, and valencia, demonstrated that a clear relationship exists between seed maturity and development of O/L ratio. Previously, this relationship was established by the discovery that the most immature seeds (those with a white mesocarp) had O/L ratios almost entirely below the HO threshold of an O/L ratio of 9 or greater (Klevorn et al, 2016). As the mesocarp colors darkened, the range of O/L ratios present were higher. Pods with white, yellow, and orange A mesocarp colors are regarded as immature. Those with orange B, brown, or black mesocarp colors are considered as mature. Additionally, the phenomenon of increased O/L ratio with increased maturity was observed within the NO control cultivars included in the earlier study.

3.2 Fatty Acid Development

Since the peanut is the seed of the plant, there are a series of compositional changes that occur with its maturation. These include the increase and then decline of the simple carbohydrates, the production of amino acids and proteins and the production of the lipids and the change in fatty acid composition that is the topic of this investigation (Pickett, 1950; Sanders, Lansden, Greene, Drexler & Williams, 1982; Schenk, 1961). In addition, the pod and subsequently the seed inside increase in size at different rates, with the pod reaching nearly full size before the seed inside begins to develop.

Each of the four peanut market types has a specific plant growth pattern which result in different pod shapes and sizes as well as the lengths of growing season. The market types are characterized by distinct characteristics that dictate how they are used as food or in food products (American Peanut Council, 2020). In this study, all the peanuts from each of six plants of each market type were analyzed to determine their stage of maturity based on mesocarp color and all were analyzed for their fatty acid content regardless of the physical size of the pod. The samples were taken at the time of commercial harvest, which was the point best considered optimum for that market type. Runner type peanuts compose the major portion of the USA peanut crop (85%) and are used for

peanut butter and for confections. In Figure 1a, the range of seed sizes for the Runner peanuts in relation to their O/L ratios is plotted. Each point represents a single seed. The colors of the plot points correspond to the maturity class of the pod that the respective seeds were taken from. There is a clear relationship between the size of the seed and the maturation as determined by pod color and in the development of the fatty acids. The most mature seed as designated by the darkest color points are clustered around 1 g in seed weight and above O/L values of 30. It was previously reported that as peanuts mature, the levels of palmitic and linoleic acid decrease and the level of oleic acid increase (Sanders et al, 1982). The genes responsible for this are two recessive alleles of **ahFAD2A** and **ahFAD2B**. Of these, **ahFAD2A** has been shown to occur at a high frequency in runner and virginia types (Barkely et al, 2010). There are functional mutations, G448A in **ahFAD2A** and 442insA in **ahFAD2B** that are responsible for the elevated levels of oleic acids in peanut lipids as they eliminate or knock down desaturase activity (Jung et al, 2000). The O/L ratio increases as the pod color darkens but the seed size may not necessarily increase. In ordinary language, a big peanut is not necessarily a mature peanut (Sanders, 2015).

3.3 Runner-type Peanuts

Peanuts of the normal oleic (NO) Runner cultivar, Tifguard, were analyzed as the control and showed an increase in O/L ratio with increasing maturity as with the HO cultivar, (Cultivar 68-17); however, the values never increased above 3 (Table 1). Runner and Virgina type peanut plants are higher yielding than Spanish and Valencia types. For this study, the NO Runner cultivar, Tifguard, over 250 pods were produced by the plants sampled. The HO Runner cultivar produced over 350 pods. For the HO cultivar of the runner type, although a clear relationship was established between maturity and development of the increased O/L ratios, most of the pods crossed the threshold of O/L greater than 9 regardless of maturity (Figure 1a). The entire range of pod colors was found to be present at harvest. To make the data manageable, 40 pods of each were randomly selected from each maturity class of these cultivars for analysis of fatty acids. The data are presented graphically in Figure 1a for comparison. A few scattered seeds of advanced maturity were seen to fall below the threshold value of 9 indicating that plants can naturally contain these mixed levels of O/L values.

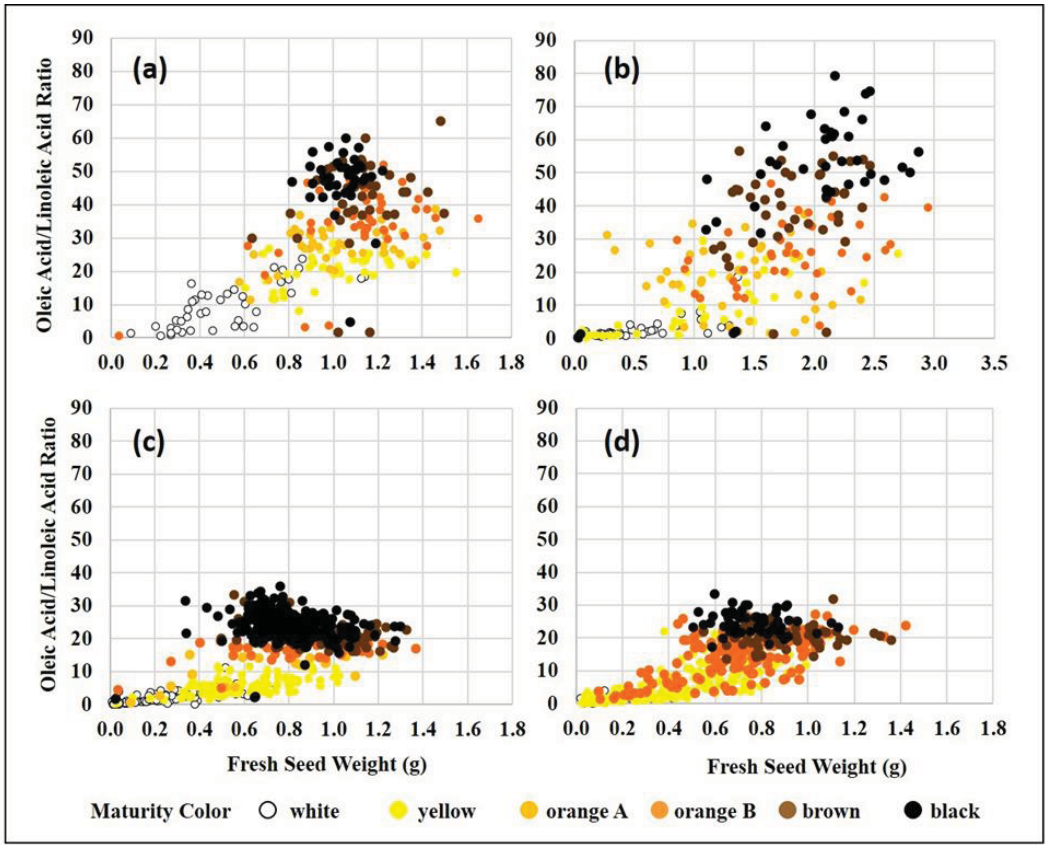

Figure 1. Variation in oleic acid-linoleic acid ratio with fresh seed weight by maturity color (a) Runner matket type-variety "68-17", (b) Virginia matket type-variety "Spain", (C) Spanish market type-variety "Ole", (d) Valencia market type-variety "Valencia 308"

Table 1. Seed weights and Oleic to Linoleic Acid ratios for the peanut market types for each maturity class

Variety	Market Type	OL Type	Maturity Color	Number of Pods	Number of Seeds	Mean Wet Seed Weight (SD) *In grams*	O/L Ratio (SD)
68-17	Runner	high	black	20	40	1.04(0.09)	47.18(8.94)
			brown	20	40	1.13(0.17)	42.39(12.23)
			orange B	20	40	1.11(0.28)	33.83(11.20)
			orange A	21	40	1.03(0.23)	27.66(5.66)
			yellow	21	40	1.01(0.23)	20.46(4.85)
			white	20	40	0.51(0.24)	9.32(6.75)
Tifguard	Runner	normal	black	20	40	1.07(0.18)	2.44(0.85)
			brown	20	40	1.16(0.23)	1.94(0.44)
			orange B	20	38	1.05(0.24)	2.08(0.49)
			orange A	21	40	0.91(0.29)	1.69(0.35)
			yellow	20	38	0.99(0.20)	1.42(0.36)
			white	21	40	0.59(0.23)	1.21(0.35)
Spain	Virginia	high	black	21	40	1.93(0.63)	49.03(19.40)
			brown	20	40	1.83(0.38)	40.96(9.41)
			orange B	24	40	1.76(0.57)	25.57(10.14)
			orange A	26	40	1.32(0.52)	17.77(9.48)
			yellow	22	40	1.02(0.64)	10.38(8.76)
			white	26	40	0.51(0.37)	2.48(3.15)
Bailey	Virginia	normal	black	22	40	1.36(0.19)	1.99(0.19)
			brown	21	40	1.28(0.17)	1.80(0.24)
			orange B	23	40	1.25(0.28)	1.46(0.21)
			orange A	23	40	1.11(0.28)	1.33(0.18)
			yellow	25	40	0.91(0.25)	1.19(0.18)
			white	22	40	0.58(0.27)	0.94(0.25)
Ole	Spanish	high	Black	134	240	0.81(0.16)	24.05(4.52)
			Brown	77	131	0.92(0.17)	21.90(3.09)
			orange B	21	38	0.81(0.28)	16.26(3.40)
			orange A	14	21	0.66(0.34)	11.26(5.10)
			yellow	61	108	0.60(0.21)	6.42(4.10)
			white	61	96	0.24(0.18)	2.11(1.91)
Pronto	Spanish	Normal	Black	9	17	0.70(0.12)	1.21(0.13)
			brown	88	146	0.78(0.17)	1.21(0.12)
			orange B	35	61	0.82(0.14)	1.10(0.08
			orange A	13	20	0.68(0.15)	1.04(0.08)
			yellow	81	115	0.47(0.20)	0.91(0.12)
			white	30	35	0.17(0.16)	0.64(0.34)
Valencia 308	Valencia	high	black	19	57	0.77(0.13)	24.60(3.35)
			brown	59	159	0.89(0.18)	20.78(3.10)
			orange B	32	92	0.77(0.24)	18.07(5.50)
			orange A	36	93	0.62(0.18)	12.47(5.88)
			yellow	76	178	0.45(0.26)	6.33(5.57)
			white	23	31	0.13(0.11)	1.36(1.12)
Valencia 309 TAN	Valencia	normal	black	1	3	0.59(0.05)	1.15(0.03)
			brown	17	39	0.74(0.15)	1.17(0.08)
			orange B	52	129	0.80(0.15	1.05(0.09)
			orange A	63	132	0.78(0.18)	0.99(0.08)
			yellow	82	140	0.49(0.27)	0.78(0.21)
			white	5	7	0.19(0.10)	0.64(0.21)

3.4 Virginia-type Peanuts

Virginia type peanuts are the largest cultivars in physical size and make up 10% of the USA peanut crop (American Peanut Council, 2020). This type is often sold as roasted in the shell peanuts ("Ballpark Peanuts") and are commonly salted. The Virginia type plants contained seeds that grew to larger sizes and the effect of immaturity is more evident (Figure 1b). The Virginia type peanut plants are also higher yielding than the Spanish or Valencia type. The NO cultivar, Bailey produced over 300 pods for the plants sampled. The HO cultivar, Spain, produced over 200 pods. As with the Runner type, a selection of 40 pods from each maturity class was analyzed and the data presented in Figure 1b. The O/L ratio increased as the pod color darkened but the seed size did not necessarily increase. As previously reported, seeds may already be to size before the optimum harvest time and not be expressing the high oleic trait but will be HO by harvest time (Klevorn et al, 2016). For this market type, very few of the very immature seeds, that is the white and yellow colors were above the threshold to be considered HO, compared to the runner type (Figure 1a). In addition, most of the smaller size seeds were not HO despite their maturity as determined by pod color. This indicates that for this market type, efficient sorting to remove small seeds is essential to prevent introduction of peanuts into finished products that will have a reduced shelf life due to onset of rancidity.

3.5 Spanish-type Peanuts

Spanish type peanuts are a minor part of the USA peanut crop (2%) (American Peanut Council, 2020). The main use of these peanuts is in candy or as roasted and salted. The Spanish type plants produced seeds that showed a close relationship between size and maturity (Figure 1c). As the Spanish plants were lower yielding, all the pods collected from the plants samples were analyzed. The peanuts of this market type tend to be relatively small with the majority being between 0.6 to 1.0 g in weight. Once the seed size increased about 0.5 g, few white pods were found. Mature (brown and black) pods appeared at that point. Yellow pods were found beyond the 0.5 g level but none above 1 g in seed weight. The largest number of the seeds were also mature from these plants at the harvest time compared to the other market types. One defining characteristic of Spanish type peanuts is their relatively short growing season (120 days after planting) compared to some runner types (150 days after planting) (Bell, Shorter & Mayer, 1991). Although the larger portion of the seeds analyzed of the Spanish type were mature at the harvest date, the O/L ratios did not reach the high values seen with the Runner and Virginia cultivars in this study. If this is a result of the shorter period of growth or genetic expression needs to be determined.

3.6 Valencia-type Peanuts

The fourth market type of peanuts is the Valencia type. Representing less than 1% of the total U.S.A. crop, most Valencia type peanuts are grown in the state of New Mexico (American Peanut Council, 2020). The primary application of these peanuts is for organic products such as peanut butter. The growing area is relatively free of plant diseases allowing for organic production (Chamberlin & Puppula, 2018). Other uses are as fresh roasted and boiled peanuts and as an ingredient in candy. The relationship between size and maturity (Figure 1d) is closer to that of the Spanish market type (Figure 1c). Like the Spanish type, all the pods collected from the sampled plants were analyzed and the data reported in the Figure 1d and Table 1. The smaller seed size of Valencia seemed to influence the number of immature seeds at harvest. There were very few white seeds and many of the seeds in the orange B category that were less than 0.5 g in size compared to the Runners (Figure 1a) and the Virginia types (Figure 1b). The very mature seeds (brown and black pods) of the Valencia type made up a smaller portion of the total than for the Spanish type. This would indicate that sorting by size would not be as effective as for the larger seeded types in removing immature kernels that have the potential for producing off flavors due to lipid oxidation. As with the Spanish type, the Valencia type did not have O/L ratios above 30 indicating that although the amount of oleic acid present in the seed was similar to that in the larger seed varieties, the linoleic acid levels were higher allowing for greater opportunities for lipid oxidation.

4. Discussion

Specifically, advanced pod maturity has been linked to increased levels of oleic acid and decreased levels of palmitic and linoleic acid (Sanders et al, 1982). A strong relationship was observed in this study between increased O/L ratios and more advanced maturity. Increased O/L ratio with increased maturity was present for all four market types yet the extent of this relationship appeared to differ among the market types. When modeling the development of O/L ratio using mesocarp color and market type as predictors, a significant interaction between mesocarp color and market type was observed. The presence of a significant interaction term confirmed the visually observed variability in the relationship between O/L ratio development with darker mesocarp colors between the four market types (Table 1). For the HO Runner market type, there was a small, but not significant increase in the O/L ratio from 1.21 (white pods) to 2.44 (black pods). For the HO Runner cultivar, even the most

immature, that is the white pods where found to be on average considered HO (9.32). This phenomenon was not observed in any of the other market types. A steady increase in the average O/L ratio through the maturity stages was reported in the HO runner cultivar with the maximum in the most mature (black) pods (47.18). Changes in O/L ratio with increased maturity were most evident in Virginia-type samples with an average O/L of 2.48 in white pods to an average of 49.03 in black pods (Table 1). Virginia-type peanuts achieved average HO O/L ratios higher than the other market types.

Spanish and Valencia-type HO peanuts followed a similar trend moving from average O/L ratios of 2.11 and 1.36 in white pods to 23.9 and 24.6 in black pods, respectively. These types both reached average O/L ratios above the defined level of 9 at the third level of maturity (Orange A) but they never reached the levels of the Runner or the Virginia types. For the NO Spanish type, the O/L ratio was found to double from 0.64 (white pods) to 1.21 (black pods). The Valencia type were very similar, that is 0.64 in the white pods to 1.15 in the black pods. That these groupings of runners and virginia types reached O/L ratios of levels twice that of the Spanish and Valencia types at maturity fits with the differences in their genetic heritages. Runner and Virginia types are of the subspecies hypogaea while Spanish and Valencia are of the subspecies fastigiata (Holbrook & Stalker, 2003).

The flavors associated with lipid oxidation have been reported to be the result of aldehydes and ketones derived from linoleic acid (Pattee, Singleton, & Johns, 1971; Wang, Adhikari, & Hung, 2017). These compounds result in flavors described as “cardboard” or “paint” in finished projects containing roasted peanuts. The reduction of linoleic acid in HO varieties is responsible for retarding or limiting the production of these objectionable flavors (O’Keefee et al, 1993). The size of peanut seeds has been associated with roasted peanut flavor and with certain distinct off flavors related to sugar levels in the seeds that change with seed maturation (Pattee, Pearson, Young & Giesbrecht, 1982). The final roast color is also influenced by peanut maturity (Sanders, Vercellotti, Crippen, & Civille, 1989). This would be a result of levels of free amino acids and sugars. These earlier studies were performed only on peanut varieties with normal oleic fatty acid profiles, as those were the only ones commercially available at that time. Although off flavors in roasted peanuts can be the result of peanut composition, curing practices, and/or storage conditions, knowledge of ways to control their onset is vital to the production of high-quality consumer products. When using peanuts of the high oleic varieties, producers should source the most mature seed possible regardless of the market type used.

5. Conclusion

The relationship between full maturity in the different market types and HO peanuts was established in this study. The data from this study shows that mixing can be present that is not necessarily the result of physical mixing or mishandling. While the size of the peanut pod is not an absolute determinate of the maturity or the fatty acid profile of the peanut seed inside, a rigorous sizing program will be advantageous to the final oil quality of peanuts especially with the larger seeded market types. This would help eliminate immature peanut seeds that are more easily susceptible to lipid oxidation resulting in unacceptable flavor experiences for consumers of products containing roasted peanuts. Careful attention to sorting peanuts for maturity as best as possible using sizing will add economic value to the peanut crop as well as ensure minimal exposure to oxidized lipids which could compromise the health of consumers.

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Disclaimer

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Developing a Software Tool to Estimate Food Transportation Carbon Emissions

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Abstract

Food transportation is an increasingly important consideration to total food sustainability in a rapidly globalizing world. To maintain the efficiency of regionalized production, food travels great distances to the consumer's plate. While this long-distance sourcing is often more sustainable from a production standpoint, the routes from origin to consumer are frequently unoptimized. To reduce emissions due to transportation, many have tried to limit the miles travelled by food items. However, the mode of travel is an equally important factor. Different modes produce vastly different emissions over equivalent distances. To effectively model these routes, a set of transportation emissions estimation tools has been created. This program uses an Excel interface to allow users to input key factors (like cargo mass, origin, and destination) and experiment with different modes and routes of travel to find the optimal transportation system for their application. This program may be used to analyze or improve the total life cycle analysis of a variety of products. In a case of the comparison of transportation modes, a salmon transportation route from the Faroe Islands (America's 2nd largest source of imported fresh salmon) to Richmond, VA, USA, resulted in a roughly 98% reduction of emissions when shipped via sea rather than flown. In a case of transportation optimization, the reciprocal trade of beef between Costa Rica and the United States was found to result in at least 158,000 kg of CO_{2eq} annually. These cases (and others) show the great need for better route optimization in food transportation systems.

Keywords: food, food miles, greenhouse gases, global warming potential, trucking, shipping, transportation

1. Introduction

Urbanization and consumer demand for non-local products has made transportation an increasingly vital section of the food economy. While these imports and exports have resulted in regional specialization and increased the dietary options of consumers, the globalization of the food economy has greatly changed the environmental impact of food systems. The current greenhouse gas emission trajectory is estimated to reach over 80 gigatons of CO_{2eq} by 2050 (Mooney, 2015). Comparatively, the widely accepted climate change budget issued by the UN Environment Program would limit carbon emissions to 54 gigatons of CO_{2eq} by 2030 (Mooney, 2015). However, even the 54 gigaton limit will result in climbing world temperatures. To reduce these effects, emissions must be limited from every source, including transportation. According to the EPA, the transportation sector produced 14% of global carbon emissions in 2010 (EPA, 2018). Out of this problem, a new buzz phrase arose – "food miles". This term refers to the distance a food item travels from production to consumer, often with a focus on sustainability. Although local sourcing has managed to cut some of the transportation emissions, the trend is mostly practiced at convenience. Many types of food are expensive and difficult to grow and maintain in an unsuitable climate. When transportation is essential for the products that an area relies upon – but does not produce – the environmental impact of lengthy travel routes is not always considered or strategized. To remedy this, travel emissions can be modeled to find the best feasible route with the lowest emissions.

While the focus of transportation optimization is often focused on distance, there are many other factors that affect the sustainability of transportation systems. A truck, train, plane, and ship used to transport the same cargo over the same distance would result in vastly different emissions. Of these, air travel is the most environmentally burdensome mode of travel. In the food sector particularly, much of the food that is flown is due to consumer demands for fresh products. Truck travel is the next most impactful option for transportation modes. This, however, is a very popular mode of transportation due to the low cost and lack of extra required infrastructure.

In 2015, shipment by HDV (heavy-duty vehicle) accounted for about 66% of total USA freight shipment by weight. Rail shipments accounted for about 9.1% of this shipped weight. By 2045, truck shipment is expected to grow in share of freight transportation by about 2% (while rail is expected to shrink by about 1.5%). Water shipment is expected to drop from 3.4% to 2.9% of total domestic shipments, while air freight change was negligible. This trend indicates a general increase in emissions, even if the total food shipped remains the same. This brings an important topic to the table – if shipping routes were altered to allow for more sustainable modes of transportation, could the environmental effects of shipping more food in the future be mitigated or reduced?

While there are many other factors that contribute to food sustainability, food sourcing results in an estimate of 9% of the life cycle emissions for food products (EPA, 2018). Depending on the route taken, this proportion can vary from <1% in local produce to about 90% in fresh-flown seafood (Farmery et al., 2015). This variety is what makes the analysis of routes vital to the environmental performance of transportation systems. Changing the origin of the food or the mode of transportation to find a more optimal pathway can play a critical role in reducing total product emissions.

In response to the environmental issues associated with the food industry, the three tools presented here are intended to model transportation routes. These tools present the opportunity for the user to use various input formats in an Excel workbook interface and then receive a CO_{2eq} estimate for a given route. The greenhouse gas emission estimate was calculated for each chosen path of travel based on the mode of travel, mass of cargo, and distance. These modeled routes can be used to examine the sustainability of food sourcing and transportation. Through better analysis and modeling of these systems, engineers and consumers alike can make more environmentally conscious decisions in transportation systems, resulting in an altogether more sustainable society.

2. Methodology

2.1 Data

The modeling of the transportation emissions requires emissions factors that are specific to common vehicle groupings. The software tools use estimates calculated from the Network for Transport Measures (NTM) to rate the emissions for each mode of transportation. To ensure simplicity for the user, the data and figures used were estimates for the most typical travel scenarios. The graph in figure 1 shows the emission ratings (per tonne-kilometer) for each mode of travel ("NTMCalc 4.0," n.d.). The chart (fig. 2) was used for the mode decision logic displayed on the initial page of the program.

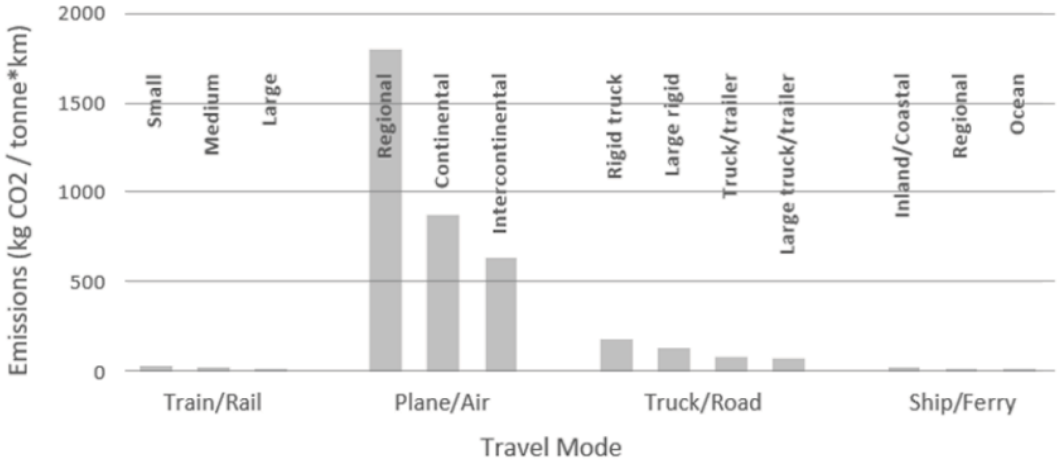

Figure 1. Emission factors for a variety of transportation modes

Emissions			Usage range
Small cargo train	35.28	($g CO_{2e}$)/(tonne*km)	Cargo mass < 90 t
Medium cargo train	22.95	($g CO_{2e}$)/(tonne*km)	90 t ≤ Cargo Mass < 110 t
Large cargo train	17.85	($g CO_{2e}$)/(tonne*km)	110 t ≤ Cargo mass < 120 t
Regional freight aircraft	1798	($g CO_{2e}$)/(tonne*km)	Distance travelled ≤ 2300 km
Continental freight aircraft	869.3	($g CO_{2e}$)/(tonne*km)	2300 km < Distance travelled ≤ 6600 km
Intercontinental freight aircraft	632.4	($g CO_{2e}$)/(tonne*km)	6600 km ≤ Distance travelled
Rigid truck	176.8	($g CO_{2e}$)/(tonne*km)	Cargo mass < 7.5 t
Large rigid ruck	129.5	($g CO_{2e}$)/(tonne*km)	7.5 t ≤ Cargo Mass < 20 t
Truck and trailer	82.52	($g CO_{2e}$)/(tonne*km)	20 t ≤ Cargo Mass < 34 t
Large truck and trailer	71.58	($g CO_{2e}$)/(tonne*km)	34 t ≤ Cargo Mass < 60 t
Inland/Coastal cargo ship	21.06	($g CO_{2e}$)/(tonne*km)	Distance travelled ≤ 2000 km
Regional cargo ship	15.732	($g CO_{2e}$)/(tonne*km)	2000 km < Distance travelled ≤ 4000 km
Ocean cargo ship	12.41	($g CO_{2e}$)/(tonne*km)	4000 km ≤ Distance travelled

Figure 2. Emission factors for transportation mode types (as used in the software tools). These are approximate estimates and the number of figures used for the mode ratings does not indicate high certainty. These data were used for calculations to give general carbon emission estimates

The data in figures 1 and 2 were compiled by the NTM from different suitable databases for each mode. The "road" transportation data was collected from the 2010 update of the Handbook of Emissions Factors for Road Transportation (or HBEFA 3.1). This handbook uses the Passenger Car and Heavy-Duty Emission Model, which uses engine mapping to dynamically monitor the emissions exiting the vehicle and compares these data to the actual power of the engine (Hausberger et al., 2009). By collecting data from a multitude of common on-road vehicles of the same hauling capacity, the HBEFA developed a set of values used to estimate the emissions based on vehicle load and distance.

"Rail"-type emission ratings were collected using similar methodology. The power required to propel a train carrying various cargo weights in common environments was compared to the emissions discharge mapping of the train to gather a multitude of data points. The data was then compiled to form a "typical" emissions rating (using average load percentages and average topographical condition factors) for the three train categories ("NTMCalc 4.0," n.d.). For the purpose of this program (which is expected to be used for loads that are less than 120 tons), it is most logical to base the type of train used – and the resultant emissions factor – on the mass loaded into an individual rail car. While the NTM was responsible for the organization of the data, the International Union of Railways (or UIC) collected the data ("NTMCalc 4.0," n.d.).

Data for waterway modes was collected in a less direct method. Since the resistance acting on the vessel has far more variation (from the effects of both load weight and buoyancy), the power simulation techniques used in the ground transportation methods was not as applicable to the calculation of water transportation coefficients. Instead, the International Maritime Organization (IMO) released statistics relating the required energy consumption of the ship to its type and deadweight ("NTMCalc 4.0," n.d.). The related emissions were then calculated from the fuel consumption required to provide that power. For the final rating, a coefficient of resistance was applied to reflect the effects of water drag on the ship (Cooper & Gustafsson, 2004).

The air data collection methods were much more variant than those of the other travel modes. For each general "type" of aircraft, the fuel consumption for a typical load per unit distance was calculated. The fuel consumption (with fuel type assumed to be Jet A-1) was related to a fuel efficiency factor that determines the carbon emissions of this type, much like the ship method ("NTMCalc 4.0," n.d.). The typical load mass used to find the total fuel usage was then taken as two parts – passenger load mass and cargo load mass. Since few planes are solely freight planes, the NTM assumes that the aircrafts used for transportation also carry humans. This dual functionality, the NTM estimates, reduces the total carbon output per unit of flighted cargo mass by 30% ("NTMCalc 4.0," n.d.). The aircrafts in the program were grouped by travel distance due to both typical aircraft size and flight pattern at these distances. These figures were collected from the International Civil Aviation Organization and compiled by the NTM. The carbon output per unit cargo mass is, like in all the other modes

discussed here, usually reduced by greater total vehicle size. Unlike the other modes, air travel emissions are very dependent on range, as the take-off and landing flight pattern result in added emissions for the total flight. To account for this, a variable emissions factor (related to both distance and unit of cargo mass) and a stagnant emissions factor (variable by only cargo mass for take-off and landing patterns) were combined to form a total emissions factor for each major plane range ("NTMCalc 4.0," n.d.).

It must be noted that the emissions factors used in this study are approximate estimates garnered from previous studies, and the number of significant figures used for the mode ratings does not indicate a level of certainty. These data are comprised of averages used only in calculations to give general carbon emission estimates. The emissions output values in the program are rounded to reflect the range of error found amongst these factor estimates and the estimates of other references, but still do not fully reflect possible error from actual transportation routes and variation in vehicle type, maintenance, or efficiency.

2.2 Program Logic

The modeling program was an Excel Macro-Enabled Workbook (*.xlsm) containing several macros and developer controls. The software tools run on a series of Visual Basic (VBA) codes. Foundationally, the codes were used to compile three pieces of information – the distance travelled, the mode of travel, and the cargo mass.

The following equation was then used to determine each route's resulting carbon dioxide emissions:

$$Em = (dist_1 * mrat_1 * mass_1) + (dist_2 * m_{rat2} * mass_2) + \ldots (dist_n * m_{ratn} * mass_n) \quad (1)$$

Where:

- Em = emissions (g CO_{2eq})
- dist = distance (km)
- m_{rat} = mode emissions rating ((g CO_{2eq}) / (tonne*km)
- mass = cargo mass (tonnes)
- 1, 2, …, n = these subscripts denote each sequential route segment

The generic setup of the program includes a single module with master equations that were utilized in each tab as well as a tab-specific code for each tool. The master equations were used to call in information from online resources, and contain equations that were used in multiple tools. This results in a system of four unique sets of code (one for each tool) and one code used to integrate the information into simple output.

Several of the master equations were needed for locational data. This was usually the first data point calculated in the tools as both distance and mass values were required to select ranges for vehicle types. As the mass and mode of transportation were provided by the user, the distance calculation was the first step in this process. The coordinates of a string address were found using data called in from a Google API (Application Programming Interface). With the input of an address, the program can relay the string of words via an internet connection to the Google Maps API (fig. 3). A set of coordinates for the address was then returned.

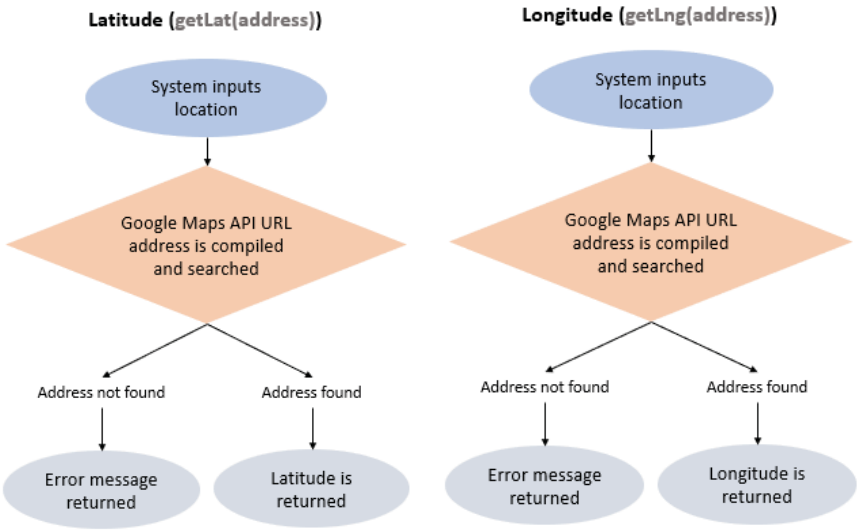

Figure 3. Logic flow of longitude/latitude acquisition

These values were then used in an equation that helps calculate the distance between two points on the globe. For this function, the latitude and longitude of both locations were inputted and run through a Haversine formula, which calculates the "as the crow flies" distance between the points (fig. 4).

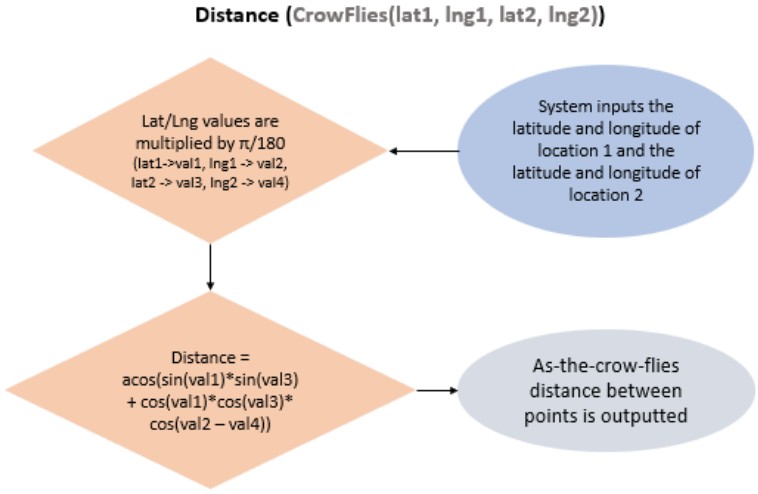

Figure 4. Logic flow of distance calculation

After the distance between points was determined, the three input values – the distance, cargo mass, and mode of transportation – were stored and the most likely mode of transportation was selected based on the usage ranges (the distance travelled or cargo mass) that were entered/selected (fig. 5). For the Plane/Air and Ship/Ferry modes, a distance range determines the rating. For example, if the cargo is traveling 4000 km directly by air, the most likely mode of travel would be by continental freight aircraft. For the Train/Rail and Truck/Road options, the usage range was based on the cargo mass. Similarly, if the user entered a cargo mass of 25 tonnes for a truck route, the most likely mode form would be a truck and trailer.

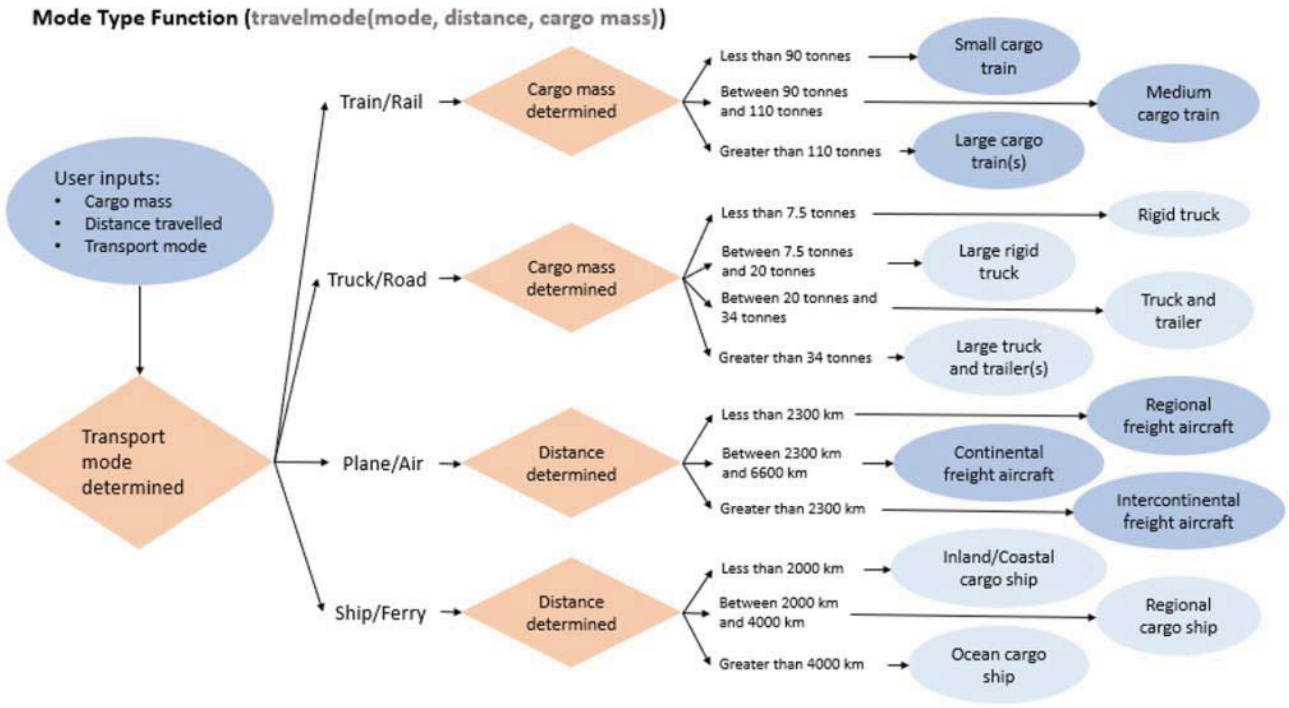

Figure 5. Logic flow of mode type selection

After the type of vehicle used was determined, a function assigns an emissions rating to the mode of travel and uses this emissions rating to calculate total emissions. This emissions value (in kg CO_{2eq}) was calculated through the multiplication of the mode emissions rating, mass of the cargo, and distance travelled (fig. 6).

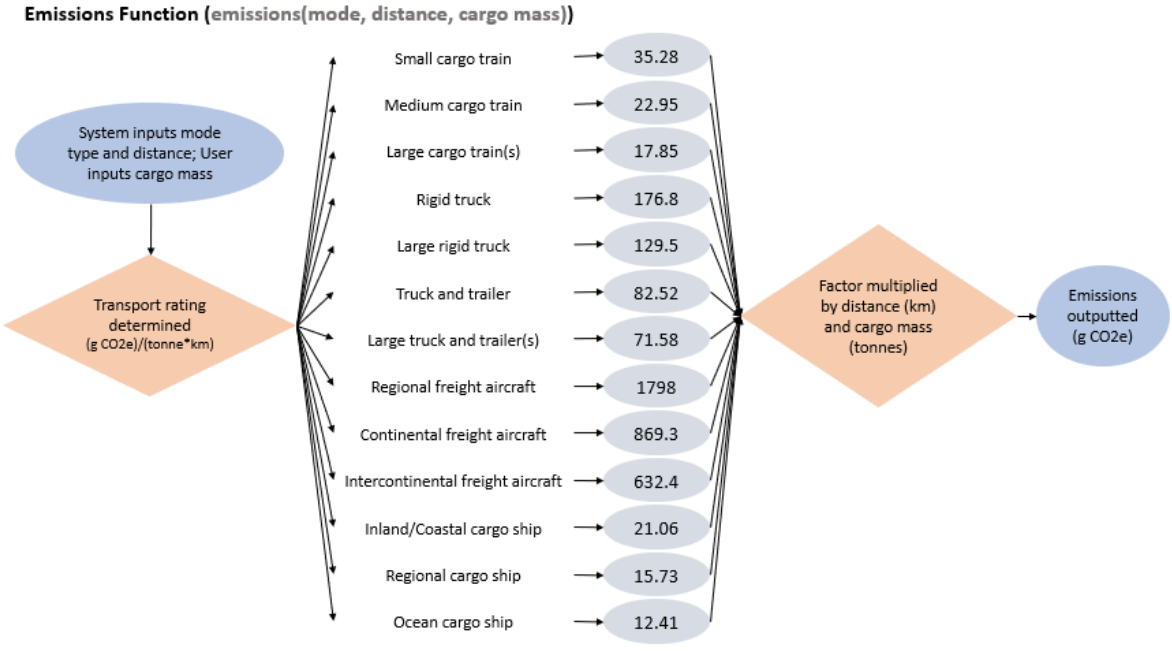

Figure 6. Logic flow of emissions factor determination

The API was also used for error checking in the software tools to prevent the calculation of distance for truck or rail travel over major bodies of water. For this function, Google Maps treats the two input addresses as a request

for road-travel directions between the two points. If road travel was not possible, a geographical barrier (like an ocean) was expected. Figure 7 shows this process. Google's directions, however, will not provide routes across some national borders (such as that between China and any neighboring country). In this case, the error checking has been overridden with keyed-in edits. For those that receive unwanted or unwarranted error reports, the function was optional. Each tool has a regular function button as well as an override button to opt out of such features.

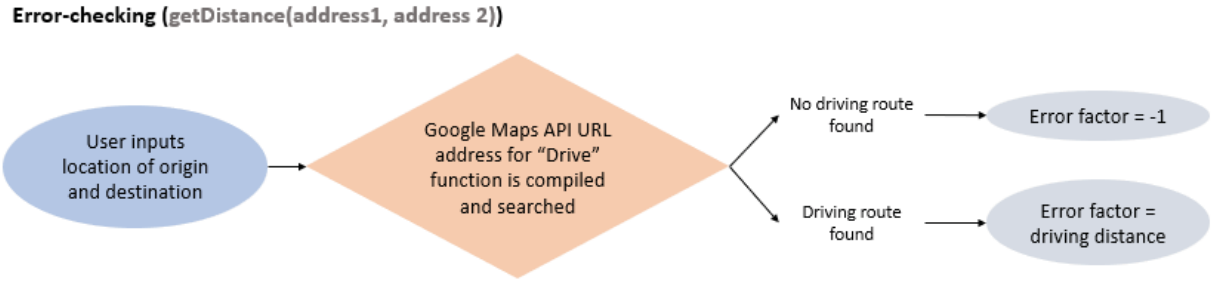

Figure 7. Logic flow of error-checking

The tab-specific codes help integrate the master formulas into different scenarios and formats. This allows for the software tools to have a resettable structure to clear any edits or entered information. This also enables the mapping of entered locations on the 2nd and 3rd tools. Most of the generic calculations were used in the tab-specific sections to ensure that the cell placement of the inputs and results were accurate.

The third tool also contains unique code to assist in finding potential ports. This function "suggests" ports from the most commonly used ports in the world (the top 100 airports and top 150 ship ports). The porting function integrates the locational codes from the module and some tab-specific port information to find ports that were accessible by road or rail, determined by the error tests provided by the distance Google API (fig. 8). By limiting ports to those that were drivable, the tool more reliably models a likely overseas transportation sequence of ground-overseas-ground travel.

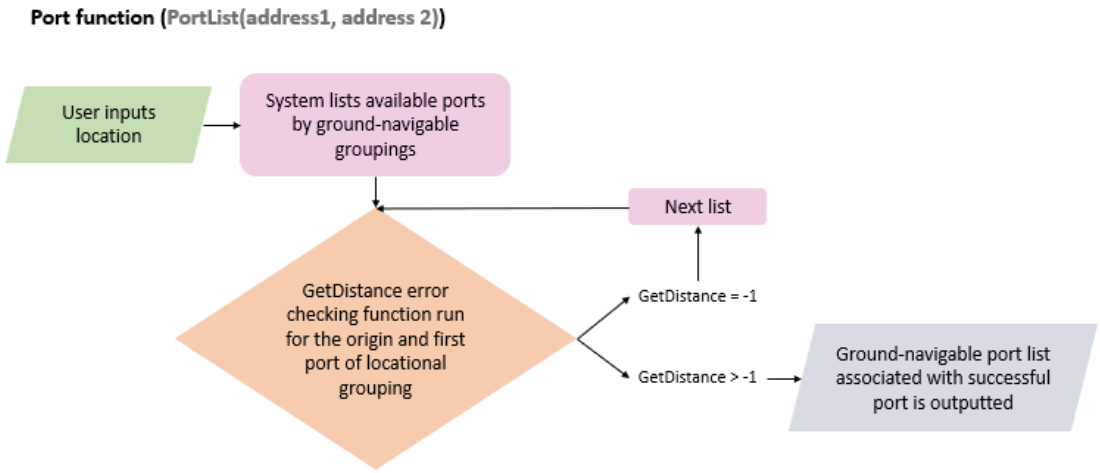

Figure 8. Logic flow of port list determination

The port-finding capabilities of the third software tool also allow for another unique feature – an optimization function. This function helps further suggest low emissions transportation routes given an origin and destination (fig. 9). Due to the great difference between road and rail emissions factors, the ground transportation sections were assumed to be via rail for lowest emissions estimation. While this assumption was not made in the overseas portion, it was extremely likely that this portion of the transportation will be via ship due to the same reasoning.

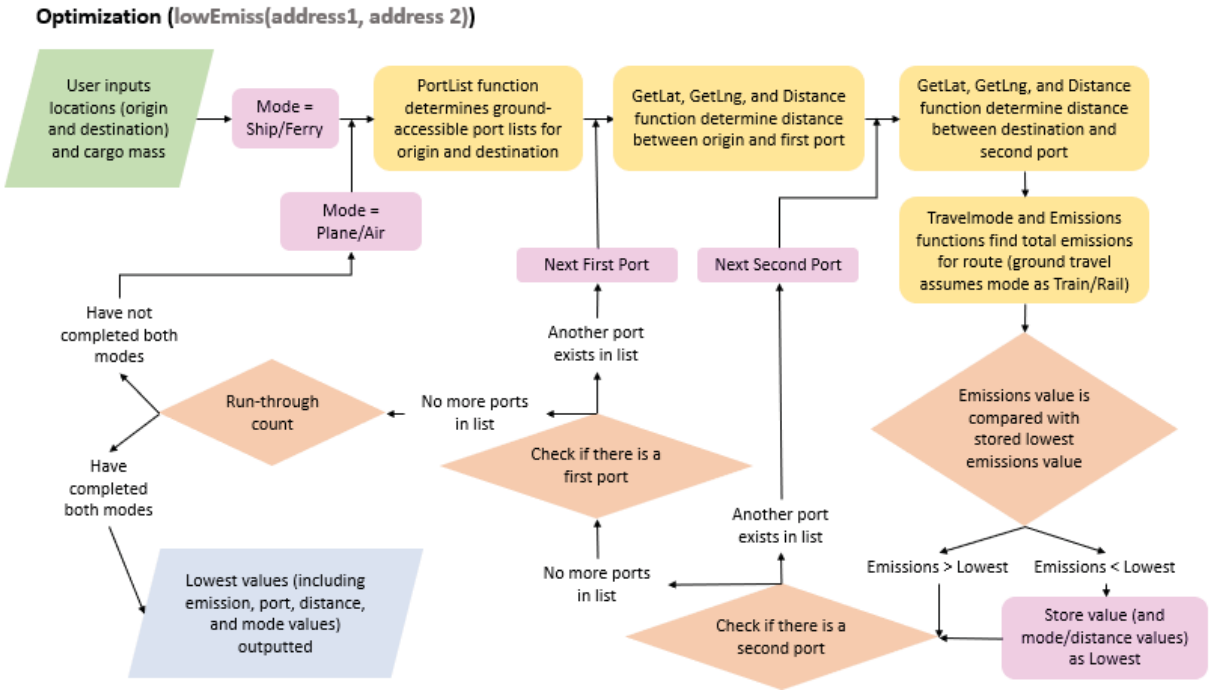

Figure 9. Optimization logic flow

3. Software Overview

3.1 Export Estimations

The export estimations tab produces a visualization of the production of food groups by region. The user may select a group from the drop-down list to display dots scaled by the mass of regional exports and the numerical values of the exports. The information may provide insight on likely origins of certain products. The layout of this page is shown in figure 10.

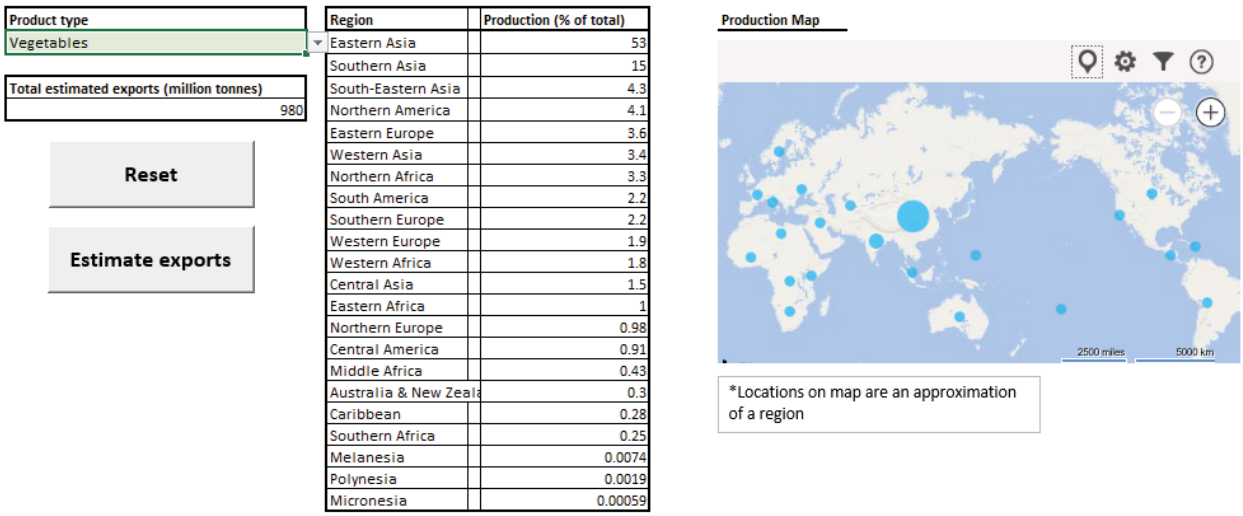

Figure 10. Software layout for export estimations

3.2 Tool 1

Tool 1 is noted for its simplicity. The user may enter the total distance travelled, the proportion travelled through each mode, and the mass of the load and the tool will return CO_{2eq} estimates (fig. 11). This tool does not allow

the user to split the trip into several segments. It also does not allow address inputs or use any distance calculation functions, but still utilizes the "most likely mode of transportation" function for each segment of travel.

Entered information		
Total distance travelled	3000	km
Cargo mass	50	tonnes
Percent by plane	25	%
Percent by train	25	%
Percent by ship	25	%
Percent by truck	25	%
Total distance travelled	100	%

Modes	Probable type of mode	Distance travelled				Carbon emissions (kg CO2e)	
Plane/Air	Regional freight aircra	750	km			67000	kg CO2e
Train/Rail	Small cargo train	750	km			1300	kg CO2e
Ship/Ferry	Inland/Coastal cargo s	750	km			790	kg CO2e
Truck/Road	Large truck(s) and trai	750	km			2700	kg CO2e
					Total	72000	kg CO2e

Reset

Solve

Figure 11. Software Tool 1 layout

3.3 Tool 2

Tool 2 is the most versatile portion of the program. This section allows inputs of checkpoint addresses, masses of cargo, and modes of transportation. The distance travelled and carbon emissions were displayed for each section of travel and summed for a total CO_{2eq} estimate. A map was also provided to visualize the route taken. This layout is shown in figure 12. All distances were measured "as the crow flies," so the map allows the user to see any geographical issues in the route taken. As in the 1st tool, the modes used were the result of the "most likely mode" function.

Reset Solve Solve (Override) Map

Entered information

Calculated information

	Address/Location	Mass of Cargo (tonnes)	Mode of Transportation	Most likely transportation form	Distance travelled (km)	Carbon emissions (kg CO2e)
Origin	New York, NY					
Point 2	Beijing, China	75	Plane/Air	Intercontinental freight aircraft	11000	520000
Point 3	Moscow, Russia	50	Train/Rail	Small cargo train	5800	10000
Point 4	Edinburgh, Germany	50	Truck/Road	Large truck(s) and trailer(s)	2510	9000
Point 5						
Point 6						
Point 7						
Point 8						
Point 9						
Point 10						
				TOTAL	19300	540000

Location map

Figure 12. Software Tool 2 layout

3.4 Tool 3

The most restrictive and structured section of the software is Tool 3. The formatting was set up to reflect a common pattern of travel for many goods – a route of ground travel from the origin to a major port, where the product is flown or shipped to another major port, from which the product travels by ground to the destination. This tool prompts the user to enter an origin and destination. The modes of travel were then selected, after which the port locations may be selected from a dropdown list. Figure 13 shows this layout. This requires the use of a function that selects two ports accessible by land, one from the origin and one from the destination. The tool then calculates an estimate of emissions for the route. Like Tool 2, the "most likely mode" and mapping functions were also used. Tool 3 was uniquely useful due to its optimization function. The user may opt to only enter in the cargo mass, origin, and destination. This tool can then suggest the lowest-emission route based on the information given.

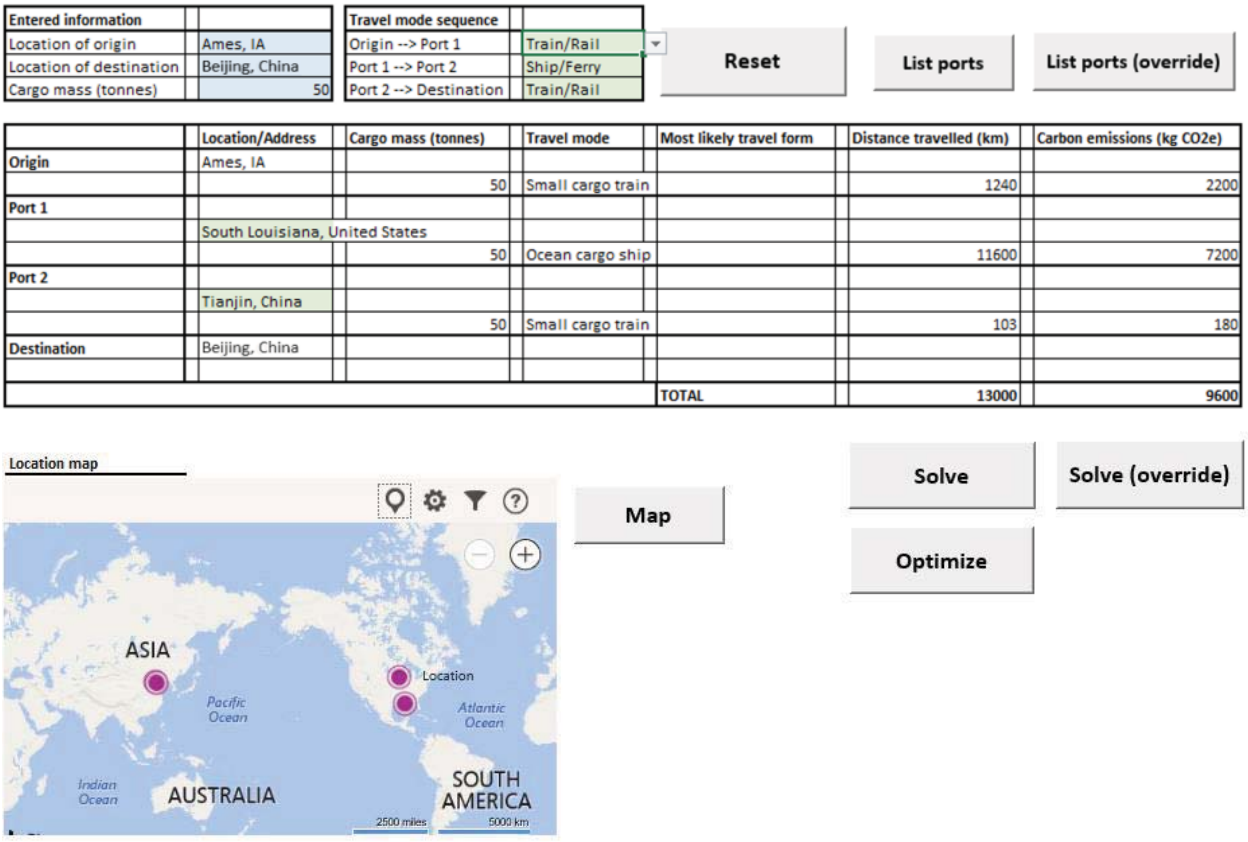

Figure 13. Software Tool 3 layout

4. Case Studies Illustrating Software Use

4.1 Case 1: Importing Goods – Effects of Varying Origins

- The term "food miles" was founded on the premise that differing origins of food items drastically change the emissions related to transportation.
- This is true to an extent -- while other factors also influence the true lifecycle carbon emissions of a product, the distance traveled by an item does impact the transportation emissions.
- The distance traveled by food is largely dependent on global supply chain trends. Regional specialization has created popular hubs for certain groups of items.
- For example, about 70% of the fresh apples consumed in Iowa were from out-of-state locations during peak Iowa harvesting season.

- The top two apple-producing states in the USA were Washington and New York, respectively, with Washington being the top apple contributor to Iowan grocery stores.
- Case study: What effect does a change in origin of a product (such as Iowan apples) have on the resulting transportation emissions?

4.2 Case 2: Effects of Modes of Travel and Relative Emissions

- While origin/destination relationships are important to the overall sustainability of product supply chains, the distance between two points is not an end-all factor.
- The effects of differing modes of transportation is often overlooked and/or underestimated. The emissions that result from equivalent routes (in terms of distance and cargo load) with differing methods of transportation can be enormously different.
- One example of this is distant overseas travel. A long-distance cargo plane results in about 50 times the CO_{2eq} emissions of a long-distance ship over the same distance with an equal cargo load.
- Fish is a product that exemplifies this idea well. The production of fresh and frozen fish is extremely similar, but distribution modes must vary to avoid the spoilage of fresh product.
- Case study: What differences can be observed in the modeling of a single route with differing vehicle types?

4.3 Case 3: Brazil to EU Beef Export Model Comparison (Direct Study Comparison)

- A wide variety of modeling methods and calculations can be used to estimate vehicle emissions.
- As there are too many factors to true vehicle efficiency to determine a single accepted estimation procedure, “true” emissions values are difficult to confirm.
- There are two primary practices for these cases. The first uses a vehicle emissions factor per unit distance and mass, while the second compares load capacity and fuel consumption with a fuel efficiency factor.
- This study utilizes the second approach to examine both the fiscal and environmental optimizations of the beef trade path between southern Brazil and northwestern Europe (Soysal et al., 2013).
- Case study: How similar are the emissions estimations calculated through different modeling methods?

4.4 Case 4: Efficiency of International Trade Trends – USA Beef Exchange

- The USA is the largest importer, largest producer, and second largest exporter of beef products.
- One of the side effects of this standing in beef trade is reciprocal trade, or trade that is back/forth between the same two nations.
- Reciprocal trade is common and often quite harmless between bordering countries – often the trades are just based on the dynamics of varying locations of production hotspots near the border.
- A less common, but much more impactful, kind of reciprocal trade is between distant countries.
- Of these reciprocal trade patterns, the Costa Rican beef exchange was especially notable. Costa Rica imported 27 million pounds of beef from the USA in 2016, but also exported 5 million pounds to the USA in 2016 (USDA ERS, “Cattle,” 2018).
- Case study: What are the environmental effects of reciprocal trade in terms of transportation emissions?

5. Results

5.1 Case 1

In this case, the effects of differing origins (namely Washington, New York, and Iowa, USA) for Iowan apple consumption was modeled using Tool 2. Although Iowa is a key part of historical apple production, the origin of

fresh apples at Iowan marketplaces is about 70% out-of-state during peak harvest times, and 85% out-of-state in the Iowa apple off-season (Pirog, 1999).

In fall harvest time, one of the top contributors of fresh apples to Iowa is the state of Washington, the top apple-producing state in the USA (Pirog, 1999). While the relationship between a top producer and top contributor may seem obvious, there are some exceptions in Iowan apple sources. New York, a closer state to Iowa, is comparable in growing conditions and is the second largest contributor to national apple production but is not as commonly seen on Iowan grocery shelves (Cornell University, 2016).

Washington is a northwestern state, so the export of apples to midwestern states is environmentally costly. A shift in Iowa apple sourcing from Washington growers to those of slightly closer states could have a quite substantial impact on systematic transportation emissions. While these states are quite distant, both have excellent apple-growing climates. As both are northern states, they have similar growing seasons and production methods, minimizing extraneous factors in comparisons.

The modeled situation is that of a 10% Iowan market sourcing adjustment from Washington apples to New York apples. Assuming the grocery store stocking mass is proportional to the average apple consumption per capita in the USA (15.3 pounds of fresh apples per year) in the city of Ames with approximately 66,000 people, about 101 thousand pounds (or about 46 tonnes) of Washington apples would be replaced by New York apples in Ames grocery stores over the course of a year (USDA ESMIS, 2012).

The modeling route used for the Washington-Iowa emissions estimation is shown in figure 14. The first portion of the route was travel via semi-truck from Yacoma, WA (the county seat of the top apple-producing county in the state) to Pasco, WA (the location of a major railway station) (USDA ESMIS 2012). Next, a typical rail route was estimated by adding “checkpoints” in Boise, ID and Cheyenne, WY, USA. The mode of travel then switches back to semi-truck from Omaha, NE, USA (another major railway stop) to Ames, IA.

Figure 14. Modele route from Washington to Iowa

The modeling route for New York-Iowa emissions estimation is shown in figure 15. This route was very similar in road/rail proportions to maintain consistency in distance modeling. The origin of the apples was estimated as Rochester, NY, USA. This is also the county seat of a top apple-producing county (Cornell University, 2016). The cargo was then carried via semitruck to Buffalo, NY, USA, where it was loaded onto rail-travel. A “checkpoint” in Cleveland, OH, USA was used to better estimate the path taken by the area railway. The cargo was then switched back to semi travel in Cedar Rapids, IA, USA, after which it was carried to the destination, Ames, IA.

Figure 15. Modeled route from New York to Iowa

Using Tool 2, the carbon emissions of both routes were modeled. The maps were shown to visualize the route and check for inconsistencies with common travel methods. In both modeling scenarios, the cargo load was estimated as 460 tonnes and adjusted to 46 tonnes post-modeling. This was done to better predict the type of mode used in each travel “leg,” as it is likely that the shipments would be made with a large mass of other

products (which would change the mass range used to choose the vehicle emissions rating). It should also be noted that the total distance travelled in the New York-Iowa route was about 1400 km, while the Washington-Iowa route was about 2400 km. The resulting adjusted emissions for the Washington and New York scenarios were 2400 and 1500 kg CO_{2eq}/year respectively, showing that the distance of travel really is an impactful factor in similar scenarios.

5.2 Case 2

While distance is the most commonly cited source of emissions disparities, the method by which the goods are transported is potentially more important for route optimization. In the case of American fish consumption, this is a very applicable and easily modeled problem. Overseas fresh fish imports are extremely common and require very inefficient travel routes due to high spoilage risk. While frozen, canned, and otherwise processed fish products may be transported via ocean travel, it is necessary to transport fresh varieties by air, resulting in far greater vehicle emissions factors.

Americans ate about 2.3 pounds of salmon per capita in 2014 (749 million pounds total) (Kantor, 2016). About 80% of this salmon was imported, with nearly 40% of salmon imports being fresh (USDA ERS, "Aquaculture," 2018). About 8.4% of American fresh salmon imports come from the Faroe Islands, the 2nd largest source of fresh imported salmon. Chosen for ease of modeling, the city of Richmond, VA has a population of about 220,000. Assuming average annual salmon consumption and import proportion in this area, about 13,600 pounds (or 6.2 tonnes) of fresh salmon is imported to the city from the Faroe Islands annually (USDA ERS, "Aquaculture," 2018).

Using Tool 2, this was a simple route to model (fig. 16). The intention of this case study was to demonstrate the effect of differing vehicles on total transportation emissions. For the sake of minimizing extraneous factors, two very similar routes have been selected – a "fresh salmon" route and a "frozen salmon" route. The Port of Virginia in Norfolk, VA is one of the top 5 North American ship ports and a common checkpoint for international imports (McCabe, 2017). Similarly, the Richmond, VA airport is the largest airport in the state and handles about 140 million pounds of cargo annually (Richmond International Airport, n.d.).

Figure 16. Potential salmon shipping routes through a combination of ship and road travel (shown in (a) and (b), respectively) and through strictly plane travel (c)

These locations lead to two natural freight routes between the origin (the Faroe Islands) and the destination (Richmond, VA). The likely ship cargo route, used for frozen, canned, or processed salmon, would result in ship

freight from the Faroe Islands to Norfolk, where a truck would likely continue the cargo route to Richmond. In the case of air travel, commonly used for fresh salmon, a plane would carry the salmon from the Faroe Islands to Richmond, where it could be distributed.

When these routes, along with a cargo mass of 6.2 tonnes, were inputted into Tool 2, extremely different emissions values were obtained. In the case of fresh fish transport, the most direct route results in a flight of about 5400 km from the Vágar airport of the Faroe Islands to the Richmond airport. This flight results in about 29,000 kg CO_{2eq}. The frozen fish transportation was a 5500 km route which results in about 560 kg CO_{2eq}. The factor of a continental aircraft (the most likely type for this air distance) was 70 times greater than the factor for an ocean watercraft at this distance, leading to this disparity.

5.3 Case 3

Differences in reporting and calculations often create a gap in information that is prone to biased exploitation. In order to find a consensus on issues such as the environmental impacts of a product line, it is of great importance to cross-reference and compare the data and methodology of a variety of research projects. A study published by the Operations Research and Logistics Group of Wageningen University examined 18 studies modeling different kinds of transportation emissions to find the distinctions that separate such projects (Soysal et al., 2013). In doing so, they designed a singular-situation modeling program to compare the fiscal and environmental impacts of supply chain changes in Brazilian beef trade.

While the financial and infrastructural research included in that project is compelling and impactful to the overarching emissions problem, the interest of this current study was focused on the comparison of emissions modeling tools. In the research of the Wageningen group, the window of focus was much narrower, bringing higher precision in likely vehicle choices and exact transportation routes. This led to the use of singular averaged fuel efficiency factors (in liters per km), estimated for typical road/ocean conditions, truck/ship sizes, and fuel types. This factor was scaled with the load capacity of the truck or ship. Then, the product of the factor, the distance travelled, and a fuel-emissions conversion factor was used to estimate the quantity of CO_{2eq} expelled during travel on a per-vehicle basis.

The route chosen in this study considered the beef exports from a small region – Nova Andradina. The beef produced in this region is likely to be shipped out of Porto de Paranaguá or Porto de Santos and unloaded at either Rotterdam or Hamburg. The exported masses in this scenario were 124 tonnes through Porto de Paranaguá and 676 through Porto de Santos (Soysal et al., 2013). The study assumes equal consumption by both end ports (Rotterdam and Hamburg).

To model a comparable case with our model, the total system was split into four separate routes. First through Port de Santos to Rotterdam, then through Porto de Santos to Hamburg, then through Porto de Paranaguá to Rotterdam, and finally through Porto de Paranaguá to Hamburg, all scaled per the masses and proportions described above (fig. 17). The emissions for these routes were 54,000 kg CO_{2eq}, 55,000 kg CO_{2eq}, 9100 kg CO_{2eq}, and 10,000 kg CO_{2eq}, respectively. This led to a total of about 128,000 kg CO_{2eq}.

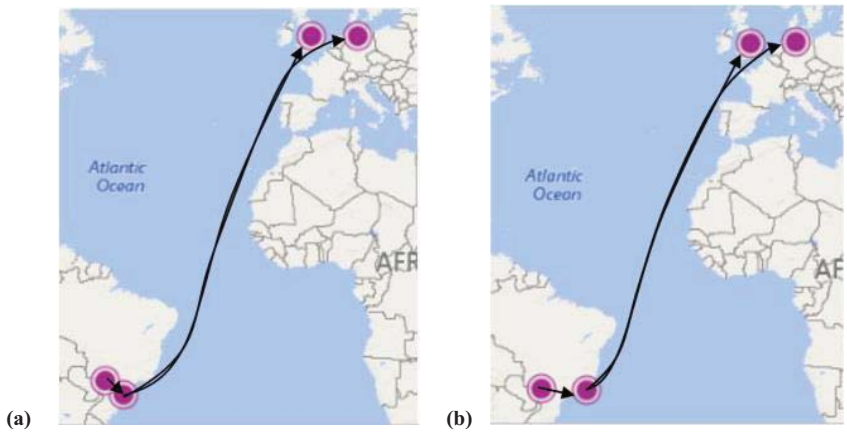

Figure 17. Routes for (a) Porto de Paranquá and (b) Porto de Santos

The results of our model were very in-line with the estimates from the Wageningen group. The group reported a "lowest cost" emissions value of 127,917 kg CO_{2eq} and a "lowest emissions" value of 113,633 kg CO_{2eq} (Soysal et al., 2013). As the vehicle emissions estimations used for this study were from on-road averages, it is expected that our modeled values would be nearest to the lowest cost scenario, which includes 100% used vehicles and no changes in infrastructure from the current transportation situation (Soysal et al., 2013).

5.4 Case 4

Beef supply and demand is of nearly matching quantities in the United States, so it is surprising that international beef trade is a common occurrence. The USA imported 3.0 billion pounds, exported 2.6 billion pounds, produced 25.3 billion pounds, and consumed 25.7 billion pounds of beef (carcass weight) in 2016 (USDA ERS, "Cattle," 2018). Intuitively, one may think that the international exchange of 650% more beef than required by the production deficit may be the result of trade with the most accessible nations (i.e., bordering countries), but USDA data shows otherwise.

Over half of imports (about 1.8 billion pounds) and over two-thirds of exports (also about 1.8 billion pounds) of processed beef are from/to countries that do not border the USA. From this, around 16 million pounds were purely reciprocal trades between non-bordering countries (USDA ERS, "Cattle," 2018). Of these reciprocal trade patterns, Costa Rican beef exchange was especially notable. Costa Rica imported 27 million pounds of beef from the USA in 2016, but also exported 5 million pounds to the USA in 2016 (USDA ERS, "Cattle," 2018). While it is not entirely clear why this practice is so common for beef products, the environmental impact of such trades is significant.

The case of Costa Rica was chosen for both the demonstration of environmental severity and ease of modeling. As it is a relatively small country, there are two major ports, Puerto Limón on the Atlantic side and Puerto Caldera on the Pacific side. Using Tool 2, both the import and export scenarios of reciprocal trade were modeled. The 5 million pounds (or about 2269 tonnes) of imported beef was estimated as having come from the two major Costa Rican ports. In this model, a baseline estimation of emissions was used to avoid overestimation. Because of this, the model assumed that all product was transported via ship/ferry travel and only includes port-to-port transportation emissions. These routes are shown in figure 18. Although geographically impossible, the "as the crow flies" distances between the closest ship ports were also used. The USA ports were selected on account of their proportion of total USA - Costa Rican import values (compiled from USA Trade Census data), so this modeling situation assumes that beef trade follows the same trading trends as total product trade. As shown below, the model only accounted for 81.5% of the imports, as the top-eight ports only represented 81.5% of total USA imports (World Port Source, 2016). Through summing the exports of these eight trade routes, the estimated emissions value for this portion of the case was 88,000 kg CO_{2eq}. If it was assumed that this case was representative of 100% of the reciprocal imports, the estimate for total reciprocal import emissions would total about 88,000 kg CO_{2eq}.

Port	Percentage of Imports	Estimated Imports (tonnes)	Estimated Emissions (kg CO2e)
The Port of Wilmingt	24.2	547.9568983	29000
Port of Philadelphia	16.2	368.1255527	20000
Port Manatee	12.4	280.1961013	12000
Port of Miami	8.6	194.3999547	7300
Port of Houston	8.0	182.0852884	7300
Port of Hueneme	4.4	99.85385312	5500
Port of Savannah	3.9	88.65753102	3500
Port of Galveston	3.9	87.57607047	3400
Total	81.5	1848.85125	88000

Figure 18. Beef transportation routes from Brazilian to American ports

The emissions value for the exports to Costa Rica were calculated in the same way. These routes are illustrated in figure 19. To maintain as much consistency as possible, the top 9 ports were used to represent 81.7% of total

reciprocal exports (World Port Source, 2016). The lowest possible emissions modeling methods were used for this model as well. Through the estimation of the export routes shown below, about 70,800 kg CO_{2eq} result from the modeled 81.7% of USA - Costa Rican reciprocal beef exports. Extrapolated to include 100% of reciprocal beef exports, this would total about 70,800 kg CO_{2eq}.

Port	Percentage of Exports	Estimated Exports (tonnes)	Estimated Emissions (kg CO2e)
Port of Corpus Christ	24.0	545.3396107	21000
Port of New Orleans	21.5	487.8627555	18000
Port of Houston	8.0	181.9390531	7300
Port of Texas City	6.1	139.3868614	5400
Port of Gramercy	5.9	132.9922527	5000
Port of Greater Bator	5.1	114.9412961	4400
Port of Port Arthur	4.4	99.20854075	3900
Port of Beaumont	3.8	86.50162839	3400
Port of Miami	2.9	65.28232066	2400
Total	81.7	1853.454319	70800

Figure 19. Beef transportation routes from American to Brazilian ports

As these data were derived from the lowest possible estimation, there are several other factors to be considered in this type of trade. First, geographical barriers are very important to non-air travel. The routes modeled here result in lower distances than true routes, as ships in a true transportation scenario would have to avoid the land through which the modeled routes pass ("as the crow flies"). Another consideration is that a significant portion of transportation emissions are the result of land travel. Transportation from producer to processor and from processor to port require much less direct routing and use either truck or train travel – both of which have larger emissions factors than ship travel. Lastly, this was also a low assumption because it was assuming all beef product was transported via ship. Some products may use air travel, resulting in far greater emissions.

In total, the reciprocal trade trends of the USA and Costa Rica result in an estimated emission value of at least 158,000 kg CO_{2eq} per year. When all the effects of this reciprocal trade are summed, the actual impact of these trends are much larger than this model shows. While economic and political incentives often drive this sort of non-bordering trade, observing the true environmental impacts of current practices reveals a great need for more sustainable trade tactics.

6. Discussion

6.1 Other Emissions Factors

Food trade trends depend heavily on a variety of factors. Spoilage, taste, consumer preference, political influences, regional climates, and economic incentives all impact the travelled routes of food items before reaching the consumer's plate. While the tools developed in this study have the capability of modeling the primary steps of transportation in the life cycle of food products, the total lifecycle of these goods (i.e., cradle-to-grave) have a variety of factors that were not addressed by this modeling system. The first, and possibly most obvious, source of emissions from food products are the actual agricultural production and processing stages. This is indirectly related to food transportation but is categorically separate from the issues of the transportation sector. Another source of emissions – possibly the most underrated – is the portion of the transportation chain that takes the food from its point of sale to the door of the consumer. Last-step LDV transportation emissions were not accurately modeled through the tools in this study, but are an important part of the total supply chain; these have been estimated to result in perhaps double the emissions of products direct-delivered via HDV (Wygonik & Goodchild, 2012).

The biggest drive for international product transportation is regional specialization. It is generally more efficient for an area to excel at the production of one item (or a few) and then engage in foreign trade than it is for an area to self-sustain. Environmentally, this concept is still applicable. While locally-sourced items may cut transportation emissions, often these emissions act as a sort of "red herring" in the grand scheme of sustainability.

The life cycle analysis (LCA) of food products contain so much more than what happens after the food is produced. In the case of sourcing problems, one key factor is the environment of the consumer area. Crop growth requires much more use of resources and subsequently creates a much larger carbon footprints. A study on tomato growth published by the French National Institute for Agricultural Research exemplified this well. When comparing the environmental impact of locally-grown tomatoes in Austria with imported Spanish tomatoes, transportation was found to be relatively unimportant in the comparison of total emissions. Overall, the LCA of conventional, multi-tunnel greenhouse tomatoes in Spain resulted in about half the CO_{2eq} emissions of the Austrian-grown conventional, greenhouse tomatoes (Theurl et al., 2013). While the transportation was the largest source of emissions in the lifecycle of the Spanish tomatoes, the Austrian greenhouse's heating requirement resulted in about double the emissions – greatly overshadowing any advantage that the locally-grown tomatoes had vis-à-vis the transportation sector (Theurl et al., 2013).

Production regionalization, however, creates problems when the transportation of fresh food is demanded. A Ghent University study observing food perceptions in Europe found that fresh fish products were thought to be significantly healthier than frozen varieties in every country studied (Vanhonacker et al., 2013). While there wasn't scientific support behind this idea, the perception is still a driving factor in the fish market. This is often at the expense of supply chain sustainability, as never-frozen fish requires quick (and environmentally costly) transportation methods. In cases like these, air travel is common, which greatly increases the emissions resulting from the route. One example of this was described in a study on carbon emissions of Australian seafood exports. Australia transports about 10% of total seafood exports via air travel (Farmery et al., 2015). Most of this seafood is flighted due to risk of spoilage for fresh fillets. In the case of salmon, the transportation of frozen fillets to the USA via sea expends about 0.7 kg CO_{2eq} per kg. The air travel required for fresh fillets, on the other hand, expends 18.3 kg CO_{2eq} per kg fish for the same route, which is about 9 times the total carbon emissions for farming and processing combined (Farmery et al., 2015). Even if the demand for freshness is maintained, there are alternatives to 100% air travel. By altering routes to introduce air-sea combination routes (where the food is only flown part of the way), the food could still reach its destination without spoiling while greatly reducing the emissions of the transport (Sims, et al., 2014).

Even for the cases of foods that do not require overseas travel, the modes of transportation contain variances that are hidden upon first observation. For the trucking and heavy-duty vehicle (HDV) options, replacement by rail travel would greatly cut emissions. The International Energy Agency predicts that a 35% replacement of HDV freight by rail freight would cut global freight emissions by 16% (Sims, et al., 2014). However, updating to rail travel is not always a simple process. For example, the railways of many areas are inflexible and difficult to expand, hindering the growth of the rail sector. The European Commission has a set goal of using only water or rail vehicles for all freight travel over 300 km (Sims, et al., 2014). While this would be an effective way to cut transportation emissions over time, the immediate fiscal and environmental cost of infrastructure production and installation would be great. It was estimated that the European rail system would have to double to accommodate this change, requiring a tremendous resource allotment to a network that is slow and costly to replace and improve.

Technology evolution, however, is not such a slow process in most other transport modes. HDV travel is the most easily and consistently improved mode of travel. Due to the shorter lifecycle of road vehicles, replacement is high. This means that outdated vehicles are not as common on road routes as on the routes of other modes. It is much more difficult and costly to replace airplanes, ships, and trains, so HDVs are a more popular target for technological improvement. While trains and ships are currently a more sustainable transportation method, trucks may begin to close the gap with focused vehicle emissions advancements and regulations.

These technological advancements are often the product of outside incentives. Inciting change is not an easy process and requires a great deal of coaxing when the fiscal benefits are hidden or non-existent. Often, governmental regulation is one of the only ways to push for development of vehicles. The immediate investment of such improvements is daunting to customers of the products, hindering the demand for more sustainable choices. Fiscal and governmental pulls have a clear history of polarizing perceptions of certain subjects – including sustainability and green initiatives. Without creating some sort of demand for greener products in the market, change can be controversial and slow.

6.2 Applications

The intended use of this software was to provide engineers, designers, and planners with a program to model transportation routes. Greater insights in product routing can help supply-chain engineers utilize the "low-hanging fruit" of addressing sourcing inefficiencies, and to greatly decrease greenhouse gas emissions in

the transportation sector. As exemplified in the case studies discussed above, these tools can help provide some guidance and insights on a wide variety of cases and questions. If used properly, this program could provide a simple and quick way to model many routes of travel to find the most sustainable (and feasible) method of product sourcing.

6.3 Uncertainty

All estimates provided by the program contain a great deal of uncertainty. The program was intended to model transportation routes for emissions comparisons, so the numbers and figures given were approximate calculations, not actual measured values. The emissions ratings for the modes of travel were averages of the most typical travel characteristics (more about this can be found in the discussion of data acquisition and at the Network for Transport Measures). The distances were all calculated "as the crow flies," so the actual transportation paths may add distance (and thus emissions) due to indirect routing.

Uncertainties may also arise from user error. Although Tools 2 and 3 were programmed to prevent Train/Rail and Truck/Road travel across oceans, the code will not always prevent unlikely or impossible routes. There were also no features in place to prevent Ship/Ferry travel across land (to allow for possible river routes). The maps on the tools were provided to help visualize any errors that may occur due to the geography of the regions.

The override buttons available in the tools may be used to troubleshoot when the error-checking boundaries prevent likely or necessary travel. These extraneous functions run the program with lowered error-checking, allowing for more travel routes. It is recommended, however, to utilize the mapping functions to determine whether there is a possible routing error before overriding.

6.4 Future Developments

As discussed in the section of extraneous emissions factors, transportation is a multifaceted issue. There is an abundance of functions and considerations that could be added to create a more useful and comprehensive software program.

First, the porting options in Tool 3 were not expansive enough to make the optimization function effective in low-population areas. The top world freight ports are often not food-focused and do not include many of the potential food ports in Central/South America which are large contributors to global agriculture. Expanding the selectable and suggested options in Tool 3 would help make more informed decisions about more realistic food transportation routes.

Second, the store-to-home step of transportation is a very important part of emissions modeling. This variable in food supply is one of the most impactful and most easily altered parts of the total lifecycle emissions scheme. Providing a way to model and compare mass delivery systems with personal consumer vehicle use could provide a great deal of insight on an unclear emissions source. This would require the addition of LDVs to the list of transport modes.

Another key feature that could possibly be integrated into future iterations of this set of software tools are time -dependent functions. Perishability is a key consideration in fresh foods and a time estimation function added to the second and third tools would help the user consider the possibility of routes from a spoilage perspective. The added emissions factors of refrigerated trucks would also aid in modeling transportation of perishables. Reflecting those factors in the tools would create a simpler way to measure the feasibility as well as a more accurate measure of emissions.

7. Conclusions

Transportation sustainability is a multifaceted problem. Economic, political, and regional (such as specialization and climate) factors all play important roles in how countries import and export goods. Nearly all foods, regardless of origin, are now quite easily accessible, often resulting in less-than-optimal transportation practices. The set of tools described in this study helped examine these issues more closely, giving insight into variations in transportation practices that could potentially help reduce carbon footprints.

These tools have a variety of functions and purposes, but all have common categories of inputs/outputs. In each, the user must either give locational or distance data, which was interpreted as a distance between points. The cargo mass was also an essential factor to the emissions results. Lastly, the user usually needs to give a mode of transportation order, which was used (along with the distance and mass) to determine the specific emissions factor used on the route. This distance, mass, and factor are multiplied to yield an emissions product in kg CO_{2eq}. These tools use an Excel file format to maintain simplicity and provide an easily understood form of emissions modeling for those that wish to find an estimate of currently practiced transportation routes.

The case studies provided here exemplify the variety of uses for these tools. Estimating the LCA of various products and finding inefficiencies in current practices are two of the most prominent possible uses. As shown in Cases 2 and 3, there are many factors that directly impact transportation emissions. Although distance (or "food miles") can contribute greatly to the total emissions, the modes by which these distances are travelled leave an equally large footprint. A change in origin of Iowan apples from the state of Washington to the state of New York resulted in a reduction of carbon emissions that was roughly proportional to the change in route distance (about 37%). However, the modeled salmon transportation route from the Faroe Islands (America's 2nd largest source of imported fresh salmon) to Richmond, VA resulted in a roughly 98% reduction of emissions when shipped via sea rather than flown, even though the flown route was slightly shorter in distance. The validity of these tools has been strengthened through comparison to a similar case study. That study, involving an emissions estimation for Brazilian beef transportation by Wageningen University, used an estimation method that compiled methods from 18 other transportation emissions projects. The comparison of the use of these tools with their final estimation resulted in a 1-11% disparity in results, depending on the method used by the Wageningen University group. This helped confirm that the emissions factors used in our tools were close to the methods and factors used in other literature. In Case 4, transportation optimization was observed. The reciprocal trade pattern of beef between Costa Rica and the United States was analyzed and found to result in at least 158,000 kg of excess CO_{2eq} annually.

Although these tools do help model one portion of the life cycle of a food product, users must be advised that transportation itself is not an end-all solution to food sustainability. Many other factors play into both the transportation sector as well as the other parts of a product lifecycle. The last portion of transportation, store-to-consumer, was not covered by this modeling program, which may lead to the underestimation of true emissions. Where and how food is produced both directly impact total emissions and indirectly affect transportation choices. Foods produced in a nearby unnatural environment requiring heat input are likely to result in more emissions from production alone than the emissions from the total lifecycle of a product from a distant natural environment. Infrastructure and the manufacturing and maintenance of vehicles also play a role in total transportation lifecycle emissions. Although trains and ships still result in less emissions, trucks are easier to replace with new technologies, speeding the potential for improvements.

Transportation emission reductions are one of the many ways that societies could help limit the rise of global carbon emissions. The food sector, driven by the globalization of the food economy, has created a rising environmental impact due to food transportation. The tools outlined in this study provides a variety of methods for users to analyze and model these transportation systems. While there are many considerable factors in the total lifecycle sustainability of an item, modeling feasible food transportation routes is clearly a step towards lessening the impacts of food transportation emissions.

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Appendix: MS Excel Program and Instructions for Use

The authors provide their MS Excel program for those readers who are interested in modeling various transport distances for a variety of food products for their specific situations of interest. The user may either run the MS Excel directly, or they can modify the programming for their specific modeling needs. This file can be downloaded (Tools.coded.xlsm) from the authors' online GitHub repository, available at: https://github.com/karosent/Food_Transport_Emissions. The reader is encouraged to work with this file in order to more fully explore the simulation's capabilities.

If the reader utilizes the authors' online GitHub repository to access the MS Excel program, they will be provided with instructions which are available as a README.pdf file on the GitHub site.

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Identification of Aroma Compounds in Freeze-dried Strawberries and Raspberries by HS-SPME-GC-MS

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Abstract

The objective of this study was to determine a method for the identification of aroma volatile compounds in freeze-dried (FD) strawberries and raspberries for quality purposes. The aroma profile was examined using headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). FD strawberries and raspberries were extracted at four different times (10,15, 20 and 30 min) and three different temperatures (40 °C, 60 °C and 80 °C) using a SPME fiber coated with 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane (DVB/CAR-PDMS) to determine optimum recoveries for aroma volatile compounds. The DVB/CAR-PDMS SPME fiber showed the best extraction of aroma volatile compounds from strawberry and raspberry at 60°C for 15 min. Twenty-nine volatile compounds were identified from the strawberry samples and 20 from the raspberry samples, including terpenes, aldehydes, esters, acids and alcohols. Select aroma compounds in FD strawberries and raspberries were quantitated using SPME and GC-MS. It is important to determine the desirable aroma active compounds in freeze-dried strawberries and raspberries for quality uses since they are becoming popular commercially.

Keywords: aroma compounds, GC-MS, HS-SPME, strawberry, raspberry

1. Introduction

Aroma is an important characteristic of fruit quality that is gaining increased attention. Aroma volatile compounds are specific to the genus and to the variety of the fruit. Although some fruits may have similar aroma compounds, each fruit has a unique aroma that depends upon the combination of the volatiles and their concentration. These volatile compounds may play a key role in determining the acceptability of products by consumers (Hadi, Zhang, Wu, Zhou & Tao, 2013)

Fresh strawberry (*Fragaria x ananassa*) is one of the most complex fruits containing an estimated 350 volatile aroma compounds. The two major dominating aroma compounds are the furanones, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) (Hadi et al., 2013; Jetti, Yang, Kunianta, Funn & Qian, 2007; Jouquand & Chandler, 2008; Forney, 2001). These give strawberry the sweet, flowery scent. Esters are significant comprising 25% to 90% of the total number of volatiles in ripe strawberry fruit with the important ones being methyl and ethyl butanoate, butyl acetate, and methyl and ethyl hexanoate (Hadi et al., 2013; Jetti et al., 2007; Jouquand & Chandler, 2008; Forney, 2001). Some other important aroma compounds are linalool, γ-decalactone and 2,3-butanedione, but these are specific to the cultivar and promote the pleasant coconut and citrus odor (Hadi et al., 2013; Jetti et al., 2007; Jouquand & Chandler, 2008). Some aldehydes and alcohols also are important and contribute to the green, unripened strawberries (Jetti et al., 2007; Jouquand & Chandler, 2008; Forney, 2001).

Approximately 300 volatile compounds have been found in fresh raspberry (*Rubus idaeus* L.*)* (Hadi et al., 2013; Du & Qian, 2010; Aprea, Biasiolli & Gasperi, 2015). Terpenoids were dominant in the volatile profiles with similar amounts of ketones, aldehydes, esters and alcohols also present (Forney, 2001). The key compounds that contribute to the raspberry aroma include raspberry ketone (4-(4-hydroxyphenyl)butan-2-one), α-ionone, β-ionone, linalool, (Z)-3-hexenol, geraniol, nerol, α-terpineol, furaneol, hexanal, β-ocimine, 1-octanol, β-pinene, β-damascenone, ethyl 2-methylpropanoate, (E)-2-hexenal, heptanal, and benzaldehyde. Raspberry ketone,

α-ionone, and β-ionone, which produce the sweet-smelling and floral aroma were determined to be the key aroma compounds in raspberry regardless of the cultivar and are characteristic of the raspberry aroma, while linalool and geraniol were detected in great amounts in some cultivars (Hadi et al., 2013; Du & Qian, 2010; Aprea et al., 2015). Since raspberry ketone has a high boiling point, it may not be detected with certain analysis techniques (Forney, 2001).

Jetti et al. (2007) used HS-SPME-GC-MS to quantify select aroma-active compounds in ten strawberry cultivars grown in California and Oregon. Each sample was adsorbed onto a 50/30 μm divinylbenzene (DVB)/Carboxen (CAR)-polydimethylsiloxane (PDMS) fiber, extracted for 60 min at 50°C, and desorbed for 3 min at 250°C in the GC injector. Aprea, Biasiolli, Carlin, Endrizzi & Gasperi (2009) assessed headspace SPME-GC-MS for the analysis of two raspberry varieties and identified 28 compounds and tentatively identified 18 compounds present in the fruits. Extraction was performed using a SPME 50/30 μm (DVB/CAR-PDMS) fiber for 30 min at 35°C and desorption was for 5 min at 250°C.

Freeze-drying has become of increasing interest among consumers (Ciurzyríska & Lenart, 2011). Freeze-drying results in a high-quality food product and extended shelf-life because of the low temperature used in processing. The flavors, aroma, and nutritional content of the original product generally stay the same making the process popular for preserving food (Ciurzyríska & Lenart, 2011). Freeze-drying is useful for fruits that are seasonal, such as strawberries and raspberries. In the last decade, FD strawberry and raspberry have become more prevalent in the food industry, for example, with dehydrated fruits being added to snacks, cereals and confectionary products.

The aim of this study was to determine the optimum extraction of volatile compounds in freeze-dried strawberries and raspberries to study their aroma profile, which is an important trait of fruit quality, by using HS-SPME-GC-MS.

2. Materials and Methods

2.1 Samples and Chemicals

Freeze-dried whole strawberries and whole raspberries were obtained from Van Drunen Farms (Momence, IL, USA). Furfural, ethyl hexanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane), gamma-decalactone, alpha-ionone, beta-ionone, and anisole (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Sodium carbonate anhydrous and methanol, HPLC grade were bought from Fisher Scientific (Hanover Park, IL, USA).

2.2 SPME Fiber and Extraction Conditions Selection

Aroma volatiles from the FD strawberry and raspberry samples were initially extracted using four types of SPME fibers, coated with different stationary phases: 100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 85 μm carboxen/polydimethylsiloxane (CAR/PDMS) and 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane on a StableFlex fiber (DVB/ CAR-DMS). These fibers were acquired from Supelco (Bellefonte, PA, USA). The fibers were conditioned before use according to manufacturer's instructions.

The sample (1.0 g) was weighed into a clear, 20 mL headspace vial (Gerstel, Linthicum, MD, USA), capped (magnetic crimp caps with PTFE septa, Gerstel, Linthicum, MD, USA), and placed on the headspace autosampler. The incubation time was 15 min at 60°C. The fiber was exposed at four different times of 10, 15, 20 and 30 min to the headspace of the sample and at three different temperatures of 40°C , 60°C, and 80° C. The desorption time was 10 min at 250°C in the GC inlet in the splitless mode.

2.3 Sample Matrix Selection

Whole freeze-dried sample (1.0 g) was weighed into a clear, 20 mL headspace vial (Gerstel, Linthicum, MD, USA), capped (magnetic crimp caps with PTFE septa, Gerstel, Linthicum, MD, USA), and placed on the headspace autosampler. In a second vial, the ground, powdered strawberry/raspberry sample (1.0 g) was weighed into a vial. In a third vial, 10 mL of distilled water was added to the sample (1.0 g). In a fourth vial, water was substituted for the salt solution (20% of sodium sulfate in distilled water). For all the trials, the headspace extraction was performed using the optimized extraction parameters (15 min, 60°C) with a 50/30 μm DVB/CAR-PDMS fiber (2 cm, Supelco, Bellefonte, PA, USA) The desorption time was 10 min at 250°C in the GC inlet.

2.4 Sample Preparation and SPME Extraction

The FD sample (1.0 g) was placed into a clear, 20 mL headspace vial and an internal standard, anisole (50 μg)

was added. A 50/30 μm DVB/CAR-PDMS fiber (Supelco, Bellefonte, PA, USA) was chosen for aroma extraction. The sample was incubated for 15 min at 60°C. The SPME fiber was then exposed at the agitation speed (400 rpm) and optimized extraction time and temperature (15 min, 60°C). Afterwards, the SPME fiber was desorbed through the injector port of the gas chromatography at 250°C for 10 min in the splitless mode.

2.5 Quantitation

Standard stock solutions of approximately 10,000 μg/mL of each select compound detected in FD strawberry (furfural, mesifurane, and gamma-decalactone) and FD raspberry (furfural, alpha-ionone and beta-ionone), and anisole (internal standard) were prepared in methanol. Working standards were prepared as mixtures so that final concentrations ranged from 0.01 μg/mL to 1000 μg/mL. The calibration curve range was as follows: 5 to 1000 μg/mL for mesifurane and furfural; 0.5 to 100 μg/mL for gamma-Decalactone; 0.01 to 5 μg/mL for α-ionone and β-ionone. Fifty microliters of anisole (IS) was also added to each standard mixture for a final concentration of 5 μg/mL. After equilibration, the standards were extracted with SPME and analyzed by GC-MS as was done for the samples. Calibration curves were generated as a plot of ratio of concentration of analyte to the concentration of internal standard vs. ratio of peak area response of analyte to internal standard. These were used to quantitate the concentration of volatile compounds in the samples.

2.6 Gas Chromatography-mass Spectrometry (GC-MS)

The analysis of aroma volatiles extracted by HS-SPME was performed using a Model 7890A gas chromatograph (GC) equipped with a 5975C (G3440A) inert mass spectrometer detector (MSD) with a triple-axis detector (G3171A) (Agilent Technologies, Santa Clara, CA, USA). The GC was coupled with a Gerstel Multipurpose Sampler (MPS) (Linthicum, MD, USA) that had a SPME fiber holder. The injector port had a 2 mm deactivated GC liner and the inlet was kept at a constant temperature of 250°C. A fused silica HP-5ms-UI column (30 m x 0.25 mm ID x 0.25 μm thick film), Agilent Technologies (Santa Clara, CA, USA) was used. Helium was the carrier gas with a flow rate of 1 mL/min.

The initial oven temperature was 40°C with a hold time of 5 min. Then the temperature rose to 230°C at 10°C/min for 5 min and increased again to 250°C at 10°C/min for 5 min.

The MSD operated in electron ionization mode at 70 ev. The MSD transfer line was set at 280°C. The ion source was heated at 230°C and the MS quads were both heated at 150°C. Solvent delay was 1.5 min. The mass acquisition range was 20 to 450 m/z.

2.7 Data Analysis

Aroma volatile compound identification and quantitation were performed using Agilent Technologies' ChemStation software (version E.02.02.). Samples were prepared in triplicate for each analysis. Microsoft Excel 2016 (Redmond, WA, USA) was used for the calculation of means, standard deviations (SD) and relative standard deviations (%RSD). Significant differences between samples were analyzed using analysis of variance (ANOVA) with Sigma Plot 14.0 (Systat Software; San Jose, CA, USA). The aroma volatile compounds were identified using the U.S. National Institute of Standards and Technology (NIST) Mass Spectral Library, v.2.3, 2017 in Chemstation. Authentic pure reference standards confirmed select volatile compounds in FD strawberry and FD raspberry samples.

3. Results and Discussion

3.1 Fiber Type Selection

A fiber's coating type and thickness is the most important feature that determines the analytical performance of SPME. The type of fiber used affects the selectivity of extraction. Generally, polar fibers are used for polar analytes and non-polar fibers for non-polar analytes similar to conventional GC stationary phases.

PDMS is best used for extracting non-polar analytes, such as volatile flavor compounds but also polar compounds using GC (Vas &Vékey, 2004; Ducki, Miralles-Garcia, Zumbé, Tornero & Storey, 2008). CAR/PDMS and PDMS/DVB have been used for extracting volatile low molecular mass and polar analytes. DVB/CAR-PDMS is composed of a layer of PDMS/DVB over a layer of CAR-PDMS and is used for odors and flavors (volatiles and non-volatiles) (Vas &Vékey, 2004; Ducki et al., 2008). FD strawberry and FD raspberry samples were initially extracted using these four SPME fibers and analyzed by GC-MS to determine which fibers achieved the greatest performance for the recovery of aroma volatile compounds at the intermediate extraction time of 15 min and extraction temperature of 60° C. Twenty-nine compounds in FD strawberry and 20 compounds in FD raspberry were extracted and tentatively identified based on their mass spectra (MS) (Table 1). These included terpenes, lactones, aldehydes, alcohols, acids, and furanones.

Table 1. Effect of fiber type on the extraction of aroma volatile compounds peak areas (mean ± %RSD, n=3) in freeze-dried strawberry and raspberry for 15 min at 60° C

	50/30um CAR/DVB/PDMS		65um PDMS/DVB		85um CAR/PDMS		100 µm PDMS	
Compound	**Strawberry**	**Raspberry**	**Strawberry**	**Raspberry**	**Strawberry**	**Raspberry**	**Strawberry**	**Raspberry**
Furfural	14287556 ± 3.62	53944896 ± 16.77	1048909 ± 17.56	4834081 ± 13.19	2450119 ± 70.95	24918746 ± 13.08	52967 ± 49.38	327748 ± 11.34
Oxime,methoxy-phenyl	ND	1431976 ± 7.70	ND	456634 ± 5.03	ND	572142 ± 4.10	ND	138760 ± 13.04
Methyl hexanoate (hexanoic acid, methyl ester)	906604 ± 14.36	ND	85330 ± 76.24	ND	214907 ± 29.27	ND	9740 ± 19.48	ND
3(2H)-furanone,2(1-hydroxy-1-methyl-2-oxopropyl)-2,5-dimethyl	4721280 ± 7.83	ND	541481 ± 8.29	ND	1021527 ± 17.87	ND	46018 ± 5.2	ND
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3770591 ± 11.67	2394817 ± 8.03	445807 ± 18.82	434187 ± 18.12	220305 ± 73.62	102113 ± 27.68	36041 ± 24.1	8276 ± 22.91
Ethyl hexanoate (hexanoic acid, ethyl ester)	3065190 ± 26.97	ND	253103 ± 30.70	ND	466822 ± 32.20	ND	20606 ± 34.4	ND
Hexyl acetate (acetic acid, hexyl ester)	2622895 ± 35.26	ND	264464 ± 63.24	ND	293391 ± 12.79	ND	25512 ± 16.8	ND
2,5-Dimethyl-4-methoxy-3(2H)-furanone (Mesifurane)	24928055 ± 2.29	ND	3507881 ± 15.31	ND	5143420 ± 12.63	ND	321602 ± 20.7	ND
Nonanal	2838635 ± 7.56	1130253 ± 9.54	271579 ± 22.81	142880 ± 12.92	103200 ± 21.55	103457 ± 1.07	39141 ± 18.4	24245 ± 13.40
4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	2554730 ± 9.82	2228400 ± 10.16	419541 ± 2.74	349589 ± 1.97	245745 ± 8.99	236018 ± 15.29	10756 ± 23.9	9177 ± 15.50
Benzyl acetate (acetic acid, phenylmethyl ester)	138063 ± 40.19		47002 ± 21.78		27632 ± 26.48		5359 ± 12.0	
5-Hydroxymethyl furfural	414631 ± 14.13	2184792 ± 21.98	228793 ± 9.48	991878 ± 14.30	35652 ± 15.05	202107 ± 17.57	7170 ± 11.4	68077 ± 11.78
Propanoic acid, 2-methyl-, 3--hydroxy-2,2,4-trimethylpentyl ester	593540 ± 8.01	366659 ± 9.53	93218 ± 1.74	73157 ± 4.56	76348 ± 3.73	58162 ± 8.89	33977 ± 9.30	22143 ± 1.85
Octyl 2-methylbutanoate	1604694 ± 25.19	ND	319792 ± 20.98	ND	161766 ± 28.54	ND	230321 ± 37.92	ND
2-furancarboxaldehyde,5-methyl	ND	300985 ± 17.89	ND	352918 ± 25.57	ND	187987 ± 13.77	ND	14855 ± 17.81
Ethanol,2-(hexyloxyl-)	ND	1173046 ± 18.79	ND	215500 ± 4.91	ND	115225 ± 4.03	ND	23213 ± 5.25
2H-pyran-2-one,tetrahydro-6-propyl	ND	2984231 ± 28.05	ND	359921 ± 31.83	ND	189780 ± 23.91	ND	124500 ± 5.95
γ-Decalactone	1289139 ± 5.02	ND	468706 ± 81.85	ND	78841 ± 23.63	ND	281219 ± 70.22	ND
2-Furanmethanol,tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-[2s-[2α,5β (R))]	1049749 ± 16.92	ND	52256 ± 3.67	ND	16402 ± 30.54	ND	70927 ± 12.96	ND
Caryophyllene	ND	47485 ± 20.69	ND	39877 ± 13.85	ND	63030 ± 30.78	ND	23648 ± 15.43
α-Ionone	ND	4097353 ± 18.96	ND	590635 ± 11.81	ND	718633 ± 13.63	ND	631754 ± 19.83
β-Ionone	ND	5489049 ± 10.47	ND	1175454 ± 6.88	ND	840167 ± 28.67	ND	953036 ± 18.21
γ-Dodecalactone	492529 ± 18.84		171680 ± 26.33		20976 ± 26.98		227406 ± 5.40	
7,9-Di-tert-butyl-oxaspiro(4,5)-deca-6,9-diene-2,8-dione	242324 ± 21.53	155720 ± 9.52	17009 ± 9.77	23496 ± 5.77	3389 ± 6.01	3611 ± 22.05	16251 ± 9.58	26290 ± 3.37
Total	**65520205**	**68187540**	**8236551**	**10040207**	**10580442**	**28311178**	**1435013**	**2395722**

ND- not detected

An examination of the total peak area for each fiber showed that the most efficient fiber is 50/30 µm DVB/CAR-PDMS, extracting eight times more than the 65 µm PDMS/DVB, about seven times more than the 85

μm CAR/PDMS and 45 times more than the 100 μm PDMS fiber for volatiles in strawberry. The fiber that was the best performer for extracting volatiles in raspberry was also 50/30 μm DVB/CAR-PDMS, extracting seven times more than the 65 μm PDMS/ DVB, two times more than the 85 μm CAR/PDMS and 28 times more than the 100 μm PDMS fiber. Extraction with the PDMS fiber was very weak compared to the other three fibers shown by the very low peak responses. The DVB/CAR-PDMS fiber extracted the most analytes (total peak area 65,520,205 for strawberry and 68,187,540 for raspberry) and proved to be very capable at extracting volatiles. This is in agreement with other studies of aroma volatile compounds, which identified the DVB/CAR-PDMS fiber as the best fiber for extraction in cocoa products, strawberry fruit, mushrooms, olive oil, and raspberry fruits and juices (Ducki et al., 2008; Jetti et al., 2007; Aisala, Sola, Hopia, Linderborg & Sandell, 2019; Sanz, Belaj, Sánchez-Ortiz, Pérez, 2018; Du et al., 2011; Aprea et al., 2009). Therefore, it was decided that this fiber would be used to establish the aroma volatile profiles of FD strawberry and raspberry.

Mesifurane and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) are two most important furanones in fresh strawberry (Jetti et al., 2007). In this study, the two major aroma compounds in FD strawberry are shown to be 2,5-dimethyl-4-methoxy-3(2H)-furanone (Mesifurane) and furfural and represent 38.1% and 21.8% of the total volatiles in FD strawberry, respectively. Furaneol was not detected in the FD strawberry samples. This may be because furaneol is heat labile and is unstable both in the presence of air and in aqueous solutions (Forney, 2001; Weerawatanakorn, Wu, Pan & Ho, 2015) or it may not be detected with this HS-SPME-GC-MS method (Jouquand & Chandler, 2008). Other furanones, 3(2H)-furanone, 2(1-hydroxy-1-methyl-2-oxopropyl)-2,5-dimethyl, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, and gamma-decalactone (2(3H)-furanone, 5-hexyldihydro-) comprise about 15.0% of the total volatiles in FD strawberry. Other compounds identified in fresh strawberries and similarly in the FD product in this study include esters, pyranones, lactones, and other minor components (Jetti et al., 2007). In his study, Forney (2001) determined that fresh strawberry contained 1-30% furanones, 10-30% alcohols with the highest total abundance being 15-70% of esters.

In FD raspberry, furfural comprises 64.9% of the total content of volatile compounds, β-Ionone 8.0% and α-Ionone 6.0% as is typical of the compounds of fresh raspberry (Hadi et al., 2013; Forney, 2001; Du & Qian, 2010). Pyranones, 2H-pyran-2-one,tetrahydro-6-propyl and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl constitute 7.6% of the total content of volatiles in FD raspberry. Other compounds found in the FD raspberry sample in this analysis include oxime, 5-hydroxymethyl furfural, aldehydes, ethanol and other minor analytes. In his study, Forney (2001) showed that fresh raspberry constituted 2-40% alcohols, 5-15% esters and ketones, 10-20% aldehydes, with the most abundance being 20-50% terpenoids.

Similar volatile compounds detected in both FD berry samples but containing different quantities are furfural, 5-hydroxymethyl furfural, nonanal, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3one, and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl. The volatile profiles of fresh berries depend on the cultivar, ripeness, environmental conditions, fruit matrices (whole, slices or homogenized) and analytical methods used for identification (Hadi et al., 2013; Jetti et al., 2007; Jouquand & Chandler, 2008; Forney, 2001; Du & Qian, 2010).

3.2 SPME Extraction Time and Temperature Selection

Extraction time and temperature are two critical variables that influence sample recovery by the SPME fibers. Therefore, these two parameters were examined to determine the optimum extraction conditions.

Extraction times were examined at 10, 15, 20, and 30 min and at three different temperatures (40°C, 60°C, and 80° C). The 50/30 μm DVB/CAR-PDMS fiber was chosen for the extraction of volatile compounds in the FD strawberry and raspberry samples because usually, the thinnest fiber is used to reduce extraction times (Vas & Vékey, 2004). This was shown in this study where most of the compounds in FD strawberry and raspberry required only 15 min of extraction time generating high peak area responses. This was similar to the findings of Ducki et al. (2008) who noticed that after 20 min there was a decline in the amount of volatiles extracted and the semi-volatiles took 30 min to be extracted with the DVB/CAR-PDMS fiber for cocoa products. Ducki et. al (2008) extracted for 15 min compounds from the cocoa products both volatile and semi-volatiles compounds. Initial results of this study showed that using 65 μm PDMS/DVB, 85 μm CAR/PDMS and 100 μm PDMS would require longer extraction times (up to 30 min) for some volatile compounds in FD strawberry and raspberry (data not shown). It was, therefore, decided that the 50/30 μm DVB/CAR-PDMS fiber was the best option for extraction of volatiles in FD strawberry and raspberry for 15 min.

Comparable to the cocoa products study of Ducki et. al (2008), it was determined that the amount of volatile compounds extracted increases greatly with the temperature of extraction. The optimum extraction temperature was at 60°C in this study and 80°C in the cocoa products study. However, 80°C was not applied for the

extraction of cocoa products since chemical changes were likely to occur in the sample at this high temperature but 60°C was used (Ducki et al., 2008).

Only eight compounds were tentatively identified at 40° C in strawberry with the lowest peak counts being for ethanol, 2-(2-butoxethoxy)- and butanoic acid, 3-methyl,-octyl ester (Table 2). Most of the peak area responses for compounds such as alcohols, acids, terpenes and some esters are higher at 80° C than at 60° C. However, at 80° C, 21 compounds are identified in strawberry out of the 29 peaks that are identified at 60° C. Mesifurane, a characteristic aroma compound for strawberry, has a higher peak response at the 60° C temperature. A decision was therefore made to use 60° C as the optimum temperature for extraction of volatiles from strawberry with the 50/30 μm DVB/CAR-PDMS fiber.

Table 2. Effect of temperature on the extraction efficiency of major compounds in FD strawberry (peak area ± %RSD), n=3. 50/30 um DVB/CAR-PDMS fiber was used, and 15 min for extraction time

Compound	40° C	60° C	80° C
Butanoic acid, ethyl ester	ND	3559721 ± 77.00	ND
Furfural	ND	19445182 ± 53.31	ND
Oxime,methoxy-phenyl	338625 ± 8.79^{a}	1440284 ± 37.24^{b}	276022 ± 15.91^{a}
Methyl hexanoate	ND	730820 ± 13.11^{a}	1670709 ± 8.59^{b}
3(2H)-furanone,2(1-hydroxy-1-methyl-2-oxopropyl)-2,5-dimethyl	ND	4452794 ± 3.21	ND
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	ND	3107453 ± 35.95	ND
Ethyl hexanoate	ND	3623295 ± 24.44	ND
Hexyl acetate	ND	634125 ± 9.51^{a}	5853577 ± 8.80^{b}
2-hexen-1-ol,acetate	18775 ± 36.05^{a}	2000819 ± 22.16^{b}	8652248 ± 6.97^{c}
3(2H)-furanone,-4-methoxy-2,5-dimethyl (Mesifurane)	451560 ± 27.03^{a}	6600808 ± 19.54^{b}	3287796 ± 48.12^{c}
1-Octanol	ND	3137311 ± 64.31^{a}	6065952 ± 6.80^{a}
Linalool	ND	1151138 ± 14.17^{a}	14688297 ± 1.81^{b}
Nonanal	332160 ± 31.96^{a}	2497489 ± 5.04^{b}	17803476 ± 10.12^{c}
4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	ND	73012 ± 33.89	ND
Ethanol,2-(2-butoxyethoxy)-	12951 ± 12.89^{a}	607885 ± 37.54^{b}	1924287 ± 20.92^{c}
L-alpha-terpineol	ND	45966 ± 44.51^{a}	3963813 ± 12.19^{b}
Decanal	ND	1746636 ± 28.76^{a}	1938806 ± 3.17^{b}
Acetic acid, octyl ester	ND	534955 ± 13.13^{a}	5113712 ± 19.21^{b}
5-hydroxymethyl furfural	ND	366275 ± 28.45	ND
Propanoic acid, 2-methyl-, 3--hydroxy-2,2,4-trimethylpentyl ester	197162 ± 4.11^{a}	538535 ± 5.02^{b}	ND
Butanoic acid, octyl ester	ND	1271621 ± 25.06^{a}	6178825 ± 44.04^{b}
Octyl 2-methylbutanoate	48756 ± 25.42^{a}	1478588 ± 11.66^{b}	456629 ± 17.79^{c}
Butanoic acid, 3-methyl, -octyl ester	12365 ± 5.54^{a}	46941 ± 16.93^{b}	2132722 ± 6.66^{c}
gamma-Decalactone (2(3H)-furanone, 5-hexyldihydro-)	ND	10689 ± 49.65^{a}	3587471 ± 6.32^{b}
1,6,10-dodecatrien-3 ol, 3,7-11 trimethyl (E)	ND	2856563 ± 8.47^{a}	29634692 ± 3.50^{b}
Hexanoic acid,octyl ester	ND	341559 ± 30.29^{a}	2113772 ± 10.52^{b}
Butanoic acid,decyl ester	ND	31801 ± 31.59^{a}	1031738 ± 20.85^{b}
γ-Dodecalactone	ND	23203 ± 12.02^{a}	2697754 ± 5.35^{b}
7,9-Di-tert-butyl-oxaspiro(4,5)-deca-6,9-diene-2,8-dione	ND	162122 ± 15.63^{a}	1124296 ± 5.79^{b}

Values followed by different letters denote significant difference $p < 0.05$ for each analyte in FD strawberry across the rows and those followed by same letters denote no significant difference.

Fourteen out of 20 volatile compounds were tentatively identified at 40° C in raspberry with the lowest peak responses being for the terpenes (caryophyllene, α-ionone, β-ionone) (Table 3). Undetected compounds were mostly the furanones. Peak area responses were higher for terpenes at 80° C, except for D-Limonene and α-ionone, which had the same responses at both 60° C and 80° C. Since there were some undetectable compounds at 80° C, it was determined that 60° C was the best extraction temperature of volatiles from raspberry with the 50/30 μm DVB/CAR-PDMS fiber.

Table 3. Effect of temperature on the extraction efficiency of major compounds in FD raspberry (peak area ± %RSD), n=3. 50/30 um DVB/CAR-PDMS fiber was used, and 15 min for extraction time

Compound	40° C	60° C	80° C
Furfural	ND	53225620 ± 15.81[a]	27834234 ± 11.59[b]
Oxime,methoxy-phenyl	416878 ± 15.20[a]	2133051 ± 16.67[b]	9876478 ± 6.08[c]
2-Furancarboxaldehyde, 5-methyl	ND	999647 ± 16.07	ND
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3one	ND	2955337 ± 8.31	ND
alpha-Phellandrene	72968 ± 6.90[a]	6652327 ± 7.51[b]	9924240 ± 27.59[c]
o-Cymene	686626 ± 40.69[a]	2270296 ± 10.50[b]	1995020 ± 23.79[b]
D-Limonene	2928818 ± 9.32[a]	6512046 ± 8.17[a]	6865122 ± 66.30[a]
2(3H)-furanone dihydro, 3-hydroxy-4,4-dimethyl	ND	1246083 ± 13.46[a]	12990448 ± 15.63[b]
Linalool	ND	156305 ± 0.63[a]	3593803 ± 96.99[b]
Nonanal	144320 ± 1.67[a]	1083346 ± 6.13[b]	4612337 ± 21.91[c]
Ethanol,2-(hexyloxyl-)	234079 ± 3.55[a]	1305104 ± 5.93[b]	ND
4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	284308 ± 4.23[a]	2206587 ± 8.26[b]	260719 ± 16.81[a]
5-Hydroxymethyl furan	ND	2279812 ± 21.50	ND
2H-pyran-2-one,tetrahydro-6-propyl	199953 ± 19.32[a]	2000300 ± 43.16[b]	ND
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	168991 ± 8.09[a]	408804 ± 3.73[b]	ND
Caryophyllene	55663 ± 28.17[a]	979518 ± 15.38[b]	2636674 ± 76.05[c]
α-Ionone	31494 ± 22.57[a]	4196839 ± 14.42[b]	42919853 ± 24.00[b]
trans-β-Ionone	86404 ± 21.02[a]	5530591 ± 11.66[b]	71217494 ± 11.45[c]
2H-pyran-2-one,tetrahydro-6-pentyl	52833 ± 19.15[a]	2059400 ± 12.87[b]	4024666 ± 28.54[b]
7,9-Di-tert-butyl-oxaspiro(4,5)-deca-6,9-diene-2,8-dione	ND	156263 ± 9.80[a]	144566 ± 5.07[a]

ND- not detected

Values followed by different letters denote significant difference $p < 0.05$ for each analyte in FD strawberry across the rows and those followed by same letters denote no significant difference.

3.3 Whole, Dry, Wet and Brine Conditions

In order to understand the influence of dry or wet matrices on the headspace analysis of aroma volatile compounds, samples were analyzed in dry (whole and powder form) and in wet (whole in water and whole in brine) matrices. Similar, select volatile compounds were identified and the peak areas were compared with the dry and wet matrices in both FD strawberry and raspberry samples.

Twenty-nine analytes were tentatively identified in the dry matrices in FD strawberry (Table 4). Eight out of 29 analytes were not detected in the water matrix, and seven out of 29 were not detected in the salt matrix. These included furfural, furanones (3(2H)-furanone,2(1-hydroxy-1-methyl-2-oxopropyl)-2,5-dimethyl, 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-, ethanol, and 5-hydroxymethyl furfural 2-(2-butoxyethoxy)-), L-alpha-terpineol, linalool, and decanal, which are soluble in water and are not able to go to the headspace for extraction even when salt was added. Of the 21 volatile compounds detected, the peak area response for the compounds extracted by the fiber in FD strawberry was slightly higher for dry matrices (11 compounds) than for wet matrices (10 compounds). Similarly, Ducki et al. (2008) reported that the amount of compounds in dry cocoa products extracted by the fiber was higher than under wet conditions. When FD strawberry was extracted as whole and powder samples, significant differences ($p < 0.05$) in the peak areas existed for 11 of the volatile compounds.

Table 4. Comparison of the effect of different matrices (whole, powder, whole and water, and whole and 20% sodium sulfate) on the SPME extraction efficiency of key volatile compounds in FD strawberry (Peak area ± %RSD), n=3. 50/30 um DVB/CAR-PDMS fiber was used with 15 min for extraction time at 60°C

Compound	Whole	Powder	Whole in Water	Whole in 20% Sodium Sulfate
Butanoic acid, ethyl ester	3559721 ± 77.00^{a}	10923952 ± 0.03^{b}	13795932 ± 9.55^{c}	15021562 ± 8.85^{b,c}
Furfural	19445182 ± 53.31^{a}	6532651 ± 40.95^{a}	ND	ND
Oxime,methoxy-phenyl	1440284 ± 37.24^{a}	1240125 ± 3.55^{a}	5742229 ± 5.63^{b}	7851299 ± 11.53^{c}
Methyl hexanoate	730820 ± 13.11^{a}	1891277 ± 0.63^{a}	1054803 ± 36.19^{a}	847980 ± 11.95^{a}
3(2H)-furanone,2(1-hydroxy-1-methy l-2-oxopropyl)-2,5-dimethyl	4452794 ± 3.21^{a}	4669995 ± 3.24^{a}	ND	ND
2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3107453 ± 35.95^{a}	6279950 ± 2.67^{b}	2946616 ± 22.22^{a,c}	1859292 ± 40.81^{c}
Ethyl hexanoate	3623295 ± 24.44^{a}	6509357 ± 6.44^{b}	5878222 ± 24.23^{b}	1757713 ± 1.33^{a}
Hexyl acetate	634125 ± 9.51^{a}	2627191 ± 2.14^{b}	3346907 ± 28.07^{b}	3263514 ± 17.82^{b}
2-Hexen-1-ol,acetate	2000819 ± 22.16^{a}	4846235 ± 0.77^{b}	1704739 ± 91.45^{a,c}	2828275 ± 11.39^{a,b}
3(2H)-furanone,-4-methoxy-2,5-dimethyl (Mesifurane)	6600808 ± 19.54^{a}	13600772 ± 0.64^{b}	1785937 ± 14.44^{c}	6980647 ± 14.51^{a}
1-Octanol	3137311 ± 64.31^{a}	3695303 ± 44.5^{a}	3592951 ± 22.32^{a}	2814700 ± 78.67^{a}
Linalool	1151138 ± 14.17^{a}	1764165 ± 1.95^{a}	ND	5950901 ± 21.24^{b}
Nonanal	2497489 ± 5.04^{a}	269386 ± 2.13^{b}	5288716 ± 31.23^{c}	1982454 ± 7.25^{a}
4H-pyran-4-one,2,3-dihydro-3, 5-dihydroxy-6-methyl-	73012 ± 33.89^{a}	134112 ± 55.83^{a}	ND	ND
Ethanol,2-(2-butoxyethoxy)-	607885 ± 37.54^{a}	588287 ±0.95^{a}	ND	ND
L-alpha-terpineol	45966 ± 44.51^{a}	36107 ± 12.30^{a}	ND	ND
Decanal	1746636 ± 28.76^{a}	3542893 ± 1.38^{b}	ND	ND
Acetic acid, octyl ester	534955 ± 13.13^{a}	489652 ± 5.20^{a}	3947927 ± 14.10^{b}	1209814 ± 31.67^{c}
5-hydroxymethyl furfural	366275 ± 28.45^{a}	164398 ± 10.76^{a}	ND	ND
Propanoic acid, 2-methyl-, 3--hydroxy-2,2,4-trimethylpentyl ester	538535 ± 5.02^{a}	313581 ± 4.21^{a}	116387 ± 1.64^{a}	341064 ± 3.53^{a}
Butanoic acid, octyl ester	1271621 ± 25.06^{a}	4269375 ± 2.36^{b}	3022711 ± 11.48^{c}	1564762 ± 47.49^{a}
Octyl 2-methylbutanoate	1478588 ± 11.66^{a}	2948636 ± 2.50^{b}	2290430 ± 23.87^{a,c}	914771 ± 50.36^{a,d}
Butanoic acid, 3-methyl, -octyl ester	46941 ± 16.93^{a}	33274 ± 24.75^{a}	20332 ± 13.04^{a}	246798 ± 66.41^{b}
gamma-Decalactone	10689 ± 49.65^{a}	13484 ± 33.24^{a}	1807659 ± 84.48^{b}	314832 ± 49.55^{c}
1,6,10-dodecatrien-3 ol, 3,7-11 trimethyl (E)	2856563 ± 8.47^{a}	2329114 ± 4.88^{a}	4910414 ± 28.55^{a}	2689401 ± 30.73^{a}
Hexanoic acid,octyl ester	341559 ± 30.29^{a}	843773 ± 4.42^{b}	840087 ± 56.96^{b}	211550 ± 26.23^{a}
Butanoic acid,decyl ester	31801 ± 31.59^{a}	24403 ± 17.63^{a}	267772 ± 27.61^{b}	124987 ± 34.45^{b}
γ-Dodecalactone	23203 ± 12.02^{a}	52063 ± 7.59^{a}	414577 ± 4.95^{b}	274804 ± 40.15^{b}
7,9-Di-tert-butyl-oxaspiro(4,5)-deca-6, 9-diene-2,8-dione	162122 ± 15.63^{a}	285203 ± 10.17^{a}	117536 ± 16.59^{a}	184061 ± 8.47^{a}

ND- not detected

Values followed by different letters denote significant difference $p < 0.05$ for each analyte in FD strawberry across the rows and those followed by same letters denote no significant difference.

Overall, there was a significant difference ($p < 0.05$) for 10 volatile compounds when adding water or salt to whole FD strawberry for extraction (Tables 4). Some compounds that exhibited higher responses when extracted in water or salt solution included oxime, methoxy-phenyl, nonanal, acetic acid, octyl ester, gamma-decalactone, butanoic acid, decyl ester, and γ-dodecalactone. Adding salt to the whole strawberry seemed to generate greater peak responses only for oxime, methoxy phenyl, nonanal, acetic acid, octyl ester, gamma-decalactone, butanoic acid, decyl ester and γ-dodecalactone. Increasing the ionic strength brought the compounds to the volatile phase.

Twenty volatile compounds were able to be identified in the dry matrices in FD raspberry (Table 5). Three out of 20 analytes were not detected in the water and salt matrices. This is because ethanol,2-(hexyloxyl-), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl and 5-hydroxymethyl furfural are very soluble in water and are unable to go to the volatile phase for extraction. Of the 17 volatile compounds detected, the peak area response was slightly higher for the dry matrices (9 compounds) than for wet matrices (8 compounds). For FD raspberry,

significant differences ($p < 0.05$) in the peak areas existed for three of the volatile compounds when extractions for whole slices and powder were compared.

Table 5. Comparison of the effect of different matrices (whole, powder, whole and water, and whole and 20% sodium sulfate) on the SPME extraction efficiency of key volatile compounds in FD raspberry (Peak area ± %RSD), n=3. 50/30 um DVB/CAR-PDMS fiber was used with 15 min for extraction time at 60°C

Compound	Whole	Powder	Whole in Water	Whole in 20% Sodium Sulfate
Furfural	65959698 ± 16.60[a]	44461187 ± 5.19[a]	6965395 ± 14.98[a]	20127299 ± 5.33[a]
Oxime,methoxy-phenyl	1032884 ± 4.59[a]	1342056 ± 8.94[a]	7758191 ± 3.41[b]	5434575 ± 9.69[b]
2-Furancarboxaldehyde, 5-methyl	1650754 ± 5.81[a]	828639 ± 6.08[b]	46203 ± 19.13[c]	600652 ± 49.96[d]
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3one	2334662 ± 6.09[a]	2591935 ± 6.33[a]	879927 ± 14.75[b]	1157732 ± 21.79[b]
alpha-Phellandrene	7171798 ± 27.89[a]	6225020 ± 4.34[a,b]	8204421 ± 16.42[b]	431660 ± 10.65[c]
o-Cymene	2464968 ± 11.27[a]	4864463 ± 2.84[a]	7626342 ± 12.65[b]	3930305 ± 28.97[a]
D-Limonene	9189485 ± 13.72[a,d]	11473078 ± 4.61[b,c,d]	10725574 ± 15.89[c]	8913517 ± 21.19[d]
2(3H)-Furanone dihydro, 3-hydroxy-4,4-dimethyl	1219108 ± 5.02[a]	1103365 ± 12.92[a]	249529 ± 65.90[b]	290056 ± 54.13[b]
Linalool	158366 ± 5.70[a]	169026 ± 3.76[a]	512848 ± 11.27[a]	1559233 ± 47.94[b]
Nonanal	2077823 ± 8.50[a]	1504867 ± 6.13[a]	3136660 ± 3.96[b]	1874053 ± 13.55[a]
Ethanol,2-(hexyloxyl-)	980123 ± 6.98[a]	370022 ± 9.87[a]	ND	ND
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	2350148 ± 6.21[a]	2612961 ± 0.87[a]	ND	ND
5-Hydroxymethyl furfural	2964082 ± 9.11[a]	3163771 ± 3.11[a]	ND	ND
2H-Pyran-2-one,tetrahydro-6-propyl	1883719 ± 27.35[a]	1871741 ± 3.67[a]	357887 ± 22.76[b]	524200 ± 42.84[b]
Propanoic acid, 2-methyl-, 3-hydroxy-2,2, 4-trimethylpentyl ester	292219 ± 5.03[a]	77190 ± 8.46[b]	57715 ± 22.35[b]	192410 ± 3.53[c]
Caryophyllene	1329522 ± 58.38[a]	999833 ± 12.40[a]	3749802 ± 58.85[a]	3220045 ± 45.20[a]
α-Ionone	204055 ± 20.31[a]	146760 ± 7.67[a]	628675 ± 15.69[b]	528521 ± 16.86[b]
trans-β-Ionone	7068983 ± 31.89[a]	4819243 ± 8.67[a]	15802506 ± 4.63[b]	17129863 ± 5.27[b]
2H-pyran-2-one,tetrahydro-6-pentyl	2177198 ± 31.20[a]	1597128 ± 9.95[a]	722914 ± 44.08[b]	1347073 ± 30.59[a]
7,9-Di-tert-butyl-oxaspiro(4,5)-deca-6,9-diene-2,8-dione	128819 ± 5.07[a]	109711 ± 20.53[a]	71121 ± 12.73[a]	78274 ± 31.93[a]

ND- not detected

Values followed by different letters denote significant difference $p < 0.05$ for each analyte in FD raspberry across the rows and those followed by same letters denote no significant difference.

Significant difference ($p < 0.05$) was determined in eight volatile compounds when adding water or salt to whole slices of FD raspberry for extraction. Volatile compounds that demonstrated higher responses when extracted in water or salt solution included o-cymene, linalool, nonanal, α-ionone, and *trans*-β-ionone. The ionic strength of the sample usually increases with the addition of salt allowing the compounds to go to the volatile phase if they are soluble in water. However, this did not make much of a difference in this experiment.

3.4 Quantitation of Select Volatiles

This HS-SPME-GC-MS method was used to quantify select key aroma compounds of interest for FD strawberry and raspberry. These included furfural, mesifurane, and gamma-decalactone for FD strawberry and furfural, alpha-ionone and beta-ionone. In general, excellent correlation coefficients were obtained for the selected aroma compound standards ($R^2 > 0.99$) with the 50/30 μm DVB/CAR-PDMS SPME fiber.

Mesifurane, the major odor-active compound, found in fresh strawberry was determined to contain the highest concentration (93.25 ± 29.5 μg/g) in the FD strawberry compared to furfural (8.27 ± 1.06 μg/g) and gamma-decalactone (9.48 ± 0.31μg/g). Unlike the FD strawberry quantity determined for furfural, FD raspberry had a much greater amount (32.20 ± 4.27 μg/g). FD raspberry contained a lower content of alpha-ionone (0.20 ± 0.02 μg/g) and beta-ionone (0.30 ± 0.04 μg/g).

4. Conclusion

This study was able to identify several volatile compounds and quantitate select, important ones in FD strawberry and raspberry that are similar to those of the fresh fruits with HS-SPME-GC-MS. The most important aroma compounds found in FD strawberry were mesifurane followed by furfural, while furfural was the major compound identified in FD raspberry followed by α-ionone and β-ionone. In fresh strawberry, mesifurane and furaneol are the major aroma compounds detected followed by esters (Hadi et al., 2013; Jetti et al., 2007; Jouquand & Chandler, 2008; Forney, 2001). The key aroma compounds contained in fresh raspberry were raspberry ketone, α-ionone and β-ionone (Hadi et al., 2013; Du & Qian, 2010; Aprea et al, 2015). Concentrations of volatile compounds in strawberry and raspberry fruits can vary depending on cultivar, fruit ripening stage, environmental conditions, different extraction and analysis techniques. This investigation is important for determining the quality of freeze-dried ingredients of strawberry and raspberry alone or when added to other food products, such as cereals, nutritional bars, or confectionary items.

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Antioxidant Activities in Sweet Potatoes Leaves Steamed with Spices

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Abstract

Sweet potato leaves contain phenolic and flavonoids compounds which give them a potential antioxidant capacity. However, these antioxidant activities can be lost during cooking due to high temperature. In the aim to enhance their antioxidant activities, sweet potato leaves were steamed with antioxidant spices. The spices were used alone or mixed: nutmeg, Guinea pepper, cloves, nutmeg-Guinea pepper, nutmeg-cloves, Guinea pepper-cloves and nutmeg-Guinea pepper-cloves. The phytochemical analysis was carried-out on total phenolic compounds and flavonoids, while the antioxidant activities were determined via free radical-scavenging and inhibition power of lipid peroxidation. The results showed that cloves total phenolic content (513.33 mg Gallic Acid Equivalent (GAE)/g dry matter (DM)) increased to 1786.67 mg GAE/g DM when mixed with Guinea pepper. Flavonoids content was high in Guinea pepper-cloves (57.17 mg Quercetin Equivalent (QE)/g DM). In sweet potato steamed leaves, total phenolic compounds content is improved by incorporating cloves (625.83 mg GAE/g DM), while flavonoids content is slightly improved by Guinea pepper (125.00 mg QE/g DM). Among spices, cloves, Guinea pepper-cloves and nutmeg IC_{50} values (0.45, 0.83 and 1.50 µg/ml respectively) did not differ significantly to that of vitamin C (1.67 µg/ml), indicating that they had a good antiradical activity. The capacity of spices and steamed sweet potato leaves with and without spices to inhibit lipid peroxidation was higher than that of Gallic acid which is the standard reference. Spices, particularly cloves and Guinea pepper can improve antioxidant activities during sweet potato leaves steaming.

Keywords: antioxidant activities, lipid peroxidation, spices, steaming, sweet potato leaves.

1. Introduction

Sweet potato leaves are the most consumed leafy vegetables in Côte d'Ivoire (Agbo et al., 2014). As the other vegetables, they are recommended to human organism well-being because they contain high levels of vitamins, minerals, fiber, and phenolic compounds. Indeed, they are consumed for their capacities to contribute to overcome anemia and other nutrients deficiencies (Vyas et al., 2009; Gerla & Pietrzak, 2014). But sweet potato leaves are consumed after cooking. However, cooking occurs nutrients and phenolic compounds losses which increase with cooking time (Gokoglu et al., 2004; Chuah et al., 2008).

Leafy vegetables are also cooked with spices to improve their taste and their flavor. Moreover, spices contain phenolic compounds which give them a potential antioxidant activity (Shobana & Naidu, 2000). Such properties could be used to prevent micronutrients losses and increase food antioxidant capacities to overcome oxidative stress due to free radicals (García-Alonso et al., 2004). Indeed, Gayathri et al. (2004), in their studies, have shown that cooking vegetables with acidulate and antioxidant spices (tamarind, turmeric and onion) increase the retention of β-carotene in vegetables but they not focused on antioxidant activities. Therefore, it is opportune to appreciate spices impact on potential antioxidant activities in sweet potato leaves during cooking.

Several studies have been conducted on fresh and cooked sweet potato leaves. Some of them revealed important antioxidant activities in fresh leaves (Mibei et al., 2012; Agbo et al., 2018). Johnson and Pace (2010) have also shown a high level of radical scavenging activity in sweet potato leaves in comparison to the other parts of the

plant. However, there were important losses while cooking leaves with water (Zoro et al., 2014; Agbo et al., 2019). Therefore, steaming seemed to be an appropriate cooking method. Indeed, Johnson and Pace (2010) indicated that blanching of sweet potato leaves for 60 s resulted in increased flavonoids retention with level like those in fresh leaves and Tang et al. (2015) also revealed that steaming was good for the retention of total phenolic compound. But these studies have not determined the possible effect of spices on antioxidant activities in the leaves. This study was conducted to evaluate the potential antioxidant activities in sweet potato leaves steamed with antioxidant spices (cloves, nutmeg, and Guinea pepper).

2. Instrumentations

The laboratory instrument used were an orbital shaker incubator (BJPX-Kansas) from Biobase Biodustry (Shandong) Co. Ltd (Shandong, China) and a spectrophotometer (UV-1800) from Beijing Rayleigh analytical instrument Co., Ltd (Beijing, China). The water bath (SWB-A) and the drying-oven (BOV-T70C) were from Biobase Meihua Trading Co., Ltd (Shandong, China).

3. Chemicals and Reagents

Chemicals reagents: methanol (CAS 67-56-1), ethanol (CAS 64-17-5), aluminium chlorure (CAS 7446-70-0), Folin–Ciocalteu reagent (CAS 5995-86-8), 2-diohenyl-1-picryhydrazyl (DPPH) (CAS 1898-66-4), were purchased from Sigma-Aldrich (St- Louis, MO, USA). Sodium carbonate (CAS 497-19-8), vitamin C (CAS 50-81-7), potassium acetate (CAS 127-08-2), ammonium thiocyanate (CAS 1762-95-4), were purchased from VWR Prolabo Chemicals (Leuven, Belgium). Gallic acid (CAS 149-91-7), quercetin (CAS 6151-25-3), linoleic acid (CAS 60-33-3) and iron II sulfate (CAS 7720-63-0) were purchased from Merk KGaA, 64271 (Darmstadt, Germany). All chemicals were analytical grade.

4. Materials and Methods

4.1 Sample Collection

Sweet potato leaves were collected in « Gouro Market » a leafy vegetables wholesale trade in Abidjan, Côte d'Ivoire. Cloves and nutmeg were also collected in the same market while Guinea pepper was harvested in a field in Dabou.

4.2 Sampling Preparation

Sweet potato leaves were collected from 3 sellers chosen randomly, then, mixed and transported to the laboratory for the analysis. The leaves were destalked, cleaned, washed under running water. Guinea pepper were burned before being dried under the sun for 1 week. Nutmeg was grated with a grater while Guinea pepper and cloves were ground with a grinder (Moulinex Lm 241025). Spices were used alone or mixed in the proportion of (1:1) for 2 spices or (1:1:1) for 3 spices. The spices mixes were nutmeg-Guinea pepper, nutmeg-cloves, Guinea pepper-cloves and nutmeg-Guinea pepper-cloves.

4.3 Steam Cooking

The treatment used in this study was steaming. Sweet potato leaves were steamed for 20 minutes with a steam cooker (Severin DG 2438) without spices and with spices. A quantity of 600g of leaves was used for each steaming. For steaming with spices, spices were mixed to fresh leaves before steaming process to the concentration of 1g for 100g of leaves. For all kind of samples, two steaming were performed. After steaming, all samples were dehydrated at 16°C during 72h in a room under air conditioner.

4.4 Analyses

4.4.1 Total Phenolic and Flavonoid Extraction and Determination

Phenolic compounds were extracted according to Bala et al. (2014). Dried samples (10 g) were soaked in 100 ml of methanol/water solution (80:20, v/v). The mixture was shaken with an orbital shaker incubator (BJPX-Kansas, Biobase) for 24 hours. After that, it was filtered with Whatman paper n°1 and the filtrate was stored in a drying-oven (BOV-T70C, Biobase) at 40°C during 24 hours for solvent evaporation. The final paste was the crude extract.

Total phenolic compounds were determined by Folin-Ciocalteu method at 765 nm and expressed as gallic acid equivalents (GAE) in milligrams per gram DM (Dry Matter) using the standard curve of gallic acid (Mc Donald et al., 2001). Total flavonoids were determined at 415 nm and expressed as quercetin equivalents (QE) in microgram per gram DM using the standard curve of quercetin (Chang et al., 2002).

4.4.2 Free Radical Scavenging Activities and Anti-radical Power Determination

Extracts free radical scavenging activities were measured based on DPPH method (Parejo et al., 2000). This test

consists to evaluate the capacity of extract to fixed DPPH free radical by the measurement at 517 nm. Vitamin C (100 µg/ml) was used as standard and the percent inhibitory activity was calculated as follow in equation 1:

$$\text{Inhibition DPPH (\%)} = \frac{\text{Abs}_c - \text{Abs}_e}{\text{Abs}_c} \times 100 \quad (1)$$

Abs_c: absorbance of the control,

Abs_e: absorbance of the extract/standard.

The sample concentration which can inhibit 50% of DPPH (IC_{50}) was determined on graphic and allowed to calculate the efficient concentration (EC_{50}), which is the sample concentration which can reduce 1 µmol of DPPH (Equation 2). The anti-radical power was then determined in equation 3 (Kroyer, 2004).

$$EC_{50} = \frac{IC_{50}}{\text{DPPH solution concentration (mg of sample / µmol of reduced DPPH)}} \quad (2)$$

IC_{50}: Sample concentration which inhibe 50% of DPPH

EC_{50}: Efficient concentration for 50% of DPPH

$$ARP = \frac{1}{EC_{50}\ (\text{µmol of reduced DPPH / mg of sample})} \quad (3)$$

ARP: anti-radical power

4.4.3 Lipid Peroxidation Inhibitory Activity Determination

Lipid peroxidation inhibitory activity was determined according to ammonium thiocyanate test with some slight modifications (Lee et al., 2009). A quantity of 0.5 ml of samples extracts at graduate concentrations (0.20 to 6 mg/ml) was mixed to 0.20 ml of linoleic acid (20 mg/ml in ethanol 99%) and 0.40 ml of phosphate buffer (50 mM; pH 7.4). The mixture was heated in a water bath at 40 °C for 15 min. Then, 0.10 ml of mixture was added to the reaction mixture (3 ml of ethanol (70 %), 0.10 ml of ammonium thiocyanate (30 mg/ml) and 0.05 ml of FeSO4 (2.45 mg/ml in HCl 3.5 % (v/v)). The absorbance was determined at 500 nm after incubation at ambient temperature during 3 min. Gallic acid (100 µg/ml) was used as standard. The inhibitory percentage of lipid peroxidation was calculated in equation 4:

$$\text{Lipid peroxidation inhibitory (\%)} = 1 - \frac{\text{Abs}_e}{\text{Abs}_c} \times 100 \quad (4)$$

Abs_c: absorbance of the control,

Abs_e: absorbance of the extract/standard.

4.4.4 Statistical Analysis

Data analysis and graphic representations were made with Graph Pad Prism 5.00 (Microsoft U.S.A). Results made in triplicate were expressed as means with standard deviation. A one-way ANOVA was performed, and means were separated using Tukey test or Dunnett test ($p \leq 0.05$). For Dunnett test, samples were compared to vitamin C used as reference.

5. Results & Discussions

5.1 Total Phenolic and Flavonoid Compounds in Spices and Sweet Potato Leaves Steamed with and without Spices

The study has evaluated the antioxidant activities in sweet potato leaves steamed with and without spices. Previously, total phenolic and flavonoids compounds have been determined in spices (alone or mixed). Total phenolic content is higher in cloves (513.33 mg GAE/g DM) than in nutmeg and Guinea pepper (Table 1). This corroborate Halvorsen et al. (2006) results which showed that cloves are on the top food products which have high antioxidant content due to phenolic compound. Moreover, Viuda-Martos et al. (2010) have revealed high phenolic content in cloves (898.89 mg GAE/g DM).

Among mixed spices, Guinea pepper-cloves have the most important total phenolic and flavonoids content which were about 1786.67 mg GAE/g DM and 57.17 mg QE/g DM, respectively. However, total phenolic and flavonoids content are decreased in nutmeg-Guinea pepper, nutmeg-cloves, and nutmeg-Guinea pepper-cloves. According to Tangkanakul et al. (2009), Guinea pepper-cloves' total phenolic content is higher than that of turmeric which is about 1340.70 mg GAE/100g. The fact that total phenolic and flavonoids content are increased

in the mixe Guinea pepper-cloves suggest that Guinea pepper exhibit a positive synergistic effect over cloves. Such synergistic effect was also revealed by Shobana & Naidu (2000) with the mixtures of onion-ginger and ginger-garlic. Moreover, the fact that in all mixes' spices containing nutmeg there is a decrease of total phenolic and flavonoids compounds suggest that nutmeg probably exhibit a negative synergetic effect over Guinea pepper and cloves.

Fresh sweet potato leaves contain important content of total phenolic and flavonoid compounds. The total phenolic content is higher than that indicated by Su et al. (2019) which is about 46.7 mg GAE/g DM in Bonita (sweet potato leaves variety). According to Sun et al. (2014) polyphenols were very important in sweet potato leaves.

However, steaming process reduce total phenolic and flavonoid compounds. Among spices, only cloves improve total phenolic (625.83 mg GAE/g DM) content in steamed sweet potato leaves (Table 2). Flavonoids rate is slightly improved by the addition of Guinea pepper and cloves. This could be due to the total phenolic and flavonoids contained in these spices. It could be also due to the breakdown of tough cell walls during steaming process (Oboh & Rocha, 2007). Moreover, Oulai et al. (2015) have revealed an increase of total phenolic during blanching from 15 to 45 minutes. Nutmeg and spices mixes containing nutmeg (nutmeg-Guinea pepper, nutmeg-cloves, and nutmeg-Guinea pepper-cloves) present lowest total phenolic compounds and flavonoids contents while added to sweet potato leaves during steaming. This is probably because these spices have low total phenolic and flavonoids content which may have inhibitory actions with other constituents of the plant.

Table 1. Total phenolic and flavonoid compounds content in spices (alone and mixed)

Spices	Total phenolic (mg GAE/g DM)	Flavonoid (mg QE/g DM)
Nutmeg	84.50 ± 10.00^{b}	51.33 ± 10.20^{b}
Guinea pepper	69.58 ± 11.37^{a}	47.83 ± 8.34^{b}
Cloves	513.33 ± 49.64^{d}	42.67 ± 3.88^{b}
Nutmeg-Guinea pepper	93.75 ± 0.25^{b}	11.08 ± 1.13^{a}
Nutmeg-cloves	223.75 ± 1.30^{c}	14.00 ± 1.09^{a}
Guinea pepper-cloves	1786.67 ± 6.11^{e}	57.17 ± 0.14^{c}
Nutmeg-Guinea pepper-cloves	140.92 ± 0.38^{c}	10.67 ± 1.23^{a}

In row, values with different letter differed significantly (Tukey test, $p \leq 0.05$).

Table 2. Total phenolic and flavonoid compounds content in sweet potatoes leaves steamed with and without spices

Sweet potatoes leaves	Total phenolic (mg GAE/g DM)	Flavonoid (mg QE/g DM)
Fresh leaves	1285.83 ± 84.27^{d}	660.00 ± 60.70^{d}
Steamed leaves	174.75 ± 17.11^{b}	117.67 ± 10.87^{c}
Leaves nutmeg	100.92 ± 1.63^{a}	21.34 ± 1.70^{a}
Leaves Guinea pepper	134.17 ± 28.76^{b}	125.00 ± 5.00^{c}
Leaves cloves	625.83 ± 91.18^{c}	121.42 ± 4.40^{c}
Leaves nutmeg-Guinea pepper	52.00 ± 0.25^{a}	5.50 ± 0.25^{a}
Leaves nutmeg-cloves	82.58 ± 0.14^{a}	33.41 ± 0.29^{b}
Leaves Guinea pepper-cloves	141.25 ± 0.25^{b}	80.08 ± 0.14^{c}
Leaves nutmeg-Guinea pepper-cloves	48.33 ± 0.14^{a}	15.00 ± 0.25^{a}

In row, value with different letter differed significantly (Tukey test, $p \leq 0.05$)

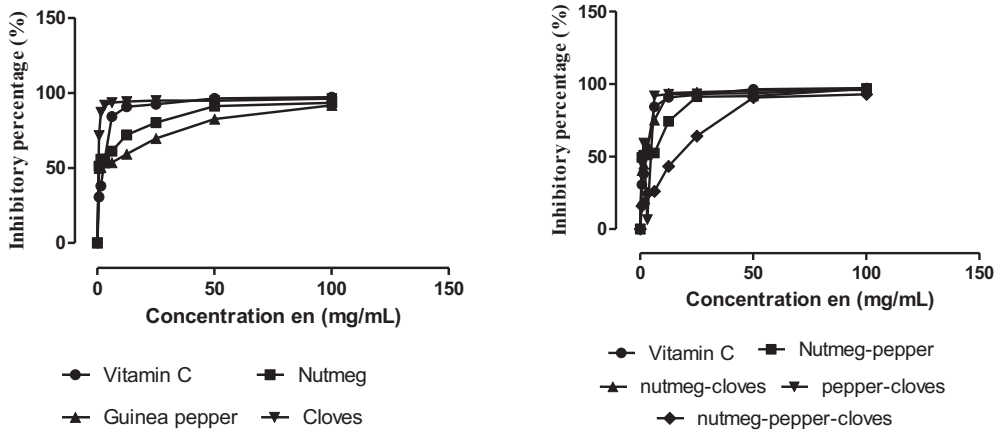
5.2 Antioxidant Activities in Spices and in Sweet Potato Leaves Steamed with and without Spices

5.2.1 Free Radical Scavenging Activities and Anti-radical Power of Spices and Sweet Potato Leaves Steamed with and without Spices

Vitamin C, spices and sweet potato leaves steamed with and without spices DPPH free radical scavenging activities are presented in figures 1 and 2. These activities increase with concentrations and suggest a profound antioxidant activity (Bala et al., 2014). Vitamin C inhibitory concentration of 50% DPPH (IC_{50}) is about 1.67 µg/ml. Among spices, cloves and Guinea pepper-cloves IC_{50} values (0.45 and 0.83 µg/ml respectively) do not differ significantly to that of vitamin C (reference), indicating a good antiradical activity and therefore an important antiradical power for cloves (290.21 µmol.ml/µg) and Guinea pepper-cloves (133.33 µmol.ml/µg)

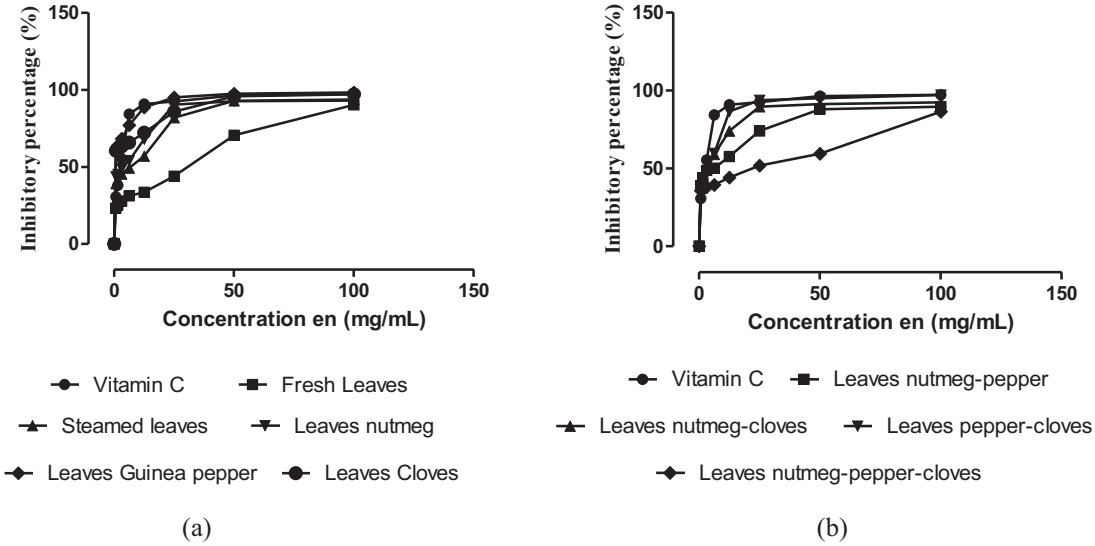
(Table 3). This is due to their total phenolic content and their positive synergetic effect. Indeed, Halvorsen et al. (2006) have indicated an antioxidant capacity of 125.55 mmol/100g in cloves. According to Ghadermazi et al. (2017), cloves potential antioxidant activities could be attributed to higher content of phenolic component such as eugenol and eugenyl acetate and to their hydrogen donating ability by which they are potent free radical scavengers.

Steaming sweet potato leaves with spices improve the antiradical activity. Indeed, sweet potato leaves steamed without spices IC_{50} value (7.83 µg/ml) become 0.27 µg/ml with Guinea pepper and 0.50 µg/ml with cloves. The antiradical power in these sweet potato leaves steamed with Guinea pepper and cloves are respectively about 373.13 and 200.00 µmol.ml/µg. This could be due to their capacity to resist to thermal denaturation (Shobana & Naidu, 2000) and to the fact that some process like microwave, steaming and boiling increase antioxidant contents of food (Halvorsen et al., 2006). However, the 3 spices mixes (nutmeg-Guinea pepper-cloves) IC_{50} value is low (16.00 µg/ml) and produced an inhibition of DPPH free radical scavenging activities in steamed leaves. This is reflected by a low IC_{50} value in sweet potato leaves steamed with nutmeg-Guinea pepper-cloves (23.17 µg/ml) and a bad antiradical power of 4.32 µmol.ml/µg. Such result is probably due to their low total phenolic content. In fact, there is a positive correlation between phenolic compound and antioxidant capacity (Tangkanakul et al., 2009). This suggests that if total phenolic compounds are low, antioxidant capacity should be also low and vice versa.



(a) (b)

Figure 1. Evolution of antiradical activities of vitamin C and spices alone (a) and mixed spices (b)



(a) (b)

Figure 2. Evolution of antiradical activities of vitamin C and sweet potato leaves steamed with one spice (a) and sweet potato leaves steamed with mixed spices (b)

Table 3. Spices and sweet potato leaves steamed with and without spices average and standard deviation IC_{50} and antiradical power (ARP)

Spices and sweet potato leaves	IC50 (µg/ml)	ARP (µmol.ml/µg)
*Vitamin C	1.67 ± 0.76^{a}	68.89 ± 30.06^{c}
Nutmeg	1.50 ± 0.50^{a}	72.22 ± 25.46^{c}
Guinea pepper	5.12 ± 2.93^{b}	23.39 ± 10.10^{b}
Cloves	0.45 ± 0.31^{a}	290.21 ± 143.61^{d}
Nutmeg-Guinea pepper	4.83 ± 1.44^{b}	21.79 ± 5.55^{b}
Nutmeg-cloves	2.17 ± 0.29^{b}	46.67 ± 5.77^{c}
Guinea pepper-cloves	0.83 ± 0.29^{a}	133.33 ± 57.74^{d}
Nutmeg-Guinea pepper-cloves	16.00 ± 0.50^{c}	6.25 ± 0.20^{a}
Fresh leaves	31.00 ± 1.80^{d}	3.23 ± 0.19^{a}
Steamed leaves	7.83 ± 0.76^{b}	12.85 ± 1.30^{b}
Leaves nutmeg	4.17 ± 0.58^{b}	24.34 ± 3.67^{b}
Leaves Guinea pepper	0.27 ± 0.01^{a}	373.13 ± 0.10^{d}
Leaves cloves	0.50 ± 0.01^{a}	200.00 ± 0.06^{d}
Leaves nutmeg-Guinea pepper	7.67 ± 1.15^{b}	13.23 ± 1.83^{b}
Leaves nutmeg-cloves	3.17 ± 0.76^{b}	32.78 ± 7.52^{b}
Leaves Guinea pepper-cloves	3.50 ± 0.50^{b}	28.97 ± 4.18^{b}
Leaves nutmeg-Guinea pepper-cloves	23.17 ± 2.93^{c}	4.36 ± 0.52^{a}

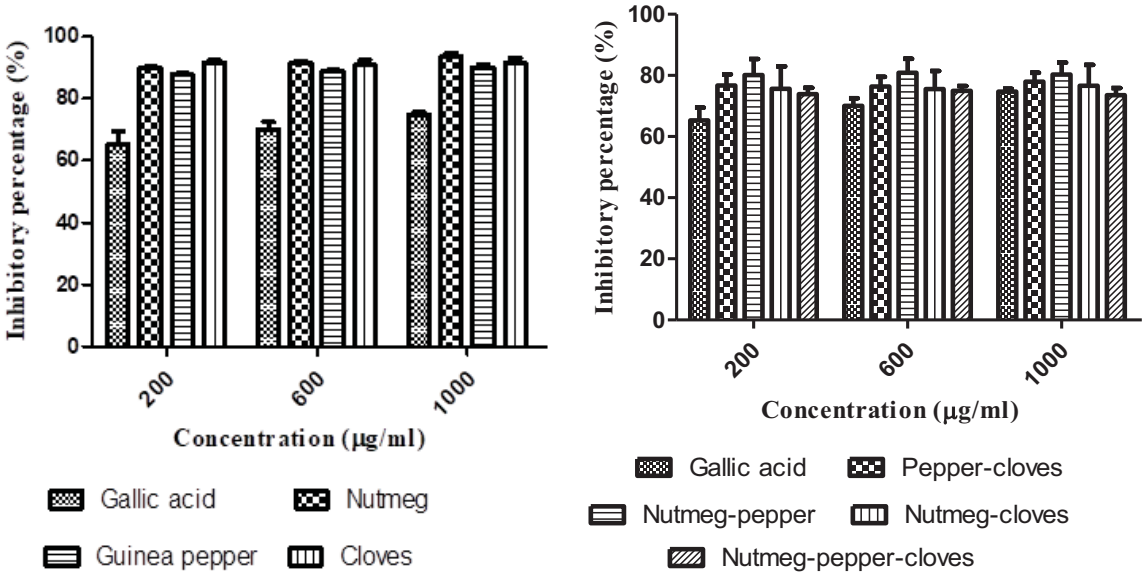
In row, value with different letter differed significantly (Dunnetts test, $p \leq 0.05$).

*Vitamin C was chosen as control in Dunnetts test.

5.2.2 Lipid Peroxidation Inhibitory Activities of Spices and in Sweet Potato Leaves Steamed with and without Spices

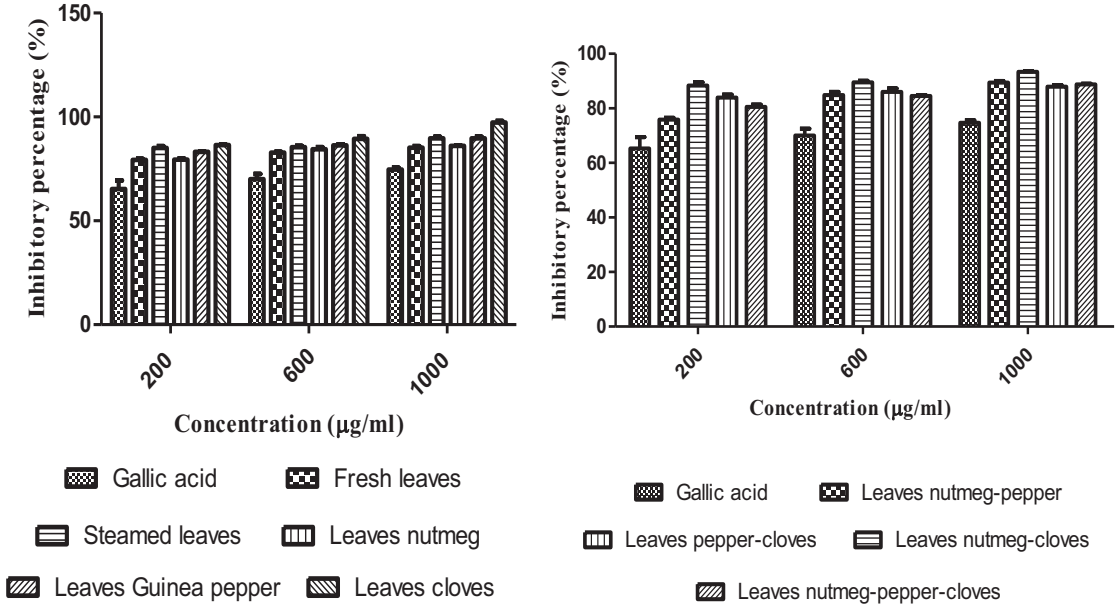
Lipid peroxidation inhibitory activity of spices and sweet potatoes leaves steamed with and without spices are upper than that of standard (gallic acid) and increase with concentration (200, 600 and 1000 µl/ml) (Figure 3 and 4). Indeed, gallic acid lipid peroxidation activity vary from 65.32 to 74.69 %. Among spices, lipid peroxidation activity is important in nutmeg and varies from 90.00 to 93.54 % (Figure 3). The fact that spices and sweet potato leaves steamed with and without spices have a lipid peroxidation inhibitory activity higher than that of gallic acid indicate their potentiality to inhibit linoleic acid lipid peroxidation. This is due to their flavonoids content which have the capacity to reduce peroxyl radicals by electrons transfer thanks to their low redox potential (Oktay et al., 2003; N'khili, 2009).

Steaming process improve fresh sweet potato leaves lipid peroxidation activity initially of 79.26 % at 200 µl/ml, to 85.02 % also at 200 µl/ml. Cloves and nutmeg-cloves also improve the lipid peroxidation rate. Indeed, in sweet potato leaves steamed with cloves, the lipid peroxidation activity varied from 86.46 to 97.34 % and in sweet potato leaves steamed with nutmeg-cloves it varied from 88.38 to 93.38 % (Figure 4). The improvement of lipid peroxidation rate during steaming could be due to this process. Moreover, Shobana & Naidu (2000) have revealed that boiling increases the effect of spices extract on lipid peroxidation.



(a) (b)

Figure 3. Evolution of lipid peroxidation inhibitory of gallic acid and spices alone (a) and mixed spices (b)



(a) (b)

Figure 4. Evolution of lipid peroxidation inhibitory of gallic acid and sweet potato leaves steamed with one spice (a) and sweet potato leaves steamed with mixed spices (b)

6. Conclusion

This study had shown total phenolic compounds, flavonoids, and antioxidant potential capacities of spices and sweet potato leaves by their high antiradical power and lipid peroxidation inhibitory activities. With such capacities, spices, particularly cloves and Guinea pepper, contribute to enhance antioxidant activities during sweet potato leaves steaming. Spices used alone have a better action. While mixed, synergetic or inhibitory actions were observed. Indeed, Guinea pepper-cloves exhibit a synergetic effect improving total phenolic compound and antiradical activities. But inhibitory actions were revealed with nutmeg-Guinea pepper, nutmeg-cloves, and nutmeg-Guinea pepper-cloves mixes. Sweet potato leaves should be steamed with spices to

increase their potential antioxidant activities. The best spices to be incorporated with sweet potato leaves are cloves used alone or mixed with Guinea pepper. This is grateful for households and will increase spices use while cooking leafy vegetables.

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Conflict of interests

The authors declare no conflict of interests.

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Physicochemical Properties and Antioxidant Activity of Mixed Oil of Safou (*Dacryodes edulis* (G. Don) H.J. Lam) from Several Trees

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Abstract

The valorization of lipids can be highlighted by industrial exploitation of safou pulp, very rich in these. However, the low yield of safou trees is lacking. It's necessary to estimate the technological potentialities of the oil obtained in order to describe the nutritional value and potential exploitation of this oil. Physical and chemical characteristics of this oil were examined. The aim of our study was to extract oil from fruits of several safou fruits and prepared a unique sample. This sample was essayed for its physicochemical properties and antioxidant activity. Results showed that the refractive index was 1.4693. The density and viscosity values were 0.9 mg/mL and 31.08 mPa/s, respectively. Acid and peroxide values were 6.17 mg KOH/g and 31.46 meq O_2/kg. Gas chromatography revealed that the major fatty acids were C16:0 (44.23%), C18:1 (30.50%), and C18:2 (19.62%). Triacylglycerols were the most important lipids (88.88% of total lipids). Spectrometric assessment of color led to the remarkable presence of the peaks associated with the visible absorption of carotenoids near 530 nm and chlorophyll pigments located between 610 and 670 nm. Antioxidant activity and DPPH radical scavenging activities of safou oil were exanimate. So, oil mixtures can be used, while varietal delimitation and mix some varieties for oil industry.

Keywords: mixing, safou, oil characteristics, antioxidant potential

1. Introduction

The safou tree, *Dacryodes edulis* (G.Don) H.J. Lam (Burseraceae family) is an important native resource of the Gulf of Guinea. It is a multifunctional plant and valued for its medicinal, nutritional, economic, and social uses. *D. edulis* is an important food commodity which has had enormous attention in central Africa (Mollet et al., 1995; Ajebesin, 2011). The pulp, the only edible part of the fruit, is particularly rich in lipids (50-70% of dry matter), indicating that safou could be an important source of oil. Proteins and sugars account for 10% and 30% of dry matter, respectively. The pulp contains minerals (3% of dry matter), and vitamins (Silou, 2012; Kadji et al., 2016). Kapseu et al. (1998) reported that one safou tree is able to produce about 40-50 Kg of oil, corresponding to 4-5 t/ha. The oil obtained of pulp presents a nutritional interest with an important proportion of linoleic (17 – 24%) and oleic (28 – 32%) acids. This oil can be used for food, pharmaceutical cosmetic industries (Ajibesin, 2011; Grigoras, 2012; Enengedi et al., 2019). Studies reveal that safou fruit can be used as an alternative source of fats and oil (Silou, 2012; Okpala, 2015; Akusu & Wordu, 2019).

However, the absence of varietal delimitation of this specie limits the optimal exploitation of safou oil. In fact, the specific taxa existing in *Dacryodes edulis* are not clearly differentiated and delimited.

The objective of the present study was therefore to extract oil from fruits collected from several safou trees in Franceville, assess the physical and chemical characteristics, and propose possible uses for this oil mixture as preliminary investigations into the scientific basis for its use for edible purposes.

2. Material and Methods

2.1 Study Area

This study was conducted from March 2019 in Franceville city (1°37'S, 13°35'E) in Gabon.

2.2 Material

Mature fruits were picked on 200 trees randomly selected in home gardens in Franceville city (Gabon). Samples of 20 fruits were manually collected for each tree and transported to the laboratory for oil extraction.

2.3 Methods

2.3.1 Oil Extraction

The extraction of lipids was made by the Soxhlet method by using multi-unit apparatus (model Soxtec System HT 1043). Five grams of dried and crushed safou pulp was placed in a cartridge of the Soxhlet apparatus for 200 samples. After a 1 hour extraction with hexane as solvent, the solvent was evaporated under reduced pressure and solvent traces eliminated by oven drying after extraction.

Apparatus characteristics:

- Maximum volume of sample: 65 ml (oil extraction cartridge measurements: 33 X 88 mm).
- Capacity: 6 samples.
- Temperature: from 20 to 285° during 10 minutes.

2.3.2 Sample Preparation

After oil extraction, a sample of 2 mL of safou oil was taken in each of 200 samples obtained. The mixture was prepared by successive returning. The mixing oil obtained was considered as the unique sample used from several analysis.

2.3.3 Oil Characteristics

Physical and chemical analysis (relative density, refractive index, viscosity, acid value, iodine index, saponification index, peroxide value, and unsaponifiable matter) of oil samples were carried out by using standard methods described by French Association for Standardization (AFNOR, 2000).

Relative density (or specific gravity)

Relative density is defined as mass per unit volume of a fluid. Oil density was determined picnometrically. The relative density of the oil was determined gravimetrically by employing the weight ratio of the oil to the equivalent amount of water according to the following formula:

$$\text{Relative density} = \frac{W2}{W1}$$

Where, W2 and W1 are the weights of oil and the equivalent amount of water, respectively.

Refractive index

The refractive value of oil was measured using a precision Abbe refractometer, having a measuring range of refractive index of 1,300 – 1,700 with the accuracy ±0.0002 at 25°C.

Viscosity

The viscosity of oil is a measure of its resistance to internal flow and an indication of its oiliness in the lubrication of surfaces. The kinematic viscosity of mixing safou oil was measured using Ostwald U-tube viscometer. The measurements were held in a controlled temperature bath at 25°C. The reference liquid was water where its viscosity (η_r) at 25° is 1.002 cP. The viscosity (η) of safou oil was calculated using formula:

$$\text{Viscosity } (\eta) = \frac{W \,.\, T \,.\eta r}{Wr \,.\, Tr}$$

Where W and T is the mass and time flow of the safou mixing oil and Wr and Tr is the mass and time flow of the water, respectively.

Spectrometric Evaluation of color

Place in a 10 mL volumetric flask, 1 g of fat previously melted at 40°C, complete to volume with carbon tetrachloride (CCl_4), the resulting solution was filtered. It measures the absorption between 400 and 700 nm at the maxima of the absorption of the main pigments of vegetable oils: carotenoids, chlorophyll (Helmy, 1990),

using a spectrophotometer (Model GENESIS 10).

Acid value

Ethanol was boiled on a water bath for a few minutes to remove dissolved gases. The boiled ethanol was neutralized by adding a few drops of phenolphthalein and about 10 ml 0.1N potassium hydroxide until a pale pink color was obtained. 2-3 g of oil was weighed into a 250 mL conical flask and 50 ml of hot previously neutralized ethanol was added. The mixture was then brought to a boil on a water bath and the hot mixture was titrated with 0.1N potassium hydroxide solution until the pink color (stable for few minutes) returned. The acid value was calculated from the relation shown in the equation:

$$\text{Acid value (mgKOH/g)} = \frac{V \,.\, N \,.\, 56.1}{W}$$

Where, V is titer value (mL), N is normality of KOH = 0.1N and 56.1 = molar mass of KOH and W is the weight of sample.

Free fatty acid (FFA)

The percentage free fatty acid (as palmitic acid) was obtained by multiplying the acid value with the factor 0.503 (Akubugwo et al., 2008). Thus, percentage FFA (as oleic acid) = 0.503 X acid value.

Saponification value

About 2 g of oil was weighed into a conical flask and mixed with 25 ml of 0.5 N ethanolic KOH. A blank was also prepared by taking 25 mL of alcoholic KOH in a similar flask. Reflux condensers were fitted to both flasks and the contents were heated in a water bath for one hour, swirling the flask from time to time. The flasks were then allowed to cool a little and the condensers washed down with a little distilled water. The excess KOH was titrated with 0.5 N HCl acid using phenolphthalein as an indicator. The saponification value was calculated using the equation:

$$\text{Saponification value (mgKOH/g)} = \frac{(a-b) \,.\, F \,.\, 56.1}{W}$$

Where, b = titer value of blank (mL), a = titer value of sample (mL), F = factor of 0.5 N HCl = 1 (in this case) and 28.05 = mg of KOH equivalent to 1 ml of 0.5 N HCl and W is weight of sample.

Peroxide value

Two grams of oil sample was weighed into a 500 mL conical flask and 10 mL of chloroform was added to dissolve the sample. This was followed by the addition of 15 ml of acetic acid and 1ml of freshly prepared saturated potassium iodide solution. The flask was immediately closed, stirred for about 1 minute, and kept at room temperature away from light for exactly 5minutes. About 75 mL of distilled water and 3ml of starch poison were added to the content of the flask and then shaken vigorously. Few drops of starch solution were added as an indicator. The liberated iodine was titrated against 0.01N sodium thiosulphate solution. The same procedure was carried out for blank and the peroxide value expressed in milliequivalent of active oxygen per kilogram of sample was calculated using

$$\text{Peroxide value (mg}O_2\text{/Kg)} = \frac{(V1-V0) \,.\, T \,.\, 1000}{W}$$

Where, V0 is the volume of the sodium thiosulphate solution used for blank, V1 is the volume of the sodium thiosulphate solution used for determination of sample, T is the normality of the sodium thiosulphate used, and W is the mass of the test sample in grams.

Iodine value

Approximately 0.2 g of oil sample was weighed into a dry 250 mL glass stopper bottle and 15ml of chloroform was added to the oil. About 25 mL of Wij's solution was then added and allowed to stand in the dark for 1 hour. Twenty mL of 10% Potassium Iodide 150 mL of water and 2 mL of starch poison at 0.5% were added and the resulting mixture was then titrated with 0.1N Sodium thiosulphate solution using starch as indicator just before the endpoint. A blank determination was carried out alongside the oil samples.

Iodine value was calculated thus:

$$\text{Iodine value} = \frac{(V2-V1) \,.\, N \,.\, 12.69}{W}$$

Where, V2 = titer value for blank, V1 = titer value for sample and 1.269 = Concentration conversion coefficient and W is weight of sample (g).

Unsaponifiable matter content

Unsaponifiable matter content of oil was determined following international chemical methods. The oil sample (5 g) was saponified with 50 mL of 2 N KOH methanolic solution for 1 hour. To the resulted mixture, 50 mL of distilled water was added. The unsaponifiable matter was extracted three times with 50 mL of hexane-ether (60:40). Organic fractions were collected, washed three times with 50 mL of distilled water, and then dried with sodium sulfate. Hexane-ether was removed in a rotary evaporator to recover the unsaponifiable matter which was then weighed.

Determination of fatty acids composition

Fatty acid composition of safou oil was determined using gas chromatography using a flame ionization detector (GC-FID) as fatty methyl ester (FAME) according to Rohman and Che man (2009) with slight modification. FAMEs were prepared by adding 1 mL of n-hexane and 400 µL of 1 M sodium methoxide to 60 µL of safou oil. The tube was stoppered, and the contents vigorously mixed with a dry water bath for 30 seconds. After this, 400 µL of 1 N hydrochloric acid and 1 mL cyclohexane were added. The organic phases were analyzed by gas chromatography on a Hewlett-Packard apparatus (HP 5890 series) fitted with a polar capillary column (Ref. HP FFAP No. 559436116, 25 m x 0.20 mm internal diameter, CA, USA) and a flame ionization detector (FID).

Analyses were carried out at a constant temperature of 235°C; injector and detector were set at 250°C; helium was used as carrier gas (160 kPa, 2 mL/min). FAMEs were identified by comparison of their retention times with those of authentic standards (Sigma Aldrich Co, St Louis, Mo., USA), and quantification was performed by internal standardization.

Determination of lipid classes

Free fatty acid (FFA), mono- (MAG), di- (DAG), and triacylglycerol (TAG) were determined by gas chromatography. Crude oil (1 g) was dissolved in 15 mL of chloroform. The extract underwent preparative column chromatography on silica gel (Merck 0.10-0.15 mm, Darmstadt, Germany). This gave three fractions: the TAG fraction was eluted with benzene, the DAG, and FFA fraction with a mixture of diethyl ether/benzene: 10: 90 (v: v) and the MAG fraction with diethyl ether. The purity of the three fractions was checked by thin-layer chromatography (TCL), using silica gel ascending development with hexane/diethyl ether 80: 20 (v: v) and visualization with chromic-sulphuric acid at 180°C. Each fraction was analyzed by gas chromatography with a FISON GC 8000 gas chromatograph equipped with an FID detector. The capillary column was 15 m CP Sil 8CB fused silica capillary (15 m x 0.25 mm internal diameter, 0.25 µm film thickness, Canada). Programmed oven temperature was used, starting at 80°C and then ramped to 360°C at 10°C/min; the detector temperature was 365°C. The carrier gas was helium. Samples (1 µL) were injected using an on-column injector. Peaks were recorded and identified against authentic standards by comparison of retention times. Peak areas were computed using internal normalization.

Antioxidant Activity Determination

The antioxidant activity of the safou oil sample was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Hatano et al, 1988; Bhatnagar *et al*., 2009). 2.5 mL of oil solution of various concentrations (0, 0.3875, 0.75, 1.5, and 3 mg/mL) prepared in methanol was added into 1.0 mL of methanolic solution of DPPH (0.3 mM) and kept in the dark at room temperature for 30 minutes. The freshly prepared solutions of DPPH at a concentration of 10^{-4} M (4 mL) were added to the sample (50 ± 1 mg). This mixture was vortexed for 20 s and absorbance was measured at 517 nm in the UV-Visible spectrophotometer (Model GENESIS 10) and then kept at room temperature. After incubation for 30 min, the decreases in absorbance at 517 nm were monitored for this sample. The radical scavenging activity (RSA) was estimated from the difference in the absorbance of the methanolic DPPH solution with and without oil (control). The percent inhibition was calculated from the following equation:

$$RSA\ (\%) = \left(\frac{A0 - A}{A0}\right) \times 100$$

Where A0 is the absorbance of the control and A is the absorbance of the samples.

Three replicates for each sample were assay.

The IC_{50} value representing the concentration of the compounds that cause 50% inhibition of radical formation

was obtained by interpolation from linear regression analysis (Stoilova et al., 2007).

The antiradical power (ARP) of extracts calculated as (Suja et al., 2005):

$$\text{ARP} = \frac{1}{EC50}$$

Antioxidant activity index (AAI) was calculated as follows:

$$\text{AAI} = \frac{Final\ concentration\ of\ DPPH\ (\mu g \cdot mL{-}1)}{IC50\ (\mu g \cdot mL{-}1)}$$

where the final concentration of the reaction was 49.79 $\mu g \cdot mL^{-1}$.

2.3.4 Statistical Analysis

For physicochemical parameters (without spectrum, fatty acid composition, lipid classes and antioxidant activity), values represented are the mean and standard deviations for three replicates. Statistical analysis was carried out by Excel version 8.0 software.

3. Results and Discussion

3.1 Physicochemical Characteristics

The physicochemical characteristics of mixing oil are presented in Table 1.

Table 1. Physicochemical characteristics

Physicochemical properties	
Parameters	Values (SD)
Relative density (20°C)	0.91 (0.006)
Refractive index (20°C)	1.4693 (0.001)
Viscosity (mPa.s)	31.08 (2.71)
Acid value (mg KOH/g)	6.17 (0.407)
Free fatty acid (%)	3.10 (0.407)
Peroxyde value (meq O_2/kg)	31.46 (3.76)
Saponifiable value (mg KOH/g)	201.49 (8.08)
Iodine value (mgI/100g)	68.48 (2.66)
Unsaponifiable matter (%)	0.72 (0.07)

SD: standard deviation

3.1.1 Physical Parameters

The physical properties that were studied are following: relative density, refractive index, kinematic viscosity, and spectrum in visible. The value of relative density was 0.91 ± 0.06 while the refractive index was about 1.4693 ± 0.00. The specific gravity and refractive index of *D. edulis* oil are within the range of those reported for most conventional edible oils (Nzikou et al., 2011; *Codex Alimentarius*, 2015); and higher than that of palm (Karleskind & Wolf, 1992; *Codex Alimentarius*, 2015).

Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. Viscosity value obtained was 31.08 mPa.s. This value is near to palm oil (34-36) viscosity and lower inside sunflower (40-44) oil (Blin et al., 2013).

Spectrometric Evaluation of color

The measurement of absorption between 400 and 700 nm provides information on the intensity of the color of the oil studied. The figure 1 represents the absorption spectrum in this area of the visible of oil sample of *Dacryodes edulis*.

The spectrum showed two (2) maximum absorbance

The visible domain of this spectrum showed two (2) maximum absorbances (1.68 and 0.96) at 420 and 660 nm, respectively.

As shown in this figure 1, the spectrum exhibits the light absorption maxima of carotenoids around 430 nm and 490 nm, and two very distinctive absorption maximum corresponding to the chlorophyll pigments between 610 and 670 nm. Mampouya (2006) also identified this double peak in the same areas in safou pulp oil from Congo.

These results corroborate those of Helmy (1990) who studying the oils extracted from seeds of citrus and cucurbits, identified absorption maxima at 400, 425, 455, 480 nm for carotenoids, at 610, 670 nm for chlorophylls.

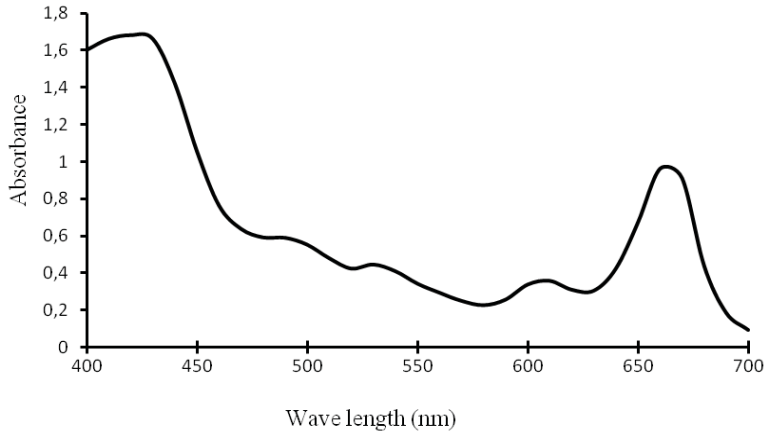

Figure 1. Spectrum in visible of safou oil

The absorption spectrum of our oil sample has another peak around 530 nm. This peak may well correspond to the unknown pigment than Helmy (1990) located at 525, 570, 575 nm. All this allows us to conclude that the fat of *Dacryodes edulis* contains carotenoids and chlorophylls which are majority and confirm the slightly greenish coloration observed to the naked eye.

3.1.2 Chemical Parameters

The chemical parameters that were studied are following: acid value, free fatty acid, peroxide value, saponification value, iodine value and unsaponifiable matter.

Acidity, which corresponds to free fatty acids (FFA) level, was around 6.17 mg KOH/g, slightly higher than the limit value of 4% indicated by *Codex Alimentarius* (2015) for crude oil. This high value probably results from increasing of oxidative degradation of oil during the long sample storage time before its extraction (more than 1 year) (Noumi et al., 2014). In the same way, the high peroxide value recorded (31.46 meqO_2/kg) was certainly due to oil alteration during storage as previously suggested by Foukou et al. (2009) for other crude oils extracted from African non-conventional oil crops. *Codex Alimentarius* (2015) proposes 10 meqO_2/kg for oil. The saponification value was relatively high (201.49 mg KOH/g) suggesting that this oil is suitable for soap making and the manufacture of lather shaving creams. The high saponification values recorded for the seed oils suggest that the oils contain high molecular weight fatty acids and low levels of impurities. This is evidence that the oil could be used in the soap making industry (Enengedi et al., 2019). Iodine value was 68.5 mgI/100g oil, close to other results obtained on *D. edulis* (Akusu & Wordu, 2019; Enengdi et al., 2019). These authors show that Iodine value range at 50.25 - 58.05. Unsaponifiable matter content was rather low (0.72%) compared to results previously published by Loemba-Ndembi & Silou (2006) and Ondo-Azi et al. (2014) on safou pulp oil.

3.2 Fatty Acid Composition

Fatty acids (FAs) are part of the lipid class. They are saturated or unsaturated (Nagy & Tiuca, 2017). The major saturated fatty acid in *D. edulis* pulp oil was palmitic acid (44.23%); the main unsaturated fatty acids were oleic acid (30.50%) and linoleic acid (19.62%) with small amounts of stearic (2.88%) and linolenic (0.78%) (Table 2). The fatty acids order (%P>%O>%L>%S>%Ln) corresponds to the profile described by several authors on safou oils (Silou et al., 2002; Ondo-Azi et al., 2014). In opposite to the other authors, Akusu & Wordu (2019) reported another fatty acid order never described in safou oil: %O>%L>%P>%S, obtained on one sample from Nigeria. It's different from four profiles described on fatty acids from safou oil from Gabon among 213 safou trees respectively (Ondo-Azi et al., 2014).

However, oil of safou pulp is therefore an important source of unsaturated fatty acids. That is an important information because polyunsaturated fatty acid-rich diet contributes to the beneficial effects on human health (Fomuso & Akoh, 2002) and should help the general population to live longer (Carvalho & Caramujo, 2018).

Table 2. Fatty acid composition of mixing safou oil

Fatty acid compositions			
Fatty acids	Percentage	Fatty acids	Percentage
C14:0	0.11	C18:0	2.88
C14:1	0.03	C18:1	30.5
C15:0	0.24	C18:2	19.62
C16:0	44.23	C18:3	0.78
C16:1	0.18	C20:0	0.14
C17:0	0.15	C20:1	0.07

Safou oil contains linoleic acid (30.5), which is an essential polyunsaturated fatty acid. This type of fatty acid helps in the prevention of vascular heart diseases and are supplied only in food. Besides, the oil of safou has a greater amount of oxidative stability unlike those with unsaturated acids (Ikhuoria & Maliki, 2009; Okpala, 2015).

3.3 Lipids Classes

Table 3 reported lipid classes, free fatty acids (FFA), Monoacylglycerols (MAG), Diacylglycerols (DAG) and Triacylglycerols (TAG) are found.

Table 3. Lipid classes of mixing safou oil

Lipid classes	
Glycerides	Percentage
FFA	5.48
MAG	ND
DAG	5.68
TAG	88.88

FFA: Free Fatty Acids, MAG: Monoacylglycerol, DAG: Diacylglycerol, TAG: Triacylglycerol, ND: Not Detectable

Regarding the lipid fractions (Table 3), triacylglycerol was the predominant lipid class (88.88% of the total lipid classes), followed by diacylglycerol (5.68%), free fatty acids (5.48%), and monoacylglycerol were present but in very low amount. The presence of free fatty acids may be due to the partial enzymatic hydrolysis of triacylglycerol during the storage of the dried safou pulps.

3.4 Antioxidant Activity

The antioxidant activity of the safou pulp oil is presented in table 4. So, inhibition of DPPH was 12.9%. The DPPH radical scavenging activity expressed as IC50 value of the safou oil mixed was 142.25 µg/mL and the ARP value is 7.10-3 (Table 4). IC50 value is inversely proportional to the antioxidant activity. According to the IC50 value, safou pulp oil can be categorized as oil with considerable antioxidant potential. Studies reported that the IC50 value of selected vegetable oil extracts, namely soy, sunflower, rapeseed, corn ranged from 29.7 to 34.0 µg/mL (Aleksander et al., 2008). The values reported were low to safou pulp oil, but pomegranate seed oil, sesame oil, and blackcurrant seed oil have a high level comparable to safou oil 78%, 70%, and 76%, respectively (Badea et al., 2015).

Table 4. Antioxidant activity in mixing safou oil

Characteristics	Amount
DPPH scavenging (%)	12.9
IC50 (µg)	142.25
ARP	7.10^{-3}
AAI	0.35

ARP: Antiradical Power, AAI: Antiradical Activity Index

The antiradical activity index (AAI) of safou pulp oil amount is 0.35. This value shows that this oil has a moderate antioxidant activity because AAI=0.35<0.5, according to Scherer and Godoy (2009). These authors established the following criteria of AAI values for plant extracts: poor activity < 0.05 < moderate < 1.0 < strong

< 2.0 < very strong.

For an edible oil, it's very important to have an antioxidant activity because it can this oil have a great interest for nutrition. It can absorb free radicals and appear to have a positive impact on cardiovascular and cancer ailments, as attributed to the Mediterranean diet (Agbiolab, 2014).

4. Conclusion

The present work envisages that oil obtained from the mixing of several 200 oil samples presents similar characteristics as separated safou oil samples. Acid value shows a greater value; this value can be reduced after refining step. So, many of the physicochemical properties of the mixing oil of safou studied have close similarities with other commercials as groundnut and palm. DPPH radical-scavenging activity obtained was 12.9%. The results of our study suggest that to cope with the weakness of raw materials to enhance this bioresource, mixing oils can be a solution. The antioxidant activity revealed shows that safou oil can be used in a diet for cardiovascular and cancer diseases.

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Effect of *Autranellacongolensis* on Lipid Profile of Rats' Brain with Experimentally Induced Alzheimer's Disease

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Abstract

Lipids are essentials components of the brain. Changes in brain lipid composition affect the physical and functional properties of the neuronal cell membrane and have been implicated in the physiopathology of Alzheimer disease (AD). We evaluated in this study the effect of hydroethanolicbark extract of *A. Congolensis* on lipid profile of rats' brain with experimentally induced AD. The experimental model consisted of female rats, which received orally for 8 consecutive weeks a single dose of 50 mg/Kg b.w./day of aluminum trichloride ($AlCl_3$) (except control group) followed by distilled water (disease control group) or doses of the extract (150 or 300 mg/Kg b.w./day) or vitamin E (100 mg/Kg b.w./day) or galanthamine (2 mg/Kg b.w. /day). Brain cholesterol, phospholipids and plasmalogenlevels and fluidity were evaluated. Brain membranes ATPase activities, Ca^{2+}, $Mg^{2+}$and glucose levels were also assayed. Significant modifications of brain lipid composition and fluidity were observed in disease control group compared with control. In addition, Na^+, K^+-ATPase and Mg^{2+}-ATPase activities significantly decreased, the level of intracellular Ca^{2+} increased, Mg^{2+} content decreased and brain glucose level was significantly higher. Standard drugs (vitamin E,galanthamine) showed a negative effect on brain lipid profile. The extract of 150 mg showed significant improvements of brain lipid profile and fluidity. It also indicated improved brain ATPase activities, ions and glucose brain homeostasis. The extract (150 mg/Kg b.w. dose) by maintaining the brain lipid composition may protect neuronal cell membraneand probably preventing the progression of AD.

Keywords: Alzheimer disease, *Autranellacongolensis*, glucose metabolism, lipids metabolism, Mg^{2+}-ATPaseandNa^+, K^+-ATPase

1. Introduction

The brain is the organ with the highest lipid content after fatty tissue (Sastry, 1985). The brain membranes are mainly composed of phospholipids, cholesterol and glycolipids which determine their fluidity, stability and permeability. The maintenance of the composition and the fluidity of the membranes are necessary factors for the proper functioning of the proteins integrated in the membranes, or even the membranes bound enzymes activities (like Na^+, K^+-ATPase and Mg^{2+}-ATPase) or receptors and ion channels(Farooqui & Horrocks, 1985). Changes in the distribution and the content of these lipids lead to a loss of functions associated with the membranes. For instance, the depletion of membrane cholesterol in primary neuron cultures induces the relocation of N-methyl D-Aspartate receptors (NMDAR) out of lipid rafts, thus inhibiting the cytotoxic response. Inversely, an enrichment of cholesterol in the membranes leads to a grouping of lipid rafts promoting the interaction of proteins and signal transduction in the cell (Marquer *et al*., 2011). In addition, decrease in phosphatidylserine levels in membranes affects acetylcholine receptor (Sunshine & McNamee, 1992), activities of Na^+, K^+-ATPase and diacylglycerol kinase (Spector & Yorek, 1985). Such changes in the levels of cholesterol and phospholipids have been observed in the brain and blood of Alzheimer's disease (AD) patients (Goodenowe *et al*., 2007; Varma *et al*., 2018; Goodenowe & Senanayake, 2019).

Alzheimer's disease (AD) is a disease affecting the brain, just as coronary disease affects the heart (Alzheimer's Association, 2019). It is mainly characterized by the presence of extracellular aggregation of amyloid-beta (Aβ) peptide and intracellular neurofibrillary tangles in the brain (Mattson *et al*., 2004). The amyloid hypothesis proposed by Hardy & Higgins remains the dominant model of the pathogenesis of AD (Selkoe & Hardy, 2016). In this hypothesis, the Aβ peptide that is generated by enzymatic cleavage of amyloid precursor protein (APP: an

integral membrane protein) interact with neuronal cell membranes (Selkoe, 1993). Two types of Aβ-membrane interactions have been found (Wong *et al*., 2009). Aβ peptide can insertinto the cell membrane and form a pore-like structure that increase the permeability of membrane and may trigger cell death signal. The second interaction is the binding of Aβ peptide onto the surface of the membrane that affects the functions or activities of membrane bound enzymes, receptors and ion channels (Wong *et al*., 2009). Many studies found that membrane lipid environment is a key factor for Aβ-membrane interactions and its toxicity (Arce *et al*., 2011; Sani *et al*., 2011). Recently, it has been demonstrated that lipid model mimic healthy and Alzheimer diseased states of the neuronal membrane interact differently with Aβ1-42(Drolle, Negoda Hammond, Pavlov & Leonenko, 2017). In fact, in lipid model mimic diseased state of neuronal membrane, the amyloid toxicity was significantly higher than in lipid model mimic healthy state of neuronal membrane (Drolle *et al*., 2017). Based on these data, it has been proposed that changes in lipid membrane due to aging and AD may trigger amyloid toxicity as new hypothesis for AD. Therefore, Drolle *et al.,* (2017) suggested that maintaining the lipid composition and structure of neural membranes in healthy state may serve as a new preventive strategy against AD.

The drugs approved for the treatment of AD like galanthamine are only symptomatic treatments. In addition, the number of people with dementia continues to increase and would reach 131.5 million by 2050 (Cummings *et al*., 2017). There is a great need to prevent or effectively treats this disease (Cummings *et al.*, 2017). Bioactive compounds such as terpenoids, flavonoids, sterols, alkaloids extracted from plants are known as important sources of potential agents for treatment or preventing AD (Jesky & Hailong, 2011; Man *et al*., 2012). The genus *Autranella* or *Mimusops* belongs to the family of Sapotaceae (Jahan *et al.*, 1995), *Mimusopscongolensis* (*Autranellacongolensis*), one of species of this genus is indigenous to Cameroon (Fokou, 2006). *Mimusops* species are constantly used in Indian traditional medicine. *Mimusopselengi* L. is traditionally used as cardiotonic or for its hypotensive and antibacterial activity. It has been indicated that, administration of ethanolic bark extract of *M. elengi*L. (100, 300, 600 mg/kg, *p.o*) in Triton WR-1339 induced hyperlipidemia rats, significantly reduce levels of triglyceride and total cholesterol while increasing HDL levels (Ghaisa *et al*., 2008). Hanumanthacha & Milind (2012), also found that ethanolic bark extract of *M. elengi* (100, 200 mg/kg *p.o*) improve memory in mice. The bark of A. *Congolensis* is used traditionally in Cameroon like *M. elengi* L. for its cardiotonic or hypotensive proprieties. Previously, phenolic compounds (24-feruloyltetracosanoic acid and (+)-catechin), sterol ((24R)-Stigmast-7,22(E)-dien-3-α-ol (chondrillasterol)) and pentacyclictriterpenoids (taraxerol 3-hexacosanoate, taraxerol 3-tetracosanoate, taraxerol 3-docosanoate, taraxerol and taraxerone) have been found as constituents of the barks of *A. congolensis* (Fokou, 2006). Most of these compounds were also found in the barks of the *Mimusops* species. Additionally, Pentacyclictriterpenoids and plant sterol are compounds able to cross the blood-brain barrier and accumulate in the brain (Pradesa *et al*., 2011; Vanmierlo *et al*., 2012). Due totheir structural similarity with cholesterol, these compounds are modulators of lipid membrane physical properties (Abboud *et al*., 2016; Haralampiev *et al*., 2017), cholesterol-dependent cellular processes and lipid metabolism (Yang *et al*., 2004). The present study, therefore evaluate the effect of *A. congolensis* on lipid profile of rats' brain with experimentally induced AD.

2. Methods

2.1 Plant Material and Preparation of Hydroethanolic Extract

The leaves and barks of *A. congolensis* were collected in the East Region of Cameroon. The plant material was identified at the National Herbarium of the Institute of Agricultural Research for Development (IRAD), Yaoundé, Cameroon. Plant barks were cut into small pieces of 2-5cm and dried in the laboratory at room temperature. The dried barks were ground to fine powder using a grinder. A sample of 200 mg fine powder was extracted with 1000 mL of ethanol: water (50:50 v/v) extractant for 48 h at room temperature, and then filtered. The obtained filtrate was evaporated and the crude extract was stored until used.

2.2 Animals and Experimental Model

2.2.1 Animals

The experiment was performed on female Wistar rats. Body weight was 250-300 g. The rats were housed four in a cage, at a constant room temperature (22 ± 1 °C) under a 12 h Light: 12h Dark (light 08.00-20.00 h) cycle and acclimated 1 week before use. Food and water were provided *ad libitum.* Animals were cared for in accordance with the principles of the *"Guide to the Care and Use of Experimental Animals"*(Committee on Care and Use of Laboratory Animals, 1985).

2.2.2 Experimental Protocol

The animals were randomly divided into 6 groups of 8 rats each (table 1). Eight rats were used as control group. The remaining rats received a single dose of aluminiumtrichloride*(p.o*). One hour later, the animals received doses of the extract or vitamin E (alpha tocopherol) or galanthamine and distilled water for control and disease control groups. Doses of aluminum, extract or reference drugs were prepared in distilled water and administered once daily (5 mL/kg b.w.*p.o*) for 8 consecutive weeks. Vitamin E served as standard drug for protecting lipid cell membrane and galanthamine served as standard drug for AD treatment. The weight of animals was monitored weekly with an electronic scale throughout the study.

Table 1. Experimental design and drug doses in different groups of rats

Groups	Name of groups	Compounds and drugs administered
NC	Normal Control	Distilled water
DC	Disease Control	Aluminium (50 mg/Kg bw/day) + Distilled water
AC150	Test group 1	Aluminium (50 mg/Kg bw/day) + hydroethanolic extract of *A. congolensis* (150 mg/Kg bw/ day)
AC300	Test group 2	Aluminium (50 mg/Kg bw/day) +hydroethanolic extract of *A. congolensis* (300 mg/Kg bw/ day)
VE100	Standard treatment 1	Aluminium (50 mg/Kg bw/day) + vitamin E (100 mg/Kg bw/ day)
GAL2	Standard treatment 2	Aluminium (50 mg/Kg bw/day) + galanthamine (2 mg/Kg bw/ day)

2.3 Brain and Blood Tissue Collection and Preparation

At the end of the experiment period, animals were sacrificed by decapitation after 12 hours of fasting. Blood samples were collected from trunk in tubes containing EDTA and centrifuged to 1500 g at 4 °C for 15 min. The supernatant containing the plasma was collected and stored at -20°C. The brains, liver, kidneys and heart were rapidly removed, weighed and thoroughly washed with isotonic saline. Each brain was mid-sagittal divided into 2 portions. The first portion (right hemisphere) was homogenized in 10 volume ice-cold (0-4 °C) buffer (50mM TRIS-HCl, pH 7.4 and 300 mM sucrose). Then, the homogenate was centrifuged at 1000 g for 10 minutes to remove nuclei and debris. The resulting supernatant were then immediately stored at -80°C and used for the enzymes activities assay and for the determination of magnesium, calcium and glucose contents. The second portion of brain (left hemisphere) was used for lipid profile analysis.

2.4 Lipid Analysis

2.4.1 Lipid Extraction

Total lipids were extracted according to the method of Folch *et al*., (1951). Briefly, the tissues were homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (0.5 g in 10 mL of solvent mixture). The homogenates were centrifuged to recover the liquid phase. Then, the solvent was washed with 0.2 volume (4 mL for 20 mL) 0.9% NaCl solution. After vortexing some seconds, the mixture was centrifuged at 2000 g to separate the two phases. The lower chloroform phase containing lipids were stored at -20 C until used.

2.4.2 Determination of Total Phospholipids Content

Total phospholipids was determined according to the method of Stewart (1980), based on the formation of a colored complex between phospholipids and ammonium ferrothiocyanate. An aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 2 mL of chloroform. One millimeter of reagent [aqueous solution of iron trichloride (27 g/L) and ammonium thiocyanate (30 g/L)] was added and the mixture homogenized in a tube shaker (Vortex) for 30 seconds, then centrifuged for 10 minutes at 6000 rpm. The absorbance of the lower chloroform phase was measured at 488 nm against the blank (2 mL chloroform + 1 mL of reagent). A standard range was carried out for quantities varying from 10 to 100 µg of phosphatidylcholine (PC). The phospholipids content were expressed as µg equivalent phosphatidylcholine (µg Eq PC) per mg of total protein (µg Eq. PC/mg protein).

2.4.3 Determination of Plasmalogens Content

Plasmalogen content was determined according to the method of Gottfried and Rapport (1962), based on the reaction of vinyl ethers content of plasmalogen with iodine. An aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 0.5 mL of methanol. Then 0.5 mL of iodine reagent (6 x 10^{-4} N iodine in 3 % aqueous KI) was added. The mixture was

stirred vigorously for 1 minute and left at room temperature for 10 minutes. After addition of 4.0 mL of 95% ethanol, the absorbance was read at 355 nm against a sample blank in which 0.5 mL of 3% KI was substituted for the iodine reagent. The molar extinction coefficient of iodine was reported to 27,500. The plasmalogen content was expressed in brain as nmoles of vinyl groups/mg total protein and in erythrocytes and plasma expressed as mmoles of vinyl groups/L (mM).

2.4.4 Determination of Cholesterol and Triglycerides Levels

Cholesterol

Cholesterol levels were determined enzymatically according to the method of Richmond (1973), following a kit protocol (Chronolab references number 101-0576; 101-0593; 101-0526, 101-0440). Briefly, an aliquot of lipid extract was taken in a glass test tube and evaporated to dryness in a stream of nitrogen. The dried lipid extract was dissolved in 0.5 mL of methanol. 10 µL of this methanolic extract was taken in a glass test tube and 1 mL of working reagent was added. The solutions were mixed and incubated for 10 minutes at room temperature (25°C), and the absorbance of the brain sample or plasma sample (10 µL plasma + 1 mL working reagent) or standard (10 µL standard cholesterol + 1 mL working reagent) were read against the blank at 505nm. The cholesterol content were expressed in brain as µg/mg total protein and expressed in plasma as mmol/L (mM).

Triglycerides

Triglycerides levels were also determined enzymatically according to the method of Fossati and Principe (1982), following a kit protocol (Chronolab reference numbers 101-0241; 101-0016; 101-0268; 101-0052 and 101-0053). Briefly, 1mL of working reagent was added to 10 µL of plasma sample or standard. The solutions were mixed and incubated for 10 minutes at room temperature (25°C), and the absorbance of the samples and standard were read against the blank at 505 nm. Triglycerides content were expressed as mmol/L (mM).

2.5 Enzymes Activities, Calcium, Magnesium and Glucose Levels Assay

2.5.1 ATPases Activities Assay

Na^+, K^+-ATPase and Mg^{2+}-ATPAses activities were assayed by a method adapted from Rohn *et al*., (1993). The method is based on determining the amount of phosphate released by enzymatic cleavage of ATP in absence or presence of ouabain. For each rat brain sample, two test tubes were used. 450 µL of assay buffer (NaCl 150 mM, KCl 15 mM, MgCl 15 mM EGTA 0.1 mMin histidineHCl-TRIS 30 mM pH 7.5) containing 50 µg of brain homogenate protein was added to the first tube. The second tube was made in same condition however, ouabain was added in the final concentration of 1.5 mM. The tubes were pre-incubated at 37°C for 10 minutes, and the assay was started with the addition of 50 µL of ATP.Na_2-TRIS (final concentration 5 mM) thus, the final volume of reaction was 500 µL. After 60 min, the reaction was terminated by the addition of 500 µL of 15% (w/v) of coldtrichloroacetic acid solution. The level of inorganic phosphate present in solution was quantified following the calorimetric method of (Baginski *et al*., 1967) and was used as a measure of ATPase activity. The absorbance values obtained were converted to activity values by linear regression using a standard curve of sodium monobasic phosphate that was included in the assay procedure. The Na^+, K^+-ATPase activity was determined by subtracting the ouabain sensitive activity (Mg^{2+} -ATPase activity) from the total ATPase activity level (first tube). The activity of ATPases was expressed as µmol of inorganic phosphate liberated from ATP by 1 mg of protein fora duration of one hour (µmol Pi/mg protein/hour).

2.5.2 Calcium, Magnesium and Glucose Levels

Calcium

Calcium ions ($Ca^{2+)}$ were measured following the kit Sigma-Aldrich protocol (Number MAK022) and expressed as µmol/mg total protein.

Magnesium

Magnesium ions (Mg^{2+}) were measured following the kit Sigma-Aldrich protocol (NumberMAK026) and expressed as µmol/mg total protein.

Glucose

Glucose levels in brain and plasma were measured following the kit protocol Sigma-Aldrich (Number GAGO20) and expressed in brain as µmol/g tissus and in plasma expressed as millimole/L (mM)

2.6 Total Protein

The total protein levels were determined by the method described by Lowry *et al*., (1951).

2.7 Statistical Analysis

The statistical package for social sciences (SPSS) software version 20.0 (Chicago-IllinoisInc.) was used and One-way analysis of variance (ANOVA) with Tukey's test was performed to compare variability amongst the groups. Significant differences were detected at 95% confidence interval and the results obtained were expressed as Mean ± Standard Deviation.

3. Results

3.1 Effect of Extract on Body Weight Variation and Relative Weightsof Brain, Liver and Heart

Table 2 shows the initial, final and body weight variation as well as the relative weights of brain, liver and heart. We observed a significant decrease in body weight and brain relative weight in disease control rat in comparison with control rats. Rats treated with extract at 150 mg/Kg b.w. showed a significant lower body weight and brain relative weight loss than disease control group. The vitamin E treated group also showed a significant lower brain relative weight loss compared to disease control group. No significant difference in liver and heart relative weight were observed between groups.

Table 2. Body weight (BW) variation and relative weights of brain, liver and heart

Groups	Initial BW (g)	Final BW (g)	Brain relative weight (g x10^{-3})	Liver relative weight (g x 10^{-3})	Heart relative weight (g x 10^{-3})
NC	274.4±19.3	281.6±13.1 (+2.3%)^{a}	7.6±0.4^{a}	31.5±2.5^{a}	3.2±0.2^{a}
DC	279.4±13.1	264.4±11.4 (-7.5%)^{b}	6.6 ±0.2^{b}	28.1±1.0^{a}	2.9±0.19^{a}
AC150	274.7±15.5	274.2±11.8 (-0.0%)^{a}	7.5±0.33^{a}	26.7±1.7^{a}	3.1±0.11^{a}
AC300	276.0±15.5	266.5±10.8 (-4.1%)^{ab}	6.7±0.3^{b}	25.0±1.8^{a}	3.3±0.12^{a}
VE100	270.0±9.5	269.25±9.2 (-0.6%)^{ab}	7.4±0.0^{a}	39.0±1.5^{a}	3.4±0.10^{a}
GAL2	279.0±15.3	278.0±13.0 (-0.3%)^{a}	6.7±0.2^{b}	30.0±0.6^{a}	3.1±0.16^{a}

NC: Normal Control rats; DC: Disease Control rats; AC_{150}: 150 mg /kg BW of the extract; AC_{300}: 300 mg /kg BW of the extract; VE_{100}: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different $p < 0.05$.

3.2 Effect of the Extract on Brain and Blood Lipid Composition Changes

3.2.1 Effect of the Extract on Brain Lipid Composition

Table 3 reveals brain lipid parameters of the rats. In disease control rat, the total cholesterol (CHL) increased significantly (+115.8%), and the total phospholipids (PHL) content were not significantly modified compared with control rats. Also, the cholesterol to phospholipid (CHL/PHL), molar ratio significantly increased (+93.6%) and the plasmalogen content significantly decreased (-35.7%) compared with control rat. In the group treated with the extract (150 mg/Kg b.w), the level of total cholesterol and the CHL/PHL molar ratio were significantly lower than those of disease control rats and statistically comparable to those of control. Plasmalogen levels were significantly higher in the group treated with the extract (150 mg/Kg b.w) than in disease control group and also statistically comparable to those of control group. The alteration of brain lipid profile in groups treated with vitamin E or galanthamine was higher than in disease control group.

Table 3. Brain lipid parameters of rats

Groups	Cholesterol (µg/mg protein)	Phospholipids (µg/mg protein)	Cholesterol/phospholipids molar ratio (mol :mol)	Plasmalogens (nM/mg protein)
NC	193.6±13.8^{a}	525.0±12.3^{a}	0.8±0.0^{a}	158.8±4.9^{a}
DC	417.9±22.1^{b}	529.5±10.0^{a}	1.6±0.0^{b}	101.9±4.0^{b}
AC150	215.3±7.8^{a}	726.9±10.9^{b}	0.6±0.0^{a}	179.5±7.8^{a}
AC300	461.9±15.9^{b}	656.0±13.5^{ab}	1.3±0.0^{b}	143.1±2.7^{ab}
VE100	424.6±9.4^{b}	392.9±21.5^{c}	2.03±0.1^{b}	94.80±3.3^{b}
GAL2	395.3±6.8^{b}	269.8±6.7^{c}	2.1±0.0^{b}	103.8±2.4^{b}

NC: Normal Control rats; DC: Disease Control rats; AC_{150}: 150 mg /kg BW of the extract; AC_{300}: 300 mg /kg BW of the extract; VE_{100}: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically

different $p < 0.05$.

3.2.2 Effect of the Extract on Blood Lipid Composition

Table 4 indicates the blood lipid parameters of the rats. In disease control rats, there were significant increases in plasma triglyceride (+104.8%) and plasma cholesterol (+54.1%) levels compared to control rats. The plasmalogens content in plasma and erythrocytes were not significantly modified between these two groups. Rats treated with the extract (150 mg/Kg b.w) showed lower cholesterol and triglycerides levels than those of disease control group and statistically similar to those observed in control group. Vitamin E treated-rats also showed decrease in plasma cholesterol and triglycerides but lower than those observed in rats treated with the extract (150 mg/Kg b.w).

Tableau 4. Blood lipid parameters of rats

Groups	Plasma triglycerides (mM)	Plasma cholesterol (mM	Plasma plasmalogens (mM)	Erythrocytes plasmalogens (mM)
NC	0.41 ± 0.003^{a}	0.85 ± 0.034^{a}	0.32 ± 0.004^{a}	0.19 ± 0.001^{a}
DC	0.84 ± 0.001^{b}	1.31 ± 0.029^{b}	0.32 ± 0.025^{a}	$0,18\pm0.005^{a}$
AC150	0.32 ± 0.002^{a}	0.84 ± 0.036^{a}	0.30 ± 0.007^{a}	0.22 ± 0.002^{a}
AC300	0.65 ± 0.011^{b}	1.52 ± 0.031^{b}	0.33 ± 0.005^{a}	0.21 ± 0.001^{a}
VE100	0.48 ± 0.004^{a}	1.09 ± 0.014^{b}	0.31 ± 0.006^{a}	0.21 ± 0.001^{a}
GAL2	0.86 ± 0.013^{b}	1.12 ± 0.034^{b}	0.30 ± 0.001^{a}	0.20 ± 0.002^{a}

NC: Normal Control rats; DC: Disease Control rats; AC_{150}: 150 mg /kg BW of the extract; AC_{300}: 300 mg /kg BW of the extract; VE_{100}: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different $p < 0.05$.

3.3 Effect of the Extract on Brain ATPases Activities and Associated Cations Contents

The results indicated a significant decreases in Na^{+}, K^{+}-ATPase (-32%) and Mg^{2+}-ATPase (-26,5%) activities, and depletion in Mg^{2+} contents in disease control rats compared to normal control rats (Table 5). The rats treated with the extract (150 and 300 mg/Kg b.w) showed a significant increase in Na^{+}, K^{+}-ATPase and Mg^{2+}-ATPase activities and concomitant increases in Mg^{2+} levels and decrease in Ca^{2+} levels. Vitamin E and galanthamine treatments also significantly increased Na^{+}, K^{+}-ATPase activity and increased the Mg^{2+} and decreased Ca^{2+} contents compared with disease control.

Table 5. Na^{+}, K^{+}-ATPase and Mg^{2+}-ATPase activities, and Mg^{2+} and Ca^{2+} levels in brain

Groups	Na^{+}, K^{+}-ATPase (µmol Pi/h/mg protein)	Mg^{2+}-ATPase (µmol Pi/h/mg protein)	Mg^{2+} (µmol/mg protein)	Ca^{2+} (µmol/mg protein)
NC	2.2 ± 0.02^{a}	4.0 ± 0.01^{a}	30.2 ± 3.7^{a}	15.9 ± 1.0^{ab}
DC	1.4 ± 0.03^{b}	2.2 ± 0.01^{b}	20.5 ± 2.2^{b}	18.4 ± 1.3^{b}
AC150	2.1 ± 0.03^{a}	5.3 ± 0.01^{a}	28.3 ± 2.3^{a}	13.8 ± 1.3^{a}
AC300	2.7 ± 0.04^{a}	4.8 ± 0.02^{a}	34.8 ± 2.2^{a}	11.6 ± 2.1^{a}
VE100	3.0 ± 0.06^{a}	3.2 ± 0.01^{a}	33.6 ± 3.9^{a}	12.4 ± 1.3^{a}
GAL2	2.6 ± 0.10^{a}	2.7 ± 0.01^{ab}	31.4 ± 3.7^{a}	10.5 ± 1.7^{a}

NC: Normal Control rats; DC: Disease Control rats; AC_{150}: 150 mg /kg BW of the extract; AC_{300}: 300 mg /kg BW of the extract; VE_{100}: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different $p < 0.05$.

3.4 Effect of the Extract on Brain and Plasma Glucose Levels

The findings revealed plasma and brain glucose contents in rats (Table 6). In disease control rats, the level of glucose in brain was significantly increased, whereas no significant change was observed in plasma glucose level compared to control rats. Rats treated with the extract at both doses (150 and 300 mg) showed significant low levels in cerebral glucose content as compared with disease control rats. Brain glucose level in rats treated with

the extract at 150 mg/Kg b.w was significantly lower than in control group. Treatments with reference drugs also lowered the brain glucose level compared with disease control group.

Table 6. Bain and plasma glucose levels

Groups	Brain glucose (µmol/g tissus)	Plasma glucose (mM)
NC	2.1±0.1[a]	5.3±0.2[a]
DC	10.6±0.3[b]	5.9±0.3[a]
AC150	1.3±0.0[c]	5.7±0.2[a]
AC300	3.0±0.1[d]	6.14±0.0[a]
VE100	5.2±0.1[d]	7.0±0.2[a]
GAL2	4.8±0.1[d]	5.6±0.3[a]

NC: Normal Control rats; DC: Disease Control rats; AC_{150}: 150 mg /kg BW of the extract; AC_{300}: 300 mg /kg BW of the extract; VE_{100}: 100 mg/kg BW of vitamin E; GAL2: 2mg/kg BW of galanthamine, Values are expressed as mean ±Standard deviation, values with different superscripts down the column are statistically different $p < 0.05$.

4. Discussion

The analyze of lipid profile of rats in this study, both at the brain and peripheral levels revealed a significant change in lipid composition in disease control rats compared to normal control. The molar ratio of CHL/TPL an accepted index of membrane fluidity (Senault *et al.*, 1990), also increased in disease control rats, indicating decreased fluidity (Senault *et al.*, 1990). These results corroborates with several studies showing the capacity of Al^{3+} to induce changes in lipid composition and the physical properties of plasma membrane. Indeed, Pandya, Dave & Katyare (2003), examined the myelin lipid profile of rats after exposure to $AlCl_3$. They observed significant increase in cholesterol (CHL) content with a decrease in the total phospholipid (TPL) content and in the TPL / CHL molar ratio. More so, changes in phospholipids composition of the myelin membrane were similar to phospholipid profiles reported in AD brains. Sarin *et al*., (1997) studied the lipid composition and various membrane-bound enzymes in different regions of monkey brain following chronic aluminum exposure indicating similar observations. In human neuroblastoma cells, Verstraeten *et al*., (2002) found that, Al^{3+} (10 to 100 µM) caused a significant loss of membrane fluidity, and increased the relative content of lipids in gel phase while promoted lipid rearrangement through lateral phase separation.

On the other hand, we found that the brain and blood lipid profiles of rats receiving extract particularly at the dose of 150 mg/Kg were statistically similar to those of control. This therefore highlights a strong effect of the extract at this dose on protecting lipid membranes or on modulating lipid metabolism. Such effects may be due to the presence of pentacyclictriterpenes (taraxerol 3-hexacosanoate, taraxerol 3-tetracosanoate, taraxerol 3-docosanoate, taraxerol and taraxerone) or sterol ((24R)-Stigmast-7,22(E)-dien-3-α-ol (chondrillasterol)) previously isolated from this plant by Fokou (2006). In fact, it has been shown that, taraxerol is able to incorporate itself in DPPC (Dipalmitoyl-phosphatidylcholine) bilayers and is more potent than cholesterol to decrease the DPPC gel phase (Rodriguez *et al*., 1997). In addition, spinasterol [(22 E, 24 S)-5α-stigmasta-7, 22-dien-3β-ol] a stereo isomer of chondrillasterol showed the ability to partially take over the role of natural cholesterol on the structural level of lipid membrane (Haralampiev *et al*., 2017). Thus, it is possible that by incorporating themselves into the brain lipid membrane, taraxerol and chondrillasterol probably in our extract displace cholesterol and therefore modified its homeostasis or metabolism. It is well known that plant pentacyclictriterpenoids or sterol affect critical regulatory pathways of lipid metabolism. In fact, the metabolism of cholesterol in cell is finely regulated by transcription factors like SREBP (Sterol Regulatory-Element Binding Proteins), by low density lipoprotein (LDL) receptors, or by ATP-binding cassette (ABC) transporters receptors (Shimano & Sato, 2017). In BV2 microglial cells, Kharrassi et al., (2014) found that spinasterol can modulate the gene expression of two nuclear receptors, the Liver X receptors (LXR -α and LXR-β) by targeting ABCA1 and ABCG1 genes. Yang *et al*., (2004) discovered that stigmasterol, inhibits the treatment of SREBP-2 and reduces the synthesis of cholesterol. Pentacyclictriterpenes have been indicated to inhibit the expression of the ACAT gene (Acyl-coenzyme A: cholesterol acyltransferase) which controls ACAT, a key enzyme that take part in the metabolism of cholesterol and plasma fatty acids (Liu *et al*., 2007). All of these may be likely biochemical mechanisms by which the compounds present in our extract have lowered the cholesterol and triglyceride contents in the brain and plasma. In addition, plasmalogen constitute a particular class of membrane glycerophospholipids (GP) having a unique structural characteristic (a vinyl ether group, -0-CH = CH-, at the sn-1 position ofglycerol backbone instead of the usual ester function). In neurons, the plasmalogens are enriched

in polyunsaturated fatty acids (PUFAs), decosahexonoic acid and arachidonic are the most abundant (Braverman & Moser, 2012). The presence of these PUFAs inplasmalogens may render them very susceptible to lipid peroxidation and oxidative damage. Therefore the high contents of total phospholipids and plasmalogen observed in the groups having received the extract could be due to the protective effect of the extract against the oxidation of the PUFAs contained in these phospholipids. It should also be noted that the vitamin E and galanthamine reference drugs used in this study enhanced brain lipid alteration. Such effect of vitamin E was also observed by other authors. In fact, Subudhi *et al*., (2009), observed that vitamin E supplementation (200 mg/kg b.w of vitamin E orally for 30 days) in hypothyroid rats altered plasma lipid profile by increasing the levels of plasma total cholesterol, non-HDL cholesterol and decreasing those of HDL-cholesterol. Vitamin E supplementation also increased lipid peroxidation and protein carbonylation. It is therefore important that, additional studies be done to better understand the effects of vitamin E supplementation or galanthamine administration in brain lipid profile in the context of AD.

The activity of membrane proteins such as Na^+, K^+-ATPase depends strongly on the surrounding lipid environment. Enzymes evaluation showed a significant reduction in the activities of Na^+,K^+-ATPase and Mg^{2+}-ATPase in disease control rats compared with normal control. Decrease in Na^+, K^+-ATPase activity has been shown as an early marker of AD (Yu *et al*., 2016). The complete hydrolytic activity of the enzyme depends on an association between the phospholipids and the cholesterol of the plasmatic membrane (Habeck *et al*., 2015). This might explain the lower Na^+, K^+-ATPase activity of disease control rats, accounted for by their low phospholipids levels and high cholesterol level compared with control rats or with rats treated with the extract. In addition, we found in disease control group an alteration of ions (Ca^{2+} and Mg^{2+}) homeostasis. This alteration may be due to decrease of brain ATPase activities which are known to modulate Ca^{2+} and Mg^{2+} homeostasis (Sanui *et al*., 2003). Mg^{2+}content can modulate the activity of Mg^{2+} kinase-dependent enzymes such as glycolysis kinase (hexokinase, phosphofructokinase and pyruvate-kinase) and therefore take part in the glucose homeostasis (Cohn & Roth, 1983). Glycolysis is also regulated during Na and K movements by the activity of phosphofructokinase (Erecinska & Dagani, 1990). All of this suggest a relationship between the activities of Mg^{2+}-ATPase, Na^+ ,K^+-ATPase and glycolysis or glucose homeostasis. We found in this study that, brain-glucose level in disease control rats was significantly elevated than in control group. Therefore, diminished cerebral ATPase activities probably account for elevated brain-glucose observed in these rats by impairing intracellular glucose utilization through glycolysis. Another potential explanation for elevated brain-glucose could be that of impaired glucose transporter (GLUT) activity, resulting to decrease brain-glucose uptake and consequent cerebral 'hypometabolism'. These results are in agreement with An et al., (2018) who found that higher brain glucose levels in AD were related to lower rates of glycolysis and lower glucose transporter 3 (GLUT3). In the rats treated with the extract, we observed a low level of glucose in brain suggesting a high rate of glucose utilization with probably high glycolytic rate and high energy-production.

5. Conclusion

We evaluated in this study the effect of hydroethanolic extract of *A. congolensis* on lipid profile of rats' brain with experimentally induced Alzheimer's disease. Our results revealed the ability of this extract to maintain brain lipid composition and fluidity at healthy state. The activities of membrane-bound ATPases, Ca^{2+} and Mg^{2+} homeostasis, and glucose metabolism in brain were also improved by this extract more than reference drugs. It was concluded that the extract may protect neuronal cellular membranes against Aβ peptide interaction and toxicity, thereby preventing the progression of AD. However, we should chemically characterize this extract and show the underlying mechanisms, by which extract compounds interfere with regulatory pathways of lipid metabolism. Furthermore, the high dose of extract (300 mg/Kg b.w) had not improved brain lipid alteration. Further studies on dose-effect of extract will be carried out to better understand this result.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NNDJ carried out the study and wrote the manuscript; NJL contributed to conception, design and analysis of data, and OJE assisted with and supervised the manuscript writing. All authors have read and approved the final manuscript.

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Traditional Fermented Butter *Smen*/*Dhan*: Current Knowledge, Production and Consumption in Algeria

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Abstract

Algerian *Smen*/*Dhan* is an ethnic dairy product which is a traditional fermented butter made from whole raw milk by empirical methods. This product constitutes a significant part of the Algerian diet and represents a gastronomical heritage that need to be preserved and protected. The aim of the present study was to investigate the place and use of this product in the Algerian culture as well as to identify the traditional methods used for its preparation. A survey was conducted on 880 households in the South and East of Algeria. The results revealed that 96.47% of the surveyed population knew what *Smen/Dhan* was, 75.22% consumed it and 47.61% prepared it at home. Concerning the production method, raw milk (cow, goat and/or sheep milk) is spontaneously fermented until coagulation. Then, the obtained coagulum (*Raib)* is churned with different tools to obtain the butter used for *Smen*/*Dhan* preparation. Then, two different preparation modes are used depending on the surveyed regions. Households from Batna, Khenchela and Setif proceed directly with butter salting while in the Biskra, Jijel, El Oued and Ouargla regions, a different process is used which first involves a heat-treatment of the butter followed by the addition of one or more ingredients. After packing in a traditional ceramic container (*Ezzir*) and other containers, a maturation step is applied with a long duration varying from one month to several years. This product is consumed as an additive to enhance the taste and aroma of some traditional dishes (*couscous*/other) and is also used in traditional medicine.

Keywords: *Smen*/*Dhan*, Algeria, survey, traditional preparation, consumption

1. Introduction

Nowadays, there is an increasing interest for foods that are linked with specific places or territories. Indeed, consumers are more and more attracted to local foods with a traditional character or image, and such local foods are often perceived as to be of higher quality, freshness, more sustainable and contributing to support the local economy (Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009). Among traditional foods, fermented foods have been consumed since antiquity and still constitute a main food source in the population's diet throughout the world. They also represent an important part of human dietary and culinary culture and most of these products are produced at household level or in small enterprise using spontaneous fermentation (El Sheikha, & Hu, 2018; Johansen, Owusu-Kwarteng, Parkouda, Paonou, & Jesperen, 2020).

Among fermented foods, dairy products are very popular due to their simple formulation. Milk as a raw material is well suited to support the growth of microorganisms because it is rich in carbon and nitrogen sources as well as micronutrients. Indeed, the ability to maintain it in a fresh state is limited, especially in warm environments and in the absence of a cold chain (Hutkins, 2006; Puniya, 2015), as these latter conditions will rapidly lead to its spoilage; that is the reason why different methods have been developed to allow its preservation for longer

periods. Among these methods, milk fermentation is one of the most ancient practices to ensure milk preservation as it guarantees the availability of safe and nutritious foods throughout the year, and prevents the risk of shortage of dairy products to consumers (Abd-El Salam, & Benkerroum, 2006).

Many types of fermented milk products exist throughout the world. Their organoleptic properties depend on different factors such as the animal species from which milk it is obtained, milk pre-treatment, conditions of fermentation and associated microbiota, and subsequent processing (Zamfir et al., 2006).

In Algeria, a large variety of dairy products are prepared with naturally fermented milk. They have played and still play a major role in people's diet, especially those from rural areas. Among them, *Lben*, *Raib*, *Zebda*, *Dhan* or *Smen* and *Jben* are the most common and are mostly marketed throughout informal circuits (Idoui, Benhamada, & Leghouchi, 2010).

Smen or *Dhan* is a traditional fermented butter which is made from raw milk, cream or butter originating from several animal species (Rajorhia, 1993; Guessas, Adjoudj, Hadadji, & Kihal, 2012; Iradukunda, Aida, Ouafi, Barkouch, & Boussaid, 2018). Its fermentation can last from a few months to several years, depending on the moisture, humidity and room temperature of the storage place (Kacem, & Karam, 2006). It is produced in many countries around the world, in Asia, the Middle-East and Africa (Afsaneh, Hosseinpour, & Mina, 2016) and its name and preparation methods differ from one region to another. In Algeria, it is known as *Dhan* or *Smen* (Bensalah, Labtar, Delorme, & Renault, 2011; Guessas, Adjoudj, Hadadji, & Kihal, 2012), *Smen* in Morocco (Tantaoui-Elaraki, & El Marrakchi, 1987; Benkerroum, & Tamime, 2004; Faid et al., 1993; Sakili, & Isoual, 2003 Iradukunda, Ouafi, Barkouch, & Boussaid, 2018), *Ghee* in India and Ethiopia (Dhurvey, Kawtikwar, & Sakarkar, 2012; Mortensen, 2016; Alganesh, & Yetenayet, 2017), *Samna* in Egypt and *Samin* in Sudan (Sserunjogi, Abrahamsen, & Narvhus, 1998).

This fermented butter is mostly used as a flavouring ingredient to improve the taste and aroma of many traditional dishes (Iradukunda, Aida, Ouafi, Barkouch, & Boussaid, 2018). It is also used in traditional medicine for reducing pain associated with the cold feeling that accompanies coughs, rheumatism and bone trauma (Sakili, & Isoual, 2003).

In Algeria, *Smen*/*Dhan* represents an ethnic heritage. It is manufactured according to an artisanal process, which is far from being perfectly known. A better knowledge of this product presents a scientific interest. In this context, the aim of the present study was thus to investigate the use and consumption of this traditional product as well as to identify how it is manufactured and stored in the Northeastern and Southeastern parts of Algeria.

2. Material and Methods

2.1 Survey Study

A field survey was conducted between May 2017 and March 2018 in different regions of the Southeast (Biskra, El Oued and Ouargla) and the Northeast of Algeria (Batna, Khenchela, Jijel and Setif) as shown in **Figure 1**. These regions were chosen based on a preliminary survey showing that *Smen/Dhan* preparation and consumption is quite common in these areas.

Families were asked about their practices and utilization of *Smen*/*Dhan* and the overall sample surveyed consisted of 880 households distributed in 71 towns from and nearby different localities, i.e., Batna, Biskra, El Oued, Jijel, Khenchela, Ouargla and Setif. The survey was mostly directed towards elder people especially women, dairy farmers, and farmers who are used to prepare *Smen*/*Dhan* and possibly market it in informal circuits in order to have a view as precise as possible on the practices associated with this traditional product.

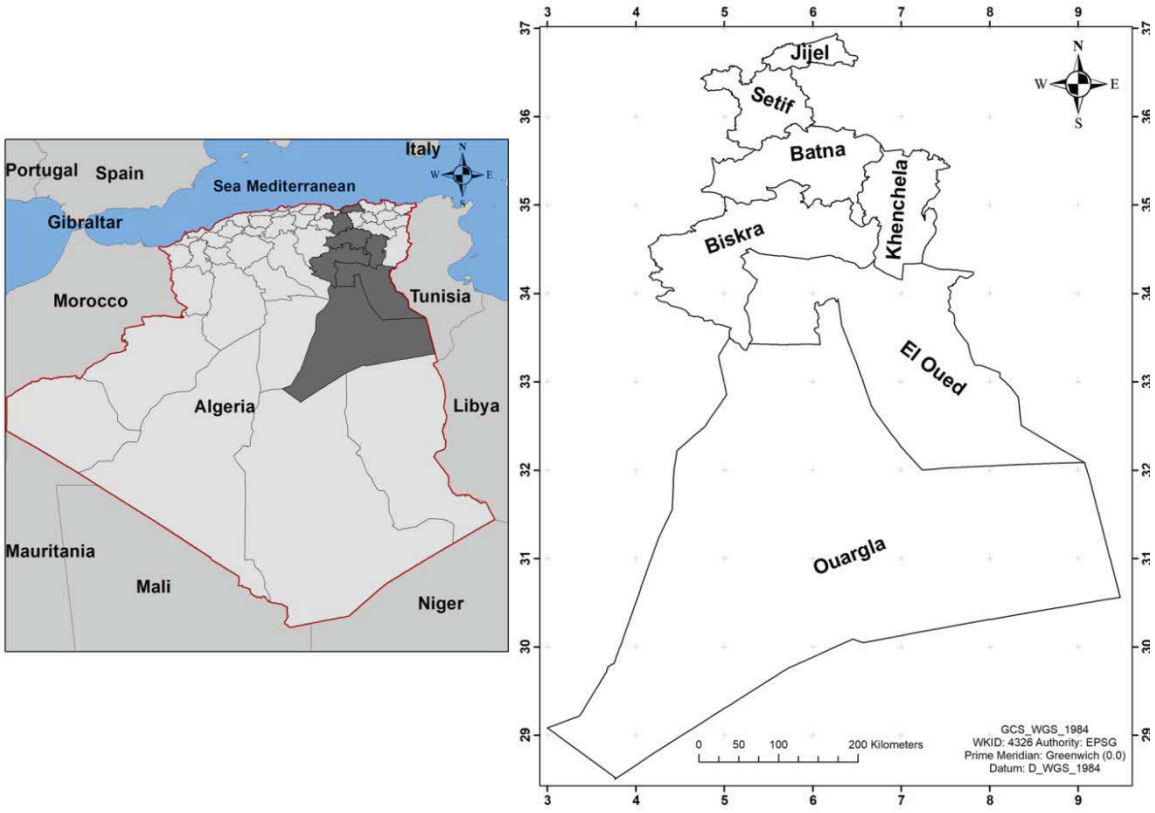

Figure 1. Detailed maps highlighting the surveyed regions of Algeria

2.2 Data Collection

Data were collected using a questionnaire and completed by face-to-face interviews. Before the beginning of the survey, the questionnaire framework and purpose was explained followed by the interview, which lasted about 15 to 20 minutes. Questions were read and if necessary, explained before collection of the answers.

2.3 General Content of the Questionnaire

The survey questionnaire contained forty-eight questions **(supplementary file 1)**. It was divided into five parts. The first part of the questionnaire contained questions about the identity of the interviewed person (gender, age, education degree, professional activity and residence). The second part dealt with the profile of the business/enterprise where the product was produced. The third part allowed to gather information on the fermented product itself, i.e. the place of the fermented product in the local culture, its local name, the geographical production area, the mode and period of consumption as well as the product use. The fourth part included questions about the product organoleptic properties (texture, color, smell, taste…) and the fifth part concerned the description of the production process, the raw materials used and their origins, as well as the equipment used.

2.4 Statistical Analysis

Statistical analysis was performed using XL STAT, version 2014. The results of this study are presented using descriptive statistics and expressed as percentage of response to each question in the survey. ANOVA test was used to analyze the variance between modalities of each surveyed variables ($p < 0.05$). Correlation analysis was used to explore the relationship between the different parameters.

3. Results and Discussion

3.1 Characteristics of the Studied Population and Link with Smen/Dhan Preparation

Our study group consisted of 880 households and the interviewed persons were aged between 19 and 99 years old. As shown in **Table 1**, the surveyed population was overwhelmingly composed of adults over 38 years of age. The average and median age of the surveyed population was 49.47 and 50 years old, respectively. Over 55% of

respondents lived in urban areas.

After subdiving the surveyed persons into 20 year age-groups, we did not observe any relationship between age groups and preparation of *Smen*/*Dhan* in households, which indicates that *Smen*/*Dhan* preparation was performed indistinctively by all age groups. This result may be explained by the fact that *Smen*/*Dhan* preparation relies on a simple method of preparation in contrast to other traditional foods, such as couscous, which need much more effort and experience in their preparation. As an example, (Bekhouche, Merabti, & Bailly, 2013) reported that couscous *lemzeiet* was mainly prepared by women that are aged between 40 and 60 years. On the other hand, we did not find any relationship between professional activity and *Smen*/*Dhan* preparation, this can be explained by the fact that the product preparation does not require a great effort, and its preparation is quick and easy.

The percentage of the surveyed females was higher than that of males, as 84.54% of the interviewed persons were females while only 15.45% were males. This observation is not surprising given the women's role in the manufacture of traditional products, including *Smen*/*Dhan*, and the fact that *Smen*/*Dhan* preparation is an activity exclusively realized by women. For all these women, the expertise or the art of *Smen*/*Dhan* making was passed on by their mothers, grandmothers or mothers-in-law as previously reported by Gagaoua and Boudchicha (2018) for ethnic meat products of the North African and Mediterranean countries. While keeping and preserving the same eating habits, the *Smen*/*Dhan* making process was considered by the interviewed persons as a precious legacy transmitted from one generation to another. In contrast, men play a relatively insignificant role in the *Smen*/*Dhan* preparation but are involved in selling tasks.

Table 1. Socio-demographic structure of the 880 surveyed persons

Surveyed population	Number	Percentage (%)
Age (years)		
19-38	202	22.95
38-58	424	48.18
59-78	226	25.68
79-99	28	3.18
Gender		
Female	744	84.54
Male	136	15.45
Region		
Urban	489	55.56
Rural	391	44.43
Professional Activity		
Housewives	701	79.65
Students	29	3.29
Civil servant	133	14.77
Retired	17	1.93

3.2 Place of the Product in Local Culture

Smen/*Dhan* is a traditional product very appreciated by the Algerian population. This product is part of the eating habits in different regions of Algeria. The results revealed that 96.47% of the surveyed population knew what *Smen/Dhan* was because of its importance in the Algerian kitchen **(Table 2)**. Even if the product was not prepared by all Algerian families (47.6% of households), its recognition by most families confirmed that this product is a traditional product of Algeria, which knowledge and utilization are transmitted from one generation to another. It is worth noting that the name used for this product, i.e. *Smen* or *Dhan* varied according to the surveyed region. Indeed, we found out that the majority of respondents used the name *Dhan* in all the surveyed regions (Batna, Biskra, El Oued, Khenchela, Setif, Ouargla), except for Jijel in which the name *Smen* was used.

Smen/*Dhan* is widely used by Algerian families. Indeed, 75.22% of the surveyed population consumed it on a regular basis. The interviewed persons also indicated that they consumed *Smen*/*Dhan* for its taste, i.e., as flavouring agent in traditional dishes, but also for the health benefits associated with its consumption.

Concerning people that did not prepare themselves *Smen/Dhan*, the purchase of *Smen/Dhan* was done in formal circuits if available on the market or informally through other households producing their own product. We noted that the price of one Kg of *Dhan* was 2000 DA in southern areas of Algeria (Biskra, El Oued, Ouargla) while it

was 800 DA in Eastern areas. This price difference can be explained by the nature of the raw material used for its preparation in these two areas. Indeed, *Smen*/*Dhan* is prepared with goat's milk in the South and cow's milk in the East and goat's milk is more expensive than cow's milk.

Table 2. Distribution of the surveyed population according to knowledge, manufacture, consumption and purchase of *Smen/Dhan* in different regions of Algeria

Regions (*Wilayas*)	Number of respondents	Knowledge (n)	Preparation (n)	Purchase (n)	Consumption (n)
Batna	100	100	46	38	77
Biskra	100	100	21	50	67
El Oued	100	100	53	42	73
Jijel	100	69	42	21	68
Khenchela	276	276	158	114	221
Sétif	104	104	48	33	79
Ouargla	100	100	51	31	77
Total	880	849	419	329	662
%	100	96.47	47.61	37.38	75.22

3.3 Traditional Preparation Steps of Smen/Dhan

Based on the survey results, we could define the different steps used for the preparation of traditional "*Smen*/*Dhan*" **(Figure 2, 3)**. These steps are described below.

3.3.1 Raw Material Used

Raw milk of different animal species can be used in order to make butter, which is then further processed to *Smen/Dhan*. Based on the survey results, it was found that, cow, goat and sheep milk or mixture of two milk types (goat and sheep milk) could be used to produce butter, and the milk type differed significantly between eastern and southern regions. Indeed, cow's milk is the most widely used milk in the eastern regions with 73.97, 100, 70% and 100% of interviewed households using cow's milk in Batna, Jijel, Khenchela and Setif localities, respectively. In contrast, in the Southern regions, goat's milk is mostly used for the preparation of *Dhan*, with 92.75%, 98.68% and 100% of interviewed households preparing *Smen/Dhan* with goat milk in Biskra, Ouargla and El Oued localities, respectively. Concerning sheep milk, its utilization is scarce and most of the time, it is used in mixture with goat milk. It is not surprising that the milk type differs between Eastern and Southern regions as it reflects the most prevalent bred animals in these two regions.

3.3.1.1 Milk Coagulation

After milk collection, raw milk is spontaneously fermented at room temperature until coagulation with a duration time varying from 12 to 120 h depending on the season at which this fermentation is performed. Indeed, in the summer season, because of higher temperatures, the fermentation time varies between 12 to 24 h while in the winter, it can last up to 120 h. In order to accelerate the fermentation process in the winter season, some people add ingredients to the milk, such as lemon juice, vinegar, *Raib* (fermented milk), yogurt, klila (cheese) and/or hot water, or leave the milk to coagulate in front of heat or the fireplace. The obtained coagulum is called *Raib* and it may be consumed as it is or processed further as described below.

3.3.1.2 Butter Making

For butter making, *Raib* is then churned. Churning can be performed with several tools either manually using the traditional *Chekoua* which is a goatskin bag, an electric churn equipped with wood, metal, paddles or a plastic bottle **(Figure 4)**.

In the past, *Chekoua* was the mostly used churning tool, but due to its decreasing availability as well as the better ease and speed of use of other tools, it disappeared gradually. Benkerroum and Tammim (2004) stated that the decreasing use of traditional churning tools was due to the fact that electric churns are faster and do not require much labour. In addition, these tools are easier to clean while *Chekoua* cleaning is more fastidious and laborious, and it can be easily contaminated by undesirable microorganisms including spoilage and pathogenic ones. On the other hand, this tool can be a source of beneficial microorganisms which could play a significant role in *Smen/Dhan* organoleptic properties, but this aspect has not been studied in detail so far.

Nevertheless, in Algeria, people still use the traditional *Chekoua*, especially people living in rural areas. Indeed, 21% of the surveyed population still used *Chekoua* for churning, while 41% and 38% use electric churns and

plastic bottles or cans, respectively. With regard to churning duration, it varied depending on the churning tool used as well as the ambient temperature, quantity of *Raib* to be processed and its fat content which is also dependent on the animal species from which milk is derived. According to the surveyed population, it can last between 1 to 2 h using a traditional *Chekoua* or a bottle and between 30 min to 1 h using an electric tool. At the end of churning, a quantity of cold or hot water can be added in the mixture according to the ambient temperature, in order to favor the agglomeration of butter grains. This trick is also used by the Moroccans (Tantaoui-Elaraki, & El Marrakchi, 1987). At the end of this process, two phases appear, a solid one called *zebda i.e.,* butter and a liquid one called *Lben, i.e.,* buttermilk. After that, the butter is collected by hand or using a strainer or a perforated ladle.

Concerning butter yield, 30 L of milk yield between 0.8 and 1 kg of butter, which is slightly lower than that reported by Alganesh and Yetenayet (2018) with 20 to 25 L of milk for 1 kg of butter. After churning, the butter is washed several times to remove *Lben* traces which constitute the aqueous phase.

3.3.2 *Smen/Dhan* Preparation

According to the surveyed households from East and South of Algeria, *Smen/Dhan* preparation is transmitted by identical method from generations. Its production is related to two main factors. First, *Smen/Dhan* is produced because it is an important ingredient in traditional foods, but also for its possible use in traditional medicine. The second reason is that *Smen/Dhan* production allows keeping the butter for longer time period. Indeed, butter transformation into *Smen/Dhan* leads to a more stable product from an organoleptic and microbiological point of view and thus prevent food losses. Benkeroum and Tamim (2004) also reported that the raw butter surplus exceeding the domestic demand, was transformed into Moroccan *Smen* for longer preservation.

It is also worth mentioning that among households preparing *Smen/Dhan* at home, 61.3 % prepared the butter themselves at home starting from raw milk as described above, while the others did not and purchased butter directly from milkmen. These milkmen are traders who collect milk from farms, and sell milk and traditional dairy products to people in the area. These traders own large churns which are used for the preparation of *Lben* and butter. This was also true for households living in urban areas of the Eastern regions (Batna, Jijel, Setif and Khenchela), which did not transform themselves the raw milk into butter, but bought directly the latter from milkmen, in contrast to households from rural areas and those from the Southern regions. These traders also manufacture and sell *Smen/Dhan* but, in general, *Smen/Dhan* preparation is performed by their mother or wife following the traditional process.

After churning, the obtained butter can be processed immediately into *Smen/Dhan,* or the latter is only produced after the accumulation of a large butter quantity in the household. Indeed, we noted that 31.6% of households used to prepare *Smen/Dhan*, immediately processed the butter, while the remaining households only prepared *Smen/Dhan* after accumulation of large butter quantities. In the latter case, butter is kept in the refrigerator or at room temperature with a small amount of salt which allows storing butter for a longer time period as salt addition reduces food water activity and thus prevents the growth of non-halotolerant undesirable microorganisms. Interestingly, we observed that *Smen/Dhan* preparation method is different according to the surveyed region. This difference was observed between the regions of Batna, Khenchela, Setif on one hand and the regions of Biskra, El oued, Jijel, Ouargla. Indeed, following butter washing, households from regions of Batna, Khenchela, Setif proceed directly with butter salting (Figure 2, see section 3.3.2.1) while in others regions of Biskra, El Oued, Jijel, Ouargla, a different process is used which first involves a heat treatment of the butter (Figure 2, see section 3.3.2.2) followed by the addition of one or more ingredients. Both methods, together with the butter acidic pH, relatively high salt (NaCl) content, and its low moisture and water activity, contribute to the microbiological stability of the obtained products, which in turn increases butter shelf life (Sserunjogi, Abrahamsen, & Narvhus,1998; Afsaneh, Hosseinpour, & Mina, 2016; Alganesh, & Yetenayet, 2017).

3.3.2.1 Butter Salting

As mentioned above, in certain localities, i.e., Batna, Khenchela, and Setif, people proceed directly to butter salting with a process similar to that of Moroccan *Smen* (Tantaoui-Elaraki & El Marrakchi, 1987; Benkerroum & Tamime, 2004). In the present study, 94% of households preparing *Smen/Dhan* with this method add directly salt to the butter and this quantity varies from one household to another. The answers of the surveyed population were very diversified, and the interviewed people did not know exactly the salt concentration used, only the fact that the butter must be well salted. According to 60% of surveyed population and based on our estimation, the salt concentration varies between 50 and 80 g/ kg of butter. It is worth mentioning that certain households (2.20%) add a quantity of matured *Smen/Dhan* (period of maturation between 2 years to 10 years) with recently prepared *Smen*/*Dhan* in order to minimize the maturation time and gives a result in a short time. Benkourroum and Tamim

(2004) also reported that the ripening period of Moroccan *Smen* could be shortened to 30 days after mixing a portion (ca.5–8%) of matured *Smen* with freshly made product.

After salt addition, butter is well kneaded allowing a homogeneous distribution of salt in the butter. People stated that the salt addition was necessary for better preservation and for taste improvement.

After salting, the salted butter is left for a certain time to get the water out, which is then removed, allowing a shelf-life extension of *Smen/Dhan* during storage. Salted butter can then be directly packaged for further maturation.

3.3.2.2 Heat Treatment

The use of heat treatment was adopted in Biskra, Jijel, El Oued and Ouargla localities. For this, the butter is heated at slow fire until melting. The melting temperature could not be defined precisely by the respondents, but they indicated that butter had to be slowly melted to avoid fatty degradation and for a better separation of *Lben* residues and impurities. This method is similar to that of *Samna* making in Egypt and to a smaller extent to Ethiopian and Indian *Ghee*. Indeed, for *Samna,* butter is heated under continuous stirring at 50 to 60°C while for Ethiopian and Indian *Ghee,* temperature can reach to 110°C at 120°C, allowing the removal of more moisture and development of specific flavour (Sserunjogi, Abrahamsen, & Narvhus,1998; Chaudhary et al., 2019). According to (Illingworth, Patil, & Tamime, 2009), *Ghee* can be defined as a clarified pure fat which is obtained exclusively from milk, cream or butter by application of heat treatment leading to an almost complete elimination of moisture and solid fats.

At boiling point, white froth, which corresponds to residual *Lben* as indicated by the interviewed persons, appears on the melted butter surface. This froth is then skimmed off with a soup spoon, followed by the addition of different ingredients, the basic one being coarse semolina called *Dchicha*. Indeed, 92.5% of the population who prepared *Smen/Dhan* using this method, declared that they added *Dchicha* to butter during boiling under continuous agitation. The quantity of added *Dchicha* was usually approximate but the majority of respondents added ~ 160 g of *Dchicha* per Kg of butter, the purpose of this addition being residual *Lben* and water absorption during cooking to obtain *Smen/Dhan* of good quality. According to (Sserunjogi, Abrahamsen, & Narvhus, 1998) who focused on *Ghee*, after separation, the fat phase is further boiled together with a piece of bread and the boiling step is stopped when the bread becomes crispy and light brown in colour.

Besides *Dchicha*, 88.75% of them also added a small quantity of salt, ~ 8 g/Kg of butter. Moreover, 41.66% of respondents were adding herbs to flavor the product. These herbs were added by people of Biska, El Oued and Ouargla while those of Jijel did not use them. The different herbs, *i.e.,* rosemary (*Salvia rosmarinus*), thyme (*Thymus* spp.), juniper (*Junipirus* spp.) and fenugreek (*Trigonella foenum-graecum*) mixtures, are added in small quantities (a pinch) to butter during cooking. Finally, 7.90% of respondents were also adding a small onion piece to the mixture. Hazra and Parmar (2014) reported that the addition of herbs can prevent oxidative rancidity of *Ghee*.

The cooking duration differed from one respondent to another with duration between 30 min and 1.5 h. This variation was justified by the time needed for cooking and decanting the *Dchicha* semolina grains. At the end of cooking, the cooked butter is clarified with the semolina at the bottom of the pan. After this heat treatment phase, the product is filtered through using a sieve or a piece of a stamen to eliminate any trace of impurities and additives followed by packaging as described below.

The cooked semolina removed of melted butter is consumed by 35% of the respondents using this method. Nowadays, cooked semolina is considered as a traditional dish and is consumed for pleasure while in the past, it was also consumed to avoid food losses in a context of food shortage.

3.3.3 Packaging, Maturation and Storage

The packaging of salted or cooked butter is then carried out in containers made of glass (53.45% of the surveyed population preparing *Smen/Dhan*), plastic (34.04%), or a traditional ceramic container called *Ezzir* (12.5%). The latter type of container was mainly preferred by people from rural areas. The butter must be well compacted to remove air, so the pot must be fully filled to minimize free space. Some people declared that they covered the top a paste of semolina to completely prevent air entry. The ripening process was conducted in a dry place, in the dark and, at room temperature. In some cases, the product was left in the refrigerator, especially in the Southern regions of Algeria, because these regions are hot areas, especially in the summer. We noted that 53.45%, 41.48% and 3.45% of the population producing *Smen/Dhan* let it to mature at room temperature, in the refrigerator, or both depending on the season while 1.59% bury the *Smen/Dhan* underground. As previously reported by Benkerroum and Tamime (2004) for *Smen* made in several Morocco regions, the pot is buried underground to ensure darkness,

anaerobiosis and to minimize temperature variations.

The maturation duration was different from one person to another, varying from one month to several years, according to the consumer preferences. As indicated by the respondents, *Smen/Dhan* has a strong characteristic taste which strength depends on the maturation time.

After *Smen*/*Dhan* maturation, most people utilize it as it is (90.42% of the population consuming *Smen*/*Dhan*) while 9.57% heated *Smen*/*Dhan* at a slow fire until melting, before filtration and storage at room temperature, to stop the *Smen*/*Dhan* maturation process.

As for all other dairy products, *Smen*/*Dhan* may undergo physico-chemical or microbiological alterations. These phenomena can take place during storage and affect its organoleptic properties (texture, taste). According to the surveyed population, most people (70%) stated that they never experienced *Smen*/*Dhan* spoilage during storage. The others stated that they had observed mould spoilage on the *Smen*/*Dhan* surface which, according to the answers of some elderly people, was due to a default encountered during *Smen*/*Dhan* preparation and the remaining presence of *Lben* in the butter before packaging. Other spoilage encountered by consumers included rancidity defects, colour defect due to mould growth and unpleasant flavour development.

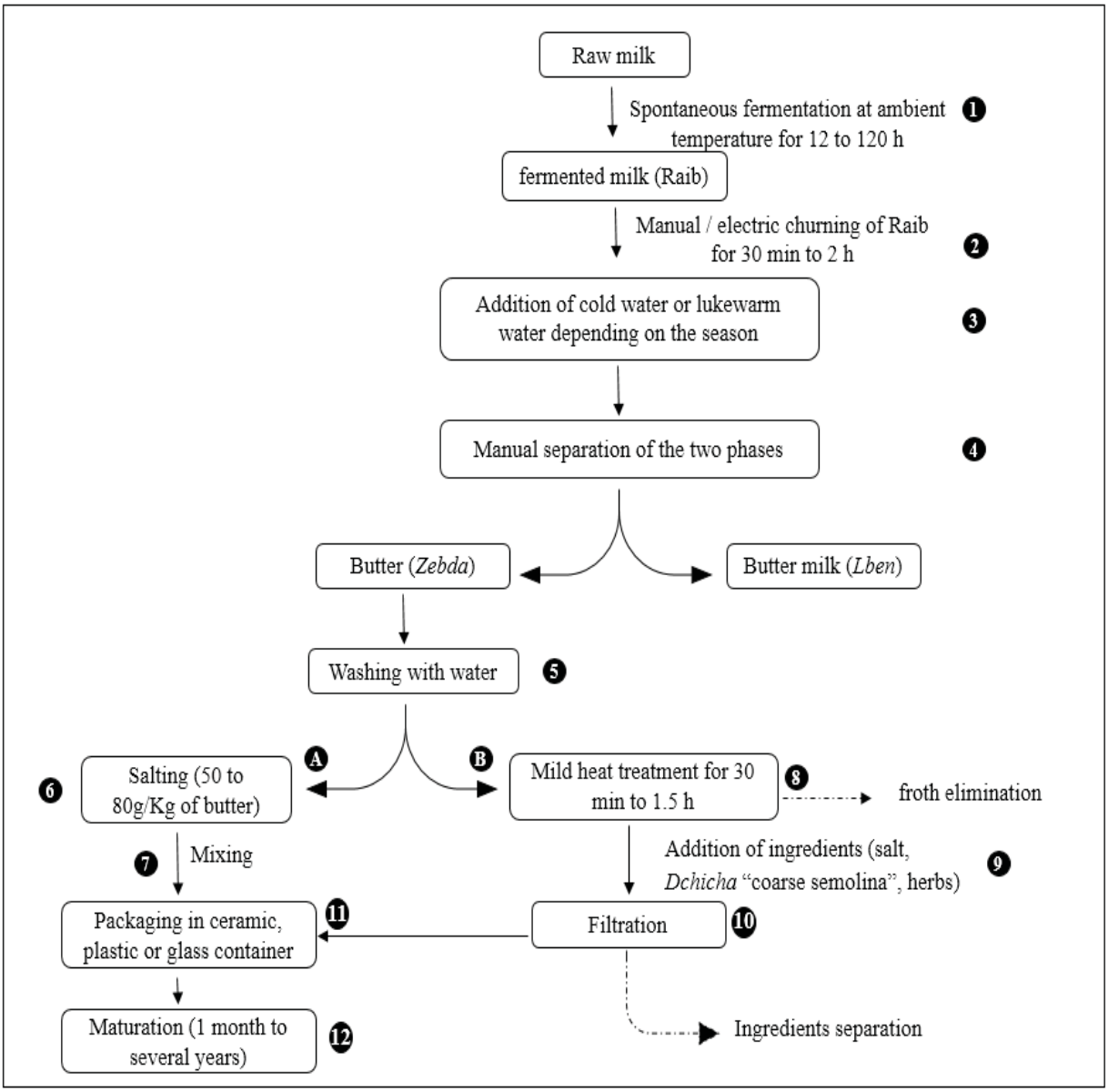

Figure 2. Diagram showing the different steps of traditional *Smen/Dhan* manufacturing

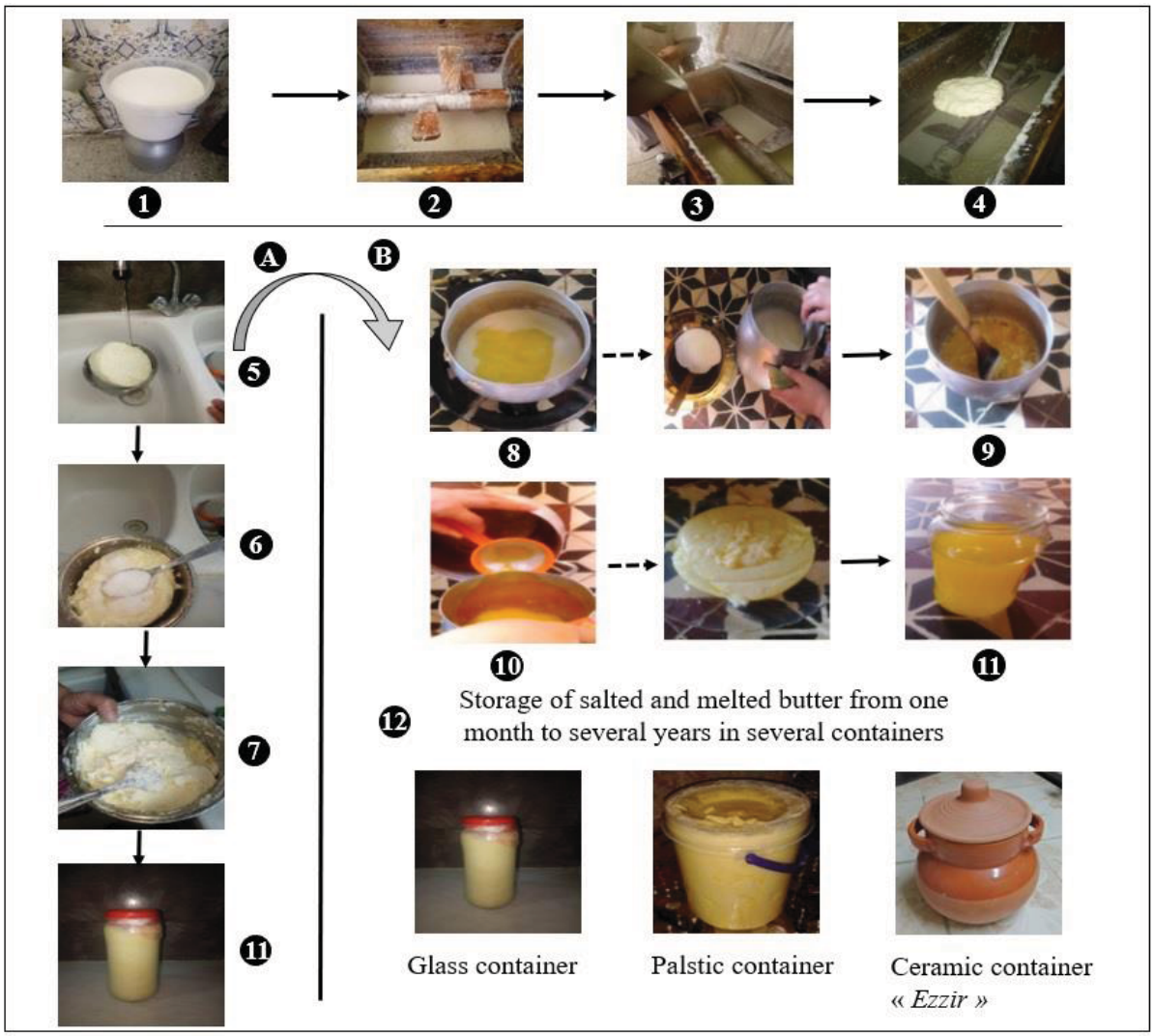

Figure 3. Photographs showing the different steps of traditional *Smen/Dhan* manufacturing

Figure 4. Different tools used for Rayeb churning (**a:** traditional *Chekoua* made from goat skin, **b_1-b_2:** traditional churn in wood, **c:** electric churn)

3.4 Smen/Dhan Consumption

Regarding the use of *Smen*/*Dhan* in the local culture, and based on the answers of the surveyed population, the majority of the respondents used it for meal preparation and also consumed it alone. The consumption has different objectives, *i.e.*, improving the taste and aroma of some traditional dishes or therapeutic purposes. Regarding culinary usage, people use *Smen*/*Dhan* with maturation age between 1 month to 2 years. Its characteristic taste depends on maturation time and this taste is stronger with a long maturation period. It is usually used at the end of cooking as a flavoring agent in some traditional dishes such as *couscous*,

chekhchoukha and *trida,* for cooking meat, and for preparation of traditional cakes such as *makrout*, *baklawa*, *mberdja*, and *rfis*. This is in agreement with previous work that stated that ripened *Smen,* because it has a strong rancid and salty characteristic taste, is mostly used as a condiment to enhance the flavor of some traditional Moroccan dishes (Benkerroum, & Tamime, 2004).

According to the results of this study, traditional *Smen*/*Dhan* ripened for more than two years is used for therapeutic purposes. This practice is much more observed in southern regions of Algeria, where it is occasionally drunk with coffee to treat cough. This practice was also reported for *Ghee* by Alganesh and Yetenayet (2017) who indicated that traditionally made *Ghee* stored for more than a year was recommended to treat chronic coughs. *Smen/Dhan* is also used to treat injuries, burns, migraine, headache, injury, hemorrhoids, flu and eczema with a superficial application. We also noted that some farmers use *Smen*/*Dhan* to treat trauma and injuries of their cows and other animals. The same applications in traditional medicine are also seen with Indian *Ghee* (Gandhi, & Lal, 2015). The therapeutic effects of *Smen*/*Dhan* have not been studied so far but concerning *Ghee*, Ahmad and Saleem (2020) showed that it has a high concentration of conjugated linoleic acid which possess antioxidant, anticarcinogenic, antidiabetic and antiatherogenic properties (Chinnadurai, Kanwl, Tyagi, Stanton & Ross, 2013).

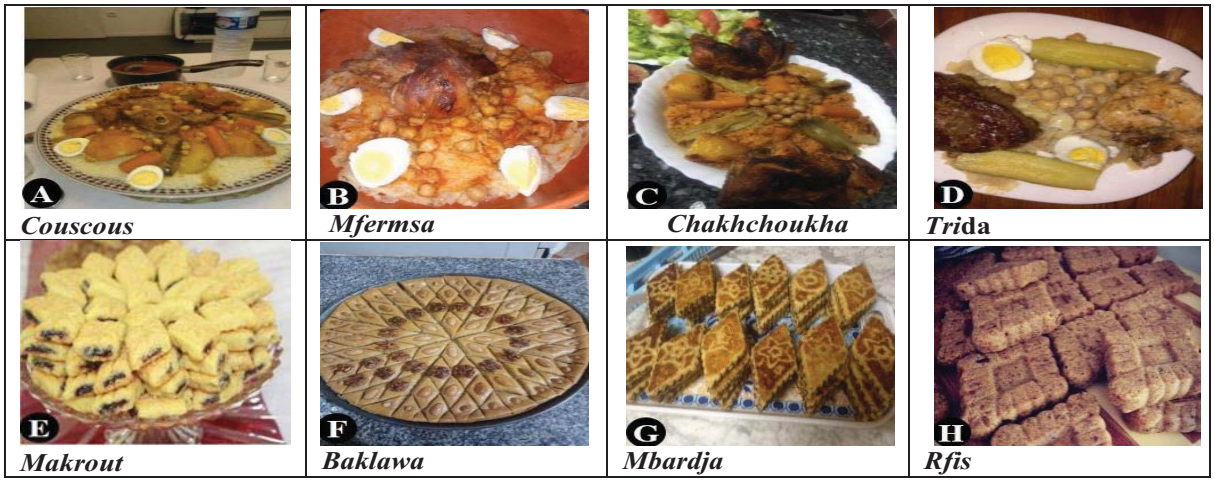

Figure 5. Photographs of some traditional foods (A, B, C, D) and pastries (E, F, G, H) prepared with *Smen*/*Dhan* matured for 1 to 24 months

4. Conclusion

Through this study, it was found that *Smen*/*Dhan* is an important product in the Eastern and Southern Algerian culture. It represents an ethnic food with a specific know-how and is part of Algerian eating habits. *Smen*/*Dhan* is prepared by housewives and takes an important place at the family level. This product is used for self-consumption, and it can be commercialized to provide an income for the family. Unfortunately, its marketing is generally in informal circuits because *Smen*/*Dhan* has not yet received any quality label despite its importance in traditional cooking and medicine. Therefore, it would be of great interest to create cooperatives that could market this product in a formal way while guaranteeing a better quality with a specific quality label. It could help local producers improving overall product quality and safety as well as to promote the gastronomic heritage of Algeria. Because the Algerian *Smen*/*Dhan* is a fermented food, it would be of great interest to study its microbial diversity and identify the key microorganisms involved in its sensory characteristics to better control fermentation processes and, thus, its quality attributes.

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Conflict of interest

The authors declare no conflict of interest.

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