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Solanum anguivi Lam. fruits' nutritional quality and potential effect on type 2 diabetes mellitus

BY

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DECLARATION

"I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Gießen for the Safeguarding of Good Scientific Practice" and the guidelines in the "Makerere University Graduate Handbook, 2013".

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DEDICATION

To my husband, Mr. Issa Sebunya, and children, Afzal, Tahir, and Khaira, for their love and support.

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"Don't focus on negative things; focus on the positive, and you will flourish."--- Alek Wek

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LIST OF ABBREVIATIONS

AA	Antioxidant activity
AAE	Ascorbic acid equivalent
BCC	Bioactive compounds content
DE	Diosgenin equivalent
dIlp	*Drosophila* insulin-like peptide (gene)
dILP	*Drosophila* insulin-like peptide
DW	Dry weight
FW	Fresh weight
FRSC	Free radical scavenging capacity
GAE	Gallic acid equivalent
HSD	High sugar diet
IPCs	Insulin producing cells
PCA	Principal component analysis
QE	Quercetin equivalent
SALF	*Solanum anguivi* Lam. fruits
T2DM	Type 2 diabetes mellitus
TAC	Total antioxidant capacity
TAL	Total alkaloid content
TFC	Total flavonoid content
TPC	Total phenolic content
TSC	Total saponin content
VCC	Vitamin C content

SUMMARY [ENGLISH]

The burden of diabetes is enormous due to its rapidly increasing global prevalence resulting from lifestyle and dietary habits changes. Although drugs have been developed to treat type 2 diabetes mellitus (T2DM), they are often accompanied by several side effects, and yet they are also expensive. *Solanum anguivi* Lam. fruits (SALF) are traditionally consumed as a remedy for T2DM. This may be attributable to the presence of bioactive compounds such as phenolics, flavonoids, saponins, alkaloids, and vitamin C in SALF. It has been documented that the nutritional quality of fruits and vegetables may vary among accessions and ripeness stages or may be affected by thermal treatments. However, the data on the influence of accession and ripeness stage and the effect of thermal treatments on the nutritional quality of SALF is scarce. There is also a dearth of data regarding the potential antidiabetic effect of dietary SALF and its underlying mechanisms of action. The present study assessed the bioactive compounds content (BCC) and antioxidant activity (AA) of different SALF accessions and determined the relationships between SALF BCC, AA, and plant morphological characteristics. The influence of the ripeness stage (unripe, yellow, orange, and red) on the BCC and AA of SALF accessions was investigated. The effect of drying temperature (35, 55, and 85 ^{o}C) and cooking method (boiling and steaming) and duration (15, 30, 45, 60, and 120 min) on the BCC and AA of SALF accessions were also investigated. The study further explored the potential of dietary SALF to prevent (prevention study) and manage T2DM-like phenotypes (therapeutic study) using the fruit fly, *Drosophila melanogaster* (*D. melanogaster*) model, whose energy metabolism has been suggested to be seemingly comparable with humans. The *D. melanogaster* has also been reported to possess a high number of genes also conserved in humans (including those for the insulin/insulin-like growth factor signaling pathway) and to develop a T2DM-like phenotype upon the intake of a high-sugar diet (HSD). Several metabolic parameters and physiological responses to nutrition have been reported to be dependent on the sex of an animal. In *D. melanogaster*, a sexually dimorphic response to HSD intake has been reported. Hence, male and female flies were used to determine whether the protective effects of SALF are sex-dependent.

In the present study, morphological characterisation, including eleven traits for leaves, stems, and fruits from 12 plants per accession, was assessed based on the descriptors of *Solanum* species. The BCC was determined by spectrophotometry (for total phenolics, flavonoids, saponins, and vitamin C), HPLC (for quantification of phenolics), and gravimetry (for total

alkaloids). The AA, assessed as free radical scavenging activity (FRSC) and total antioxidant capacity (TAC) was also determined by spectrophotometry. For the therapeutic and prevention studies, male and female *D. melanogaster* were separately exposed to HSD to induce a T2DM-like phenotype. Flies fed on SY10 medium served as the control. In the T2DM therapeutic study, flies were pre-exposed to HSD or control diet for 10 days, followed by an exposure to either HSD supplemented with 5 (HSD+SALF5) or 10 (HSD+SALF10) mg/ml SALF, HSD, or control diet until day 24. To assess whether a T2DM-like phenotype may be prevented by an exposure to HSD supplemented with SALF, male and female flies were reared on control, HSD, HSD+SALF5, or HSD+SALF10 for 24 days. The effect of HSD and HSD+SALF on weight, climbing activity, glucose, and triglyceride contents, survival, and gene expression of the PPARγ co-activator 1α (PGC-1α) fly homolog *Srl* and the *Drosophila* insulin-like peptides (*dIlp*) 3 and 5 were investigated. The weights and climbing activity of the flies were assessed on day 24, while survival was recorded every other 2-3 days when the flies were switched to fresh medium. Furthermore, glucose and triglyceride contents as well as mRNA levels of *Srl* and *dIlp* 3 and 5, were analyzed.

The morphological characteristics of *Solanum anguivi* Lam. varied among the accessions investigated in this study. The BCC and AA of the fruits (SALF) significantly differed among the accessions. All investigated SALF accessions were rich in total phenolics, flavonoids, saponin, vitamin C, and alkaloids, compared to foods that have been documented as rich sources of these compounds. Clusters derived for the accessions based on morphological characteristics or chemical content had some similarities. The ripeness stage significantly affected the BCC and AA of SALF, and this significantly differed among accessions. The highest total contents of phenolics and flavonoids, and the highest AA were present at the unripe stage. Additionally, the highest total flavonoid and saponin contents were present at the orange stage, and the highest vitamin C (for some accessions) and alkaloid contents were present at the red stage. Comparison with the red stage of SALF accessions at the unripe stage contained higher amounts of total phenolics (up to 39% higher), flavonoids (up to 15% higher), and AA (by 49-94%). SALF at the red stage, however, had 17-79% higher total alkaloids than the unripe stage, while at the orange stage up to 45% higher total saponins were present compared to the unripe stage. The HPLC results revealed that during ripening, the content of chlorogenic and caffeic acids reduced, gallic acid and rutin increased, while the effect on quercetin levels varied among the accessions. The regression models showed that SALF AA

was mostly affected by total phenolic content (TPC) and vitamin C content (VCC) at the unripe stage; TPC and total flavonoid content (TFC) at the yellow stages; TFC and total saponin content (TSC) at the orange stage; and TFC and total alkaloids at the red stage.

Thermal treatments significantly affected the BCC and AA of SALF. The drying temperature that resulted in the highest increase of TPC and AA in the SALF samples was 35 ^{o}C (2-fold), while 85 ^{o}C resulted in little to no increase of TPC and a significantly lower AA than the control (raw/undried SALF). Chlorogenic acid, caffeic acid, and rutin contents were higher at a drying temperature of 35 ^{o}C versus 85 ^{o}C, while the reverse was true for the gallic acid content. The effect of the drying temperature on the quercetin content varied with accession and ripeness stage. Drying significantly reduced the VCC and TFC compared to the control, and the drying temperature with their highest retention was 35 ^{o}C. The effect of drying on total alkaloids and saponins was dependent on the accession and ripeness stage. Similar to drying, both steaming and boiling significantly reduced the VCC. The cooking parameters also significantly reduced the TSC, except 15 min of boiling, which did not affect it. The total alkaloids in SALF were significantly reduced by steaming and increased by boiling. However, a very long cooking duration (120 min) increased the alkaloids 1.5-fold by steaming and 2-fold by boiling. Total flavonoids were not affected by both steaming and boiling, except for 15 min of boiling, which significantly increased the content. Boiling and steaming increased the TPC and AA of SALF up to 4-fold and 3-fold, respectively. Overall boiling resulted in the highest TPC and AA increment of SALF among the investigated thermal treatments in this study, with 15 min (of boiling) as the shortest duration with the highest TPC and AA. However, SALF that was boiled (for 15 min) and then dried (at 35 ^{o}C) had higher AA than SALF that was either boiled or dried only. The study revealed positive correlations between AA and the total contents of phenolics, flavonoids, and saponins SALF as well as negative correlations between AA and the contents of vitamin C and alkaloids were shown.

The T2DM therapeutic study revealed that SALF supplementation in HSD did not alter the weights, fitness, and triglyceride levels of male and female flies in comparison with the HSD-fed and control-fed flies. The HSD-fed female flies exhibited elevated glucose levels, which significantly decreased in a dose-dependent manner upon exposure to SALF-supplemented HSD. This glucose-lowering effect of SALF may be due to the presence of saponins and phenolics, which have been associated with hypoglycaemic effects in mice. Female flies fed on a SALF-supplemented HSD exhibited a significant increase in survival compared to

corresponding HSD-fed and control diet-fed flies. To unravel the underlying protective mechanism of SALF, central genes of the fly's energy metabolism were investigated. The mRNA levels of *Srl* decreased in HSD-fed female flies compared to the control-fed, while no effect was observed in females exposed to HSD+SALF. This observation may also explain the unaltered weights and fitness of female flies exposed to HSD+SALF as upregulation of *Srl* has been associated with weight gain and increased fitness. While the expression of *dIlp3* was not changed in all groups of female flies, *dIlp6* was significantly induced in female flies by HSD+SALF5 relative to the control, suggesting a central role for *dIlp6* in mediating the induction of survival of *D. melanogaster* by SALF. An increased survival following exposure to HSD+SALF in male flies previously reared on HSD was also observed but without any effects on the glucose levels suggesting that dietary SALF counteracts the HSD-mediated decrease in survival.

Similar to the therapeutic study, the weights, triglyceride, and fitness levels of flies in the T2DM prevention study were not affected. However, both male and female flies exposed to HSD supplemented with SALF exhibited higher survival rates than flies exposed to HSD only, which was, however, not reflected in the glucose levels. SALF may protect the flies from a HSD-induced decrease in survival. This lower survival in HSD-fed flies may be similar to premature mortality that has been documented for humans suffering from T2DM. The increased survival of flies exposed to HSD supplemented with SALF may be attributed to the presence of phenolics such as chlorogenic acid and quercetin, which have been reported to increase lifespan in *D. melanogaster*. In the present study, *D. melanogaster* fed on HSD+SALF benefited from the consumption of SALF, as reflected in the significant increase in survival.

In conclusion, morphological characteristics of *Solanum anguivi* Lam. accessions may be used to predict SALF accessions with similar BCC and AA. The unripe stage of SALF had the highest AA and TPC and thus may have the highest health-promoting properties. Combined thermal treatments (boiling for 15 min and drying at 35 ^{o}C) may mediate the strongest health benefits as they resulted in the highest AA increment of all investigated thermal treatments. The dietary intake of SALF significantly lowered the glucose levels and increased the survival of *D. melanogaster*, which was, however, not sex-dependent. Additional studies in higher organisms, including humans, are needed to unravel the underlying mechanisms of the therapeutic and preventive effects of dietary SALF and its potential use in the management and treatment of T2DM.

ZUSAMMENFASSUNG [DEUTSCH]

Aufgrund veränderter Lebens- und Ernährungsgewohnheiten und der damit verbundenen weltweit steigenden Prävalenz von Typ2 Diabetes mellitus (T2DM), gibt es eine zunehmende Belastung der Sozialsysteme insbesondere des Gesundheitssysteme. Zur Behandlung des T2DM wurden verschiedene Medikamente entwickelt, die für die Therapie auch zur Verfügung stehen. Diese weisen jedoch häufig Nebenwirkungen auf und sind teuer, was in ärmeren Ländern ein Problem darstellt. *Solanum anguivi* Lam. Früchte (SALF) werden traditionell als Heilmittel bei T2DM konsumiert. Diese potentielle Wirkung kann möglicherweise auf die hohen Gehalte bioaktiver Verbindungen wie Phenole, Flavonoide, Saponine, Alkaloide und Vitamin C in SALF zurückgeführt werden. Die Nährwertqualität von Obst und Gemüse kann - je nach Anbau und Reifestadium - variieren oder auch durch eine thermische Behandlungen beeinträchtigt sein. Es liegen jedoch nur wenige Daten über den Einfluss der einzelnen Akzessionen und des Reifestadiums sowie der Wirkung thermischer Behandlungen auf die Qualität von SALF vor. Daten zur potenziell antidiabetischen Wirkung von SALF sowie mögliche zugrunde liegende Wirkmechanismen sind nur marginal vorhanden. In der vorliegenden Arbeit wurde der Gehalt an bioaktiven Verbindungen (BCC) und die antioxidative Aktivität (AA) von SALF sowie die Beziehungen zwischen SALF, BCC, AA und den morphologischen Eigenschaften der Pflanze untersucht. Insebsondere wurde der Einfluss des Reifestadiums (unreif, gelb, orange und rot) auf die BCC und AA der verschiedenen SALF-Akzessionen genauer analysiert. Auch der Einfluss der Trocknungstemperatur (35, 55 und 85 ^{o}C), der Kochmethode (Kochen und Dämpfen) und die Dauer (15, 30, 45, 60 und 120 min) auf die BCC und AA der verschiedenen SALF-Akzessionen wurde ermittelt.

Weiterhin wurde in der vorliegenden Arbeit das mögliche präventive l (Präventionsstudie) sowie das therapeutische (therapeutische Studie) Potenzial unter Verwendung des Modells der Fruchtfliege *Drosophila melanogaster* (*D. melanogaster*) genauer untersucht. *D. melanogaster* entwickeln unter einer zuckerangereicherten Diät einen dem T2DM ähnlichen Phänotypen. Der Energiestoffwechsel der Fruchtfliege ist bis zu einem gewissen Grad mit dem des Menschen vergleichbar. So sind in *D. melanogaster* eine Vielzahl an Genen des Energiestoffwechsels konserviert, die auch im Menschen zu finden sind (z.B. Gene, die im Insulin/Insulin-ähnlichen Wachstumsfaktor-Signalweg eine Rolle spielen). Stoffwechselparameter und physiologische Reaktionen auf die Ernährung scheinen auch vom Geschlecht eines Tieres abzuhängen und auch für *D. melanogaster* wurde eine sexuell dimorphe Reaktion auf die Aufnahme von HSD

gezeigt. Daher wurden in der vorliegenden Arbeit die Untersuchungen sowohl an männlichen als auch an weiblichen Fliegen durchgeführt, um eine mögliche geschlechtsabhängige Schutzwirkung von SALF zu identifizieren.

In der vorliegenden Arbeit wurde die morphologische Charakterisierung, einschließlich elf Merkmale für Blätter, Stängel und Früchte von 12 Pflanzen pro Akzession, basierend auf den Deskriptoren von Solanum-Arten bewertet. Der BCC wurde durch Spektrophotometrie (für Gesamtphenole, Flavonoide, Saponine und Vitamin C), HPLC (für die Quantifizierung von Phenolen) und Gravimetrie (für Gesamtalkaloide) bestimmt. Neben der AA, bestimmt als Radikalfängeraktivität (FRSC) wurde auch die Gesamtantioxidanskapazität (TAC) durch Spektrophotometrie bestimmt. Für die Therapie- und Präventionsstudie wurden männliche und weibliche Fliegen getrennt einer HSD ausgesetzt, um jeweils einen T2DM-ähnlichen Phänotyp zu induzieren. Als Kontrolle dienten Fliegen, die mit einem Kontroll-Medium (SY10) gefüttert wurden. Für die T2DM-Therapiestudie wurden die Fliegen für 10 Tage mit einer HSD oder einer Kontrolldiät gefüttert. Anschließend wurden die Tiere entweder auf einer HSD ergänzt mit 5 (HSD+SALF5) oder 10 (HSD+SALF10) mg/ml SALF, einer HSD oder einer Kontrolldiät bis Tag 24 gehalten. Um zu beurteilen, ob ein T2DM-ähnlicher Phänotyp, der durch eine HSD-Exposition induziert wurde, durch eine SALF-Supplementierung, verhindert werden kann, wurden männliche und weibliche Fliegen auf Kontrolle, HSD, HSD+SALF5 oder HSD+SALF10 für 24 Tage gehalten. Die Wirkung von HSD und HSD+SALF auf Gewicht, Kletteraktivität, Glukose- und Triglyceridgehalte, Überleben und Genexpression von *Srl*, dem PPARγ Co-Aktivator 1α (PGC-1α) Fliegenhomolog sowie auf die Expression der *Drosophila* insulinähnlichen Peptide (*dIlp*) *3* und *5* wurde in der vorliegenden Arbeit untersucht. Die Anzahl der lebenden Tiere wurde alle 2-3 Tage im Rahmen des Futterwechsels dokumentiert, während die Gewichte und die Kletteraktivität an Tag 24 des Versuchs ermittelt wurde. Die Glucose- und Triglyceridgehalte sowie die mRNA-Spiegel von *Srl*, *dIlp 3* und *5* wurden in den Fliegen analysiert.

Die morphologischen Eigenschaften von *Solanum anguivi* Lam. variieren zwischen den in der vorliegenden Arbeit untersuchten Akzessionen. BCC und AA von SALF unterscheiden sich signifikant zwischen den Akzessionen. Im Vergleich zu Nahrungsmitteln, die als reichhaltige Quelle für die entsprechenden Verindungen gelten, wiesen die untersuchten SALF-Akzessionen hohe Gehalte an Gesamtphenolen, Flavonoiden, Saponin, Vitamin C und Alkaloiden auf. Auf den entsprechenden Akzessionen basierenden morphologischen

Eigenschaften bzw. enthaltenen bioaktiven Verbindungen wurden Cluster abgeleitet, die Ähnlichkeiten in diesen Parametern aufwiesen. Das Reifestadium von SALF beeinflusste signifikant BCC und AA, wobei dies signifikant von der entsprechenden Akzession abhängig war. Die höchsten Gesamtgehalte an Phenolen und Flavonoiden und die höchsten AA konnten im unreifen Stadium der Frucht detektiert werden, die höchsten Flavonoid- und Saponin-Gesamtgehalte wurden im orangenen Reifestadium, die höchsten Vitamin C- (für einige Akzessionen) und Alkaloid-Gehalte im roten Reifestadium ermittelt. Im Vergleich zum roten Reifestadium zeigte sich, dass im unreifen Stadium höhere Gehalte an Gesamtphenolen (bis zu 39 % mehr), Flavonoiden (bis zu 15 % mehr) und AA (um 49-94%) zu detektieren waren. Im Vergleich zum unreifen Stadium wies SALF im roten Reifestadium jedoch 17-79% höhere Mengen an Gesamtalkaloiden und im orangefarbenen Reifestadium bis zu 45% höhere Gesamtsaponine auf. Im Rahmen der HPLC-Analyse zeigte sich, dass mit zunehmender Reife der Früchte die Konzentrationen an Chlorogen- und Kaffeesäure abnahmen, die Konzentrationen an Gallussäure und Rutin zunahmen, während die Wirkung auf die Quercetin-Gehalte zwischen den Akzessionen variierte. Die Regressionsmodelle zeigten, dass AA im unreifen Stadium hauptsächlich vom Gesamtphenolgehalt (TPC) und Vitamin C-Gehalt (VCC) beeinflusst wird, vom TPC und Gesamtflavonoidgehalt (TFC) im gelben Reifestadium, vom TFC und Gesamtsaponingehalt (TSC) im orangefarbenen Reifestadium und vom TFC und Gesamtalkaloide im roten Reifestadium von SALF abhängig ist.

Thermische Behandlungen beeinflussten die BCC und AA von SALF erheblich. Bei einer Trocknungstemperatur von 35 °C wurde in den SALF-Proben der höchste Anstieg von TPC und AA detektiert (2fach). Bei 85 °C wurde ein geringer Effekt auf die TPC sowie eine signifikant niedrigere AA im Vergleich zur Kontrolle (rohes/ungetrocknetes SALF) festgestellt. Der Gehalt an Chlorogensäure, Kaffeesäure und Rutin war bei 35 °C höher als bei 85 °C, während das Gegenteil für den Gehalt an Gallussäure zu verzeichnen war. Die Auswirkung der Trocknungstemperatur auf den Quercetin-Gehalt variierte sowohl durch die verschiedenen Akzessionen als auch Reifestadien. VCC und TFC wurden durch das Trocknen im Vergleich zur Kontrolle signifikant reduziert, wobei eine Trocknungstemperatur von 35 °C die höchste Retention aufwies. Die Wirkung des Trocknens auf die Gesamtalkaloide und Saponine war abhängig von der jeweiligen Akzession sowie vom Reifestadium. Sowohl das Dämpfen als auch das Kochen reduzierte den VCC in SALF erheblich. Auch die einzelnen Kochparameter führten zu eienr signifikanten Abnahme des TSC, mit Ausnahme der Kochzeit

von 15 Minuten, wo kein Einlfuss auf den TSC zu beobachten war. Die Gesamtalkaloide in SALF wurden durch Dämpfen signifikant reduziert und durch Kochen erhöht. Eine sehr lange Kochdauer (120 min) erhöhte den Alkaloid-Gehalt beim Dämpfen um das 1.5-Fache, beim Kochen um das 2-Fache. Dämpfen und Kochen hatten keinen Einfluss auf die Gesamtflavonoide in SALF, lediglich bei einer Kochzeit von 15 Minuten zeigte sich ein signifikant erhöhter Gehalt der Gesamtflavonoide. Durch Kochen und Dämpfen wurden wowohl der TPC als auch die AA von SALF um das 4-fache bzw, 3-fache erhöht. Die höchsten TPC- und AA-Zunahmen bei SALF wurde bei der thermischen Behandlungen durch Kochen induziert, wobei das Kochen für eine Dauer von 15 Minuten mit den höchsten TPC und AA in Verbindung stand. Wurden die beiden thermischen Methoden kochen (15 Minuten) und trocknen (bei 35°C) kombiniert, zeigte SALF eine höhere AA als ausschließlich gekochtes oder ausschließlich getrockenetes SALF. Im Rahmend der vorliegenden Studie konnte eine positive Korrelation zwischen AA und dem Gesamtgehalt an Phenolen, Flavonoiden und Saponinen SALF und für die Gehalte an Vitamin C und Alkaloiden eine negative Korrelation gezeigt wurden.

Die Ergebnisse der therapeutischen T2DM-Studie zeigten, dass durch die vier Behandlungen (HSD, HSD+SALF5, HSD+SALF10 und Kontrolldiät) weder das Gewicht, noch die Fitness und der Triglyceridspiegel von männlichen und weiblichen Fliegen verändert wurde. Die HSD-gefütterten weiblichen Fliegen wiesen erhöhte Glukosespiegel auf, die bei einer Exposition gegenüber SALF-supplementierter HSD dosisabhängig signifikant abnahmen. Diese blutzuckersenkende Wirkung von SALF könnte auf das Vorhandensein von Saponinen und Phenolen zurückgeführt werden, da diese Verbindungen bei Mäusen mit einer hypoglykämischen Wirkung in Verbindung gebracht wurden. Weibliche Fliegen, die einer SALF-supplementierten HSD gehalten wurden, zeigten einen signifikanten Anstieg der Überlebensrate im Vergleich zu entsprechenden HSD- und mit Kontrollfutter gefütterten Fliegen. Um den zugrunde liegenden Schutzmechanismus von SALF aufzuklären, wurde die Expression zentraler Gene des Energiestoffwechsels der Fliege untersucht. Die mRNA-Spiegel von *Srl* nahmen bei HSD-gefütterten weiblichen Fliegen im Vergleich zu den Kontrollen ab, während bei HSD+SALF-exponierten Weibchen keine Wirkung beobachtet wurde. Diese Beobachtung kann möglicherweise auch das nicht beeinflusste Gewicht sowie die nicht beeinträchtigte Fitness weiblicher Fliegen erklären, die mit HSD+SALF gefüttert wurden, da eine Hochregulierung von *Srl* mit einer Gewichtszunahme und erhöhter Fitness in Verbindung

gebracht wurde. Während die Expression von *dIlp3* in allen Gruppen weiblicher Fliegen nicht verändert war, wurde *dIlp6* bei weiblichen Fliegen durch HSD+SALF5 im Vergleich zur Kontrolle signifikant induziert, was auf eine zentrale Rolle von *dIlp6* in einer SALF-induzierten Steigerung der Lebensspanne von *D. melanogaster* hindeutet. Eine Erhöhung der Überlebensrate durch eine HSD+SALF-Exposition bei zuvor mit HSD aufgezogenen männlichen Fliegen wurde ebenfalls beobachtet, jedoch ohne Auswirkungen auf den Glukosespiegel. Dies deutet möglicherweise darauf hin, dass eine SALF-Supplementierung der HSD-vermittelten Reduktion der Lebensspanne entgegen wirken kann. Diese Wirkung scheint jedoch unabhängig von einer Wirkung auf den Glukosespiegel zu sein.

In der T2DM-Präventionsstudie waren weder das Gewicht, noch die Triglyceridspiegel und das Fitnessniveau durch eine SALF-Supplementierung der Fliegen beeinflusst. Jedoch zeigten sowohl männliche als auch weibliche Fliegen, die eine HSD mit SALF erhielten, eine höhere Überlebensrate, als Fliegen, die nur HSD erhielten, was sich allerdings nicht in den Glukosespiegeln widerspiegelte. In *D. melanogaster* kann SALF vor einer durch eine gesteigerte Glukoseaufnahme bzw. durch erhöhte Glukosesiegel induzierten Verringerung des Überlebens entegenwirken. Möglicherweise ist das in den Fliegen beobachtete geringere Überleben mit einer vorzeitigen Sterblichkeit vergleichbar, wie sie für Menschen mit T2DM dokumentiert wurde. Diese protektiven Wirkungen von SALF können möglicherweise auf die vorhandenen bioaktiven Substanzen wie beipieslweise von Phenolen wie Chlorogensäure und Quercetin zurückgeführt werden, für die beschrieben wurde, dass sie die Lebensdauer von *D. melanogaster* erhöhen können.

Zusammenfassend kann festgestellt werden, dass sich die im Rahmen der vorliegenden Arbeit ermittelten morphologischen Merkmale von *Solanum anguivi* Lam. Akzessionen nutzen lassen, um SALF-Akzessionen mit ähnlichen BCC und AAs vorherzusagen. Das unreife Stadium von SALF wies die höchsten AA und TPC auf und vermittelt daher möglicherweise die höchsten gesundheitsfördernden Eigenschaften. Kombinierte thermische Behandlungen (Kochen für 15 Minuten und Trocknen bei 35 °C) können möglicherweise den Nutzen für die Gesundheit noch verstärken, da ihre Anwendung zu den höchsten AA von SALF führten. Bei *D. melanogaster* resultierte die Aufnahme von SALF in einem signifikant niedrigeren Glukosespiegel und einer erhöhten Überlebenrate, wobei die beobachteten Wirkungen nicht geschlechtsabhängig waren. Für die weitere Aufklärung potenziell therapeutischer und präventiver Wirkungen von SALF bei T2DM sind weitere Untersuchungen an höheren

Organismen, einschließlich dem Menschen, notwendig, um zugrundeliegende Mechanismen weiter aufzuklären und einen potentiellen Einsatz als Antidiabetikum aufzuklären und zu prüfen.

CHAPTER ONE

INTRODUCTION

1.1. Background

Non-communicable diseases (NCDs), also known as chronic diseases, result from a combination of genetic, physiological, environmental, and behavioural factors, such as tobacco use, physical inactivity, the harmful use of alcohol, and unhealthy diets (WHO, 2018). NCDs (diabetes, cardiovascular diseases, cancer, and chronic respiratory disease) are collectively responsible for 71% of all deaths worldwide (WHO, 2018). The social burdens associated with chronic diseases include; prolonged disability, diminished resources within families, reduced productivity, and tremendous demands on health systems (WHO & World Economic Forum, 2011). NCDs may increase individual and household impoverishment, which deters social and economic development (WHO, 2018); thus, NCDs are associated with poverty and may hinder poverty reduction initiatives (WHO, 2020).

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder of fuel homeostasis characterized by insulin resistance and pancreatic β-cell dysfunction resulting from chronic hyperglycemia (I. Hameed et al., 2015). It has also been associated with sub-clinical inflammation, oxidative stress, and dyslipidemia (I. Hameed et al., 2015; Tangvarasittichai, 2015). The burden of diabetes is enormous due to its rapidly increasing global prevalence, and the changes in lifestyle and dietary habits, possibly due to urbanization (I. Hameed et al., 2015; Nolan et al., 2011). Diabetes may lead to long-term pathogenic conditions such as micro-and macro-vascular complications, neuropathy, retinopathy, nephropathy, and consequently decreases the quality of life and increases the rate of mortality (Bahadoran et al., 2013; Evans et al., 2002; Nolan et al., 2011; Santaguida et al., 2005; Spranger et al., 2003). The first-line treatment of T2DM is metformin, which inhibits gluconeogenesis (synthesis of glucose in the body from non-hexose precursors (R. W. Hanson & Owen, 2013), through the activation of AMP-activated protein kinase (AMPK) (Marín-Peñalver et al., 2016). Other oral drug classes include insulin secretagogues, which increase insulin secretion, α-glucosidase inhibitors that reduce hyperglycemia through delayed carbohydrate absorption and digestion, thiazolidinediones, which increase glucose uptake through increased insulin sensitivity and dipeptidyl peptidase-4 (DPP4) inhibitors, which increase insulin secretion and inhibit glucagon (Marín-Peñalver et al., 2016). However, various side effects associated with T2DM drugs have

been reported, such as diarrhoea, anorexia, weight gain, abdominal discomfort, hypoglycemia, and nausea (Jung et al., 2006; Marín-Peñalver et al., 2016).

For centuries, plants have been traditionally used as medicine to treat several ailments, including diabetes, as they were available and considered safe (Abu-Odeh & Talib, 2021; Sathasivampillai et al., 2017). *Solanum anguivi* Lam. fruits (SALF) are vegetables commonly consumed in Australia, Africa, and Asia and are traditionally believed to reduce the risk of diabetes (Bukenya & Carasco, 1995; Elekofehinti et al., 2012; Jayanthy et al., 2016). The therapeutic effect of SALF extracts in diabetic mice has been previously reported (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011), further suggesting its potential to treat T2DM. The SALF antidiabetic effects have been attributed to the presence of bioactive compounds such as phenolics, flavonoids, vitamin C, saponins, and alkaloids (Abbe et al., 2019; Dan et al., 2014). *Solanum anguivi* Lam. (*S. anguivi*) is an ethnomedicinal plant belonging to the family *Solanaceae.* It is commonly known as "forest bitter berry" or "African eggplant" (Kaunda & Zhang, 2019), although the latter is also used for *Solanum aethiopicum* and *Solanum macropcarpon* (R. Y. Yang & Ojiewo, 2013). It grows mostly in the wild and tropics, but sometimes, e.g. in Uganda and Côte d'Ivoire, it is a semi-cultivated vegetable (Bukenya, 1993). However, data on how much SALF is produced is scarce. *S. anguivi* is a diverse plant as it has been reported to have different accessions (Stedje & Bukenya-Ziraba, 2003). The SALF are usually consumed in raw form or after thermal treatments such as boiling (Abbe et al., 2019), drying (and then milling) (Bamba et al., 2020), and steaming.

1.2. Problem statement

Goal 3 of the UN sustainable development goals (SDGs) is to ensure healthy lives and promote well-being for all of all ages (UN, 2015). Chronic diseases such as T2DM are a threat to human life, as they may lead to premature deaths (WHO, 2016). The burden of T2DM on the global health system and economy may result from costs that may be direct (e.g. lengthy and exorbitant medical costs) or indirect (e.g. loss of productivity and untimely deaths) (Nolan et al., 2011; WHO, 2016), which may consequently suppress development (WHO, 2018). The high cost of T2DM management drugs in low-income countries often forces millions of people into poverty, which further stifles development (WHO, 2018). Although some households may afford the T2DM treatment (e.g. in high-income countries), these drugs still have a challenge of various side effects, with some withdrawn from the market (Meriga et al., 2017). Thus, safer

and more affordable approaches are needed for the treatment of T2DM in combination with the drugs. Concomitantly, the reduction of the global burden of T2DM may include approaches that positively affect T2DM modifiable risk factors. Among the multiple risk factors underlying the incidence and progression of T2DM, diet is the main modifiable factor (Bahadoran et al., 2013). Dietary-based strategies have been reported as effective in the prevention and management of T2DM (Asif, 2014) and are also cost-effective (Franz et al., 1995). Research on underutilized fruits such as SALF, which are believed to treat T2DM was thus necessary, and their low agricultural costs would make them easily grown in low-income settings, thus rendering them accessible for potential health benefits.

Various studies have shown that the health-promoting properties of fruits and vegetables are due to the presence of bioactive compounds, whose content and antioxidant activity (AA) may be influenced by factors such as accession, ripeness stage, and thermal treatments. Stedje & Bukenya-Ziraba (2003) reported that SALF accessions differed genetically; however, the information on their morphological variations was limited. Simultaneously, the data on the variation of bioactive compounds content (BCC) and AA of SALF among its accessions and the whether the effect of ripening on the SALF BCC and AA varies with accession was scarce. Furthermore, literature on the effect of thermal treatments on the BCC and AA of SALF was scanty, with limited information documented by Abbe et al. (2019) and Bamba et al. (2020). This study sought to investigate the variation in morphological characteristics, BCC, and AA of SALF accessions. The ripeness stage with the highest BCC and AA, and whether this differed among SALF accessions was also assessed. Additionally, the study aimed to determine the thermal treatment(s) (drying temperature, cooking method, and cooking duration) that resulted in the highest BCC and AA in unripe and ripe SALF accessions.

Although the T2DM therapeutic effects of SALF extracts have been reported in mice (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011), information on the therapeutic potential of dietary (whole) SALF was still lacking. The efficacy recorded for extracts may be higher than actual food due to a higher concentration of the BCC in the former than the latter. Thus the therapeutic potential of dietary SALF needed to be investigated since SALF is usually consumed as food (Bukenya-Ziraba, 2004). Simultaneously, information on the potential of dietary SALF to prevent T2DM in any model organism was scarce. The possibility of bioactive compounds in foods to affect every step of the body's processes from gene to protein-altering metabolic pathways justifies the use of more accessible systems compared to humans, such as

model organisms, to elucidate the mechanisms of these complex interactions (Rubio-Aliaga, 2012). Additionally, the complex ethical issues and costs involved in human studies justify the use of model organisms. The energy metabolism of the fruit fly, *Drosophila melanogaster* (*D. melanogaster*), is reportedly comparable to that for humans (Morris et al., 2012; Musselman et al., 2011; Skorupa et al., 2008), as it involves insulin-like peptides and a glucagon-like peptide, adipokinetic hormone (AKH) (Staats, Lüersen, et al., 2018), whose roles are similar to insulin and glucagon, respectively. The similarity of the *D. melanogaster* insulin signalling with that in humans and the possession of a high number of genes conserved in humans (including those for the insulin/insulin-like growth factor signaling pathway) makes the former a suitable model for diabetes research (Lushchak et al., 2014; Staats, Lüersen, et al., 2018; van Dam et al., 2020). Additionally, the development of a T2DM-like phenotype including hyperglycemic and insulin-resistant states, and reduced lifespan upon high sugar diet (HSD) intake in *D. melanogaster* is well documented (Morris et al., 2012; Rovenko et al., 2015; van Dam et al., 2020). This study, thus, aimed to investigate the potential of dietary SALF in preventing and/ or managing a T2DM-like phenotype using the *D. melanogaster* model. As sex may influence the pathogenesis of numerous diseases in animals, including T2DM in humans (Chella Krishnan et al., 2018; Tramunt et al., 2020), the study simultaneously investigated whether the protective effects of SALF supplementation in HSD were sex-dependent. The *D. melanogaster* has been reported to exhibit sexually dimorphic responses to HSD (Chandegra et al., 2017).

1.3. General objective

To investigate the nutritional quality of dietary SALF and its potential to prevent and/ or manage T2DM-phenotype using the *D. melanogaster* model.

1.3.1. Specific objectives

i) To analyze the morphological characteristics, BCC, and AA of SALF accessions.

ii) To assess the influence of the ripeness stage on the BCC and AA of SALF accessions.

iii) To determine the effect of thermal treatments on the BCC and AA of SALF accessions.

iv) To investigate the therapeutic effects of different concentrations of dietary SALF in male and female *D. melanogaster* with a T2DM-like phenotype.

v) To investigate the preventive effects of different concentrations of dietary SALF against the development of a T2DM-like phenotype in male and female *D. melanogaster*.

1.3.2. Hypotheses

i) The BCC and AA of SALF significantly differ among the accessions.

ii) The ripeness stage significantly influences the BCC and AA of SALF accessions.

iii) Thermal treatments significantly affect the BCC and AA of SALF accessions.

iv) Dietary SALF dose-dependently manages T2DM-like characteristics in both male and female *D. melanogaster.*

v) Dietary SALF dose-dependently prevents a T2DM-like phenotype in both male and female *D. melanogaster.*

1.4. Significance of the study

This study shall have practical implications in nutrition and health aspects. The findings from the study may guide consumers on the SALF accession and ripeness stage with the highest nutraceutical potential, that is, the SALF accession(s) and ripeness stage with the highest content of a bioactive compound of interest and/ or AA.

The present study may provide information that would guide consumers on the drying temperature and cooking (boiling and steaming) duration that would result in the highest content or retention of bioactive compounds and AA of SALF for increased potential nutraceutical benefits for consumers as well as manufacturers of bioactive compound supplements.

The study provides information for the first time about the potential of dietary SALF to prevent the pathogenesis of T2DM-like phenotype and its therapeutic effects in flies with T2DM-like phenotype. This may guide future studies that seek to elucidate these benefits in higher model organisms such as mice and, eventually, humans.

CHAPTER TWO

LITERATURE REVIEW

2.1. Bioactive compounds

Bioactive compounds may be defined as essential and non-essential compounds that occur in nature, are part of the food chain, and can regulate metabolic functions leading to beneficial health effects (Biesalski et al., 2009; Galanakis, 2017). Bioactive compounds may be present in plant or animal food sources. Some of the examples of bioactive compounds include flavonoids, polyphenols, carotenoids, vitamins, minerals, choline, coenzyme Q, glucosinolates, and taurine (Hamzalioğlu & Gökmen, 2016). Bioactive compounds may present therapeutic potential with influence on energy intake (Siriwardhana et al., 2013), modulate metabolic processes, and demonstrate positive properties such as antioxidant effect, inhibition of receptor activities, and the inhibition or induction of enzymes and gene expression (Carbonell-Capella et al., 2014; D. I. Santos et al., 2019). This thesis focuses on plant-derived bioactive compounds, as discussed in the sections below.

2.2. Factors that may influence the contents of the bioactive compounds in SALF

The synthesis and degradation of bioactive compounds are regulated by the interaction of genetic, agronomic, and environmental factors (Leskovar et al., 2009). However, defining the optimum pre-harvest (genetics, agronomy, and environment) factors to maximize the biosynthesis of specific bioactive compounds is complex and difficult (Pérez-Gregorio et al., 2014).

2.2.1. Genetics

Different varieties, cultivars, or accessions of the same plant species grown under the same conditions, have been previously reported to have different contents of bioactive compounds and AA. Accumulating evidence has suggested that genotype may have a profound influence on the content of the bioactive compounds (Benvenuti et al., 2004; Milivojević et al., 2010). Li et al. (2012) reported that the significant differences in carotenoid content and AA among 20 cultivars and breeding lines of *Solanum lycopersicum* L (tomatoes) were due to genetic differences. Hanson et al. (2006) and Okmen et al. (2009) similarly reported significant differences in the TPC and AA of *S. melongena* (eggplant) accessions and cultivars,

respectively. Genetic variance among SALF accessions has been previously reported by Stedje & Bukenya-Ziraba (2003). However, it has not been documented whether the BCC and AA of SALF differ among its accessions. Although the BCC in some plants has been reported to correlate with their morphological characteristics (Boucheffa et al., 2019; Dalir & Safarnejad, 2017), the relationship between the morphological characteristics, BCC, and AA of SALF has been not reported.

2.2.2. Agronomic factors

2.2.2.1. Ripeness stage

Various studies have shown that the ripeness stage may either increase or decrease the contents of plant bioactive compounds. Dan et al. (2014) showed that phenolics, catechic tannins, quinones, and ascorbic acid of SALF decreased with ripening, while sterols, polyterpenes, flavonoids, saponins, and coumarins increased. There was no variation in coumarins during the ripening of SALF. Saponins, flavonoids, sterols, and polyterpenes were rich in orange and red SALF, while polyphenols and catechin tannins were rich in green and yellow berries. Similarly, Abbe et al. (2019) reported that as SALF ripened, its tannin, vitamin C, and polyphenols contents decreased, as well as the FRSC, while the flavonoid contents increased. These results showed how the SALF ripeness stage influences some of the BCC and AA. However, it has not been reported whether the ripening affects the saponin and alkaloid contents and whether the influence of the ripeness stage on the BCC and AA of SALF differs among the accessions.

2.2.2.2. Soil

Soil texture (sand, loam, or clay) and organic matter can have significant effects on plant growth and plant composition through changes in cation exchange capacity, soil aeration, and water infiltration (Leskovar et al., 2009). Similarly, soil structure (degree of aggregation, size, and form of aggregates) greatly influences water movement, aeration, and porosity. The direct combined effect of soil texture and structure on mineral concentration can be very significant. Studies have reported the effect of various amounts of specific soil nutrients on the overall nutritional content of fruits and vegetables. The BCC may vary widely in response to nitrogen fertilization rates, partly due to differences in cultivars, soil type, methods and timing of application, N sources, as well as rainfall and irrigation management (Leskovar et al., 2009). Low nitrogen supply was reported to increase the content of some phenolic compounds (rutin, a caffeic acid glycoside, and a caffeic acid derivate) and total ascorbic acid in tomatoes (Bénard

et al., 2009), while high N fertilization rates may decrease ascorbic acid (Leskovar et al., 2009). A study by Aina et al. (2019) also showed a strong relationship between the levels of macronutrients like nitrogen, phosphorus, and potassium in the soil and the level of phenolics, flavonoids, beta-carotene, and lycopene contents in tomatoes.

2.2.2.3. Climatic factors

Plants of the same species grown in different environments have been reported to contain different BCC resulting from differences in their geographical zones (Dong et al., 2011; W. Liu et al., 2016). Differences in the BCC may also be observed in plants from the same species and same growing region but different years of harvest. The differences may result from changes in temperatures and wind patterns associated with climate change, which affect precipitation and consequently the plant architecture, flowering, fruiting, and the BCC (S. Kumar et al., 2017). Seasonal changes may also affect the BCC of plants. Milivojević et al. (2010) observed significant differences in flavanol contents and TPC of black currant cultivars, which also differed throughout the year due to changes in the weather conditions. The altered production of secondary metabolites as a result of temperature stress is an expression of the self-defence mechanism by medicinal plants, such as increased biosynthesis of phenolic compounds in low temperatures (Bilger et al., 2007; W. Liu et al., 2016). Different researchers reported that variations in climatic factors might elicit different BCC values for fruits from the same species.

2.2.2.4. Post-harvest handling

Postharvest handling, such as storage, may lead to changes in both the quality and BCC of plants (H. Li, Tsao, et al., 2012). Postharvest storage may affect flavonoid and phenolic contents and AA of fruits and vegetables (Ayala-Zavala et al., 2004). Storing freshly harvested *Solanum melongena* (eggplants) in varied temperature and light-controlled chambers for ten days significantly affected their TPC (Cortbaoui & Ngadi, 2015). The TPC substantially degraded in eggplants exposed to elevated temperatures (30 ^{o}C), while an increase was observed in those that were maintained at 10 ^{o}C in the absence of light. Concellón et al. (2012) also noted elevated TPC in eggplants stored at a lower temperature. These findings revealed that postharvest handling of fruits and vegetables might alter their BCC, and thus, the methods employed should be considered before BCC analyses.

2.2.2.5. Processing and preparation

The health-promoting capacity of fruits and vegetables also depends on their processing history, which may affect the content, activity, and bioavailability of the bioactive compounds (Nicoli et al., 1999). Previously, consumption of unprocessed vegetables such as green leafy vegetables was promoted due to the anticipated detrimental effects of processing on their AA (Tarwadi & Agte, 2003). However, evidence has emerged showing a possible enhancement of the bioavailability and extractability of some bioactive compounds in some cooked vegetables (Link & Potter, 2004; Miglio et al., 2008), such as phenolics (Ifie & Marshall, 2018) and carotenoids (Chan et al., 2014; Gärtner et al., 1997; J. Shi & Le Maguer, 2000) in cooked tomatoes.

The cooking and processing methods that may be subjected to some vegetables before consumption include steaming, boiling, baking, roasting, and frying (van Boekel et al., 2010). These methods may, however, variously affect the BCC and AA of the vegetables, and the magnitude of these impacts may depend on the food matrix and the cooking and processing parameters such as temperature and time (Al-juhaimi et al., 2018). Miglio et al. (2008) showed that water-cooking treatments (boiling and steaming) rather than frying; better preserved the polyphenols, carotenoids, glucosinolates, and ascorbic acid than in carrots, courgettes, and broccoli. The cooked vegetables also had significantly higher AA than the raw ones, a phenomenon attributed to food matrix softening and increased extractability of compounds in the former, which may have partially contributed to increased antioxidant compounds (Miglio et al., 2008). Dolinsky et al. (2016) reported that heat treatment significantly impacted the AA and the soluble and hydrolyzable polyphenolic contents of most vegetables analyzed, either increasing or decreasing the concentration of these compounds.

There are some reports on the effect of boiling and drying SALF. Abbe et al. (2019) observed that boiling SALF from different ripeness stages for 5 or 10 min reduced total phenolics, flavonoids, vitamin C and FRSC, an effect that increased with boiling time. However, the data on the impact of boiling on the saponin and alkaloid contents and TAC is scarce. SALF may also be steamed prior to consumption. For example, in Uganda, SALF is usually wrapped in banana leaves and placed on top of bananas (*matooke*). The SALF is separately wrapped in banana leaves and steamed until the bananas are ready (approximately 2 hours). However, there are no previous reports on the effect steaming has on the SALF BCC and AA.

Alternatively, SALF may be traditionally processed into powder by sun-drying for 2 to 3 days (Bamba et al., 2020) and then milled. However, sun-drying may cause biological and physical contamination (Arslan & Özcan, 2012) and, therefore, preferring oven-drying because it is faster and safer (Agiriga et al., 2015). Bamba et al. (2020) determined the effect of oven-drying temperature (60 ^{o}C and 80 ^{o}C for 24 hours, or 100 ^{o}C for 8 hours) on the TPC and TAL of SALF. The TPC increased with temperature, while the TAL was not significantly affected. However, the effect of drying temperature on the SALF total flavonoids, saponins, vitamin C, and AA, as well as the effect of low oven temperature on the SALF BCC and AA, has not been documented.

2.3. The *D. melanogaster* as a model for type 2 diabetes mellitus.

A model organism is an organism in which biology can be studied, and whole parts or its whole biological process resembles that for humans or any other species of interest (Rubio-Aliaga, 2012). Numerous models have been developed to understand the pathophysiology of diabetes, its complications, and its treatment. However, invertebrate model organisms have become a cornerstone of various fields of biological and biomedical research, including diabetes (Wilson-Sanders, 2011). The fruit fly, *D. melanogaster,* is one of the most commonly used invertebrate models. It is a very versatile and potent model used in recent years for studies in nutrition, metabolism, and metabolic disorders such as T2DM.

The *D. melanogaster* possesses various similarities with mammals, which makes it suitable for nutritional intervention studies. The fly's organs such as the heart, lung, kidney, liver, gonads, intestines, and hormones such as insulin-like peptides, which are produced from the fly's central and peripheral nervous system, have similar functions as those in mammals (Staats, Lüersen, et al., 2018). The digestion and absorption of food in the fruit fly occur mainly in the midgut. Similar to mammals, dietary macronutrients are broken down by hydrolases (as mammalian intestinal enzymes) prior to absorption, such as the breakdown of starch to monosaccharides by the fly's α-amylases (Commin et al., 2013; Staats, Lüersen, et al., 2018). The dietary lipids and proteins are digested by the lipases that are secreted into the midgut lumen (Horne & Haritos, 2008; Sieber & Thummel, 2009, 2012), and peptidases such as trypsin (Davis et al., 1985; Lemaitre & Miguel-Aliaga, 2013), respectively, as in humans. To control their metabolism and development, *D. melanogaster* possess a complex neuroendocrine system (similar to humans), which produces peptide and steroid hormones, such as insulin-like

peptides.

2.3.1. Types of insulin-like peptides in adult *D. melanogaster*

The *D. melanogaster* possesses eight homologous insulin-like peptides (dILP1−8). The dILP1, 2, 3, and 5 are mainly produced in 14 beta cell-like insulin-producing cells (IPCs) found in the fly's brain (Brogiolo et al., 2001; Nässel et al., 2013; Okamoto et al., 2009). The dILP3 is also produced in the muscle cells of the midgut and the dILP5 in the ovary and renal tubules (Nässel et al., 2013; Veenstra et al., 2008). The dILP6 is mainly produced in adipose cells of the fat body (Okamoto et al., 2009; Slaidina et al., 2009), dILP7 in the abdominal neuromeres of the fused thoracic-abdominal ganglia (Brogiolo et al., 2001; Miguel-Aliaga et al., 2008; C. H. Yang et al., 2008) and dILP8 in the ovary (Chintapalli et al., 2007; Nässel et al., 2013). The expression of dILP4 in the adult tissues is not yet known (Semaniuk et al., 2021). Furthermore, only dILP2, 3, 5, 6, and 7 have established roles in adult fly physiology (Nässel et al., 2013). Noteworthy, the *D. melanogaster* fat body performs functions of both adipose and liver as in mammals, including the storage and mobilization of energy reserves such as glycogen and fat (Ugur (Alfa & Kim, 2016; Arrese & Soulages, 2010; Ugur et al., 2016).

2.3.2. Glucose homeostasis in *D. melanogaster*

Similar to mammals, insulin-like peptides are released in response to high levels of circulating sugar, while a glucagon-like molecule, adipokinetic hormone (AKH), is released in response to low levels of circulating sugar (Graham & Pick, 2017; Haselton et al., 2010; S. K. Kim & Rulifson, 2004; G. Lee & Park, 2004). It stores excess energy in the form of glycogen, and lipids, which are mobilized when energy is needed, such as during exercise and nutrient depletion (Baker & Thummel, 2007; Palanker et al., 2009; Teleman, 2010). Unlike humans, upon consumption of food, simple sugars are passively taken up from the digestive tracts directly into the insect's fat body, where it is then converted to trehalose, a nonreducing sugar (Graham & Pick, 2017). Trehalose may be stored and/ or released into the hemolymph as the primary circulating sugar in insects. However, some studies have reported that unlike circulating glucose, trehalose levels do not respond strongly to dietary sugars (Mattila & Hietakangas, 2017; Ugrankar et al., 2018). Hemolymph contains very low levels of glucose as compared to trehalose. However, elevated glucose levels may be induced if the flies are exposed to high sugar diets (Graham & Pick, 2017). The high circulating trehalose levels provide sufficient energy that is needed for insect flight muscle and brain function (Becker et

al., 1996; Mattila & Hietakangas, 2017). Previous studies have shown that the genes associated with circulating glucose levels do not affect trehalose levels, which suggested that glucose and trehalose levels are independently regulated (Mattila & Hietakangas, 2017; Ugrankar et al., 2018).

Drosophila's insulin signalling is very similar to the human insulin pathway, and it has been used to study many different aspects of diabetes (Álvarez-Rendón et al., 2018). In humans, the release of insulin from the pancreatic β-cells is dependent on glucose-sensing by GLUT1 and/ or GLUT2. Entry of glucose into the β-cells triggers glycolysis and the subsequent release of ATP from the mitochondria. The increase in ATP results in the closure of K_{ATP} channels in the cell membrane, causing the cell to depolarize, which activates Ca^{2+} channels and consequent insulin exocytosis from the β-cells (detailed discussion in section 2.4 of this thesis). Similar to human β-cells, *Drosophila* IPCs respond to glucose with an influx of Ca^{2+} and action potentials as in humans (Fridell et al., 2009; Graham & Pick, 2017; Kréneisz et al., 2010). This response is triggered by a K_{ATP} channel activator, glibenclamide, thus implicating K_{ATP} channels in the response. Furthermore, the GLUT1 in fruit flies has also been suggested to perform similar roles as reported in mammals (Graham & Pick, 2017; Park et al., 2014).

Several studies have further reported the similar roles played by insulin and dILP. Consumption of a glucose-rich diet has been reported to induce the secretion of dILP2, 3, and 5 (S. K. Kim & Rulifson, 2004; Nässel et al., 2013; Song et al., 2017). A reduction in the expression of *dIlp3* and *dIlp5* in the IPCs in response to lower dietary sugar levels (but not amino acid starvation) has been reported, indicating that *dIlp* levels can respond to specific nutritional cues as insulin in humans (Colombani et al., 2003; Ikeya et al., 2002). Additionally, genetic ablation of IPC may result in diabetic phenotypes in fruit flies, including a significant increase in circulating glucose and trehalose and a moderate increase in stored lipids (Broughton et al., 2005; Rulifson et al., 2002). This further showed the similarities in the roles of dILP and insulin. As in humans, the binding of dILP to the insulin/IGF-like tyrosine kinase receptor (INR) activates phosphoinositide 3-kinase (PI3K)-dependent signalling pathways via the *Drosophila* insulin receptor substrate (IRS) Chico, which then promotes the activation of the serine/threonine-specific protein kinase AKT(Álvarez-Rendón et al., 2018). This, therefore, suggests the conserved glucose-sensing mechanism and signalling pathways that trigger dILP and insulin release in *Drosophila* and humans, respectively. Overall, dILP signalling in target tissues has anabolic effects, increases nutrient storage, and supports growth (Diegelmann et al., 2017;

Nässel et al., 2015; Oldham, 2011; Staats, Lüersen, et al., 2018).

Synthesis of AKH occurs in α-cell-like corpus cardiacum (CC) cells that are part of the ring gland directly connected to the fly's IPCs and heart (S. K. Kim & Rulifson, 2004). Similar to the release of glucagon from pancreatic α-cells in mammals, the CC cells respond to low hemolymph sugar levels by activating adenosine monophosphate-activated kinase (AMPK), which results in Ca^{2+}-dependent AKH release (Staats, Lüersen, et al., 2018). Reportedly, AKH counterbalances insulin actions by activating glycogen phosphorylase, reducing fat body glycogen and thereby increasing hemolymph sugars (Gäde & Auerswald, 2003; S. K. Kim & Rulifson, 2004; G. Lee & Park, 2004). Further, AKH elicits the mobilization of glycogen and lipid stores in target tissues via binding to the G protein-coupled AKH receptor (Staats, Lüersen, et al., 2018). Mutation of the *Akh* gene or the gene encoding its receptor (*AkhR*), or the ablation of CC cells, results in obesity, hypoglycemia, and lipid mobilization defects (Alfa & Kim, 2016; S. K. Kim & Rulifson, 2004; G. Lee & Park, 2004). These findings showed similarity in the roles played by AKH in *D. melanogaster* and glycogen in humans.

2.3.3. The *D. melanogaster* models for T2DM

Defects in insulin signalling pathways in *D. melanogaster* lead to phenotypes that are analogous to diabetic states in mammals (Alfa & Kim, 2016). In *Drosophila*, insulin resistance may be generated by rearing flies on a high-sugar diet (HSD) (Morris et al., 2012; Musselman et al., 2011) or a high-fat diet (HFD) (Birse et al., 2010). Musselman et al. (2011) reported that exposure of *D. melanogaster* larvae to HSD resulted in dILP compensation, that is, increased mRNA levels of *dIlp2*, *3*, and *5*. However, the authors observed that after sustained HSD, *dIlp* expression decreased, and the larvae developed a hyperglycemic state, which is characteristic of insulin resistance. Morris et al. (2012) also reported that exposure of flies to HSD resulted in decreased insulin/IGF-like signalling (IIS) activity, with great downregulation of *dIlp2* and *3* mRNA levels, while *dIlp5* levels were only slightly lowered. Flies with reduced dILPs have also been reported to exhibit delays in development, increased hemolymph glucose and trehalose levels, as well as higher whole-body glycogen and lipid content (Rulifson et al., 2002). Genetic models have also been developed. For example, the adult flies heterozygous for the mutant InR allele InR^{05545} *(*InR^{05545}/InR^{+}*)* have a reduced *InR* activity, and loss-of-function mutation in *Akt1* (Park et al., 2014).

2.3.4. Feasibility of using *D. melanogaster* model in T2DM studies

In addition to the good conservation of signalling pathways and cellular processes as compared to humans, other advantages of using the fruit fly as a model organism include the easy husbandry, their low cost, and rapid reproduction and lifecycle (10-day regeneration time) (Rubio-Aliaga, 2012; Staats, Lüersen, et al., 2018). The *D. melanogaster* has a short lifespan (2-3 months) (Y. Sun et al., 2013) as compared to other models such as mice (251-961 days depending on the strain (R. Yuan et al., 2011)). The short time required for the flies to attain adulthood within a few days enables studies that require an adult model to start within a short period and end faster, given the short lifespan of the fruit fly. This permits many study replications without consuming a lot of time and resources. Furthermore, fruit flies produce a vast number of eggs (800) (Rubio-Aliaga, 2012), allowing a higher sample number for optimal power of experiments (Staats, Lüersen, et al., 2018). The *D. melanogaster* model renders genetic manipulation easy (Helfand & Rogina, 2003; Y. Sun et al., 2013), which may be done by injecting the eggs with DNA to create transgene flies (Rubio-Aliaga, 2012).

The *D. melanogaster* model has some limitations, such as its small size, dictating extra caution to prevent accidents such as broken wings or legs when handling them. Additionally, the effects of drugs in the fly may differ strongly from humans, such as the conversion of pro-toxins to toxins in the liver (Prüßing et al., 2013).

2.4. *Solanum anguivi* Lam. fruits: Their potential effects on type 2 diabetes mellitus [1]

Abstract

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder of glucose homeostasis associated with a status of insulin resistance, impaired insulin signalling, β-cell dysfunction, impaired glucose and lipid metabolism, sub-clinical inflammation, and increased oxidative stress. Consuming fruits and vegetables rich in phytochemicals with potential antidiabetic effects may prevent T2DM and/or support a conservative T2DM treatment while being safer and more affordable for people from low-income countries. *Solanum anguivi* Lam. fruits (SALF) have been suggested to exhibit antidiabetic properties, potentially due to the presence of various phytochemicals, including saponins, phenolics, alkaloids, ascorbic acid, and flavonoids. For the saponin fraction, antidiabetic effects have already been reported. However, it remains unclear whether this is also true for the other phytochemicals present in SALF. This review article covers information on glucose homeostasis, T2DM pathogenesis, and also the potential antidiabetic effects of phytochemicals present in SALF, including their potential mechanisms of action.

Keywords: Type 2 diabetes; *Solanum anguivi* fruits; antioxidants; pathogenesis of diabetes; bioactivity; oxidative stress; antidiabetic; glucose homeostasis

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2.4.1. Introduction

Diabetes is a chronic metabolic disorder that is illustrated by either insufficient production or the lack of response to insulin, a key hormone in the regulation of the body's metabolism (Rodrigues, 2016). The burden due to diabetes is enormous, owing to its rapidly increasing global prevalence, the devastating damage it can do to many body organs, and the direct and indirect costs (Ala Alwan, 2010). The estimated global prevalence of diabetes in people aged 20 - 79 years has risen from 6.4% (285 million) in 2010 to 9.3% (463 million) in 2019, and it is predicted to increase to 10.9% (700 million) by 2045 if there is insufficient action to address the pandemic (IDF, 2019). Based on the World Bank income classification, high-income countries had the highest diabetes prevalence in 2019 at 10% (95.2 million), while low-income

countries had the least at 4% (14.5 million) (IDF, 2019).

People suffer from different types of diabetes, including type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus, monogenic diabetes, and secondary diabetes (IDF, 2013). T2DM is the most common type of diabetes (IDF, 2013), and will, therefore, be the focus of this review article. The present review provides information on glucose homeostasis and how T2DM ensues (pathogenesis of T2DM). A few studies (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011) suggest antidiabetic properties of *Solanum anguivi* Lam. fruits (SALF), due to the presence of bioactive phytochemical compounds. *Solanum anguivi* Lam. is an ethnomedicinal plant belonging to the family Solanaceae and genus *Solanum* Lam (United States Department of Agriculture., 2021). It is native to Africa, probably occurring in all non-arid tropical African regions (Bukenya, 1993), and it has also been reported to be present in Asia and Australia (Jayanthy et al., 2016). It grows mostly in the wild, but sometimes, e.g., in Uganda and Ivory Coast, it is a semi-cultivated vegetable (Bukenya, 1993). The plants are consumed as leafy and/ or fruity vegetables (Denton & Nwangburuka, 2011). However, only limited data regarding its antidiabetic effect is available, which will be summarized in this review, as well as the potential mechanisms of action for phytochemicals present in SALF that may lead to antidiabetic effects.

2.4.2. Glucose homeostasis

The pancreas maintains blood glucose levels within a very narrow range of 4.0–6.5 mmol/L (Hindmarsh & Geertsma, 2017) mediated through the opposing and balanced actions of the hormones glucagon and insulin, referred to as glucose homeostasis (Röder et al., 2016). Glucagon and insulin are synthesized from the pancreatic α- and β- cells of the islets of Langerhans, respectively (Xavier, 2018). The systemic glucose homeostasis is achieved by the coordinated functions of different organ systems, including the skeletal muscle, the liver, the endocrine pancreas, the adipose tissue (Figure 1) (Henriksen, 2010), and the hypothalamus is responsible for the neural regulation of these organ systems (Henriksen et al., 2011).

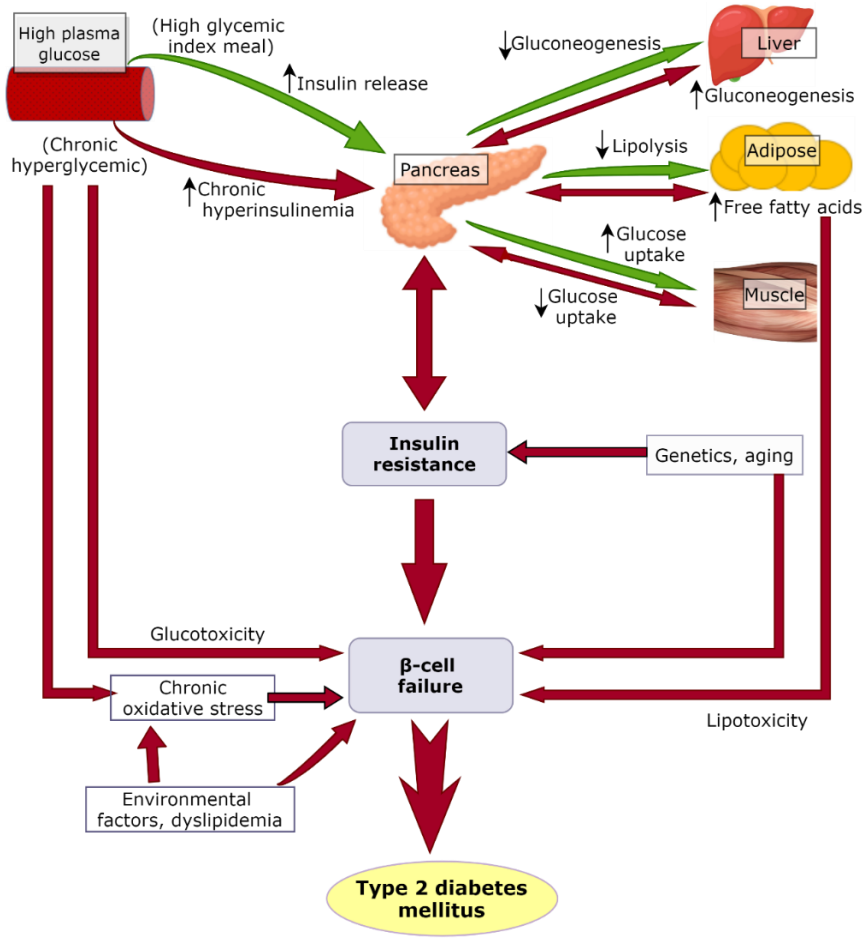


Figure 1. Normal glucose homeostasis and the pathogenesis of type 2 diabetes mellitus. This was modified, according to Ludwig (2002). The green arrows show normal glucose homeostasis, while the red arrows show the pathogenesis of type 2 diabetes mellitus. The black upward arrows represent an increase, while the downward ones represent inhibition. High plasma glucose may result from a high glycemic meal, or it may be during chronic hyperglycemia, leading to increased insulin production or chronic hyperinsulinemia, respectively. The events that follow are shown by the green and red arrows, respectively. The figure was drawn via https://app.diagrams.net/, and the pancreas, liver, and muscle pictures were obtained from www.freepik.com.

2.4.2.1. Insulin and glucagon as mediators of the glucose homeostasis

The main stimulus for the insulin release from the pancreatic β-cells is an elevated blood glucose level following the ingestion of glucose or a high-glycemic-index meal (≥65 on the glucose scale (Sacks et al., 2014)) (Tripathy & Chavez, 2010). The circulating plasma glucose is taken up into the β-cells through the facilitative glucose transporter (GLUT)-2 in an insulin-independent manner (Y. Chen et al., 2016; Rorsman & Braun, 2013) (Figure 2). Once in the β-cell, glucose undergoes glycolysis and mitochondrial glucose oxidation, leading to increased adenosine triphosphate (ATP)/ adenosine diphosphate (ADP) ratio and the subsequent closure of ATP-sensitive potassium (K^+) channels (K_{ATP} channels).

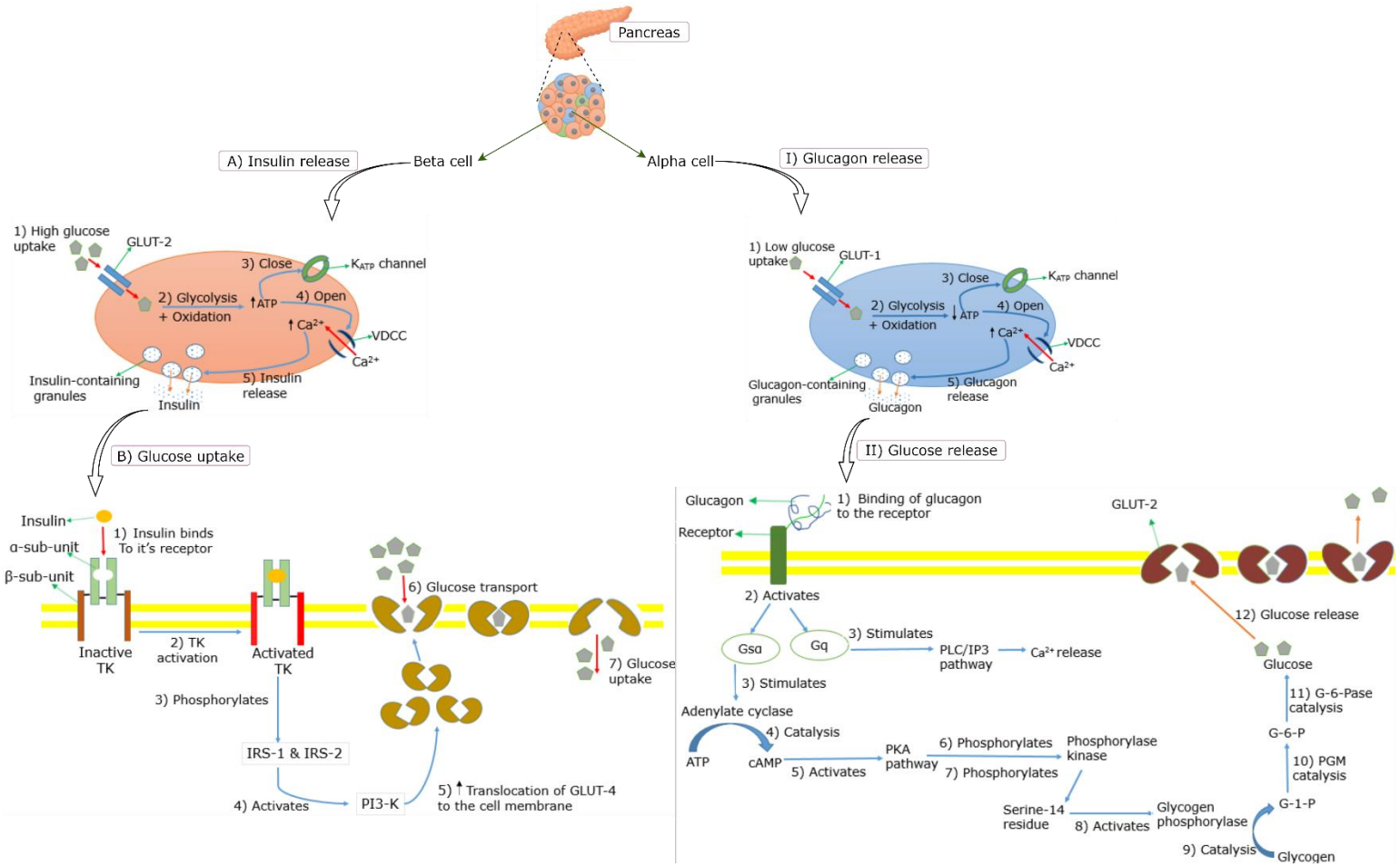


Figure 2. Insulin and glucagon secretion mechanisms. (A) = insulin release during high glucose concentration, (B) = insulin-dependent glucose uptake, (I) = glucagon release during low glucose concentration, (II) conversion of glucagon to glucose. GLUT = glucose transporter, VDDC = voltage-dependent calcium channel, ATP = adenosine triphosphate, TK= tyrosine kinase, IRS = insulin receptor substrate, PI3-K = phosphatidylinositol-3-kinase, cAMP = cyclic adenosine mono- phosphate, PKA = protein kinase A, G-1-P = glucose-1-phosphate, PGM = Phosphoglucomutase, G-6-P = glucose-6-phosphate, G-6-Pase = glucose-6-phosphatase, PLC = phospholipase C and IP3 = inositol 1,4,5-triphosphate. The events are shown by numbered steps using arrows: Red = entry, blue = resulting to, orange = exocytosis; while black upward arrow = increased content. The green arrows were used for labelling.

This leads to the depolarization of the membrane, followed by the opening of voltage-dependent calcium (Ca^{2+}) channels (VDCCs), resulting in the influx of Ca^{2+} and the eventual release of insulin (Y. Chen et al., 2016; Henquin, 2000; Philip Newsholme & Krause, 2012; Röder et al., 2016). Insulin binds to the α-subunit of the insulin receptor, which enables ATP to bind to the β-subunit of the insulin receptor, which in turn triggers the phosphorylation of the tyrosine kinase (P Newsholme et al., 2007; Wilcox, 2005) (Figure 2). Several intracellular proteins are then phosphorylated on tyrosine residues, such as insulin receptor substrates (IRS) 1 and 2, leading to the activation of phosphatidylinositol-3-kinase (PI3-K) (Henriksen, 2013; Tremblay et al., 2001). This subsequently increases the translocation of GLUT-4 molecules on the outer membrane of the insulin-responsive tissues (Ghosh & Collier, 2012; Wilcox, 2005), leading to increased glucose uptake. Insulin-mediated signalling further lowers blood glucose by reducing hepatic glucose output (gluconeogenesis) by increasing the storage of glucose as glycogen in the liver (glycogenesis) and inhibiting the release of free fatty acids (FFAs) from adipose tissue (lipolysis) through promoting fat synthesis (lipogenesis) in the adipose tissue (Klover & Mooney, 2004; Munir et al., 2013). Moreover, the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) promotes glucose uptake through an increased insulin sensitivity of the muscle, and a lower level of circulating lipids through an up-regulated storage of triglycerides (Ferré, 2004).

Glucagon plays a vital role in maintaining glucose homeostasis by promoting the breakdown of glycogen to glucose (glycogenolysis) and gluconeogenesis and inhibiting glycogenesis, thereby acting as a glucose-mobilizing hormone (Hædersdal et al., 2018; Rix et al., 2000). It is released from pancreatic α-cells when blood glucose levels start to decrease (Ghosh & Collier, 2012). Similar to insulin secretion, the release of glucagon is triggered by Ca^{2+} entry through VDCCs. During a hypoglycemic state, low levels of glucose are taken up by GLUT-1 into the cell membrane of α-cells, which subsequently induces glycolysis resulting in low levels of ATP (Müller et al., 2017; Rix et al., 2000), being followed by the closure of the K_{ATP} channels, and thus, reduced efflux of K^+ (Figure 2). Consequently, VDCCs open, allowing an influx of Ca^{2+} which triggers the release of glycogen from the α-cells (Müller et al., 2017; Rix et al., 2000). Glucagon binds to the glucagon receptor, leading to a sequence of events (Adeva-Andany et al., 2016; Berg et al., 2002; Christophe, 1995; Grigorenko et al., 2020; Jiang & Zhang, 2003; Venugopal et al., 2018) that convert glycogen to glucose (Figure 2). In addition to promoting glycogenolysis, glucagon inhibits glycogenesis in the liver simultaneously (Venugopal et al., 2018).

2.4.3. Type 2 diabetes mellitus

T2DM usually occurs in adults but is increasingly seen in children and adolescents (Temneanu et al., 2016). In T2DM patients, the pancreas produces and releases insulin, but the cells become resistant so that the insulin is ineffective, a state that is referred to as insulin resistance [IR]. Thus, the provided insulin may be insufficient to compensate for IR over time, a state that is referred to as relative insulin deficiency (ID) (Association American Diabetes, 2009). Both IR and ID lead to high blood glucose levels. T2DM patients also exhibit an impaired regulation of glucagon secretion, which is reflected in high levels during fasting in response to an oral intake of glucose (Knop et al., 2007; Lund et al., 2016; Reaven et al., 1987; Rix et al., 2000). The underlying mechanisms of hyperglucagonemia are currently not clear, but it may result from the impaired suppressive effect of insulin on the α-cells due to hypoinsulinemia and IR (Dunning & Gerich, 2007; Hamaguchi et al., 1991; Rix et al., 2000).

2.4.3.1. Pathogenesis of T2DM

T2DM is characterized by two fundamental defects: Impaired insulin action (IR) in skeletal muscle, liver, and impaired adipocyte and β-cell function (Figure 1). It is caused by a combination of genetic factors related to impaired insulin secretion and IR, by environmental factors, such as obesity, lack of exercise, and stress, as well as by aging, indicating that T2DM is a multifactorial disease (Ghosh & Collier, 2012; Kaku, 2010). Several mechanisms for T2DM pathogenesis have been proposed, as described below.

2.4.3.1.1. Oxidative stress and T2DM

Oxidative stress is defined as the excess formation and/ or insufficient removal of highly reactive molecules, that is, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Johansen et al., 2005). The imbalance between the generation of ROS or RNS and the activity of the antioxidant defences causes oxidative stress (Beier & Arteel, 2010; Škrovánková et al., 2012). Mitochondria are integral to normal cellular function as they are responsible for energy production in eukaryotes, calcium homeostasis and also play a key role in the regulation of apoptosis (Duchen, 2000; Osellame et al., 2012). Thus, alterations in mitochondrial function are often associated with T2DM, thus reflecting the centrality of energy homeostasis in β-cell physiology (J. P. Silva et al., 2000). Clinical and experimental studies have shown that oxidative stress, through free radical generation, plays a major role in the onset of diabetes (Asmat et al., 2016; Maritim et al., 2003; Okoduwa et al., 2013). In high-sugar diets,

mitochondria have more substrate available to generate ATP, due to the increased supply of glucose (Hurrle & Hsu, 2017), resulting in an overproduction of their natural by-product, ROS (Henriksen et al., 2011). The increased ROS levels damage the infrastructure of the cell and induce mitochondrial stress (Hurrle & Hsu, 2017) (Figure 1). The elevated ROS levels may also induce mitochondrial fission, which has been reported to cause mitochondrial dysfunction and IR in the skeletal muscle (Boucher et al., 2014; Jheng' et al., 2012). Hyperglycemia may also stimulate oxidative stress by the generation of ROS during the process of glycation, the non-enzymatic process through which glucose forms covalent adducts with plasma proteins, forming glycation end-products (Kennedy & Lyons, 1997).

2.4.3.1.2. Insulin resistance

The predisposing factor and best indicator for the development of T2DM in the future is IR (Hurrle & Hsu, 2017) (Figure 1). IR is classified into three categories, which are impaired insulin response in target tissues, diminished insulin secretion by β-cells, and insulin antagonists in the plasma (Pearson et al., 2016). IR is associated with an impaired insulin-dependent GLUT-4 translocation to the plasma membrane, which primarily arises from multifactorial defects in the normal engagement of the canonical insulin signalling cascade (Czech, 2017; Henriksen, 2002, 2010, 2013).

Obesity has been proposed as an underlying cause for the development of IR (Dragano & Marques, 2016). Chronic overfeeding leads to the elevated ability of adipose tissue to store the excess nutrients as triglycerides (possibly due to impaired insulin action), resulting in increased concentrations of circulating FFAs and abnormal redistribution of lipids to other organs, including the liver and skeletal muscle (Dragano & Marques, 2016). Elevated FFAs and intracellular lipids are linked to the onset of peripheral and hepatic IR (Bergman & Mittelman, 1998; Boden & Shulman, 2002). This may result from the inhibition of insulin signalling by the FFAs and intracellular lipids, leading to a reduced insulin-stimulated muscle glucose transport, possibly due to a decrease in the translocation of the GLUT-4 (Bergman & Mittelman, 1998; Boden & Shulman, 2002). Increased amounts of adipose tissue and visceral fat in obesity lead to ectopic fat accumulation in the liver, muscle, and pancreas, and thus, IR ensues (Falk Petersen & Shulman, 2006).

Aberrant hepatic insulin action is hypothesized to primarily drive IR, given that higher circulating insulin levels are necessary to adequately control the blood glucose levels (Santoleri

& Titchenell, 2019). In patients with T2DM and obesity, insulin fails to regulate hepatic metabolism appropriately, leading to excess production of glucose despite accelerated rates of lipid synthesis, a condition commonly referred to as selective hepatic IR (Brown & Goldstein, 2008). Hepatic IR is generally represented by the impaired suppression of hepatic glucose production, which is associated with an elevated hepatic triglyceride content, a known characteristic of non-alcoholic fatty liver disease (NAFLD) (Petersen et al., 2017; Roden, 2006). Other abnormalities associated with hepatic insulin resistance that may cause dysregulation of the glucose metabolism include the progression of simple steatosis (NAFLD) to fibrosis and non-alcoholic steatohepatitis (NASH) (Petersen et al., 2017; Roden, 2006).

2.4.3.1.3. Pancreatic β-cell dysfunction/failure

Several mechanisms describing the pathogenesis of pancreatic β-cell dysfunction/failure have been reported. Hyperglycemia and high amounts of saturated fats in circulation from diets or lipolysis of body fat have been suggested to trigger β-cell dysfunction, as well as IR (Cerf, 2013) (Figure 1). Chronic hyperglycemia and elevated FFAs lead to β-cell dysfunction through various mechanisms, including the generation of ROS, increased intracellular $Ca^{2+,}$ mitochondrial uncoupling, alterations in metabolic pathways, and the activation of endoplasmic reticulum stress (Cerf, 2013; Chang-Chen et al., 2008). Chronic exposure of β-cells to FFA is associated with impaired glucose-stimulated insulin secretion, a down-regulation of insulin gene expression resulting in reduced insulin synthesis, and ultimately causing apoptosis of the β-cells (Chang-Chen et al., 2008). Chronic hyperglycemia causes an increased metabolic demand towards the β-cells, which undergo compensatory insulin hypersecretion to maintain normoglycemia (Prentki & Nolan, 2006). This may lead to increased β-cell mass and function (Chang-Chen et al., 2008), consequently, to β-cell exhaustion and failure resulting in the development of T2DM (Kasuga, 2010)(P Newsholme et al., 2007; Sivitz & Yorek, 2010). Compensatory β-cell mass expansion may also be stimulated by increased FFAs consumption through increased production of glucagon-like peptide 1 (GLP-1) and its receptors as observed in dogs on a high-fat diet (Van Citters et al., 2002). In addition to chronic hyperglycemia and elevated FFAs, obesity is a major risk factor for T2DM as it desensitizes glucose recipient organs to the action of insulin (obesity-induced IR), leading to increased insulin demand resulting in β-cell dysfunction (Cerf, 2013).

2.4.4. Antioxidants and T2DM

Free radicals generated during biological oxidation reactions are reactive and simultaneously

start the chain reaction, which may lead to damage or even to the death of cells (Rao, 2016). An antioxidant is a substrate that prevents the oxidation of a molecule by neutralizing a free radical through the donation of an electron or by transferring a hydrogen atom, and thus, reducing its damaging potential (Lobo et al., 2010). Antioxidants are classified as either primary/chain-breaking/radical-trapping (slow-down/block autoxidation by competing with the propagation reactions) or secondary/preventive (interfere with the initiation process) (Hermund, 2018; Ingold & Pratt, 2014; Valgimigli & Pratt, 2012). The primary antioxidants (e.g., phenolic compounds, such as caffeic acid and tocopherol) rapidly react with peroxyl radicals preventing their reaction with oxidizable substrates and consequently the propagation of the autoxidation chain (Hermund, 2018; Valgimigli & Pratt, 2012). Secondary antioxidants (e.g., polyphenols, including flavonoids, such as quercetin (Jomova et al., 2012)) may prevent the occurrence of Fenton-type chemistry by blocking redox-active metal ions in an oxidized form (e.g., Fe^{3+}) through metal chelation (Amorati & Valgimigli, 2015; Hermund, 2018).

2.4.4.1. Endogenous and exogenous antioxidants in humans

The antioxidant defence grid in living systems consists of antioxidant molecules that act at different levels and are classified as the first-line, second-line, third-line, and fourth-line (Ighodaro & Akinloye, 2018; Lobo et al., 2010; Willcox et al., 2004). These are radical suppression or prevention, radical scavenging, radical-induced damage repair, and adaptation (utilization of the signals required for free radical production by reacting to prevent the formation or reaction of the radicals) (Ighodaro & Akinloye, 2018), respectively. First-line antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Ighodaro & Akinloye, 2018; Pham-Huy et al., 2008). Second-line includes hydrophilic antioxidants (such as ascorbic acid, uric acid, phenolics, and glutathione) and lipophilic antioxidants (such as vitamin E and ubiquinol) (Dal & Sigrist, 2016; Ighodaro & Akinloye, 2018). Third-line includes proteolytic enzymes, lipases, DNA repair enzymes, and transferases (Ighodaro & Akinloye, 2018; Lobo et al., 2010; Willcox et al., 2004).

The human endogenous antioxidant defence against free radicals and oxidative stress includes enzymatic antioxidants, such as SOD, which catalyzes the dismutation of superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or H_2O_2, as well as CAT and GPx, which both remove H_2O_2 (Asakura & Kitahora, 2018; Pham-Huy et al., 2008), and non-enzymatic antioxidants, such as lipoic acid, glutathione, L-arginine, and coenzyme Q10 (Pham-Huy et al., 2008; Pizzino et al., 2017). However, these endogenous antioxidants may not be sufficient in

some cases, such as chronic exposure to free radicals, due to smoking and consumption of high nutrient diets. Thus, exogenous (dietary) antioxidant consumption may help in the prevention of diseases associated with oxidative stress (Kurutas, 2016). Sources of dietary antioxidants include herbs, spices, medicinal plants (Elekofehinti, Kamdem, Bolingon, et al., 2013), fruits, and vegetables (Wilson et al., 2017). Due to the presence of antidiabetic phytochemicals (Jung et al., 2006), medicinal plants are used as antidiabetic remedies worldwide (Patel et al., 2012). Fruits and vegetables are also considered protective due to various phytochemicals that are mainly responsible for the plant's colour, smell, flavour, and bitterness (Miglio et al., 2008), such as polyphenols, alkaloids, and saponins. Phytochemicals are defined as bioactive plant chemicals that may provide desirable health benefits that lower the risk of developing major chronic diseases (R. H. Liu, 2004), including T2DM. This may be achieved by reducing cholesterol absorption, by directly lowering fasting blood glucose levels, e.g., by inhibiting cortisol (Jones et al., 1999; McAnuff et al., 2005), and by stimulating the immune system under different conditions (J. Sun et al., 2002). The antioxidant activity of foods correlates with the presence of phytochemicals (J. Sun et al., 2002). Thus, in addition to the enzymatic antioxidants (CAT, SOD, GPx) in humans that scavenge free radicals, some phytochemicals also act as complementary antioxidants due to their electrophilicity, ability to promote the gene expression of antioxidant enzymes and to positively modulate the actions of antioxidant enzymes (Malireddy et al., 2012).

2.4.5. *Solanum anguivi* Lam. fruit's antidiabetic properties and potential mechanisms of action

Various researchers (Andabati & Muyonga, 2014; Dan et al., 2014; Elekofehinti, Kamdem, Bolingon, et al., 2013; Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Oyeyemi et al., 2015) have reported the presence of phytochemicals in SALF, which include phenolics, flavonoids, saponins, alkaloids, coumarins, and vitamin C. The phenolics in SALF include gallic acid, chlorogenic acid, caffeic acid (Elekofehinti, Kamdem, Bolingon, et al., 2013), phenolic acids (Stommel & Whitaker, 2003), and tannins (Oyeyemi et al., 2015), as well as rutin and quercetin as representatives of the flavonoids (Elekofehinti, Kamdem, Bolingon, et al., 2013). Triterpenoid saponins (Oyeyemi et al., 2015) and steroidal saponins or glycosides, such as anguiviosides A to C (Zhu et al., 2000), III, XI, XV, and XVI (Honbu et al., 2002) have also been reported to be present in SALF. In addition, the steroidal glycoalkaloids solamargine, anguivine, and isoanguivine have been described to be present in the SALF (Ripperger & Himmelreich, 1994; R. Y. Yang & Ojiewo, 2013). There is controversy about whether *Solanum*

indicum Linn. (*S. indicum*) is the same as *Solanum anguivi* Lam. (*S. anguivi*). *S. indicum* has been reported as a synonym for *Solanum anguivi* by some authors (Bahgat et al., 2008; Bukenya-Ziraba, 2004; Burkill, 1985; D'Arcy, 1992), while others have described them as different species (Kaunda & Zhang, 2019; Stommel & Whitaker, 2003; Waller & Yamasaki, 1996). Controversy also exists regarding the safety of *S. indicum L.* fruit (SILF). Several authors have reported that SILF is safe and thus, may be consumed as a vegetable (Abdel-Aziz et al., 2011; Bahgat et al., 2008; D'Arcy, 1992; Epoh et al., 2019; Jayanthy et al., 2016; N'Dri et al., 2010), while one author has reported that it is a poisonous berry (Kaunda & Zhang, 2019). Similar to SALF, SILF has been reported to contain steroidal saponins/glycosides (isoanguivine, protodioscin, solasonine, solamargine, and indiosides A–E), terpenoids, vitamin C, phenolics (gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin), flavonoids (rutin, quercetin, isoquercitrin), glycoalkaloids (solamargine, solasonine) and coumarins (E. E. Nwanna et al., 2014; V. Sharma et al., 2017; Yahara et al., 1996). The similarities between the phytochemicals present in SALF and SILF may, therefore, indicate similarities in their antidiabetic properties.

Previous studies have shown that SALF extracts possess antioxidant abilities in vitro, such as radical scavenging capacity (Andabati & Muyonga, 2014; Elekofehinti, Kamdem, Bolingon, et al., 2013), reducing properties (Fe^{3+} to Fe^{2+}), and iron-chelating abilities (Elekofehinti, Kamdem, Bolingon, et al., 2013; Elekofehinti & Kade, 2012). SALF extracts have also been reported to inhibit lipid peroxidation (Elekofehinti, Kamdem, Bolingon, et al., 2013), which may be due to the presence of saponins as they have been reported to inhibit lipid peroxidation in diabetic rats through the restoration of SOD and CAT (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013). Blood-glucose-lowering effects have also been exhibited in diabetic rats having been administered SALF extracts (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011). The antidiabetic properties (antioxidant activities, inhibition of oxidative stress, and blood-glucose-lowering effect) of SALF may be attributed to the presence of various phytochemicals in SALF. However, only one class of phytochemicals present in SALF, that is, saponin, has been studied for its antidiabetic effects (Elekofehinti et al., 2012; Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). Since the antidiabetic properties of the other SALF phytochemicals (phenolics, flavonoids, and alkaloids) have not been documented, this paper discusses their potential antidiabetic effects and underlying mechanisms of action (summarized in Figure 3) in the context of other medicinal plants with similar phytochemical patterns.

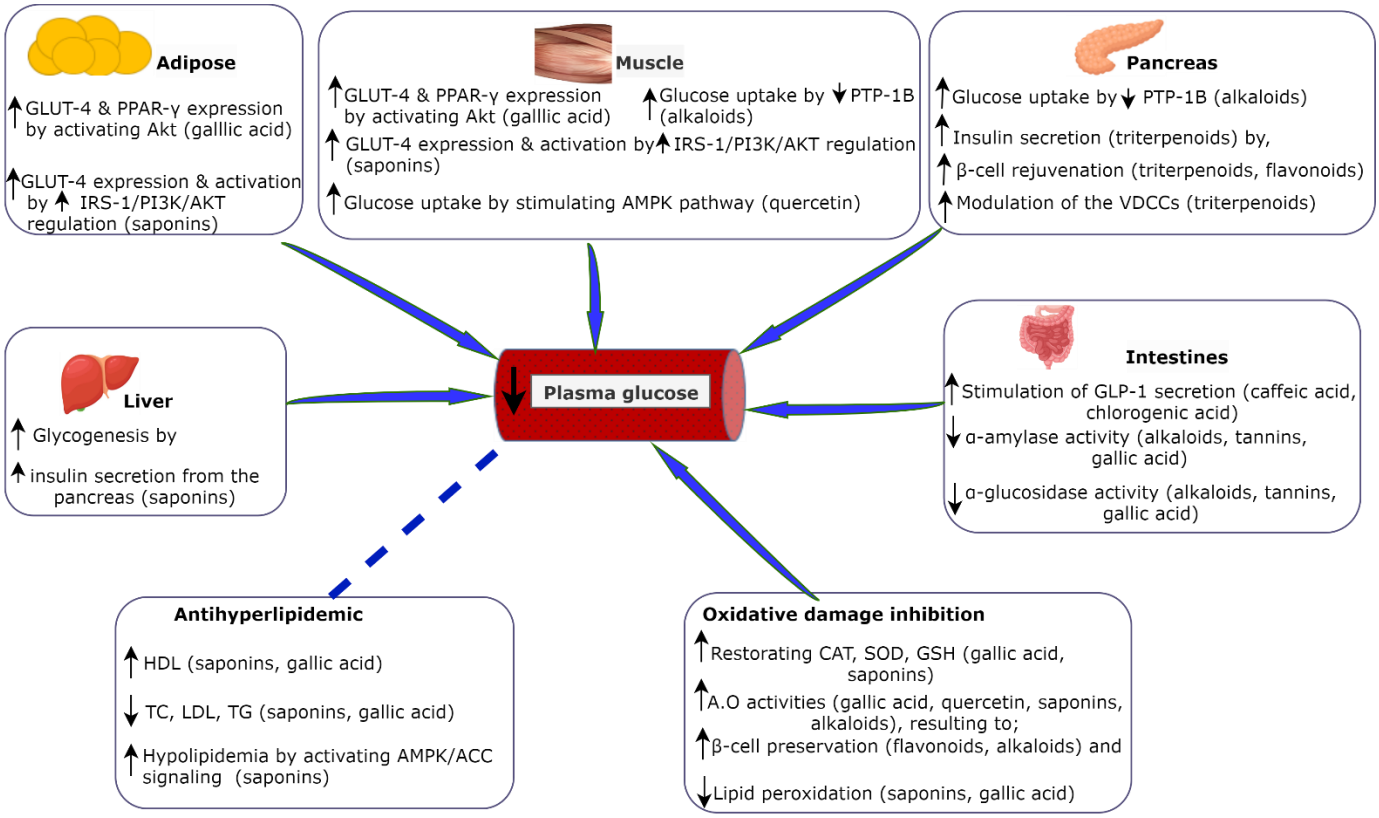


Figure 3. Potential mechanisms of action by *Solanum anguivi* Lam. fruit phytochemicals in their antidiabetic effects. PPAR = peroxisome proliferator-activated receptors, GLUT = glucose trans- porter, IRS = insulin receptor substrate, AMPK = adenosine monophosphate-activated kinase, ACC = acetyl-CoA carboxylase, PI3-K = phosphatidylinositol-3-kinase, VDCCs = voltage-dependent calcium channels, GLP = glucagon-like peptide, PTP-1B = protein tyrosine phosphatase-1B, CAT = catalase, SOD = superoxide dismutase, GSH = glutathione, A.O = antioxidant, upward black arrows = increased, downward black arrows = inhibition of, blue arrows = mechanisms of action resulting in reduced plasma glucose levels, stripped blue line = relationship between hypoglycemia and hypolipidemia. The figure was drawn via https://app.diagrams.net/, and the pancreas, liver, muscle, and intestine pictures were obtained from www.freepik.com.

2.4.5.1. Saponins

The total saponin content of SALF has been stated as 1.3 mg/100 g dry weight (DW) and the triterpenoid content as 0.3 mg/100 g DW (Oyeyemi et al., 2015); however, no reference standard (n.r.s) was used for both analyses. Other authors have also reported the presence of saponins in SALF, however, not the total saponin content (Dan et al., 2014; Karthika & Poongodi Vijayakumar, 2017). Saponins extracted from SALF have been stated to exhibit antioxidative properties in vitro, such as scavenging radicals, reducing [Fe^{3+} to Fe^{2+}], and iron-chelating abilities (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013). Furthermore, SALF saponins have been reported to exhibit antidiabetic properties in diabetic rats, including the reduction of blood glucose levels (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013) and the inhibition of oxidative stress (Elekofehinti et al., 2012; Elekofehinti, Kamdem, Kade,

Adanlawo, et al., 2013; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), which may both be due to their ability to restore the endogenous antioxidant levels (i.e., SOD and CAT levels) (Elekofehinti et al., 2012; Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), as well as their antioxidative activities (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013).

The antidiabetic effect of SALF saponins may also be referred to as their antihyperlipidemic properties and their ability to cause weight loss in diabetic rats (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). SALF saponins have also been reported to restore the plasma lipid profile in these rats, being reflected in lower levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), and increased levels of high-density lipoprotein (HDL) (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). Relative ID and IR may negatively affect the lipid profile as insulin plays a critical role in lipid homeostasis (Vergès, 2015). In T2DM patients, elevated plasma levels of TG and lipoprotein lipase (LPL), as well as decreased HDL levels, have been found, the latter being associated with defective LPL catabolism of TG-rich lipoproteins (Bassali et al., 1997). Similar to diabetic rats, being exposed to SALF saponins (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), an improvement of glucose homeostasis in the absence of weight gain has been suggested to result in lowered TG and increased HDL levels (Bassali et al., 1997). In contrast, other authors have suggested that hyperglycemia may not cause dyslipidemia, but rather abnormalities in insulin action, and hence, a hypoglycemic effect may not improve the lipid profile per se (Goldberg, 2001; Haffner et al., 2000).

Another possible mechanism through which saponins from SALF may result in hypoglycemia may be through the regeneration of islets of Langerhans as suggested for *Solanum nigrum* (*S. nigrum*) after being administered to diabetic rats (Umamageswari et al., 2017). Triterpenoid saponins in SALF may also cause hypoglycemic and hypolipidemic effects by activating GLUT4 through improved IRS-1/PI3K/AKT regulation, and activated adenosine monophosphate-activated kinase/acetyl-CoA carboxylase (AMPK/ACC) signalling, respectively, as shown in diabetic mice by *Stauntonia chinensis* triterpenoids (Xu et al., 2018). Additionally, SALF triterpenoid saponins may lower plasma glucose levels by improving insulin secretion as a result of the improved modulation of VDCCs, and thus, increasing glycogenesis, and β-cell rejuvenation, as reported for *Primula denticulate* (S. Singh et al., 2014) and *Momordica cymbalaria* Fenzl (Konri et al., 2014) triterpenoids.

2.4.5.2. Phenolics and flavonoids

The total phenolic content (TPC) for SALF has been reported as 17.1 mg gallic acid equivalent (GAE)/g dry weight (DW) (Elekofehinti, Kamdem, Bolingon, et al., 2013), 1.52 mg/100 mg DW (1.52%) (n.r.s) (Oyeyemi et al., 2015), and from unripe to very ripe stage, 9.6 to 5.5 mg/g DW (n.r.s) (Dan et al., 2014) and 11.6 to 4.5 mg/g GAE DW (Abbe et al., 2019), respectively. The total flavonoid content (TFC) of SALF has been documented as 9.5 mg QE/g DW (Elekofehinti, Kamdem, Bolingon, et al., 2013), 0.5 mg/100 mg DW (0.5%) (n.r.s) (Oyeyemi et al., 2015) and 141.3 to 455.0 mg QE/100 g DW from unripe to ripe stage, respectively (Abbe et al., 2019). Elekofehinti et al. (Elekofehinti, Kamdem, Bolingon, et al., 2013) described the contents for the SALF phenolic compounds gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin as 17.5, 21.9, 16.6, 14.7, and 7.4 mg/g, respectively. Stommel et al. (Stommel & Whitaker, 2003) reported the contents (µmol/100 g DW) of SALF chlorogenic acid isomers (1117 - 6232), isochlorogenic acid isomers (70 - 226), hydroxycinnamic acid amide conjugates (14 -286), caffeic acid derivatives (45 – 155), and acetylated chlorogenic acid isomers (316 - 1148). The tannin content of SALF has been documented as 0.17 mg/100 mg (n.r.s) (Oyeyemi et al., 2015) and 0.19 to 0.09 mg tannic acid equivalent/100 g DW, from unripe to the ripe stage, respectively (Abbe et al., 2019). Extracts from SILF and *Solanum melongena* (*S. melongena*) have been stated to exhibit antidiabetic properties through inhibiting α-amylase and α-glucosidase enzymes, which were attributed to the present phenolics (Y. I. Kwon et al., 2008; E. E. Nwanna et al., 2014). The TPC and TFC of SILF were reported as 3.8 mg GAE/g DW and 1.7 mg quercetin equivalent (QE)/g DW, respectively (E. E. Nwanna et al., 2014), while the TPC and TFC for *S. melongena* were only reported for the skin and pulp separately. Glucose is a product from the hydrolysis of starch (Mikawlrawng, 2016), which is catalyzed by the enzymes α-amylase found in saliva and pancreatic juices, and α-glucosidase found in the epithelium of the small intestine (Bharti et al., 2018). Hence, α-amylase and α-glucosidase inhibitors slow the digestion of starch in the small intestine, which decreases the amount of glucose entering the bloodstream leading to an improved insulin response (Sieniawska, 2015). Previous studies reported the hypoglycemic effects of SILF (Borgohain et al., 2016) and *S. melongena* (E. Nwanna, 2013), and these may have been mediated through inhibiting the α-amylase and α-glucosidase due to the presence of phenolic compounds. The TPC in SALF (mg GAE/g DW) is substantially higher than in SILF (E. E. Nwanna et al., 2014), suggesting inhibiting properties of SALF regarding α-amylase and α-glucosidase activity, which may be mediated by tannins as similar effects have been reported for tannin-containing *Terminalia*

chebula Retz (D. Y. Lee et al., 2017).

Phenolics present in SILF, *S. nigrum*, *S. melongena*, have been reported to possess antioxidant effects (A. Hameed & Akhtar, 2018; Y. I. Kwon et al., 2008; E. Nwanna, 2013; E. E. Nwanna et al., 2014), which may also be true for SALF phenolics. Thus, in addition to saponins, the antioxidative effect of SALF extracts may be induced by the synergistic action of saponins and phenolics. Polyphenols, such as gallic acid may also be responsible for SALF's antidiabetic effects. They may also be mediated through both a reduction of plasma glucose levels and oxidative stress damage, by restoring antioxidant enzymes, inhibiting α-amylase and α-glucosidase, as well as by maintaining a healthy lipid profile as already shown in diabetic rats for *Hibiscus sabdariffa* gallic acid (Alegbe et al., 2019). Furthermore, SALF gallic acid may increase the expression of GLUT-4 and insulin sensitivity proteins, such as PPAR-γ, through the activation of AKT as demonstrated for *Emblica officinalis* derived gallic acid in diabetic mice (Variya et al., 2020), consequently leading to increased cellular glucose uptake. Recent studies (Domínguez Avila et al., 2017; Wang et al., 2021) have shown that polyphenols increase GLP-1, suggesting them to be used together with GLP-1 agonists for the treatment of T2DM (Collins & Costello, 2021; K. S. Kim & Jang, 2015). GLP-1, an incretin hormone produced from proglucagon in the intestine and brain (Jin, 2008; K. S. Kim & Jang, 2015; Rix et al., 2000), stimulates insulin release, the proliferation, and neogenesis of pancreatic β-cells, and inhibits glucagon release, food intake, and gastric emptying (Brubaker & Drucker, 2004; Jin, 2008; Lerche et al., 2008). Potentially SALF may stimulate GLP-1 secretion through its polyphenols, such as caffeic and chlorogenic acids (Jokura et al., 2015; Montoya et al., 2014; Wang et al., 2021).

Flavonoids from SALF may also possess antidiabetic effects. The hypoglycemic effect and the regeneration of islets of Langerhans in diabetic rats administered with extracts of *S. nigrum* were referred to as flavonoids in the extract (Umamageswari et al., 2017) whose TFC has been reported as 3.61 mg QE/g DW (Veerapagu et al., 2018). This could also apply to SALF flavonoids, which potentially exhibit antioxidant properties, protect against oxidative damage and restore pancreatic cells, which result in decreased levels of glucose in the blood. SALF quercetin may increase glucose uptake in skeletal muscles by stimulating the insulin-independent AMPK pathway, which has been demonstrated by quercetin-containing *Vaccinium vitis-idaea* in vitro (Eid et al., 2010).

2.4.5.3. Alkaloids

Alkaloids have also been reported to possess antidiabetic properties (Al-Ashaal et al., 2018). Although SALF has been shown to possess alkaloids (0.05 mg/100 mg DW or 0.05% (Oyeyemi et al., 2015)), there is very limited literature on the antidiabetic effects of alkaloids from *Solanum* fruits. However, SALF alkaloids may lower blood glucose levels, as shown for *Aerva lanata* alkaloids in diabetic rats (Agrawal et al., 2013). This may be through inhibiting α-amylase and α-glucosidase activities as suggested for *S. melongena* alkaloids (Asano et al., 1997). SALF alkaloids may also lower blood glucose levels by inducing glucose uptake through inhibition of protein tyrosine phosphatase-1B (PTP-1B) (a major negative regulator for insulin receptor signalling (O. Kwon et al., 2007)) as demonstrated in C2C12 skeletal muscle cells by alkaloids from *Veratrum nigrum* (Kang et al., 2015) and *Catharanthus roseus* (Tiong et al., 2013), and in β-TC6 pancreatic cells by alkaloids from *Catharanthus roseus* (Tiong et al., 2013). Additionally, SALF alkaloids may also alleviate H_2O_2-induced oxidative damage in β-cells as shown by alkaloids from *Catharanthus roseus* in diabetic rats due to their radical scavenging capacity (Tiong et al., 2013).

2.4.6. Conclusions

Some studies have documented the antidiabetic effects of SALF. For one group of phytochemicals present in SALF, the saponins, the antidiabetic effect, and the underlying mechanism have been documented. As SALF also contains other phytochemicals, such as phenolics, flavonoids, and alkaloids, its antidiabetic effect may also refer to these compounds, which have been shown to decrease blood glucose levels through, e.g., an up-regulation of GLUT-4 and PPARγ, restoration of enzymatic antioxidants and β-cell regeneration in other settings. However, to unravel the precise underlying mechanisms of the potential antidiabetic effects of SALF, further studies are essentially needed. They would provide information on whether the SALF antidiabetic properties may be due to a potential synergistic action of saponins and other phytochemicals present or refer to the saponin fraction only. Consequently, the results may also provide valuable information on the potential use of SALF in T2DM management.

CHAPTER THREE

RESEARCH PAPERS

Chapter 3 is composed of five sections. Each section consists of a paper that has been either published, submitted, or yet to be submitted for publication. These include:

a). Morphological characteristics, bioactive compounds contents, and antioxidant activity of fourteen accessions of *Solanum anguivi* Lam. **Published** in the *Journal of Applied Botany and Food Quality* (Nakitto, A. M. S., Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H.).

b). Influence of accession and ripeness stage on the bioactive compounds content and antioxidant activities of *Solanum anguivi* Lam. fruits. **Submitted** to the *International Journal of Fruit Science* (Nakitto, A. M. S., Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H.).

c). Effect of thermal treatments on the bioactive compound contents and antioxidant activity of *Solanum anguivi* Lam. fruits. **Submitted** to the *Food Science and Technology International* (Nakitto, A. M. S., Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H.).

d). *Solanum anguivi* Lam. fruit preparations counteract the negative effects of a high-sugar diet on the glucose metabolism in *Drosophila melanogaster*. **Published** in the *Food and Function* journal (Nakitto, A. M. S., Rudloff, S., Borsch, C., and Wagner, A. E.).

e**).** A supplementation with dried *Solanum anguivi* Lam. fruit counteracted the decreased survival of *Drosophila melanogaster* exposed to a high-sugar diet. **To be submitted** to the *Food and Nutrition* journal. (Nakitto, A. M. S., Rudloff, S., and Wagner, A. E.).

3.1. Morphological characteristics, bioactive compounds content, and antioxidant activity of fourteen accessions of *Solanum anguivi* Lam.[2]

Abstract

Solanum anguivi Lam. fruits (SALF) have been shown to exhibit antidiabetic properties. This may be attributable to the presence of bioactive compounds such as phenolics, flavonoids, saponins, alkaloids, and vitamin C in SALF. The nutritional quality of fruits and vegetables may vary among accessions. This study aimed to assess the bioactive compounds content (BCC) and antioxidant activity (AA) of SALF accessions and to determine the relationships between SALF BCC, AA, and the plant morphological characteristics. Eleven morphological traits for leaves, stems, and fruits from 12 plants per accession were characterized based on existing descriptors of *Solanum* species. The BCC was determined by spectrophotometry (for total phenolics, flavonoids, saponins, and vitamin C), gravimetry (for total alkaloids), and HPLC (for the quantification of phenolic compounds). The AA, determined as free radical scavenging activity (FRSC) and total antioxidant capacity (TAC), was also determined by spectrophotometry. Morphological characteristics of the leaves, stems, and fruits of *Solanum anguivi* Lam. varied among its accessions. Total phenolics (8.0 - 12.4 mg gallic acid equivalent/g dry weight [DW]), flavonoids (0.9 - 2.1 mg quercetin equivalent/g DW), alkaloids (81.4 - 127.7 mg/g DW), saponins (51.1 - 124.8 mg diosgenin equivalent/g DW), vitamin C (3.6 - 6.4 mg ascorbic acid equivalent/g DW), FRSC (3.4 - 11.5%) and TAC (1.2 - 4.6 mg quercetin equivalent/g DW) differed significantly among the accessions. HPLC analysis showed that chlorogenic acid represented the highest content of the analyzed phenolic compounds in SALF. AA had positive correlations with phenolic, flavonoid, and saponin contents (r = 0.739, 0.230, and 0.246, respectively) and a negative correlation with vitamin C (r = -0.222). A regression model derived for factors associated with AA had a moderately high adjusted r^2 (0.600), and it suggested that total phenolics and vitamin C mostly affected the SALF AA. Cluster analysis showed that morphological characteristics could be used to predict accessions with similar BCC and AA. Accessions high in total phenolics provided the highest AA and, therefore, may mediate substantial health benefits.

Keywords: *Solanum anguivi*, antioxidant capacity, bioactive compounds, morphological characteristics, accession.

[2] Published in the *Journal of Applied Botany and Food Quality*. Nakitto, A. M. S., Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H., 2021. Morphological characteristics, bioactive compounds content, and antioxidant activity of different accessions of African eggplant (*Solanum anguivi* Lam.). *Journal*

of Applied Botany and Food Quality. https://doi.org/10.5073/JABFQ.2021.094.027.

3.1.1. Introduction

Fruits and vegetables are particularly protective against diseases associated with oxidative stress due to the possession of bioactive compounds that have antioxidative properties. Several clinical and experimental studies have shown that oxidative stress plays a major role in the onset of non-communicable diseases (NCDs) such as type 2 diabetes mellitus (T2DM) and hypertension (Maritim et al., 2003; Zhou et al., 2003). Collectively, NCDs (cardiovascular diseases, cancer, diabetes, and chronic respiratory disease) are responsible for 71% of all deaths worldwide (WHO, 2018).

Solanum anguivi Lam. fruits (SALF) may alleviate diseases such as hypertension, atherosclerosis, and diabetes (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). This may be attributed to bioactive compounds present in SALF such as phenolics, flavonoids, saponins, and alkaloids (Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015). *Solanum anguivi* belongs to the family *Solanaceae* and genus *Solanum* L (United States Department Of Agriculture, 2020). It is native to Africa and has also been reported to occur in Asia and Australia (Bukenya & Carasco, 1995; Jayanthy et al., 2016; Nakitto et al., 2021). It is commonly known as "forest bitter berry" or "African eggplant", although the latter also refers to *Solanum aethiopicum* (*S. aethiopicum)* and *Solanum macropcarpon* (*S. macropcarpon)* (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Kaunda & Zhang, 2019). *Solanum anguivi* Lam. (*S. anguivi*) plants are consumed as leafy and/ or fruit vegetables (Denton & Nwangburuk, 2011).

The bioactive compounds content (BCC) and morphological characteristics of some *Solanum* fruits have been reported to vary among their accessions (P. M. Hanson et al., 2006; Sseremba et al., 2017; Tembe et al., 2020). Although the BCC and antioxidant capacity (AA) of SALF have been reported (Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015), the information on their variations among the accessions is scarce. Some studies (Osei et al., 2010; Sseremba et al., 2017; Tembe et al., 2020) have reported variations in some morphological (flower, stem, fruit, and leaf) characteristics in *S. anguivi* accessions. However, information on variations in other morphological characteristics among *S. anguivi* accessions as well as the relationship between morphological characteristics and BCC and AA is lacking. This information would equip researchers and consumers with the knowledge to identify *S. anguivi* accessions better and to possibly predict SALF accessions with similar BCC and AA based on

their morphological characteristics.

This study assessed the BCC and AA of fruits of *S. anguivi* accessions and determined whether these were related to plant morphological characteristics. Additionally, the study determined the relationships between the BCC and AA of SALF. Regression models were derived to determine the BCC that may mostly affect the AA of SALF.

3.1.2. Materials and methods

3.1.2.1. Chemicals and reagents

The reference standards (quercetin, gallic acid, L-ascorbic acid, diosgenin), HPLC grade - methanol, ethanol, sulphuric acid, acetic acid, and ammonium hydroxide; and analytical grade - thiourea, trichloroacetic acid, Folin-Ciocalteu reagent, 1-diphenyl-2-picrylhydrazyl (DPPH), vanillin, dinitrophenyl hydrazine, hydrated copper (II) sulfate, potassium acetate, sodium bicarbonate, and aluminium chloride, were all from Sigma-Aldrich (Munich, Germany).

3.1.2.2. Sample collection and experimental design

A preliminary survey was carried out to determine the *Solanum anguivi* Lam. accessions in Mukono district (Uganda), where they have been documented to grow (Stedje & Bukenya-Ziraba, 2003). Nabiyagi village (GPS 0.472336, 32.802484) was randomly selected from Mukono district as the study area, and a quadrat random sampling technique was used to identify the occurrence of *Solanum anguivi* Lam. accessions. Briefly, two tape measures were aligned to form x and y axes on a randomly selected area in Nabiyagi village. Using randomly generated numbers, two numbers were selected randomly to form x and y coordinates. Upon finding the location of the given coordinates, two meters were measured from it in four directions (north, east, west and south) to form a quadrat. The plants within the quadrat were then examined to determine any *Solanum anguivi* Lam. accessions present. This procedure was repeated randomly throughout the village. Fourteen *Solanum anguivi* Lam. accessions were obtained in the study area. Identification of the accessions was carried out with reference to the documented phenotypic characteristics of *Solanum anguivi* Lam. (Bukenya & Carasco, 1995), while authentication was carried out by an expert taxonomist at the Department of Botany, Makerere University (Uganda). Samples were obtained from 12 plants per accession in May 2018. Branches from each accession were plucked, and the morphological characteristics were then assessed on the same day. Mature unripe fruits were collected from 12 plants per accession, pooled, and then dried to form flour as described under “sample preparation for

chemical analysis". Mature unripe fruits were selected based on size, which was a size that was similar to that for the next ripeness stage (yellow) of the fruits. This entire process was replicated after two weeks to obtain second flour samples for each accession, and thus each accession had two flour samples collected independently.

3.1.2.3. Morphological characterization

Morphological characterization was based on the descriptors of tomatoes and eggplants (ECPGR, 2008), with modifications. Accessions in this study were given codes (based on their morphological characteristics) for identification purposes. The accessions were characterized based on the amount and colour of pubescence on the leaves and stems and the colours of the leaves, stems, and fruits. Fruits were further characterized based on size/diameter (very small = < 0.8 cm, small = > 0.8 - 1.2 cm, intermediate = >1.2 - 1.8 cm, large = > 1.8 cm), shape, top (style scar area) appearance, venations, and also the average number of fruits per twig.

3.1.2.4. Sample preparation for chemical analysis

The fruits (SALF) were sorted to remove those with damages on the pericarp. The stalks were then plucked off, and the fruits were washed with distilled water and patted dry with a cotton cloth. Mature fruits (50) of similar sizes obtained from 12 plants for each accession were cut into four parts and discarded those infested with pests. The sliced samples were dried in an oven [Infrared Food Oven GL-2A, Guangzhou Itop Kitchen Equipment Co, Ltd. Guangdong, China (Mainland)] at 40 ^{o}C for 16 hr. The samples were then milled (Wonder Mill, Pocatello, Idaho) at a "pastry" setting to obtain fine flour. The flours were stored in sealed plastic bottles at -20 ^{0}C until analysis. This entire process was replicated to obtain a second independent set of flours per accession from the fruits that were collected the second time.

3.1.2.5. Extraction and quantification of total bioactive compounds content and antioxidant activity of SALF

Extraction was carried out using 80% methanol (D. Kim et al., 2003) as described by Makkar (2003), with modifications (0.2 g of dried and powdered SALF was extracted in 20 ml 80% methanol three times [10 min each], and the extracts were pooled together for BCC analyses). Each accession had two extracts from the two independent flour samples.

All absorbances were measured using an ultraviolet spectrophotometer (Perkin-Elmer 3100, Artisan Technology Group 101E Mercury Drive, Champaign, IL, USA), and 80% methanol

was used for the blanks. All quantities were expressed on a dry weight (DW) basis. The total phenolic content (TPC) was measured using the Folin-Ciocalteu reagent (FCR) method (Singleton et al., 1998), estimated from a gallic acid (≥ 98%, Sigma-Aldrich, Germany) standard curve (0.1 mg stock solution, 0.02 - 1.0 µl) and expressed as gallic acid equivalent (GAE). Total flavonoids content (TFC) was determined as described by Kumar, Rajkapoor, & Perumal (2012), estimated from a quercetin (≥ 95%, Sigma-Aldrich, Germany) standard curve (0.1 mg stock solution, 0.02 - 1.0 µl), and expressed as quercetin equivalent (QE). Total saponin content (TSC) was determined using the method of Hiai, Oura, & Nakajima (1976), estimated from a diosgenin (≥ 93%, Sigma-Aldrich, Germany) standard curve (10 - 100 µg/ml) and expressed as diosgenin equivalent (DE). Vitamin C content was determined using the method of Omaye et al. (1979), estimated from an ascorbic acid (≥ 99.7%, Sigma-Aldrich, Germany) standard curve (0.02 mg/ml stock solution, 0.063 - 0.5 ml), and expressed as ascorbic acid equivalent (AAE). Total alkaloid content (TAL) was determined using the method by Harborne (1973) and computed as mg/g.

The AA of SALF was determined by free radical scavenging capacity (FRSC) and total antioxidant capacity (TAC). The sample extracts were allowed to react with the stable free radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (≥ 90%, Merck, Germany) assay according to Brand-Williams et al. (1995). Briefly, to 3.9 ml of 6×10^{-5} mol/l (24 mg/l) DPPH in methanol, 0.1 ml SALF extract was added. The mixture was vortexed and kept in the dark for 30 min. The FRSC was calculated as:

$$\text{Free radical scavenging capacity (\%)} = ((Absorbance\ blank - Absorbance\ of\ sample) \times 100)/(Absorbance\ of\ blank) \quad \text{Eq. (1)}$$

A quercetin standard curve was prepared with FRSC (%) against concentration (1-10 µg/ml), and the total antioxidant capacity (TAC) of the samples was subsequently estimated from the quercetin standard curve and expressed as QE.

3.1.2.6. Quantification of phenolic compounds using HPLC

The HPLC analysis was carried out to assess the variation of phenolic compounds in SALF. These included gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin, as reported by Elekofehinti, Kamdem, Bolingon, et al. (2013). HPLC analysis was carried out for all accessions except for GP1, which was excluded due to the small sample quantity. To 0.5 g of SALF powders, 5 ml of methanol was added to extract phenolics as described by Elekofehinti, Kamdem, Bolingon, et al. (2013) for 30 min under constant shaking (IKA Vibrax VXR, IKA-

Labortechnik, Staufen i.Br., Germany). The extracts were then filtered through Whatman filter papers (GE Healthcare-UK Limited, Chalfont St. Giles, UK), which were then dried (Speedvac Plus SC11A, Savant, Farmingdale, NY, USA), redissolved in 1 ml methanol, and filtered through a 0.45 µm syringe filter (Carl Roth). HPLC analysis was then performed on an Agilent LC 1100-System as described by de Araújo et al. (2014) with slight modifications to reflect the instrument specifications and sample volume. An Eclipse Plus C18 column (4.6×250 mm, 5 µm) with matching guard was used with solvent A (10% methanol, 0.9% trifluoroacetic acid, TFA), solvent B (75% methanol, 0.25% TFA), and a flow rate of 0.5 mL/min. The gradient program set-points were 15 - 30%B (11 min); 30 - 99%B (11 min, 8 min steady), 99 - 10%B (3 min), return to initial values (2 min); all relevant peaks were eluted between minutes 8 and 30. For UV detection, the wavelength was set to detect the different phenolic compounds in one run. Thus, 310 nm was used, except for the time of 8 - 12 min (280 nm) and from 24 onwards (250 nm). To quantify the phenolic and flavonoid compounds, five calibration curves (gallic acid, chlorogenic acid, caffeic acid, rutin, quercetin, with RT = 8.5, 17.4, 21.0, 25.2 and 28.2 min, respectively) with seven concentrations ($R^2 > 99.99$) were applied. All samples were run in duplicate (injection volume of 5 µL).

3.1.3. Statistical analysis

Statistical analyses were carried out using SPSS (Version 21, IBM, Armonk, NY, USA). The BCC and AA experiments had two independent extracts per sample, and the analyses were done in triplicates, while the HPLC experiments had one extract per sample with analyses done in duplicate. The BCC and AA data were proven for normality of distribution (Kolmogorov - Smirnov, and Shapiro - Wilk). The results were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was carried out to determine statistically significant differences in sample means, and the *post-hoc* Duncan test was used to separate the means at $p < 0.05$. No statistical analyses were carried out for the HPLC results. Pearson's correlation was carried out to determine linear relationships between AA and BCC. Equations for the prediction of TAC were derived using multilinear regression. A principal component analysis (PCA) was applied for BCC and AA to determine the contribution of different variables (per component) to the variance of data. Categorization of accessions based on chemical or morphological data was carried out using hierarchical (Ward's method) and two-step cluster analyses, respectively.

3.1.4. Results

3.1.4.1. Morphological characteristics

Morphological characteristics for leaves and stems are summarized in Table 1. All accessions had green leaves (not shown), while 64.3% had green stems. Pubescence was mostly purple (71.4%) in leaves and white and dense (57.1%) in stems. Half of the accessions had fruits with two colours (a combination of either light green, dark green, white, or cream), with each colour occupying half of the fruit from the scars (Table 2 and Figure 4). The fruits were mostly small (64.3%), spherical (92.7%), and flat at the top (style scar area) (64.3%). Dark or very dark green fruits had sparse parallel venation, while white or light greenish cream fruits had no venation. The twigs had an average of eight fruits.

Table 1. Morphological characterization of leaves and stems of fourteen *Solanum anguivi* Lam. accessions

Accession	**Leaf**		**Stem**		
code	Pub. distr.	Pub. colour	Pub. distr.	Pub. colour	Colour
LV1	Dense	White	Dense	White	Green and purple
GV1	Sparse	White	Sparse	White	Green and purple
GV4	Intermediate	Purple and white	Dense	Purple and white	Green
CP1	Intermediate	Purple and white	Dense	Purple and white	Green
GC1	Very dense	Purple and white	Very dense	Purple and white	Green
GC2	Dense	Purple and white	Dense	Purple and white	Green
GC3	Dense	Purple and white	Dense	Purple and white	Green and purple
GC4	Very dense	Purple and white	Very dense	Purple	Green and purple
GC5	Sparse	Purple and white	Intermediate	Purple and white	Green
WP1	Very dense	Purple and white	Very dense	Purple	Green
GV2	Intermediate	White	Dense	White	Green
GV3	Sparse	White	Sparse	White	Green and purple
GP1	Intermediate	Purple and white	Dense	Purple and white	Green
GC6	Dense	Purple and white	Dense	Purple and white	Green

Pub. distr. = Pubescence distribution, Pub. Colour = Pubescence colour

Table 2. Morphological characterization of fruits of fourteen *Solanum anguivi* Lam. accessions

Accession code	Colour	Size	Shape	Fruit top	Venation	Av. twig
LV1	Light green	Small	Spherical	Flat	Dense, reticulate	9
GV1	Very dark green	Very small	Spherical	Cuspidate	Sparse, parallel	8
GV4	Dark with light green	Very small	Spherical	Flat	Intermediate, reticulate	8
CP1	White	Small	Spherical	Flat	None	12
GC1	Light green with white	Small	Spherical	Cuspidate	Sparse, parallel	12
GC2	Dark green with white	Small	Spherical	Cuspidate	Sparse, parallel	12
GC3	Dark with light green	Small	Spherical	Flat	Intermediate, reticulate	8
GC4	Light green with white	Small	Spherical	Flat	Intermediate, reticulate	8
GC5	Dark green with cream	Intermediate	Ovoid	Elliptic	Intermediate, reticulate	8
WP1	White	Small	Spherical	Flat	None	5
GV2	Dark green	Large	Spherical	Flat	Sparse, parallel	1
GV3	Dark green	Small	Spherical	Flat	Sparse, parallel	6
GP1	Light greenish cream	Small	Spherical	Conical	None	8
GC6	Light green with white	Intermediate	Spherical	Flat	Intermediate, reticulate	10

Size= fruit size diameter (very small = < 0.8 cm, small = > 0.8 - 1.2 cm, intermediate = >1.2 - 1.8 cm, large = > 1.8 cm); Av. twig = average number of fruits per twig; fruit top = style scar area.

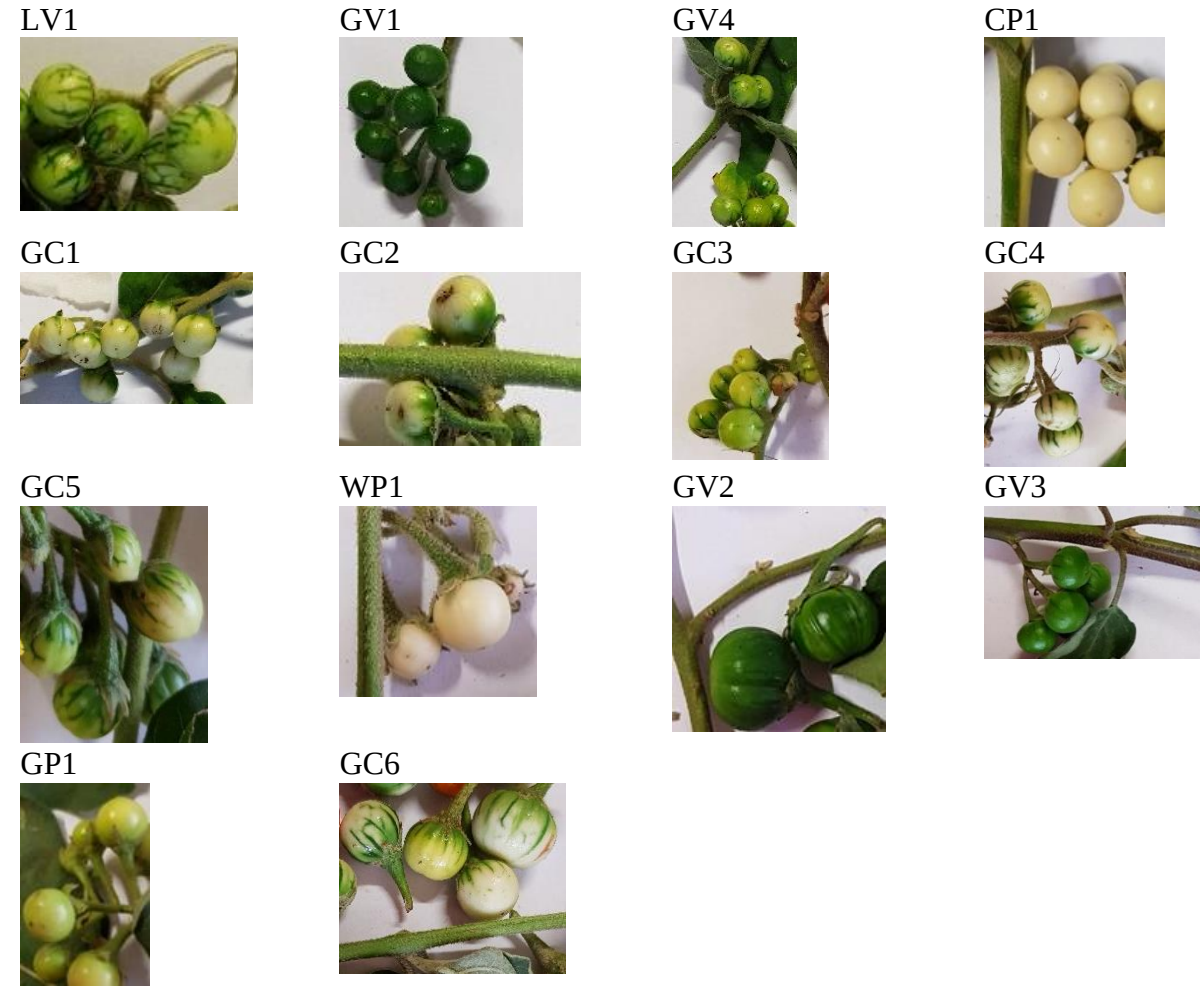


Figure 4. Photographs of fruits for fourteen accessions of *Solanum anguivi* Lam. Photographs are not to scale.

3.1.4.2. Bioactive compounds content and antioxidant activities of SALF accessions

The BCC significantly differed among accessions (Table 3). The total phenolics were highest in accession GP1 and least in WP1, while the total flavonoids were highest in GV2, GV3, and GP1 and least in CP1 and WP1. The total saponins were highest in GC2 and GV3 and least in GC5 and GV2. The total alkaloids were high and not significantly different among ten accessions, with the least content in CP1 and GC1. Vitamin C was highest in GV3 and GC2 and least in GC3 and LV1 accessions.

Table 3. Bioactive compounds content of fruits from different *Solanum anguivi* Lam. accessions

Accession code	TPC (mg GAE/g)	TFC (mg QE/g)	TSC (mg DE/g)	TAL (mg/g)	Vitamin C (mg AAE/g)
LV1	10.30±0.32 [c]	1.58±0.05 [b, c]	101.17±1.64 [c]	108.04±1.81 [a, b, c]	3.56±0.19 [g]
GV1	11.13±0.18 [b]	1.73±0.04 [b]	115.43±1.68 [b]	123.94±9.96 [a]	4.29±0.40 [f]
GV4	10.52±0.17 [b, c]	1.28±0.05 [c, d]	87.91±3.30 [e]	105.46±1.71 [a, b, c]	4.27±0.21 [f]
CP1	9.60±0.27 [d, e, f]	0.89±0.16 [e]	114.64±1.91 [b]	91.42±3.66 [c, d]	5.01±0.24 [c, d, e]
GC1	9.55±0.08 [d, e, f]	1.56±0.09 [b, c]	76.02±5.80 [f]	81.38±2.58 [d]	4.31±0.21 [f]
GC2	10.0±0.17 [c, d, e]	1.53±0.07 [b, c]	124.83±0.83 [a]	111.81±6.20 [a, b]	6.05±0.13 [a, b]
GC3	9.59±0.17 [d, e, f]	1.69±0.05 [b]	104.48±1.41 [c]	113.51±12.46 [a, b]	4.17±0.07 [f, g]
GC4	9.33±0.03 [e, f]	1.55±0.05 [b, c]	91.63±3.22 [d, e]	122.55±7.97 [a]	4.38±0.13 [e, f]
GC5	9.46±0.20 [d, e, f]	1.72±0.22 [b]	51.13±1.48 [g]	97.77±5.84 [b, c, d]	4.31±0.12 [f]
WP1	8.04±0.28 [g]	1.00±0.06 [d, e]	77.15±3.45 [f]	112.57±4.08 [a, b]	4.69±0.15 [d, e, f]
GV2	10.10±0.19 [c, d]	2.07±0.09 [a]	58.51±1.81 [g]	92.26±7.68 [c, d]	5.20±0.18 [c, d]
GV3	10.15±0.26 [c, d]	2.06±0.15 [a]	121.92±2.62 [a, b]	118.24±8.67 [a, b]	6.40±0.11 [a]
GP1	12.38±0.09 [a]	2.07±0.11 [a]	97.30±2.44 [c, d]	113.21±2.1 [a, b]	5.28±0.32 [c, d]
GC6	9.16±0.26 [f]	1.47±0.06 [b, c]	89.97±2.23 [d, e]	127.70±1.67 [a]	5.5±0.27 [b, c]

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. The fruit batches were obtained twice (two weeks apart) to obtain two independent flour samples per accession. Values are the mean ± SEM of two independent experiments measured in triplicates. The means were computed on a dry weight basis. Means within the same column with different superscripts are significantly different at $p < 0.05$. TPC = total phenolic content, GAE = gallic acid equivalent, TFC = total flavonoid content, QE = quercetin equivalent, TSC = total saponin content, DE = diosgenin equivalent, TAL= total alkaloid content, AAE = ascorbic acid equivalent.

The HPLC analysis showed variations in the phenolic acids and flavonoid compounds among SALF accessions (Table 4). Regarding the AA, both the FRSC and TAC significantly differed among the SALF accessions, with accession GP1 registering the highest, followed by GV1 and least in WP1 (Table 5).

Table 4. HPLC quantification of phenolic acid and flavonoid compounds in *Solanum anguivi* Lam. fruit accessions

Accession code	Gallic acid (µg/g)	Chlorogenic acid (µg/g)	Caffeic acid (µg/g)	Rutin (µg/g)	Quercetin (µg/g)
LV1	53.93	21.37	15.06	36.69	35.11
GV1	21.16	244.28	19.54	65.22	28.11
GV4	46.08	30.61	12.92	15.45	10.71
CP1	39.79	103.10	19.28	36.32	35.34
GC1	23.71	192.96	14.61	28.93	10.77
GC2	55.29	22.93	14.22	27.26	28.04
GC3	20.60	58.84	6.64	13.81	30.57
GC4	37.21	16.00	16.18	10.43	36.09
GC5	49.00	156.45	24.56	72.02	10.93
WP1	44.37	111.83	15.36	20.25	27.99
GV2	50.57	304.33	36.95	15.29	14.47
GV3	70.00	120.95	28.37	28.75	11.75
GC6	58.68	59.77	20.87	20.83	11.72

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. Fruits for each accession were dried, and one extract was obtained for each accession. Values are the means of duplicate analyses from one extract per accession. The means were computed on a dry weight basis. Statistical analysis was not carried out on the data.

3.1.4.3. Antioxidant activity and its relation with the BCC in SALF

The TAC and FRSC had similar significantly strong positive correlations with TPC (r = 0.74) but low positive correlation with TFC (r = 0.23) and TSC (r = 0.25). Vitamin C significantly correlated negatively (r = -0.22) with FRSC, simultaneously registering a statistically insignificant negative correlation with TAC.

Principal component analysis (PCA) grouped the data into three components, which cumulatively explained 78.8% variation in the data (Figure 5). Components 1 (FRSC, TAC and TPC), 2 (TSC and TAL) and 3 (TFC and VCC) accounted for 39.8%, 21.9% and 17.1% variance in the data, respectively.

Table 5. Antioxidant activity of fruits from *Solanum anguivi* Lam. accessions

Accession code	Total antioxidant capacity (mg QE/g DW)	Free radical scavenging capacity (%)
LV1	2.68±0.14 [c, d]	7.29±0.36 [c]
GV1	3.69±0.10 [b]	9.35±0.21 [b]
GV4	2.43±0.04 [d, e]	6.4±0.09 [d, e]
CP1	2.85±0.07 [c]	7.2±0.18 [c, d]
GC1	2.31±0.07 [e, f]	6.19±0.16 [e]
GC2	1.60±0.07 [h]	4.4±0.16 [g]
GC3	2.60±0.11 [c, d, e]	6.78±0.23 [c, d, e]
GC4	2.63±0.16 [c, d, e]	6.96±0.39 [c, d, e]
GC5	1.91±0.05 [g]	5.24±0.12 [f]
WP1	1.20±0.03 [i]	3.37±0.06 [h]
GV2	2.05±0.08 [f, g]	5.29±0.17 [f]
GV3	1.44±0.14 [h]	3.98±0.34 [g]
GP1	4.58±0.14 [a]	11.51±0.33 [a]
GC6	2.00±0.15 [g]	5.36±0.37 [f]

Solanum anguivi Lam. fruits were obtained from 12 plants per accession. The fruit batches were obtained twice, two weeks apart, to obtain two independent flour samples per accession. Values are the mean ± SEM of two independent experiments measured in triplicates. The means were computed on a dry weight basis. Means within the same column with different superscripts are significantly different at $p < 0.05$. QE = quercetin equivalent, DW = dry weight.

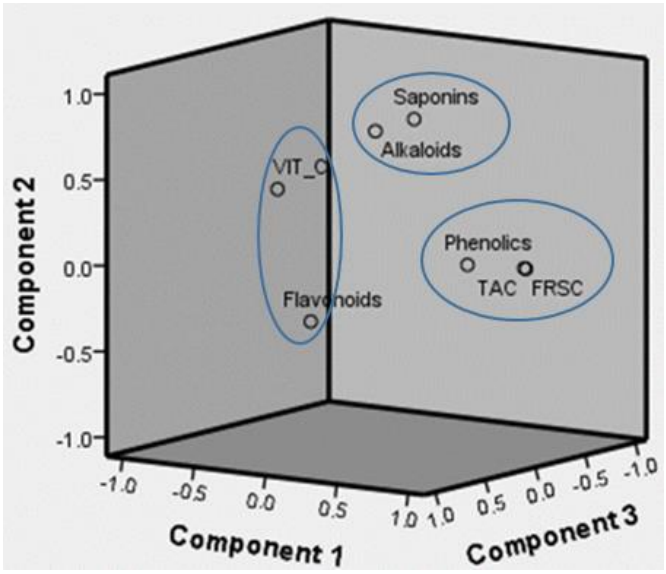


Figure 5. Components derived from principal component analysis of *Solanum anguivi* Lam. fruits based on their bioactive compounds content and antioxidant activities. TAC = total antioxidant capacity, FRSC = free radical scavenging capacity, phenolics = total phenolic content, flavonoids = total flavonoid content, saponins = total saponin content, alkaloids = total alkaloid content, vit_C = vitamin C content. The components cumulatively accounted for 78.8% variation of the data. Component 1 = free radical scavenging capacity, total antioxidant capacity, and total phenolic content; component 2 = total saponin and total alkaloid contents; and component 3 = Total flavonoid and vitamin C contents.

Regression models for factors that affected SALF AA the most were derived. Model 1 was for TAC mg QE/g DW (Eq. 2), and model 2 was for FRSC (%) (Eq. 3).

i) Model 1

$$\text{TAC (mg QE/g)DW} = -3.087 + 0.661[TPC(mg\ GAE/g\ DW)] - 0.230[Vitamin\ C\ (mg\ AAE/g\ DW)] \quad \text{Eq (2)}$$

Where, r = 0.784, r^2 = 0.615, adjusted r^2 = 0.600 and the unstandardized coefficients of the constant, phenolics and vitamin C were statistically significant at p < .001, .001 and .005 respectively.

ii) Model 2

$$\text{FRSC \%} = -6.356 + 1.561[TPC(mg\ GAE/g\ DW)] - 0.608[Vitamin\ C\ (mg\ AAE/g\ DW)] \quad \text{Eq (3)}$$

Where r = 0.788, r^2 = 0.621, adjusted r^2 = 0.607 and the unstandardized coefficients of the constant, phenolics and vitamin C were statistically significant at p < .005, .001 and .005 respectively.

3.1.4.4. Cluster analysis

Cluster analysis based on morphological characteristics revealed that leaf and stem pubescence colours and fruit colour were the most important for categorizing SALF accessions (Figure 6).

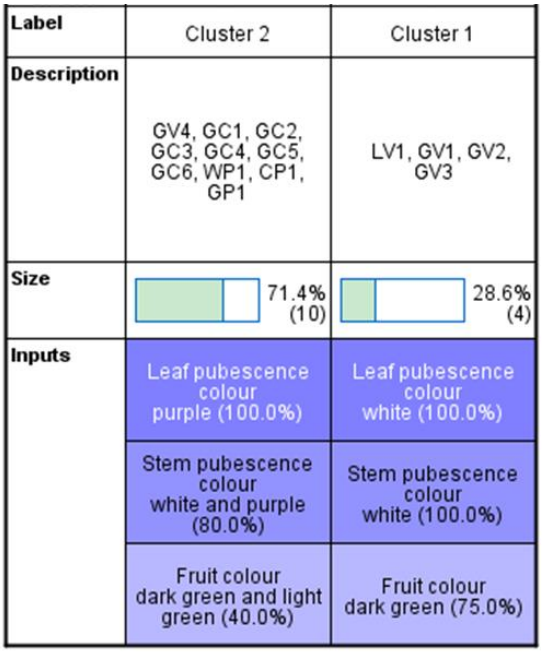


Figure 6. Two-step cluster analysis of fourteen accessions of *Solanum anguivi* Lam. based on their morphological characteristics.

Cluster analysis based on the chemical compositions grouped the SALF accessions into four (Figure 7). Cluster 1 accessions had low contents of TFC, TPC and TAC, with moderate to low vitamin C and TSC. Cluster 2 accessions had high TFC, TPC, TSC, and TA, with low vitamin C. Cluster 3 accessions had low TPC, TSC, TA, vitamin C, and TAC, with moderate TFC. Cluster 4 accessions had the highest TFC, TSC, TA, and vitamin C; moderate levels of TPC, and the lowest TAC.

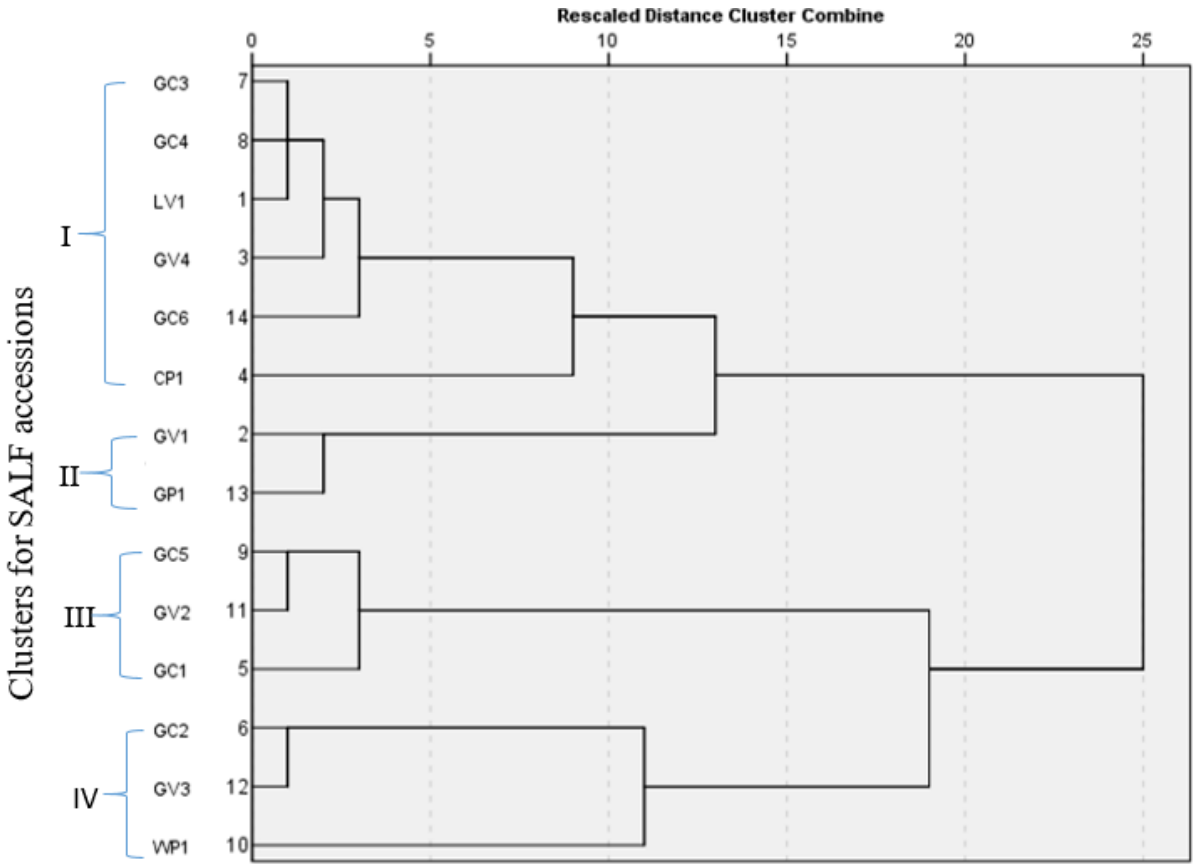


Figure 7. A dendrogram representing diversity and clusters of fourteen *Solanum anguivi* Lam. fruit accessions based on their bioactive compounds content and total antioxidant capacity. The dendrogram was obtained using hierarchical cluster analysis (Ward's minimum variance).

3.1.5. Discussion

3.1.5.1. Morphological characteristics

The morphological characteristics varied among the accessions (Tables 1 and 2). The fruits from 13 out of 14 accessions were spherical, similar to results obtained by Osei et al. (2010), who reported a round shape in SALF. The leaves in this study had sparse to dense pubescence, while Osei et al. (2010) reported the absence of leaf pubescence in SALF accessions and absent to sparse in *S. aethiopicum* and *S. macrocarpon* accessions. The size of the fruits in the present

study ranged from very small (< 0.8 cm) to large (> 1.8 cm), with none to intermediate venations. Contrary, Osei et al. (2010) reported only a small size with stripes for SALF, without quantifying them. Further, Osei et al. (2010) did not define the small size of SALF. Sseremba et al. (2017) and Tembe et al. (2020) neither reported the fruit colours and shapes nor the leaf and stem pubescence distribution of SALF accessions. Both studies gave general information on *Solanum* species. Furthermore, data on the appearance of the fruit top (style scar area), the average number of fruits per twig, and the amount and type of venations on the fruits are scarce. The morphological variations in the present study may be attributed to genetic differences in the accessions. The analysis of molecular variance (AMOVA) by Stedje & Bukenya-Ziraba (2003) showed that the variance within *S. anguivi* accessions based on DNA data was great (90.42%). The present study, therefore, adds further information to the knowledge regarding morphological variations of *S. anguivi* accessions.

3.1.5.2. Bioactive compounds contents

Total phenolic contents of SALF in this study (8.0 - 12.4 mg GAE/g DW) (Table 3) were lower than those reported by Elekofehinti, Kamdem, Bolingon, et al. (2013) (17.1 mg GAE/g DW) and Andabati & Muyonga (2014) (32.7 mg GAE/g DW), although the latter's reference standard is different from that employed in the present study. Although Dan et al. (2014) and Oyeyemi et al. (2015) reported TPC of SALF with no reference standard, 9.6 and 15.2 mg/g DW, respectively, the former falls in the range of the present study while the latter is higher. Comparable to the current study, Hanson et al. (2006) reported significant differences in TPC among *S. melongena* accessions, and the present findings fall within their range (7.4 - 14.3 mg chlorogenic acid equivalent/g). The variations in TPC of SALF by different researchers may be due to differences in the accessions, reference standards, analysis methods, agronomic or environmental factors. Tolić et al. (2017) further attributed high variations and discrepancies of phenolic content during different growing seasons to fluctuations in air temperatures, sunlight, and rainfall intensity. The HPLC results revealed that the contents of phenolic compounds in SALF varied among the accessions (Table 4). Chlorogenic acid content was the highest of the quantified phenolic compounds in the SALF accessions. Possibly, the differences in TPC among accessions in this study stemmed from variations in the proportions of phenolic compounds contained in individual accessions as previously reported by Stommel & Whitaker (2003) for *S. melongena* genotypes. Plums and apricots were ranked among the top 100 richest polyphenol sources (Pérez-Jiménez et al., 2010). Comparatively, the TPC range of SALF in this study was higher than for plums (5.6 mg GAE/g DW) (Miletić et al., 2013) and apricots

(4.7 mg GAE/g DW) (Miletić et al., 2013). Thus, empirically, all the SALF accessions in this study were rich in phenolics. Phenolics have been reported to have antidiabetic effects such as hypoglycemic and β-cells regeneration abilities (Gandhi et al., 2011). All accessions in this study were rich in TPC, and they may be recommended for dietary consumption for potential health benefits.

Total flavonoid contents in this study (0.9 - 2.1 mg QE/g DW) (Table 3) were lower than those given earlier by Elekofehinti, Kamdem, Bolingon, et al. (2013) (9.5 ± 0.5 mg QE/g DW), Andabati & Muyonga (2014) (7.2 mg CE/g DW) and Oyeyemi et al. (2015) (4.6 mg/g DW, no reference standard). Probably these differences are due to genotypic or accession variations, differences in environmental factors, extraction and analysis methods, or reference standard compounds. In addition, HPLC results of the contents of the flavonoid compounds suggested variation in the rutin and quercetin contents amongst the SALF accessions (Table 4). The differences in TFC among the SALF accessions in this study may thus have been due to variations in the contents of the flavonoid compounds. Similar to the findings in this study, Kaur et al. (2014) reported significant differences in the TFC of eggplant cultivars (0.35 - 3.24 mg QE/g DW), and the present findings are within their range. Onions are reportedly rich sources of flavonoids (Kozłowska & Szostak-Węgierek, 2018). The TFC of onion cultivars has been reported as 1.3 - 2.1 mg QE/g DW (K. Sharma et al., 2014), which is similar to the values in the present study. Thus, SALF accessions in this study may potentially be high sources of flavonoids. Flavonoids have a broad range of biological activities, such as the potential management of diabetes due to their hypoglycemic effects (J. S. Lee, 2006). All accessions in this study may thus be recommended for potential antidiabetic benefits.

Total saponin contents in this study (51.1 - 124.8 mg DE/g DW) (Table 3) were much higher than reported by Oyeyemi et al. (2015) (10 mg/g DW, no reference standard). This may be due to differences in genotype or accession, extraction conditions, and environmental factors (such as soil nutrition, climate). Similar to the present study, Agoreyo et al. (2012) reported significant differences in TSC among *S. melongena* fruit varieties (0.0 - 0.1 mg/100 g), which were lower than the range in the present study. The findings in this study showed that SALF accessions were rich in TSC because their values were higher than saponin-rich fenugreek genotypes (9.0 - 17.0 mg DE/g DW) (Arivalagan et al., 2013). SALF saponins *in vitro* and *in vivo* have been reported to have antioxidant and antidiabetic properties (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). All

accessions may thus be recommended for potential antidiabetic benefits.

Total alkaloid contents of SALF accessions in this study were higher (81.4 - 127.7 mg/g DW) (Table 3) than reported by Oyeyemi et al. (2015) (0.5 mg/g DW, no reference standard) in SALF. The variations in the results may also be due to differences in genotypes or accession, extraction, or analysis methods used. Significant differences in TAL were also observed in *S. melongena* varieties (1.0 - 1.2 mg/100 g DW) by Agoreyo et al. (2012), although they were much lower than what was obtained in the present study. However, Kalebar et al. (2019) reported that the TAL from *S. macranthum* fruits was 99.2 mg/g DW, which is comparable with the range within this study. Importantly, alkaloids may be used for the management of diabetes, as shown for *S. torvum* glycoalkaloids (Al-Ashaal et al., 2018), and thus, all accessions in this study may be recommended due to their substantial TAL.

Vitamin C content of SALF in this study was higher (3.6 - 6.4 mg AAE/g DW*)* (Table 3) than documented by Dan et al. (2014) (0.3 mg/g). Hanson et al. (2006) similarly obtained significant differences in ascorbic acid content in *S. melongena* accessions (0.56 - 1.29 mg/g), although they were also lower than what was observed in the present study. Genetic variations among SALF accessions might explain the differences in vitamin C content recorded in this study. Tomatoes are reportedly rich in vitamin C (Lykkesfeldt et al., 2014), with a VCC range of 2.0 - 5.2 mg/g DW (Hallmann, 2012), which falls within the range of the present study. All SALF accessions in this study are, therefore, potentially rich sources of vitamin C. Vitamin C has been reported to be a potent reducing agent and scavenges free radicals in biological systems (Arrigoni & De Tullio, 2002). However, it has also been reported to exhibit pro-oxidant and mutagenic effects (Arrigoni & De Tullio, 2002).

3.1.5.3. Antioxidant activity

In the current study, the FRSC (3.4 - 11.5%) and TAC (1.2 - 4.6 mg QE/g DW) (Table 5) differed from results by Andabati & Muyonga (2014) (11.4 mg VCE/g DW). Elekofehinti, Kamdem, Bolingon, et al. (2013) reported the AA of SALF as $IC_{50} = 275.0$ µg/ml. Differences in extraction conditions, analysis methods, or reference standards may cause divergence in TAC values or difficulty in comparisons between studies. Concomitant with the findings in the present study, significant differences in TAC were recorded among cultivars of eggplants (2664 - 8247 µmol TE/kg) (Okmen et al., 2009). The various researchers used different methods, reference standards, and analytical methods (fresh weight basis, per volume), which limits

comparisons with the present study findings. Free radicals are a major factor in the propagation stage of the oxidation process (Elekofehinti, Kamdem, Bolingon, et al., 2013) that may lead to oxidative stress. Consumption of fruits or vegetables with the potential for scavenging free radicals due to the presence of bioactive compounds with antioxidant properties could inhibit the spread of oxidation in the body. In the present study, accession GP1 had the highest TAC and FRSC and may, therefore, possess the highest nutraceutical benefits.

The AA of SALF in this study had associations with some BCC. The positive correlation between TSC and AA in this study is comparable with results by Elekofehinti, Kamdem, Kade, Adanlawo, et al. (2013), who showed that SALF saponins had dose-dependent FRSC. Correlation between TPC and FRSC of SALF in this study was strong and higher than reported for *S. melongena* cultivars (r = 0.52) (Okmen et al., 2009) and *S. melongena* accessions (r = 0.44) (P. M. Hanson et al., 2006). The negative correlation between vitamin C and FRSC in the present study may show pro-oxidant properties of vitamin C in SALF. Similarly, Peyrat-Maillard et al. (2001) reported that the addition of vitamin C to malt rootlet extracts led to an antagonist effect on their TAC. Vitamin C pro-oxidant properties are reportedly more evident under some circumstances, such as the presence of metal ions (iron and copper) and adequate pH (alkali) conditions (Arrigoni & De Tullio, 2002). Dan et al. (2014) and Oyeyemi et al. (2015) reported the presence of iron (467.7 and 22.2 mg/100g DW, respectively) and copper (0.1 and 1.4 mg/100g DW, respectively) in SALF. Further studies investigating the antagonistic effect of vitamin C in SALF and its possible relationship with iron and copper contents are recommended.

The derived regression models in the present study showed that amongst the analyzed BCC, total phenolics and vitamin C contents mostly affected the AA of SALF. The models (Eq. 2 and Eq. 3) derived in this study were good, as indicated by their moderately high adjusted r^2. The models may explain 60% variance of the SALF AA.

3.1.5.4. Cluster analysis

Cluster analysis was carried out to assess whether the morphological characteristics of SALF may be used to predict SALF accessions with similar BCC and AA. The clusters formed based on chemical compositions (Figure 7) agree with the correlation results and the AA regression models in this study. Cluster 1 was characterized by low phenolics and flavonoids, which may explain their low TAC. Furthermore, the accessions that had moderate VCC and low saponin

contents had the least TAC, and those with low VCC and moderate TSC had significantly higher TAC than the former. Cluster 2 accessions had chemical compositions similar to the correlation results obtained in this study, that is, high phenolic, high flavonoid, high saponin, low VCC, and high TAC. Although cluster 3 accessions were characterized by low vitamin C and high flavonoid contents, the low phenolic and saponin contents may have resulted in the low TAC. The low TAC in cluster 4 may be due to the high VCC, whose negative effect may have suppressed the positive contribution on TAC by the moderate and high amounts of phenolics and saponins, respectively. Therefore, BCC and TAC may be good predictors for variation among SALF accessions, given that the results from the cluster analysis were coherent with the correlation and regression analyses. Comparison between cluster analysis based on morphological characteristics (Figure 6) and that based on chemical compositions (Figure 7) showed that 35.7% of the accessions, that is, 5 (GV4, GC3, CP1, GC4, and GC6) out of the 14, appeared together in both analyses. The clusters formed based on morphological characteristics may predict some accessions with similar chemical compositions.

3.1.6. Conclusion

The morphological characteristics of *Solanum anguivi* Lam. varied among the accessions. All accessions were rich in phenolics, flavonoids, saponins, alkaloids, and vitamin C. However, the BCC and AA significantly differed among the SALF accessions. Chlorogenic acid was the most dominant of the analyzed phenolic compounds in the fruits. High amounts of total phenolic, flavonoid, or saponin contents may lead to high AA, while high vitamin C content may negatively affect the AA of SALF. The results, therefore, guide on which accession one may consume to benefit from a given bioactive compound. The association between the morphological characteristics and the chemical compositions for some SALF accessions suggested that the morphological characteristics of *Solanum anguivi* Lam. could be used to predict accessions with similar BCC and AA.

3.2. Influence of accession and ripeness stage on the bioactive compounds content and antioxidant activities of *Solanum anguivi* Lam. fruits [3]

Abstract

Fruits and vegetables contain bioactive compounds that are beneficial for the prevention of oxidative stress-related diseases. *Solanum anguivi* Lam. fruits (SALF) possess bioactive compounds such as phenolics, alkaloids, saponins, flavonoids, and vitamin C. It has been documented that the ripeness stage influences the nutritional quality of fruits. However, limited information on the effect of the ripeness stages (unripe, yellow, orange, and red) on the bioactive compounds content (BCC) and the antioxidant activity (AA) of SALF is currently available. This study investigated the effect of ripening on the BCC and AA of different SALF accessions. The BCC was determined by spectrophotometry (for total phenolics, flavonoids, saponins, and vitamin C), HPLC (for quantification of phenolics), and gravimetry (for total alkaloids). The AA, determined as free radical scavenging activity (FRSC) and total antioxidant capacity (TAC), was also determined by spectrophotometry. Total phenolics (7.6 - 22.6 mg gallic acid equivalent/g dry weight [DW]), flavonoids (1.3 - 4.1 mg quercetin equivalent/g DW), saponins (44.8 - 152.5 mg diosgenin equivalent/g DW), vitamin C (2.2 - 6.4 mg ascorbic acid equivalent/g DW), alkaloids (141.2 - 296.9 mg/g DW), FRSC (1.5 - 66.2%) and TAC (0.1 - 14.2 mg quercetin equivalent/g DW) significantly differed at the four ripeness stages. Fruits in the unripe stage were rich in phenolics, flavonoids, and AA, the orange stage in saponins and flavonoids, and the red stage in vitamin C (for some accessions) and alkaloids. During the ripening process, the contents of chlorogenic and caffeic acids decreased, gallic acid and rutin increased, while the effect on the quercetin content differed among the accessions. An interaction between the ripeness stage and accession was postulated. The AA had strong positive correlations with total flavonoids and phenolics ($r = 0.72$ and 0.81, respectively) and a moderate negative correlation with total alkaloids ($r = -0.67$). Regression models for factors associated with AA at the different ripeness stages had high adjusted r^2 values ($r = 0.75$ to 0.89). The models showed that SALF AA was mostly affected by phenolic and vitamin C contents at the unripe stage, phenolic and flavonoid contents at the yellow stage, flavonoid and saponin contents at the orange stage; and flavonoid and alkaloid contents at the red stage. Overall, fruits in the unripe stage had the highest AA and total phenolics and thus may have the highest health-promoting properties.

Keywords: *Solanum anguivi*, bioactive compounds, antioxidant capacity, free radical

scavenging capacity, accession, ripeness stage

[3] This content has been submitted to the *International Journal of Fruit Science*.

3.2.1. Introduction

Fruits and vegetables are among the most important sources of bioactive compounds for the human diet (Oz & Ebru, 2017). Bioactive compounds possess many health benefits such as antioxidative, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic, and anti-inflammatory effects (Schreiner & Huyskens-Keil, 2006). Consumption of fruits and vegetables has, therefore, been associated with the prevention of chronic and degenerative diseases (Saldana et al., 2010). Fruits from *Solanaceae* plants are important as they contain various bioactive compounds, which are suggested to have medicinal properties (Simonne et al., 2011). *Solanum*, a widespread plant genus of the family *Solanaceae*, has over 1000 species worldwide with at least 100 indigenous species in Africa and adjacent islands, which include several valuable crop plants and some poisonous ones (Jaeger & Hepper, 1986). The fruits of *Solanum anguivi* Lam. have been reported to treat various diseases, including hypertension, atherosclerosis, and diabetes (Bukenya & Carasco, 1995; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013).

The bioactive compounds content (BCC) in fruits of the same species may vary with the genotype and ripeness stage (S. K. Lee & Kader, 2000). Different varieties or cultivars amongst plants of the same species, grown under the same conditions, have been previously reported to differ in the BCC and antioxidant activity (AA) due to genetic differences (Benvenuti et al., 2004; Milivojević et al., 2010; Okmen et al., 2009). Various studies have similarly shown that ripening may boost or diminish the BCC of fruits. For example, total phenolic content (TPC) significantly differed at the different ripeness stages of apples (K. M. da Silva et al., 2018) and tomatoes (Bhandari & Lee, 2016). Both K. M. da Silva (2018) and Bhandari & Lee (2016) further showed that the differences during ripening were influenced by the variety for apples and cultivar for tomatoes. On the contrary, Amira et al. (2012) reported that although TPC for date fruits differed significantly with the ripeness stage, the effect was not significantly different among the cultivars. In addition to the genotype and ripeness stage, environmental factors such as climate, soil properties, as well as agricultural practices may affect the BCC and AA of fruits and vegetables (S. Kumar et al., 2017; S. K. Lee & Kader, 2000; Leskovar et al., 2009). Therefore, the BCC and AA of fruits for the same species as reported by different

authors may vary due to variations in the factors mentioned above. To analyze the effect of the ripeness stage on fruits of the same plant species, factors that may confound the results, such as location and agricultural practices, should be similar for the plants under investigation.

Solanum anguivi Lam. fruits (SALF) have been reported to possess phenolics, flavonoids, saponins, alkaloids, vitamin C, and tannins (Abbe et al., 2019; Dan et al., 2014; Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015). However, the effect of the ripeness stage has only been investigated on the total contents of phenolics, flavonoids, tannins, and vitamin C (Abbe et al., 2019; Dan et al., 2014). However, data on the effect of ripening effect on SALF total saponin and alkaloid contents is scarce. Furthermore, there is a dearth of information on the effect of ripening on the AA, TPC, total flavonoid content (TFC), total alkaloid content (TAL), total saponin content (TSC), and vitamin C content (VCC) among different accessions of SALF. This study, therefore, investigated the influence of the ripeness stage on the BCC (total contents of flavonoids, phenolics, vitamin C, saponins, and alkaloids) and AA (free radical scavenging capacity and total antioxidant capacity) of different accessions of SALF. This would establish the ripeness stage at which SALF may be consumed for potentially high health benefits. The study also determined the associations between the BCC and AA of SALF.

3.2.2. Materials and methods

3.2.2.1. Reagents and chemicals

The reference standards (gallic acid, quercetin, diosgenin, L-ascorbic acid), HPLC grade ethanol, acetic acid, ammonium hydroxide, sulphuric acid, methanol; and analytical grade 1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid, vanillin, hydrated copper (II) sulphate, potassium acetate, thiourea, sodium bicarbonate, dinitrophenyl hydrazine aluminium chloride and Folin-Ciocalteu reagent, were all purchased from Sigma-Aldrich (Munich, Germany). The reference standards (gallic acid, caffeic acid, rutin, chlorogenic acid, and quercetin) for HPLC quantification of phenolic and flavonoid compounds were obtained from Sigma-Aldrich, Steinheim, Germany).

3.2.2.2. Sample collection

All the SALF samples in this study were obtained from the same geographical location to eliminate or minimize the effects of other variables such as climatic factors (rainfall, wind patterns, and temperature). The samples were collected from Nabiyagi village (GPS 0.472336,

32.802484), Mukono district (Uganda), in March 2019. The fruits at four ripeness stages (unripe, yellow, orange, and red) (Abbe et al., 2019; Dan et al., 2014) were collected from four *Solanum anguivi* Lam. accessions named in this study as GP1, WP1, CP1, and GC1. The colours of SALF at the unripe stage differed among the accessions. Unripe fruits of GP1 were light greenish-cream, WP1 and CP1 were white, while GC1 was light green and white with venations. The SALF colours for the rest of the ripeness stages under investigation were similar among the accessions.

3.2.2.3. Experimental design

Fifty fruits per ripeness stage were randomly collected from 12 plants per accession and prepared on the same day. This process was replicated the second time by obtaining fruit samples from the accessions two weeks after the first collection. The samples were prepared to obtain flours, which were analyzed for TPC, TFC, TSC, VCC, TAL, and AA, as well as for the contents of the phenolic compounds.

3.2.2.4. Sample preparation

Procedurally, the fruits were sorted to remove those with damaged pericarps. Stalks were plucked off the fruits, the latter washed with distilled water, and then patted dry with a cotton cloth. The fruits were cut into four parts, and those with pests were discarded. The cut fruits were dried in an oven (Infrared Food Oven GL-2A, Guangzhou Itop Kitchen Equipment Co, Ltd. Guangdong, China) at 40 ^{o}C for 16 hr. The samples were then milled (Wonder Mill, Pocatello, Idaho) at a "pastry" setting to obtain fine flours that were then stored in sealed plastic bottles at -20 ^{o}C until further analysis. All samples had two flours obtained independently.

3.2.2.5. Extraction of SALF for BCC and AA analyses

Using 80% methanol (D. Kim et al., 2003), the bioactive compounds were extracted from the SALF powders as described by Makkar (2003), with slight modifications. Briefly, SALF powder (0.2 g) was weighed into a falcon tube, and 80% methanol (20 ml) was added. The falcon tube was then put into an ultrasonic water bath (Bransonic series, M 2800-E; Branson Ultrasonics Co., Danbury, CT) and subjected to ultrasonic treatment at room temperature for 10 min. The water in the ultrasonic water bath was prevented from getting warm by replacing half of it every 2 min (to maintain it at room temperature 24 - 25 ^{o}C). The contents of the falcon tubes were cooled on ice and then centrifuged (Fisher Scientific 225, Pittsburg, PA, USA) at 3000 x g for 10 min. The supernatant was collected into another falcon tube and kept on ice.

An equal volume of 80% methanol was added to the remaining pellet, and this was also subjected to ultrasonic treatment for the same duration as the first extraction. The contents were centrifuged and the supernatant collected as described above. This procedure was repeated for the third time. All the three extracts were pooled together and stored at -20 ^{o}C until further analysis. Each sample had two extracts from the two independent flour samples.

3.2.2.6. Quantification of BCC and AA of SALF

The extracts of the SALF samples were analyzed for total contents of phenolics, flavonoids, saponins, vitamin C, and alkaloids, as well as AA. The analysis of total phenolics was by the Folin-Ciocalteu reagent method (Singleton et al., 1998) and total flavonoids as described by Kumar, Rajkapoor, & Perumal (2012). The total saponins were determined by the method of Hiai, Oura, & Nakajima (1976), vitamin C content was determined as described by Omaye et al. (1979), while total alkaloids were determined by the method of Harborne (1973). The total contents of phenolics, flavonoids, saponins, vitamin C, and alkaloids were expressed as gallic acid equivalent (GAE), quercetin equivalent (QE), diosgenin equivalent (DE), ascorbic acid equivalent (AAE), and mg/g, respectively. The BCC and TAC analyses were carried out in triplicates for two independent extracts per sample.

The AA was analyzed by determining the free radical scavenging capacity (FRSC) of the samples, using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to Brand-Williams et al. (1995). A quercetin standard curve was prepared with the FRSC (%) against the quercetin concentration (1 - 10 µg/ml), and the TAC of the samples was subsequently estimated from the quercetin standard curve and expressed as mg QE/g. The FRSC was calculated as:

$$\text{Free radical scavenging capacity (\%)} = ((Absorbance\ blank - Absorbance\ of\ sample) \times 100)/(Absorbance\ of\ blank) \quad \text{Eq. (1)}$$

All the absorbances in this study were measured using an ultraviolet spectrophotometer (Perkin-Elmer 3100, Artisan Technology Group 101E Mercury Drive, Champaign, IL, USA), and 80% methanol was used for the blanks. The BCC and AA were expressed on a dry weight (DW) basis.

3.2.2.7. HPLC quantification of phenolic compounds

The HPLC quantification was carried out to assess the effect of the ripening on the individual

phenolic compounds in SALF. The phenolics reportedly present in SALF are gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin (Elekofehinti, Kamdem, Bolingon, et al., 2013). For phenolic compounds extraction, methanol (5 ml) was added to SALF powders (0.5 g) as described by Elekofehinti, Kamdem, Bolingon, et al. (2013), and subjected to constant shaking (IKA Vibrax VXR, IKA-Labortechnik, Staufen i.Br., Germany) for 30 min. The rest of the procedure is as described in section 3.1.2.6 of this thesis.

3.2.3. Statistical analysis

Statistical analyses and graph presentations were carried out using Statistical Package for the Social Sciences (SPSS) software (Version 21, IBM, Armonk, NY, USA) and GraphPad Prism (Version 8.4.1, GraphPad Software, San Diego, CA, USA). The BCC and TAC data were proven for normality of distribution (Kolmogorov–Smirnov, and Shapiro-Wilk) or transformed if not normally distributed using a two-step approach described by Templeton (2011). Briefly, step 1 involves transforming the variable into a percentile rank that results in uniformly distributed probabilities; and step 2 applies the inverse-normal transformation to the results of step 1 to form a variable consisting of normally distributed z-scores. The means were compared using ANOVA to determine statistically significant differences in sample means of BCC and TAC data. The *post-hoc* Duncan test was used to separate the means at $p < 0.05$. The results were expressed as mean ± standard error of the mean (SEM). No statistical analyses were carried out for the HPLC results. A generalized linear model (GLM) was used to determine the effect size (partial eta squared) for accession, ripeness stage, and interaction between accession and ripeness stage on the BCC and TAC. Pearson correlation analysis was carried out to determine linear relationships between the BCC (independent variables) and TAC or FRSC (dependent variables). Principal component analysis (PCA) was applied for BCC, AA, and ripeness stage to determine the contribution of different variables to the variance of data and to further determine the association between the BCC, AA, and ripeness stage. The PCA rotation method used was Varimax, with correlations below 0.3 excluded. Multilinear regression by the step-wise method was carried out to derive models for AA, and all the data used were normalized as described above. Significance was accepted at $p < 0.05$ for all statistical analyses.

3.2.4. Results and discussion

3.2.4.1. Bioactive compounds content of SALF accessions at different ripeness stages

3.2.4.1.1. Phenolics and flavonoids

Phenolics have been reported to have antidiabetic (Tresserra-rimbau et al., 2019) and free radical scavenging properties (Dudonné et al., 2009). Flavonoids, a sub-group of phenolics, may prevent coronary heart disease and possess antioxidative, hepatoprotective, anti-inflammatory, antiviral, and anticancer activities (S. Kumar & Pandey, 2013). The TPC of SALF in this study (7.6 - 22.6 mg GAE/g DW) (Table 6) was comparable to that for black chokeberries (19.54 - 52.9 mg GAE/g DW) (Tolić et al., 2015), which are recorded as fruits with the highest TPC (Pérez-Jiménez, Neveu, Vos, & Scalbert, 2010). In this study, TPC decreased with ripening progression in 3 SALF accessions (WP1, CP1, and GP1) and was unaffected in 1 accession (GC1). The TPC trend from the unripe to the red stage differed among the accessions. Whereas TPC reduced from unripe to the yellow stage, it increased at the orange and then red stages for GP1, WP1, and GC1, while CP1 registered a reduction from yellow to red stage. For all accessions, TPC was highest at the unripe stage, while the contents at the orange and red stages were not significantly different. Accession GP1 had the highest TPC as similarly reported for the unripe stage in section 3.1 (Table 3) of this thesis. Contradictorily, Table 3 displayed that accession WP1 had the least TPC at the unripe stage, while the current study (Table 6) showed accession GC1. The differences probably arose from the variations in the weather conditions at which the fruits were obtained. The results obtained by Abbe et al. (2019) and Dan et al. (2014) conform with the present findings, where TPC decreased from unripe to red stage 11.6 to 4.5 and 9.6 to 5.5 mg GAE/ g DW, respectively. The TPC for unripe and yellow stages reported by Abbe et al. (2019) and Dan et al. (2014) were covered within this study's range contrary to their lower values obtained at the orange and red stages.

The TFC significantly differed (1.3 - 4.2 mg QE/g DW) among ripeness stages for all the accessions (Table 6). Evidently, TFC for all accessions decreased from unripe to the yellow stage, increased at the orange stage, and then decreased (GP1, CP1, GC1) or remained constant (WP1) at the red stage. The TFC was highest at the unripe and/ or orange stage(s) and least at the yellow stage. Accession GP1 had the highest TFC at all ripeness stages as similarly reported for the unripe stage in section 3.1 (Table 3) of this thesis. Similar to results in Table 3, accession CP1 had the least TFC. Contradictorily, Abbe et al. (2019) reported an increase in the SALF TFC through the ripeness stages, from 1.4 - 4.5 mg QE/g DW, for unripe to red stages,

respectively. The findings in this study were agreeable with the results by Bhandari & Lee (2016), who reported that TFC in *Solanum lycopersicum* L. cultivars significantly differed at the different stages of ripening.

Table 6. Bioactive compounds content of SALF accessions at different stages of ripeness

Accession code	Ripeness stage	TPC mg GAE/g	TFC mg QE/g	TSC mg DE/g	TAL mg/g	VCC mg AAE/g
GC1	Unripe	$8.4\pm0.23^{A,B\ f,g}$	$1.73\pm0.14^{A\ f,g}$	$86.97\pm1.99^{A\ f}$	$165.85\pm2.5^{D\ e}$	$3.42\pm0.09^{C\ g}$
	Yellow	$7.63\pm0.23^{B\ g}$	$1.28\pm0.01^{C\ i,j}$	$80.71\pm1.8^{B\ g}$	$189.9\pm4.01^{C\ c,d}$	$4.6\pm0.16^{B\ d,e,f}$
	Orange	$8.57\pm0.3^{A\ f,g}$	$1.64\pm0.02^{A\ g,h}$	$69.11\pm1.48^{C\ h}$	$216.8\pm3.77^{B\ b}$	$4.8\pm0.24^{B\ c,d,e,f}$
	Red	$9.15\pm0.4^{A\ e,f}$	$1.47\pm0.03^{B\ h,i}$	$87.66\pm2.64^{A\ f,g}$	$296.94\pm5.78^{A\ a}$	$5.35\pm0.12^{A\ b,c,d,e}$
CP1	Unripe	$14.62\pm0.49^{A\ c}$	$1.32\pm0.05^{B\ i,j}$	$134.08\pm2.55^{B\ c}$	$156.29\pm3.88^{B\ e,f,g}$	$6.02\pm0.17^{A\ a,b}$
	Yellow	$9.68\pm0.13^{B\ e,f}$	$1.26\pm0.05^{C\ j}$	$144.51\pm1.93^{A\ b}$	$190.88\pm3.78^{A\ c,d}$	$4.55\pm0.67^{B\ e,f}$
	Orange	$8.67\pm0.32^{C\ f,g}$	$2.79\pm0.08^{A\ d}$	$148.59\pm1.9^{A\ a,b}$	$192.86\pm4.33^{A\ c,d}$	$4.51\pm0.21^{B\ e,f}$
	Red	$8.88\pm0.17^{C\ e,f,g}$	$1.44\pm0.04^{B\ i,j}$	$126.9\pm2.52^{B\ d}$	$199.79\pm3.82^{A\ c}$	$3.95\pm0.39^{B\ f,g}$
WP1	Unripe	$12.88\pm0.44^{A\ d}$	$1.89\pm0.03^{A\ f}$	$58.28\pm0.77^{A\ i}$	$161.04\pm4.07^{B\ e,f}$	$2.19\pm0.33^{D\ h}$
	Yellow	$9.77\pm0.3^{B\ e,f}$	$1.7\pm0.04^{B\ g}$	$52.1\pm1.36^{B\ j}$	$149.88\pm4.53^{B\ f,g,h}$	$3.2\pm0.24^{C\ g}$
	Orange	$9.46\pm0.38^{B\ e,f}$	$2.09\pm0.05^{A\ e}$	$56.47\pm0.69^{A\ i,j}$	$190.17\pm3.67^{A\ c,d}$	$4.02\pm0.23^{B\ f,g}$
	Red	$10.18\pm0.18^{A,B\ e}$	$2.08\pm0.13^{A\ e}$	$44.75\pm2.13^{C\ k}$	$188.7\pm4.87^{A\ c,d}$	$5.44\pm0.11^{A\ b,c,d}$
GP1	Unripe	$22.57\pm0.64^{A\ a}$	$4.09\pm0.04^{A\ a}$	$83.03\pm1.31^{C\ f,g}$	$146.69\pm4.05^{B\ g,h}$	$5.52\pm0.15^{B\ a,b,c}$
	Yellow	$13.83\pm1.19^{C\ c,d}$	$3.76\pm0.06^{B\ b}$	$111.05\pm2.46^{B\ e}$	$141.23\pm3.7^{B\ h}$	$5.68\pm0.24^{B\ a,b}$
	Orange	$16.01\pm0.26^{B\ b}$	$4.16\pm0.08^{A\ a}$	$152.51\pm2.07^{A\ a}$	$182.23\pm8.21^{A\ d}$	$6.39\pm0.23^{A\ a}$
	Red	$16.77\pm0.46^{B\ b}$	$3.49\pm0.07^{B\ c}$	$122.23\pm1.44^{A,B\ d}$	$191.37\pm5.54^{A\ c,d}$	$5.9\pm0.1^{B\ a,b}$

Values are the mean ± SEM of two independent experiments measured in triplicates. The fruits in each independent experiment were obtained from 12 plants per accession per ripeness stage. Results refer to the corresponding dry weights of SALF. Significant differences between all samples for each compound are shown by different lower case letters, while those per accession are shown in upper case letters. TFC = total flavonoid content, QE = quercetin equivalent, TPC = total phenolic content, GAE = gallic acid equivalent, TSC = total saponin content, DE = diosgenin equivalent, TAL = total alkaloid content, VCC = Vitamin C content, AAE = ascorbic acid equivalent.

The phenolic acids (gallic acid, caffeic acid, and chlorogenic acid) and flavonoid compounds (rutin and quercetin) were differently affected by ripening (Table 7). Comparisons among the phenolic acids revealed that both chlorogenic acid and caffeic acid decreased with ripening, while gallic acid decreased from the unripe to the yellow stage and then increased at the orange and red stages. The trend of the phenolic compound with the highest content may influence the TPC trend the most. The results in this study showed that chlorogenic acid had the highest content, and its trend during ripening was similar to the TPC trend.

Table 7. HPLC characterization of phenolic acids and flavonoid compounds at different ripeness stages of SALF accessions.

Accession code	Ripeness stage	Gallic acid (µg/g)	Chlorogenic acid (µg/g)	Caffeic acid (µg/g)	Rutin (µg/g)	Quercetin (µg/g)
GC1	Unripe	13.68	681.90	57.99	39.95	11.66
	Yellow	12.44	688.29	32.06	106.93	20.66
	Orange	17.47	858.02	35.61	142.64	22.58
	Red	26.15	635.71	45.61	113.89	16.94
CP1	Unripe	17.57	1949.84	105.47	18.79	13.20
	Yellow	14.47	1044.47	55.60	22.20	10.97
	Orange	14.84	1090.00	50.47	62.37	11.33
	Red	25.58	1037.96	57.62	64.64	12.23
WP1	Unripe	14.93	1755.04	95.62	61.29	13.82
	Yellow	11.59	1011.14	52.30	166.63	11.72
	Orange	12.77	907.31	39.05	187.49	12.68
	Red	17.02	999.15	31.45	169.87	13.20
GP1	Unripe	23.52	2554.91	116.56	55.85	16.19
	Yellow	17.00	1149.52	69.14	212.06	12.73
	Orange	17.08	990.52	50.44	263.41	25.61
	Red	32.65	1027.05	35.27	215.07	48.65

The fruits were obtained from 12 plants per accession per ripeness stage. Values are the means of duplicate measurements from one extract per sample of the *Solanum anguivi* Lam. fruit accessions per ripeness stage. The means were computed on a dry weight basis. No statistical analysis was carried out on the data.

Concurring with the findings in this study, Mennella et al. (2012) reported that the TPC and chlorogenic acid contents of ten eggplant lines, *S. sodomaeum*, *S. aethiopicum*, and *S. integrifolium* decreased with ripening. The decline in TPC during ripening may be due to the oxidation of phenolics by polyphenol oxidase, which has been reported to increase with fruit ripening (Amira et al., 2012; Rodríguez et al., 2016) as observed in olives (Ortega-García et al., 2008). Polyphenols in plants are generally involved in defence against ultraviolet radiation or aggression by pathogens (Pandey & Rizvi, 2009). The integration of phenolic esters into cell walls is an important mechanism by which plants strengthen their cell walls, defend themselves against pathogens, and protect the cells against membrane damage by oxidative stress (Amira et al., 2012). The decrease in the TPC is possibly attributed to the presence of free esters of phenolic acids at the unripe stage, which may aid the progressive binding of the phenolic acids to the cell walls during ripening (Amira et al., 2012), thus resulting in lower free phenolic compounds at the red stage.

Consistent with the present study, Anton et al. (2017) and Slimestada & Verheulb (2009) reported that the type of phenolic compound and their contents, and the TPC, significantly differed among tomato cultivars at the different ripeness stages. Similarly, Anton et al. (2017) showed that the unripe stage of the tomato cultivars had the highest chlorogenic acid content, which continuously decreased during ripening. However, some differences in the phenolic contents were observed among cultivars such as Gartenfreude, which was the only cultivar with increased accumulation of naringenin chalcone and phloretin dihexoside from unripe to red stage while other cultivars had their maximum quantity at the half-ripe stage. Regarding the flavonoid compounds during ripening of SALF, rutin content increased from unripe to the orange stage and then reduced at the red stage, while the quercetin content trend was inconsistent in the accessions.

Both the accession and ripeness stage significantly affected the TPC and TFC of SALF (Table 8). The significant interaction between accession and ripeness stage showed that TPC and TFC of SALF at the different ripeness stages were significantly influenced by the accession. The high adjusted r^2 values indicated that the models may explain 84% TPC and 91% TFC variance in SALF. Similar to the present study, Anton et al. (2017) reported a significant effect of cultivar and ripeness stage on the TPC of tomatoes. Contrary, the authors (Anton et al., 2017) reported no significant interaction between the two variables.

3.2.4.1.2. Saponins

SALF saponins have been documented to have hypoglycemic, anti-peroxidative, anti-hyperlipidemic effects (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), as well as antioxidant properties (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013). In the present study, the TSC of SALF ranged from 44.8 - 152.5 mg DE/g DW (Table 6). The soybeans TSC (5.6% or 56 mg/g DW) (John Shi et al., 2004), which have been reported as a very rich source of saponins (Kregiel et al., 2017; Lásztity et al., 1998), falls within this study's TSC range. Thus, SALF may be considered a rich source of saponins.

In the present study, the effect of ripening on the TSC of SALF differed among the accessions. Variably, the TSC increased from the unripe to the orange stage for accessions CP1 and GP1 and then decreased (for CP1) or remained constant at the red stage (for GP1). For accession WP1, TSC decreased from the unripe to the yellow stage, then increased at the orange stage, and then decreased at the red stage. The TSC for GC1 decreased from the unripe stage until the

orange stage and then increased at the red stage. Overall, the TSC was highest at the orange stage for accessions WP1, CP1, and GP1, and the unripe and red stages for GC1. The differences in the trends for TSC among the accessions in this study may be attributed to genetic differences. Consistent with the results in section 3.1 (Table 6), TSC at the unripe stage was highest in accession CP1 and least in WP1. Oyeyemi et al. (2015) reported the saponin content of unripe SALF as 12.9 mg/g DW (no reference standard), which was much lower than the TSC of all the unripe accessions in this study. A phytochemical screening carried out by Dan et al. (2014) showed that ripening increased the saponin content of SALF. This is similar to the trends exhibited by accessions CP1 and GP1 of the present study from the unripe to the orange stage. Similarly, the saponin content of *Solanum gilo* and *Solanum aethiopicum* fruits was observed to increase during ripening (Agoreyo et al., 2015). There is scarce information regarding the effect of ripening on saponin contents of *Solanaceae* fruits and vegetables. The enormous health benefits of saponins justify future studies related to factors that may influence the contents.

The partial eta square analysis showed that the accession and ripeness stage had significant independent effects on the TSC (Table 8). However, the effect of the ripeness stage on the BCC and AA of SALF was significantly dependent on its accession. The very high adjusted r^2 of the model showed that it may explain 94% variance in the TSC of SALF.

Table 8. Effect sizes of the variables (ripeness stage and accession) on the bioactive compounds content and antioxidant activity of SALF

Chemical composition	Accession	Ripeness stage	Accession*ripeness stage	Adjusted r^2
TFC	0.90	0.67	0.58	0.91
TPC	0.82	0.47	0.44	0.84
TSC	0.94	0.52	0.76	0.94
TAL	0.69	0.79	0.46	0.83
VCC	0.62	0.17	0.56	0.71
FRSC	0.92	0.73	0.29	0.92
TAC	0.92	0.70	0.26	0.92

Partial eta squared was carried out to assess the effect sizes of the variables. All the displayed effect sizes of the variables and variable interactions in the table were significant at $p < 0.05$. TFC = total flavonoid content, TPC = total phenolic content, TSC = total saponin content, TAL = total alkaloid content, VCC = Vitamin C content, FRSC = free radical scavenging capacity, TAC = total antioxidant capacity, * = represents an interaction.

3.2.4.1.3. Alkaloids

The health-promoting properties of alkaloids include antidiabetic (B. Sharma et al., 2010), antibacterial, antihypertensive, and anticancer effects (Kuete, 2014). The TAL in this study increased with ripening all SALF accessions, from an average of 141.2 mg/g DW at the unripe stage to 296.9 mg/g DW at the red stage (Table 6). Although the findings in section 3.1 (Table 3) showed that TAL was highest in both accessions WP1 and GP1, and least in CP1 and GC1, the present results (Table 6) showed that TAL was highest in WP1 and least in CP1. The differences were possibly due to difference in climatic conditions. The TAL of SALF in the current study was higher than documented for tea leaves [47.6 mg/g DW (Wong et al., 2013)], which have been defined by Kurek (2019) as rich sources of alkaloids. The SALF TAL reported by Oyeyemi et al. (2015) (0.05 % or 0.5 mg/g DW, with no reference standard) was, however, less than the results of this study. Contradictorily, a phytochemical screening of SALF by Dan et al. (2014) showed an absence of alkaloids at all the ripeness stages.

The increased TAL during the ripening of SALF in the present study may have resulted from an increase in the glycoalkaloid (GA) contents such as solamargine (Ripperger & Himmelreich, 1994), as reported in *S. sodomaeum, S. aethiopicum, S. integrifolium,* and ten eggplant lines (Mennella et al., 2012). However, little is known about the biosynthetic pathway of GAs and the factors that regulate their levels in plants (Siddique & Brunton, 2019). Agreeable with the current findings, Bagheri et al. (2017) reported increased GA (solasonine) content of eggplants with ripening; however, the reason for this increase was also unknown to the researchers. On the contrary, the alkaloid contents in *Solanum lycocarpum* (Pereira et al., 2021) and *Solanum lycopersicum* (Friedman, 2002) decreased with ripening. Alkaloids are produced by plants for resistance to challenges such as insects and pests (Siddique & Brunton, 2019), and it has been previously hypothesized that their reduction during ripening was to attract seed dispersers at the ripe/red stage (Cipollini & Levey, 1997). This hypothesis was possibly based on the bitterness of the alkaloids (Bassoli et al., 2007), which were suggested to be less in the ripe fruits, given their preference by animals compared to the unripe fruits. Since the TAL in eggplants and SALF increased from unripe to red stage, this hypothesis may explain why they are consumed unripe rather than the red stage by humans (less bitter when unripe) (Bukenya & Carasco, 1995; Mennella et al., 2012). However, the hypothesis does not explain the increase of alkaloids during ripening of SALF, as well as eggplants. More studies are, therefore, necessary to elucidate the factors that influence the increase or decrease of alkaloids in *Solanaceae* fruits, including SALF.

Accession and ripeness stage significantly affected the TAL of SALF (Table 8), with the effect of ripening significantly influenced by the accession and vice-versa. The high adjusted r^2 of the model indicated that it may explain 83% TAL variance in SALF.

3.2.4.1.4. Vitamin C

Vitamin C has various health benefits. It may protect DNA, proteins, and lipids from oxidative damages through the scavenging of free radicals (Turck et al., 2017). It also increases the bioavailability of iron by reducing non-heme iron from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form, which is more easily absorbed in the intestine, and thus vitamin C indirectly protects against anaemia (Lane & Richardson, 2014). In this study, the VCC of most SALF accessions (GP1, GC1, and WP1) significantly increased with ripening, from 2.2 - 5.5 mg AAE/g DW at the unripe stage to 6.4 mg AAE/g DW at the orange stage for GP1 or 5.4 mg AAE/g DW at the red stage for GC1 and WP1 (Table 6). However, the VCC of accession CP1 decreased with ripening, from 6.0 to 3.9 mg AAE/g DW, for unripe to red stage. The current findings are inconsistent with the results in section 3.2 (Table 3), which showed that the VCC in the unripe accessions CP1, WP1, and GP1 were similar and significantly higher than the content in GC1.

The VCC of SALF at the red stage in this study was similar to ripe tomatoes (5.1 - 6.4 mg/g DW) (Borguini et al., 2013), which have been defined as rich VCC sources (Lykkesfeldt et al., 2014). Similarly, Valšíková-Frey et al. (2018) and Bhandari and Lee (2016) reported an increased VCC with ripening for tomato cultivars. In contrast, Chaudhary et al. (2018) showed a higher VCC in unripe tomatoes than red ones, which is similar to SALF accession CP1 of the present study. A similar trend as accession CP1 was also observed by Abbe et al. (2019) and Dan et al. (2014), who reported a decline in SALF VCC from unripe to ripe as 0.3 to 0.1 mg/g fresh weight and 0.3 to 0.1 mg/g DW, respectively; with significant differences between ripeness stage. The increased VCC of SALF accessions (GP1, GC1, and WP1) in this study may largely stem from its increased biosynthesis through the Smirnoff-Wheeler pathway, as documented for tomatoes (Laing et al., 2019). However, the decreased VCC for accession CP1 may have ensued from the degradation of ascorbate, culminating in the formation of oxalic acid, threonic acid, and oxalyl threonic acid (Laing et al., 2019). Genetic differences may dictate the variations in the VCC trends exhibited among the accessions in the current study.

Both accession and ripeness stage significantly affected the VCC of SALF (Table 8), and accession had a greater impact on the VCC than the ripeness stage. Simultaneously, the

significant interaction between accession and ripeness stage showed that VCC of SALF at the different ripeness stages was significantly influenced by accession. This model had a good adjusted r^2 (Table 8), which showed that it explained 71% VCC variance in SALF.

3.2.4.1.5. Antioxidant activity of SALF accessions at different ripeness stages

The AA (FRSC and TAC) of SALF greatly decreased during ripening for all the SALF accessions (Table 9).

Table 9. Free radical scavenging capacity and total antioxidant capacity of SALF accessions at different stages of ripeness.

Accession	Ripeness stage	FRSC (%)	TAC (mg QE/g)
GC1	Unripe	$6.39\pm1.85^{A\,f}$	$1.12\pm0.39^{A\,f}$
	Yellow	$2.08\pm0.24^{A,B\,g}$	$0.19\pm0.05^{A,B\,g}$
	Orange	$2.29\pm0.63^{B\,g}$	$0.24\pm0.14^{A,B\,g}$
	Red	$1.47\pm0.12^{B\,g}$	$0.06\pm0.03^{B\,g}$
CP1	Unripe	$22.72\pm1.63^{A\,c,d}$	$4.65\pm0.36^{A\,c,d}$
	Yellow	$9.4\pm1.63^{B\,f}$	$1.77\pm0.35^{B\,f}$
	Orange	$8.19\pm0.48^{B\,f}$	$1.49\pm0.1^{B\,f}$
	Red	$8.4\pm0.96^{B\,f}$	$1.58\pm0.21^{B\,f}$
WP1	Unripe	$40.59\pm1.48^{A\,b}$	$8.63\pm0.33^{A\,b}$
	Yellow	$20.11\pm0.65^{B\,d,e}$	$4.12\pm0.14^{B\,d,e}$
	Orange	$17.09\pm0.7^{C\,e}$	$3.48\pm0.16^{C\,e}$
	Red	$21.38\pm0.48^{B\,d}$	$4.37\pm0.1^{B\,d}$
GP1	Unripe	$66.17\pm3.15^{A\,a}$	$14.18\pm0.7^{A\,a}$
	Yellow	$37.41\pm0.49^{B\,b}$	$7.92\pm0.12^{B\,b}$
	Orange	$37.9\pm0.95^{B\,b}$	$7.96\pm0.22^{B\,b}$
	Red	$26.22\pm1.83^{C\,c}$	$5.38\pm0.37^{C\,c}$

Values are the mean ± SEM of two independent experiments measured in triplicates. The fruits in each independent experiment were obtained from 12 plants per accession per ripeness stage. Results refer to the corresponding dry weights of SALF. FRSC = free radical scavenging capacity, TAC = total antioxidant capacity, QE = quercetin equivalent.

The FRSC ranged from 6.39 to 66.17% at the unripe stage compared to 1.47 to 26.22% at the red stage. Concurrently, the TAC at the unripe stage ranged from 1.12 to 14.2 mg QE/g DW versus the red stage with 0.06 to 5.38 mg QE/g DW. The unripe stage had the highest AA for all accessions, which was also observed for TPC. At all the ripeness stages, AA was highest in accession GP1 and least in GC1, which was also observed for their TPC. This pattern between TPC and AA was also recorded in section 3.1 of this thesis. Abbe et al. (2019) similarly reported a decrease in SALF FRSC from the unripe (87%) to the red stage (71%). Fategbe et

al. (2013) also reported significantly different FRSC among the eggplant cultivars, which reduced from the unripe to the ripe stage.

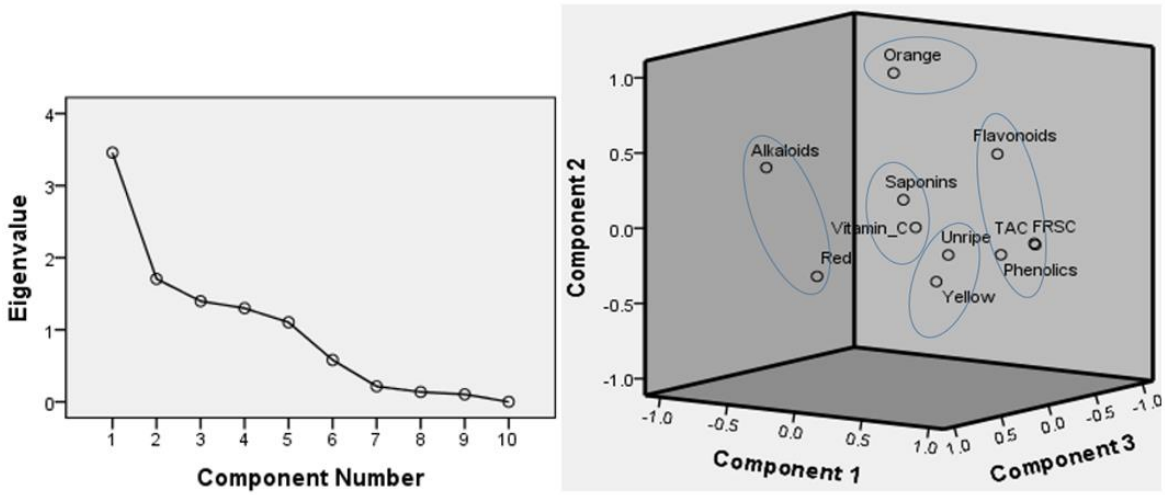
Accession and ripeness stage had significant independent effects on TAC and FRSC (Table 8). Although the interaction between accession and ripeness stage was weak, it was significant. The AA of SALF at different ripeness stages was further significantly influenced by accession. This model, however, had a high adjusted r^2, which indicated that it may explain 92% AA variance in SALF.

3.2.4.2. Relation between antioxidant activity and bioactive compounds content in SALF

3.2.4.2.1. Correlation and principal component analysis

The correlation between FRSC and the BCC and also between TAC and BCC were similar. Computations revealed that AA correlated positively with TFC (r = 0.612) and TPC (r = 0.820) and negatively with TAL (r = -0.578). The strong positive correlations of TPC with AA may indicate that phenolics contributed the most to the AA of SALF. The red stage, which had the highest TAL, also had the least TPC and thus possibly resulting in the low AA. This may explain the apparent negative correlation between AA and TAL. Comparable to this study, a significant positive correlation (r = 0.68) between TPC and FRSC was reported for tomato cultivars at different ripeness stages (Anton et al., 2017) and also in 10 eggplant lines, *S. sodomaeum*, *S. aethiopicum,* and *S. integrifolium* (Mennella et al., 2012).

The PCA based on BCC, AA, and the ripeness stage generated five components based on (i) Kaiser‘s criterion of Eigenvalues > 1, and (ii) Catell‘s scree graph spot of abrupt level out (Grané & Jach, 2013) (Figure 8a). The components accounted for a cumulative variance of 88.7% (Figure 8b). Components 1, 2, 3, 4, and 5 explained 31.9, 15.7, 15.6, 12.8, and 12.7 % variance of the data, respectively. The PCA results are comparable with the results from the correlation and BCC analyses. Component 1 showed that an increase in TFC and TPC led to an increased TAC and FRSC, while component 3 showed that TAL was highest at the red stage.



a) b)

Figure 8. A scree graph and components formed from the principal component analysis. a) A scree graph showing the number of principal components derived with an Eigenvalue greater than 1 (5 components). b) A representation of the five components derived from the principal component analysis on a 3-component axis. Component 1 = TAC, FRSC, phenolics, and flavonoids; component 2 = orange; component 3 = red stage and alkaloids, component 4 = saponins and vitamin_C; and component 5 = unripe and yellow stages. TAC = total antioxidant capacity, FRSC = free radical scavenging capacity, phenolics = total phenolic content, flavonoids = total flavonoid content, saponins = total saponin content, alkaloids = total alkaloid content, vitamin_C = vitamin C content; unripe, yellow, orange and red = stages of ripeness.

3.2.4.2.2. Regression analysis

Regression models for AA were derived in the study. Three factors including the statistical significance of the independent variables (statistical significance of *F*-ratio in the derived ANOVA table), the significance of the unstandardized coefficients of the independent variables in the model; and significance and strength of the adjusted r^2 (to determine how well the regression model fits the data) were evaluated. The regression models were based on the formula in equation 4.

$$Y_A = b_0 + b_1X_1 + b_2X_2 + b_{nth}X_{nth} \quad \text{Eq. (4)}$$

Where; Y_A = Predicted mean AA, b_0 = constant, and b_1, b_2, and $bnth$ = regression unstandardized coefficients for independent variables X_1, X_2, and X_{nth}, respectively.

The overall regression models for TAC and FRSC (irrespective of accession and ripeness stage) (equations 5 and 6, respectively) had high adjusted r^2 (0.791 and 0.792, respectively), which showed that they both explained 79% variance for SALF AA.

$$TAC\ (mg\ GAE/g\ DW) = 2.603 + 0.572[TPC\ (mg\ GAE/g)] + 0.905[TFC\ (mg\ QE/g)] - 0.026[TAL\ (mg/g)] - 0.441[VCC\ (mg\ AAE/g)] \quad \text{Eq. (5)}$$

$$FRSC\ (\%) = 14.556 + 2.58[TPC\ (mg\ GAE/g)] + 3.949[TFC\ (mg\ QE/g)] - 0.126[TAL\ (mg/g)] - 1.913[VCC)] \quad \text{Eq. (6)}$$

The regression equations 5 and 6 showed that TPC, TFC, TAL, and VCC were the most significant in the quantification of the TAC and FRSC in SALF. Since the adjusted r^2 for the TAC and FRSC models were not significantly different, only the TAC regression models at different ripeness stages were derived, as shown below (equations 7 to 10).

a) Unripe stage

$$TAC\ ((mg\ GAE)\ /\ g) = -2.318 + 1.009[TPC\ ((mg\ GAE)\ /\ g)] - 1.141[VCC\ ((mg\ AAE)\ /\ g)] \quad \text{Eq. (7)}$$

b) Yellow stage

$$TAC\ ((mg\ GAE)\ /\ g) = -4.525 + 0.505[TPC\ ((mg\ GAE)\ /\ g)] + 1.660[TFC\ ((mg\ QE)\ /\ g)] \quad \text{Eq. (8)}$$

c) Orange stage

$$TAC\ ((mg\ GAE)\ /\ g) = -6.673 + 4.931[TFC\ ((mg\ QE)\ /\ g)] - 0.039[TSC\ ((mg\ DE)\ /\ g)] \quad \text{Eq. (9)}$$

d) Red stage

$$TAC\ ((mg\ GAE)\ /\ g) = 8.306 + 2.158[TFC\ ((mg\ QE)\ /\ g)] - 0.043[TAL\ ((mg)\ /\ g\ DW)] \quad \text{Eq. (10)}$$

The regression models (equations 7 to 10) showed the BCC that mostly affected the TAC of SALF at the different stages of ripeness. Consistent with the findings in section 3.1 of this thesis, the AA at the unripe stage was mostly affected by the total phenolics and vitamin C. The models had high adjusted r^2 values at the unripe, yellow, orange, and red stages, which showed that 87%, 89%, 78%, and 75% variance in TAC of SALF, respectively, may be explained by the models.

3.2.5. Conclusion

The total flavonoids, phenolics, saponins, vitamin C, alkaloids and AA significantly varied with the SALF ripeness stage and accession. All the BCC and AA of SALF at the different ripeness stages were significantly influenced by the accessions. The unripe stage was the richest in TPC, TFC, and AA, the orange stage in TFC and TSC, and the red stage in VCC (for most accessions) and TAL. Furthermore, regression models with high adjusted r^2 were derived in the study, which showed the BCC that mostly affected the AA of SALF at the different stages of ripeness. The study findings provide information on the ripeness stage with potentially the highest nutraceutical benefits. More studies are recommended to elucidate the factors that affect the saponin and alkaloid contents of fruits, including SALF, as the available information is limited.

3.3. Effect of different thermal treatments on the bioactive compounds content and antioxidant capacity of *Solanum anguivi* Lam. fruits [4]

Abstract

The bioactive compounds content (BCC), total antioxidant capacity (TAC), and thus the potential health-promoting capacity of fruits and vegetables may depend on their processing history. *Solanum anguivi* Lam. fruits (SALF) are consumed as vegetables and have been traditionally used to manage diabetes, possibly due to the possession of bioactive compounds, including phenolics and saponins. Data on the effect of thermal treatments on the BCC and TAC of SALF is currently limited. The present study investigated the effect of drying temperature (35, 55, and 85 ^{o}C), traditional cooking method (boiling and steaming) at 100 ^{o}C, and cooking duration (15, 30, 45, 60, and 120 min) on the BCC and TAC of SALF. BCC was analyzed by spectrophotometry (total phenolic (TPC), flavonoid (TFC), saponins (TSC), and vitamin C (VCC) contents), and gravimetry (for total alkaloid content (TAL)), while HPLC was used to examine the effect on the phenolic compounds. The TAC was analyzed by spectrophotometry. The effect of drying on the BCC and TAC of SALF significantly varied with drying temperature, accession, ripeness stage, cooking method, and cooking duration. Drying at 35 ^{o}C increased the TPC and TAC of unripe SALF more than 2-fold and retained the highest VCC and TFC. Chlorogenic acid, caffeic acid, and rutin contents were higher at 35 than 85 ^{o}C, and vice-versa for gallic acid. The effect of drying on saponins and alkaloids was dependent on the accession and ripeness stage. The effect of cooking on the BCC and TAC of SALF was significantly influenced by the cooking method and duration. Boiling and steaming increased the TPC and TAC 4-fold and 3-fold, respectively. Cooking reduced the TSC and did not affect TFC. Exceptionally, 15 min of boiling did not affect the TSC and increased the TFC. The TAL was decreased by steaming and increased by boiling. However, a very long duration of boiling and steaming (120 min) greatly increased the alkaloids 2-fold and 1.5-fold, respectively. All the thermal treatments negatively affected vitamin C. Overall, the thermal treatment that resulted in the highest TPC and TAC of SALF was boiling (for 15 min), which may result in the highest health benefits of SALF upon consumption.

Keywords: *Solanum anguivi*, drying temperature, cooking duration, boiling, steaming, ripeness stage, accession, bioactive compounds, antioxidant capacity

[4] This content has been submitted to the *Food Science and Technology International* journal.

3.3.1. Introduction

Consumption of fruits and vegetables with high contents of antioxidant bioactive compounds is reported to increase the antioxidant capacity of serum/plasma (Y. J. Zhang et al., 2015) and consequently protects against various diseases associated with oxidative stress, such as type 2 diabetes mellitus (T2DM) (Asmat et al., 2016) and cardiovascular diseases (CVD) (Lin et al., 2016). The health-promoting capacity of fruits and vegetables likely depends on their processing history, which may consequently affect the content and antioxidant activity of their bioactive compounds (Nicoli et al., 1999). Various vegetables are consumed after being cooked or processed by employing various methods such as steaming, boiling, baking, roasting, and frying (van Boekel et al., 2010). Cooking and processing food may alter its antioxidant activity positively or negatively. Changes in bioactive compounds upon cooking may, therefore, originates from two antagonistic phenomena. Thermal degradation (van Boekel et al., 2010) or loss of water-soluble bioactive compounds (S. Lee et al., 2018) reduces their concentrations, while matrix softening increases bioactive compound extractability, and thereby apparently increases concentration (Gry et al., 2007; Nayak et al., 2015). Although drying increases the shelf life of the fruits, it may affect the presence and stability of heat-sensitive bioactive compounds such as polyphenols and ascorbic acid (Rawson et al., 2011). The overall effect of cooking or processing of food on bioactive concentration, therefore, depends on the parameters used, such as temperature and duration, the structure of the food matrix, and the chemical nature of the specific compound (Dolinsky et al., 2016; Miglio et al., 2008; Palermo et al., 2014).

Solanum anguivi Lam. belongs to the *Solanaceae* family and *Solanum* L. genus (United States Department of Agriculture., 2021). The consumption of *Solanum anguivi* Lam. fruits (SALF) as a vegetable has been documented in Africa, Asia, and Australia (Bahgat et al., 2008; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Nakitto et al., 2021). They are also traditionally used in the treatment of several diseases, including chronic respiratory diseases (Bector et al., 1971; Bukenya & Carasco, 1995), hypertension (Kakudidi et al., 2016), and diabetes (Ssenyange et al., 2015). The suggested therapeutic properties of SALF have been linked to its ability to inhibit oxidative stress (Elekofehinti & Kade, 2012) and to mediate blood glucose-lowering effects (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011). The observed health benefits of SALF have been attributed to the presence of bioactive compounds such as phenolics, saponins, alkaloids, and flavonoids (Abbe et al., 2019; Dan et al., 2014; Oyeyemi et al., 2015). The fruits of *Solanum anguivi* Lam. may be consumed either fresh/raw (Abbe et al., 2019), boiled (Bukenya & Carasco, 1995), steamed, or as a powder supplement

in other foods (Bamba et al., 2020). The bioactive compounds content (BCC) and antioxidant activity (AA) of uncooked SALF have been reported (Abbe et al., 2019; Dan et al., 2014; Elekofehinti, Kamdem, Bolingon, et al., 2013; Elekofehinti & Kade, 2012). However, there is limited data on the effect of cooking and drying on its BCC and AA. Abbe et al. (2019) reported the effect of boiling for 10 and 15 min on total phenolics, flavonoids, tannins, vitamin C, and free radical scavenging capacity (FRSC) of SALF, but the effect on its saponin and alkaloid contents, as well as on the total antioxidant capacity (TAC), has not been documented. The effect of sun-drying and oven-drying at 60, 80, or 100 ºC on the SALF total phenolics and alkaloids was shown by Bamba et al. (2020). However, data on the effect of these drying conditions on other bioactive compounds, as well as the effect of the steaming on all the BCC and AA is scarce.

The present study explored the effects of drying temperature, traditional cooking method (steaming and boiling), and cooking duration on the BCC (total phenolic, flavonoid, saponin, alkaloid, and vitamin C contents) and the TAC of SALF. The study also examined the associations between drying and cooking parameters with BCC and TAC of SALF.

3.3.2. Materials and methods

3.3.2.1. Chemicals and reagents

The chemicals and reagents used in this study included HPLC grade -methanol, ethanol, sulphuric acid, acetic acid, and ammonium hydroxide; analytical grade -thiourea, trichloroacetic acid, Folin-Ciocalteu reagent, 1-diphenyl-2-picrylhydrazyl (DPPH), vanillin, dinitrophenyl hydrazine, hydrated copper (II) sulfate, potassium acetate, sodium bicarbonate, and aluminium chloride, and the reference standards (quercetin, gallic acid, L-ascorbic acid, diosgenin). These were all purchased from Sigma-Aldrich (Munich, Germany).

3.3.2.2. Sample collection and identification

The SALF samples were obtained from Nabiyagi village (GPS 0.472336, 32.802484), Mukono district (Uganda), in September 2019, two weeks apart. The accessions were identified based on literature (Bukenya & Carasco, 1995) and authentication from an expert taxonomist at the Department of Botany, Makerere University (Uganda). The fruits at two ripeness stages, unripe and ripe (red) (Abbe et al., 2019; Dan et al., 2014), were collected from two accessions named in this study as WP1 and GP1. The colours of unripe WP1 and GP1 were plain white and light greenish-cream, respectively (Figure 1).

3.3.2.3. Experimental design

To determine the effect of thermal treatments on SALF BCC and TAC, the samples were either dried, boiled, or steamed. Further, the effect of drying temperature on the phenolic compounds was investigated. Fifty fruits from 12 plants per accession per ripeness stage were randomly collected and subjected to thermal experiments on the day of harvest. Unripe and ripe samples from WP1 and GP1 accessions were air-dried (B. Master, Tauro, Camisano Vicentino, Italy) at 35 ^{o}C, 55 ^{o}C, or 85 ^{o}C (low, medium, and high temperatures, respectively) for 16, 6, and 3 hours (durations that obtained a moisture content of < 10%), respectively. The unripe stage, which had higher TAC than the ripe stage in the preliminary experiments, of one accession (WP1) was subjected to the cooking experiments. Unripe SALF was boiled and steamed (at 100 ^{o}C) as described by Ahmed and Ali (2013) for either 15, 30, 45, 60, or 120 min. Specifically, 200 g of unripe SALF were placed in 1000 ml boiling water (at 100 ^{o}C) contained in a stainless steel pan (for boiling experiments), while 200 g of SALF were placed on top of the boiling water (steaming experiments), and the pan covered. Fresh (uncooked and undried) SALF was used as the control. The second batch of fruits was obtained from the plants two weeks after the first harvest, and these procedures were subsequently replicated the second time to obtain a second sample per treatment. The samples were analyzed for total contents of phenolic, flavonoids, vitamin C, saponins, alkaloids, and AA. Further, the effect of drying temperature on the phenolic compounds was investigated.

3.3.2.4. Sample preparation for chemical analysis

The fruits were sorted to retain those with undamaged pericarps, whose stalks were then plucked off. The fruits were washed with distilled water and dried with a cotton cloth. Before the start of the drying experiments, each fruit was sliced into four pieces to fasten the drying process and to identify pest-infested fruits, which were subsequently discarded. The dried samples were then milled (Wonder Mill, Pocatello, Idaho) at a “pastry” setting to obtain fine flour, which was then sealed in plastic bottles and stored at -20 ^{o}C until analysis. The fruits were left whole for the steaming and boiling treatments. However, before extraction, the cooked samples were cut into four pieces, and the pest-infested fruits were discarded.

3.3.2.5. Extraction and quantification of total contents of the bioactive compounds and antioxidant activity of SALF

Aqueous methanol (80%) (D. Kim et al., 2003) was used for the extraction of bioactive

compounds from SALF. The extraction process was as described by Makkar (2003), with slight modifications. For the drying experiments, the dried and powdered SALF of 0.2 g was extracted in 20 ml 80% methanol three times [10 min each], and the extracts were subsequently pooled together to perform the total BCC and AA analyses. For the cooked SALF, the weight used for extraction contained 0.2 g dry weight to retain the solute to solvent ratio as the samples for the drying experiments.

The total flavonoids content (TFC) was as described by Kumar, Rajkapoor, & Perumal (2012), estimated from a quercetin standard curve, and expressed as quercetin equivalent (QE). Total phenolic content (TPC) was determined according to the method by Singleton et al. (1998), estimated from a gallic acid standard curve and expressed as gallic acid equivalent (GAE). The method by Hiai, Oura, & Nakajima (1976) was used to determine the total saponin content (TSC), which was estimated from a diosgenin standard curve and expressed as diosgenin equivalent (DE). The vitamin C content (VCC) was analyzed as described by Omaye et al. (1979), estimated from an ascorbic acid standard curve, and expressed as ascorbic acid equivalent (AAE). Total alkaloid content (TAL) was determined using the method by Harborne (1973) and computed as mg/g. The AA was determined according to Brand-Williams et al. (1995) by analyzing the DPPH free radical scavenging capacity (FRSC), which was subsequently used to analyze the total antioxidant capacity (TAC) of SALF. The FRSC was calculated as:

$$\text{Free radical scavenging capacity (\%)} = \frac{(Absorbance\ blank - Absorbance\ of\ sample) \times 100}{Absorbance\ of\ blank} \qquad \text{Eq. (1)}$$

A quercetin standard curve was prepared with FRSC (%) against concentration (1 - 10 µg/ml), and the TAC of the samples was subsequently estimated from the quercetin standard curve and expressed as QE.

The BCC and TAC analyses for the two independent extracts per sample were done in triplicate per extract. All absorbances were measured using an ultraviolet spectrophotometer (Perkin-Elmer 3100, Artisan Technology Group 101E Mercury Drive, Champaign, IL, USA), and 80% methanol was used for the blanks. All quantities were expressed on a dry weight (DW) basis.

3.3.2.6. Quantification of phenolic compounds using HPLC

Purposely, HPLC quantification was carried out to assess the effect of drying temperature on the individual phenolic compounds reportedly present in SALF (gallic acid, chlorogenic acid,

caffeic acid, rutin, and quercetin (Elekofehinti, Kamdem, Bolingon, et al., 2013)). The HPLC analysis was conducted for only the dried samples (at 35, 55, and 85 ^{o}C), except for unripe WP1 dried at 35 ^{o}C, which was excluded due to its limited quantity for extraction. The SALF powders (0.5 g) were extracted in 5 ml methanol according to Elekofehinti, Kamdem, Bolingon, et al. (2013) for 30 min under constant shaking (IKA Vibrax VXR, IKA-Labortechnik, Staufen i.Br., Germany). The rest of the procedure is as described in section 3.1.2.6 of this thesis.

3.3.3. Statistical analysis

Statistical analyses were carried out using SPSS (Version 21, IBM, Armonk, NY, USA), while graphs were obtained using GraphPad Prism (Version 8.4.1, GraphPad Software, San Diego, CA, USA). The BCC and TAC data were proven for normality of distribution (Kolmogorov–Smirnov, and Shapiro - Wilk) or transformed if not normally distributed (using log10). One-way ANOVA was carried out to determine statistically significant differences in sample means of BCC and TAC data, and the *post-hoc* Duncan test was used to separate the means at $p < 0.05$. The results were expressed as mean ± standard error of the mean (SEM). No statistical analyses were carried out for the HPLC results. Pearson's correlation was carried out to determine linear relationships between TAC and BCC. Principal component analysis (PCA) was applied to determine the contribution of BCC, TAC, drying temperature, cooking method, and durations to the variance of the data. The PCA was also carried out to further assess the relationships between the variables. Multilinear regression by the step-wise method was performed to assess the BCC that significantly influenced the TAC at different drying temperatures and the control. Partial eta squared was also carried out to investigate the effect size of the variables on the BCC and TAC of SALF.

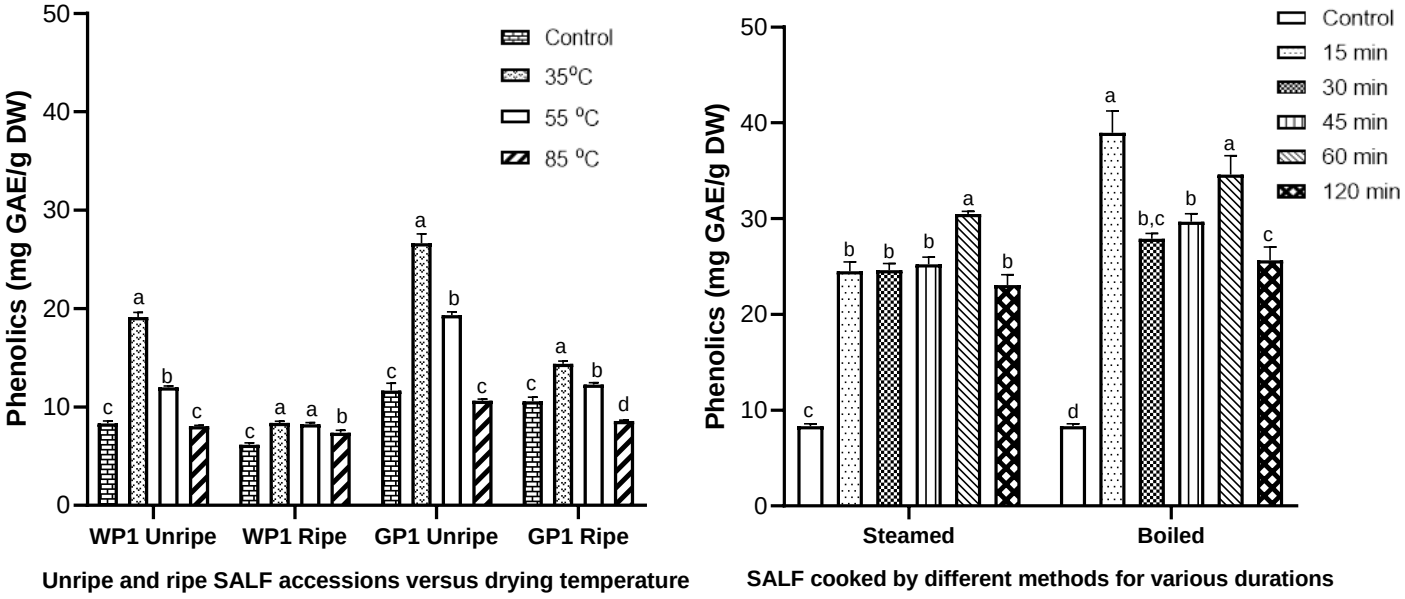
3.3.4. Results and discussion

3.3.4.1. Effect of thermal treatments on the BCC, phenolic compounds, and AA of SALF

3.3.4.1.1. Total phenolics and phenolic compounds

The TPC of both unripe and ripe SALF of both GP1 and WP1 accessions significantly differed with drying temperature (Figure 9a). The unripe stage of both accessions dried at low temperature had significantly higher TPC than the ripe (red) stage, consistent with the findings in section 3.2 (Table 6) of this thesis. Drying at 35 and 55 ^{o}C significantly increased the TPC for all the thermally treated samples as compared to the controls, with the highest increment at 35 ^{o}C. Compared to the control,

TPC of SALF dried at 85 °C was significantly higher for ripe WP1, lower for ripe GP1, but not significantly different for both unripe GP1 and WP1. The lower TPC in control was probably a consequence of enzymatic degradation during preparation since the enzymes were still active in fresh samples, as postulated by Hossain et al. (2010). Drying at 35 and 55 °C likely disrupted the cell wall matrix and subsequently released the bound phenolics (Arslan & Özcan, 2010; Călinoiu & Vodnar, 2020; Lang et al., 2019; Roshanak et al., 2016) hence the higher TPC than the control. Bound phenolics are formed when phenolic compounds are attached to macromolecules such as structural proteins, cellulose, and pectin by covalent bonds in the cell wall matrix (Shahidi & Yeo, 2016). Although drying at 55 °C may have released some bound phenolics, the lower TPC compared to SALF dried at 35 °C suggested that some free phenolics were thermally degraded to non-phenolic compounds such as CO, CO_2, H_2O, or methane (Johnston et al., 1988), as reflected by the lower TPC with increased drying temperature. Further reduction of TPC at 85 °C suggested increased thermal degradation of SALF phenolics.



(a) **(b)**

Figure 9. Effect of thermal treatments on the total phenolic contents of SALF. Total phenolic contents of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 °C, or remained undried (control), and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). GAE = gallic acid equivalent, DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

Consistent with the present results, drying increased the TPC of onions (Arslan and Özcan, 2010) while Arkoub-Djermoune et al. (2016) showed that TPC was higher in baked (77.94 mg GAE/g DW) or grilled (59.56 mg GAE/g DW) eggplants than fresh eggplants (48.38 mg GAE/g DW). Roshanak et al. (2016) also reported higher TPC in green tea dried at 60 °C than

at 80 ºC and, these were both significantly higher than the TPC for fresh tea leaves. On the contrary, Bamba et al. (2020) reported that the SALF TPC increased with oven-drying temperatures, 60, 80, and 100 ºC, at 0.4, 1.4, and 1.8 mg GAE/g DW, respectively; however, they did not report the TPC in fresh SALF.

The phenolic acid contents (Table 10) were in the order chlorogenic > caffeic acid > gallic acid. The contents of phenolic acids chlorogenic acid and caffeic acid decreased with increased temperature for both unripe and ripe SALF. Increased drying temperature, however, resulted in increased gallic acid content as the values at 35 ºC are lower than at 85 ºC for both unripe and ripe SALF. This showed that the effect of drying temperature on the phenolic compounds differed. The trend of the SALF TPC with increased temperature may mostly be influenced by the phenolic compound(s) with the highest levels, such as chlorogenic acid, which had the highest levels in this study as well as a similar trend as TPC.

Table 10. HPLC quantification of phenolic acids and flavonoid compounds of SALF as affected by drying temperature

Sample	Ripeness stage	Temp ºC	Gallic acid µg/g	Chlorogenic acid µg/g	Caffeic acid µg/g	Rutin µg/g	Quercetin µg/g
WP1	Unripe	55	28.02	757.31	50.93	21.81	11.77
		85	12.88	244.15	20.9	25.39	14.33
	Ripe	35	10.21	489.17	27.38	138.57	12.92
		55	13.19	408.2	19.45	116.53	14.78
		85	22.9	284.89	9.15	87.52	11.65
GP1	Unripe	35	33.41	1620.56	109.36	96.71	13.75
		55	50.86	1403.25	85.68	21.11	16.28
		85	70.84	244.28	39.22	31.35	14.37
	Ripe	35	25.04	469.81	57.21	224.55	14.33
		55	23.11	242.96	20.2	91.79	13.89
		85	35.58	158.25	12.5	89.93	11.99

Values are the means of duplicate analyses from one extract per sample of *Solanum anguivi* Lam. fruits accessions WP1 and GP1. The fruits were obtained from 12 plants per accession and pooled together according to their ripeness stages. The means were computed on a dry weight basis. Temp = temperature. No statistical analysis was carried out on the data.

Drying at 35 and 55 ºC may have stimulated the release of matrix-bound phenolics such as chlorogenic acid and caffeic acid, hence showing higher TPC due to an increased amount of free phenolics. Possibly too, drying at 85 ºC accelerated the formation of bound phenolics as demonstrated in black rice dried between 20 and 80 ºC by Lang et al. (2019) or activation of

polyphenol oxidase, which reportedly activates the degradation of phenolics through oxidation (R. Li et al., 2018). For some SALF phenolics such as gallic acid, the bound compounds may require a high temperature such as 85 ^{o}C to be released from the matrix, hence the higher levels than at 35 ^{o}C.

Steaming and boiling significantly increased the TPC in unripe SALF (accession WP1) more than 2-fold (Figure 9b). Notably, steaming at 15, 30, and 45 min significantly increased the SALF TPC as compared to the control, and the values at these durations were not significantly different. Further increase of steaming time to 60 min resulted in the highest TPC increase (30.5 mg GAE/g) by steaming, while 120 min lowered the TPC to values similar to SALF steamed at 15, 30, and 45 min. Boiling for 15 min increased the TPC 4-fold (39.0 mg GAE/g) as compared to the control. Increased boiling time to 30 and 45 min elevated the TPC 2.5-fold in comparison to the control, although it was lower than the TPC at 15 min of boiling. However, further increase in boiling time to 60 min increased the TPC 4-fold as recorded at 15 min. Comparable to steaming, boiling for 120 min increased TPC analogous to the control but lower than the TPC observed at 60 min. Overall, TPC was in the order boiled > steamed > raw/control. Boiling and steaming increased the TPC in SALF apparently due to the softening of the tissues or opening of the cell-matrix, which enhances both the extractability and solubility of the phenolic components (Nayak et al., 2015). Further, the increased TPC may have stemmed from a more efficient release of phenolic compounds bound to intracellular proteins, dietary fibre, and altered cell wall structures forming the corresponding free phenolic compounds (Chumyam et al., 2013). Relatedly, the heat treatments may have inactivated polyphenol oxidases, thus preventing oxidation and polymerization of polyphenols (R. Li et al., 2018). These effects were influenced by duration as the TPC in steamed samples was highest at 60 min and that for boiled was at 15 and 60 min. In contrast, Abbe et al. (2019) reported higher TPC in fresh SALF (11.632 mg GAE/g DW) than in SALF boiled for 10 and 15 min (8.0 and 7.0 mg GAE/g DW, respectively). Similar to the present findings, the TPC for boiled or steamed eggplants (Chumyam et al., 2013) and edible leaves (Gunathilake et al., 2018) was higher than recorded for the raw ones.

3.3.4.1.2. Total flavonoids and flavonoid compounds

The TFC of ripe and unripe SALF accessions significantly decreased gradually with increased drying temperature (Figure 10a). The highest TFC was observed in the controls, while the drying temperature with the highest TFC retention was 35 ^{o}C. The reduced TFC of dried SALF

may have resulted from thermal degradation of heat unstable flavonoids or complexation of the initially free flavonoid compounds with some structural constituents, which increased with drying temperature. Comparably, Arkoub-Djermoune et al. (2016) reported a significant reduction of TFC in dry heated (baked and grilled) eggplants, which was similarly attributed to thermal degradation. Contrary to this study, Roshanak et al. (2016) reported a significantly higher TFC in oven-dried green tea leaves than fresh ones.

The HPLC results showed the effects of drying temperatures on SALF flavonoid compounds rutin and quercetin (Table 10). The rutin content was reduced with increased drying temperature, possibly due to thermal degradation. The effect of drying temperature on the quercetin content seemed to vary with the accession and ripeness stage.

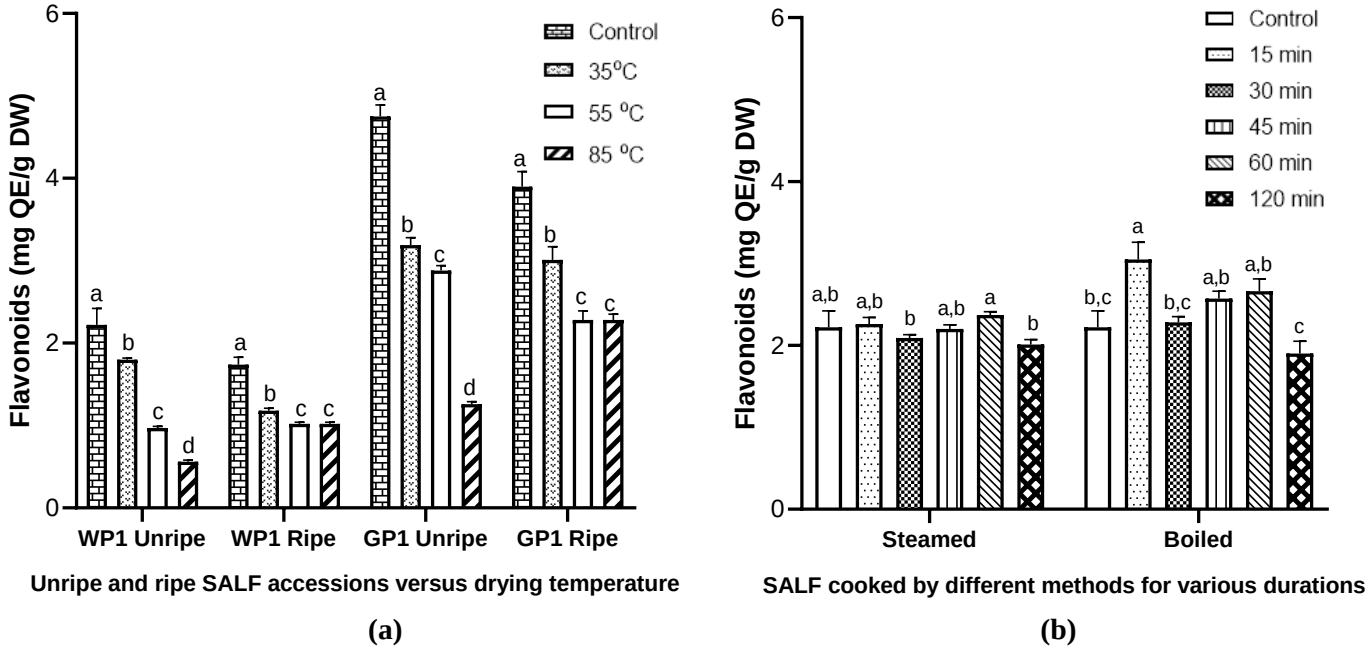


Figure 10. Effect of thermal treatments on the total flavonoid contents of SALF. Total flavonoid contents of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 °C, or remained undried (control), and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). QE = quercetin equivalent, DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

Steaming unripe WP1 SALF for all durations in this study had no significant effect on the TFC (Figure 10b) as compared to the control. Boiling duration did not affect the SALF TFC, except for 15 min, which recorded a significant increase compared to the control. The results showed that steaming and boiling did not destroy the TFC of SALF, unlike drying. This suggested that boiling and steaming were better than drying at retaining flavonoids. In contrast, Alide et al. (2020) reported that boiling duration did not have a significant effect on the TFC of garlic.

3.3.4.1.3. Saponins

The effect of drying on the total saponin content of SALF varied among the accessions and ripeness stages (Figure 11a). The TSC of SALF accessions WP1 dried at low temperature was significantly higher at the unripe than the ripe stage, while the reverse is true for accession GP1 as similarly observed in section 3.2 of this thesis (Table 6). Drying significantly reduced the TSC of ripe GP1 and unripe WP1 and GP1, yet no significant effect was observed in ripe WP1. The reduction in TSC of ripe GP1and unripe WP1 and GP1 may be associated with thermal damage of the most dominant saponin compounds that are likely heat-sensitive, while the presence of mostly heat-stable saponins in ripe WP1 may explain why they were not significantly affected by drying.

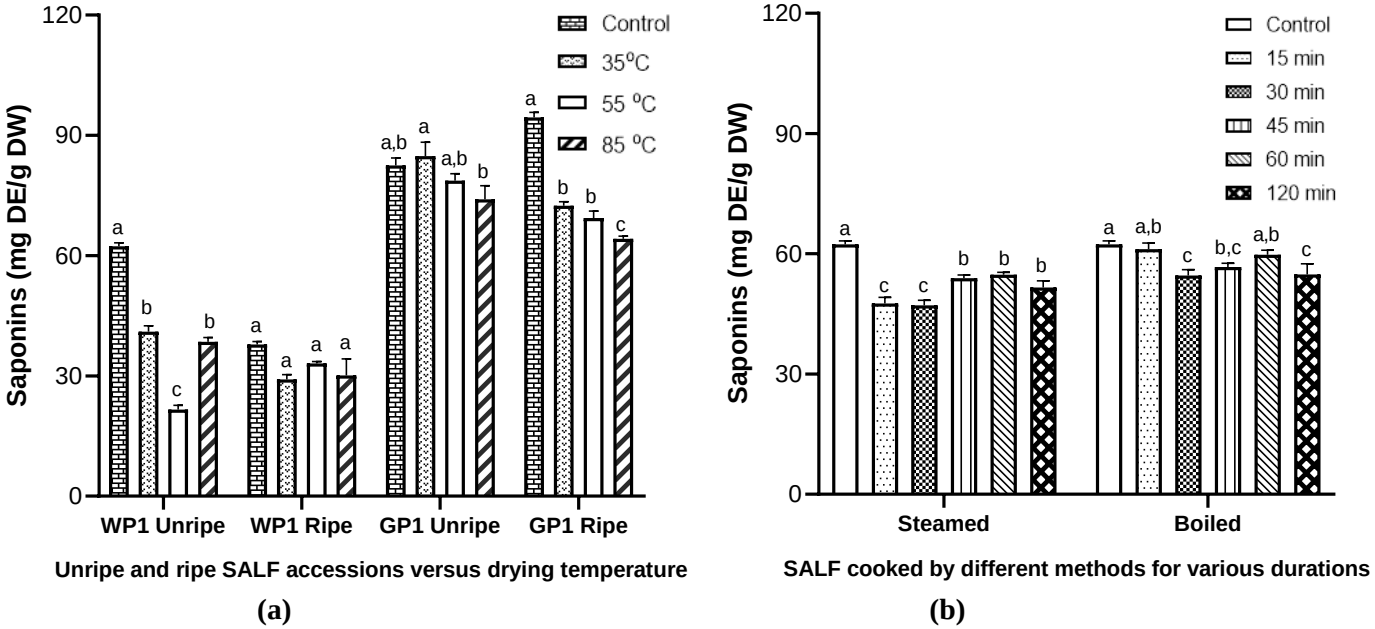


Figure 11. Effect of thermal treatments on the total saponin contents of SALF. Total saponin contents of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 °C, or remained, and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). DE = diosgenin equivalent, DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

Similar to drying, steaming and boiling negatively affected the TSC of SALF significantly (Figure 11b), except boiling for 15 or 60 min where it was similar to the control. The reduction of TSC in boiled and steamed SALF may be attributed to leaching into the cooking water during boiling or the ambient humidity during steaming, as reported by Mhada et al. (2020) for boiled and steamed quinoa seeds, respectively. Similarly, Ruiz et al. (1996) showed that the saponin content of lentils reduced with boiling; however, in contrast, that for chickpeas remained unchanged.

3.3.4.1.4. Alkaloids

The effect of drying temperature on the TAL varied with accession and ripeness stage (Figure 12a). The TAL of both accessions that were dried at low temperature was higher at the ripe than the unripe stage, as similarly reported in section 3.2 (Table 6) of this thesis. The highest TAL was recorded in the controls for all samples, and it generally diminished with increased drying temperature. However, the TAL for the ripe WP1 control was not significantly different from SALF dried at 55 ^{o}C and 85 ^{o}C, while the TAL for the unripe GP1 control was not significantly different from that at 85 ^{o}C. The decrease in TAL with increased drying temperature for some samples may have resulted from the thermal degradation of alkaloids in SALF as suggested by Nie et al. (2018) for *Solanum tuberosum* L. (potato) glycoalkaloids. Bamba et al. (2020) showed that drying at 60, 80, and 100 ^{o}C had no significant effect on SALF TAL, and this is similar to only ripe WP1 at 55 and 85 ^{o}C in the present study.

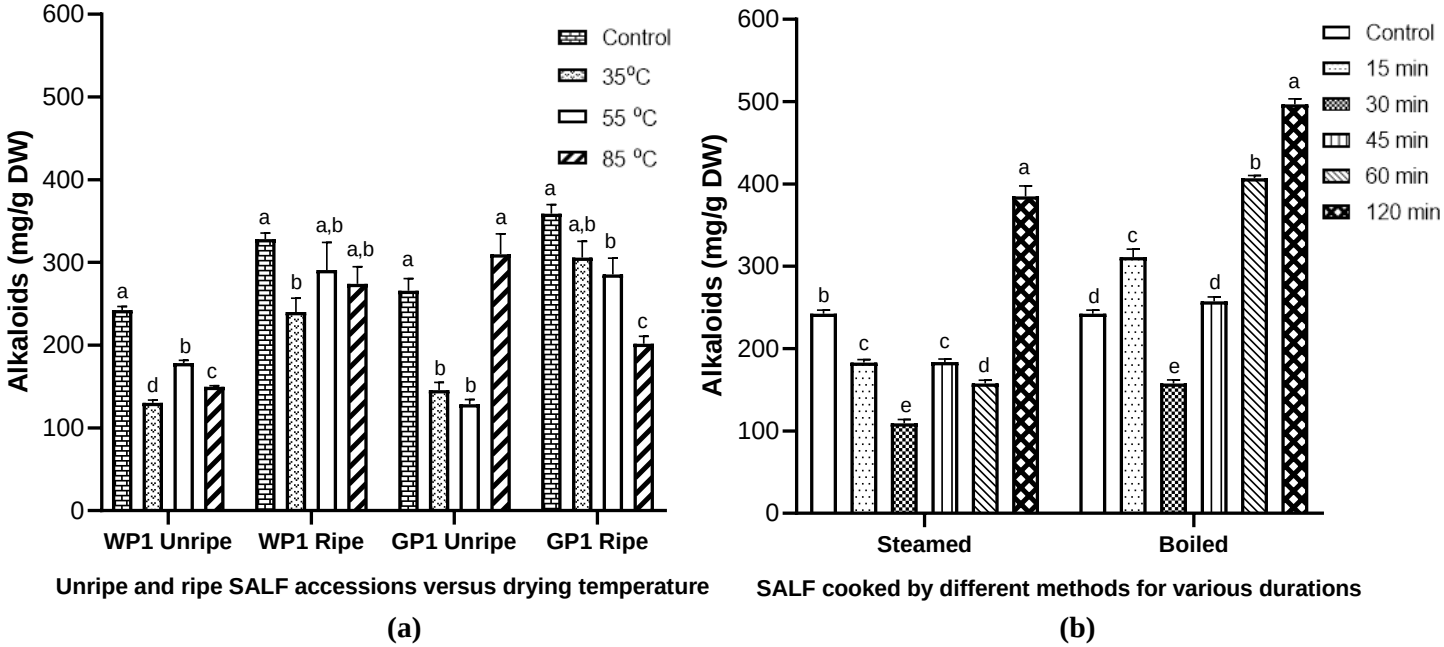


Figure 12. Effect of thermal treatments on the total alkaloid contents of SALF. (a) Total alkaloid contents of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 ^{o}C, or remained undried (control), and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

Steaming led to a reduction of the TAL while boiling increased it (Figure 12b). However, prolonged steaming (120 min) and boiling (60 and 120 min) significantly increased the TAL 1.5-fold, 1.5-fold, and 2-fold, respectively, compared to the control. The greater TAL in SALF that was boiled for a long duration may have accrued from the softening of the SALF matrix due to its direct contact with heated water, thereby increasing the TAL extractability. Similarly,

steaming may have been achieved the same effect as SALF boiled for 60 min by steaming for double the duration (120 min). Long boiling and steaming durations may have resulted in the formation of more extractable alkaloid intermediates hence the higher TAL than the control. Contradictorily, Abeshu and Kefale (2017) reported that the alkaloid content of boiled lupin beans was significantly lower than the control. However, the variance observed in the present findings may be due to the differences in the processing parameters. Abeshu and Kefale (2017) boiled the lupin beans for 30 min in a dish cooker and then dried them for three days at 50 ºC before TAL analysis.

3.3.4.1.5. Vitamin C

Drying significantly reduced the VCC in all samples, and this effect increased with drying temperature (Figure 13a). As similarly observed in section 3.2 of this thesis (Table 6), the VCC of GP1 and WP1 dried at a low temperature was significantly higher at the ripe than at the unripe stage.

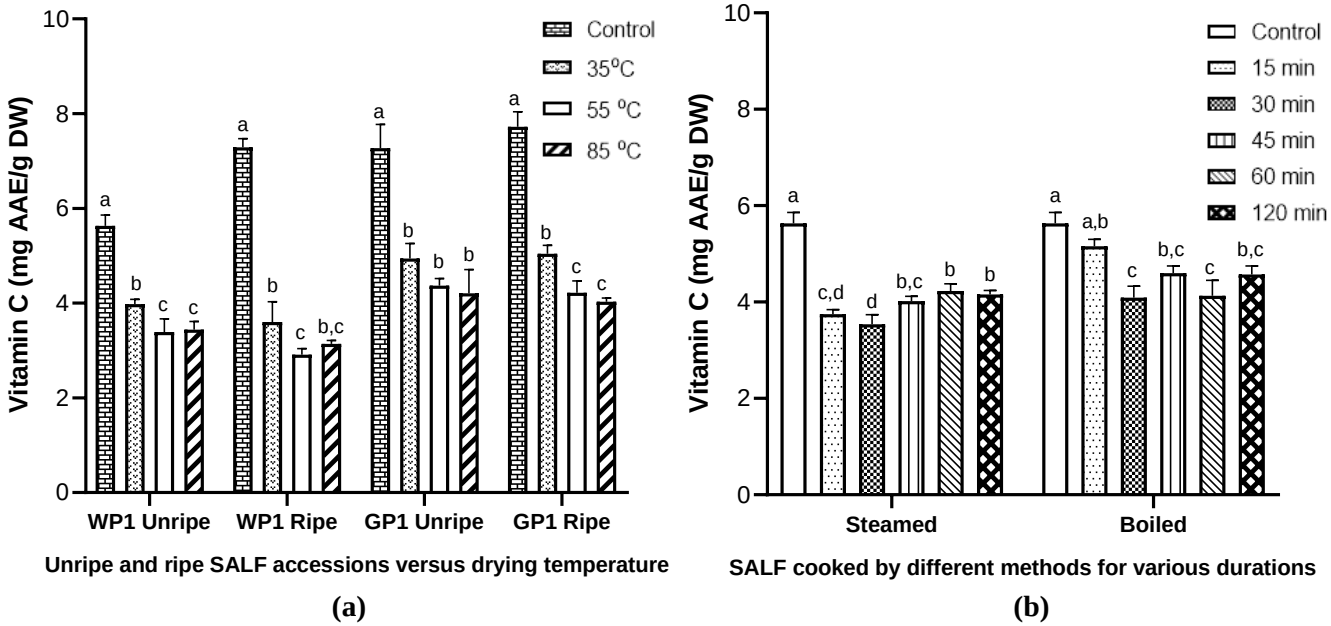


Figure 13. Effect of thermal treatments on the vitamin C contents of SALF. Total vitamin C contents of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 ºC, or remained undried (control), and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). AAE= ascorbic acid equivalent, DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

Drying at 35 ºC resulted in the highest vitamin C retention, irrespective of accession and ripeness. Vitamin C is a temperature-sensitive vitamin that is easily degraded by elevated temperatures (Tian et al., 2016), with a higher reaction rate in high than low temperatures (Orikasa et al., 2008; P. H. S. Santos & Silva, 2008), and hence the more decrease of VCC with

increased drying temperature as shown in this study. The same procedure may also have resulted in the accelerated oxidation of ascorbic acid to dehydroascorbic acid (Arkoub-Djermoune et al., 2016; Chuah et al., 2008). Likewise, Arkoub-Djermoune et al. (2016) showed significantly lower VCC in heated eggplants rather than in fresh ones. In addition, Marfil et al. (2008) reported a decrease in VCC of tomatoes with increased drying temperature, which was attributed to degradation.

Steaming and boiling of unripe WP1 led to a significant reduction of the SALF VCC, except for boiling for 15 minutes, which retained it (Figure 13b). The loss of VCC in this study may indicate leaching and/ or thermal degradation due to boiling and steaming since vitamin C is water-soluble and easily degraded by cooking (S. Lee et al., 2018; Tian et al., 2016). Comparable with the present findings, Abbe et al. (2019) reported that the VCC in SALF was significantly higher in the uncooked (0.3 mg/g) than boiled (10 and 15 min, that is, 0.13, 0.08 mg/g, respectively) SALF. Some scholars (de Oliveira et al., 2013; Tian et al., 2016; G. F. Yuan et al., 2009) have shown that vitamin C is more retained by steaming than boiling. However, Lee et al. (2018) reported that the effect of the cooking method on VCC varied among vegetables. The authors (Lee et al., 2018) documented that VCC (fresh weight basis) was higher in steamed than boiled broccoli and potatoes, higher in boiled than steamed carrots, no significant difference between steamed and boiled spinach and sweet potatoes, and completely undetectable by both methods for chard. However, in the current study, analysis based on fresh weight (FW) (data not shown) resulted in higher VCC in steamed than boiled SALF, while analysis based on DW showed higher VCC in boiled than steamed. This showed that the documented effects of cooking methods on vitamin C may vary on the basis of analysis (DW or FW).

3.3.4.1.6. Total antioxidant capacity

The TAC in this study significantly differed among the accessions and ripeness stages of the dried samples (Figure 14a). In agreement with findings in section 3.2 (Table 6), the TAC of the accessions dried at low temperature was higher in the unripe than in the ripe stage. Drying at 35 ^{o}C resulted in higher TAC for all samples compared to their control, while drying at 85 ^{o}C reduced the TAC. The TAC trend for the dried samples in this study was similar to that for TPC. This may suggest that TPC was the major contributor to the TAC of SALF.

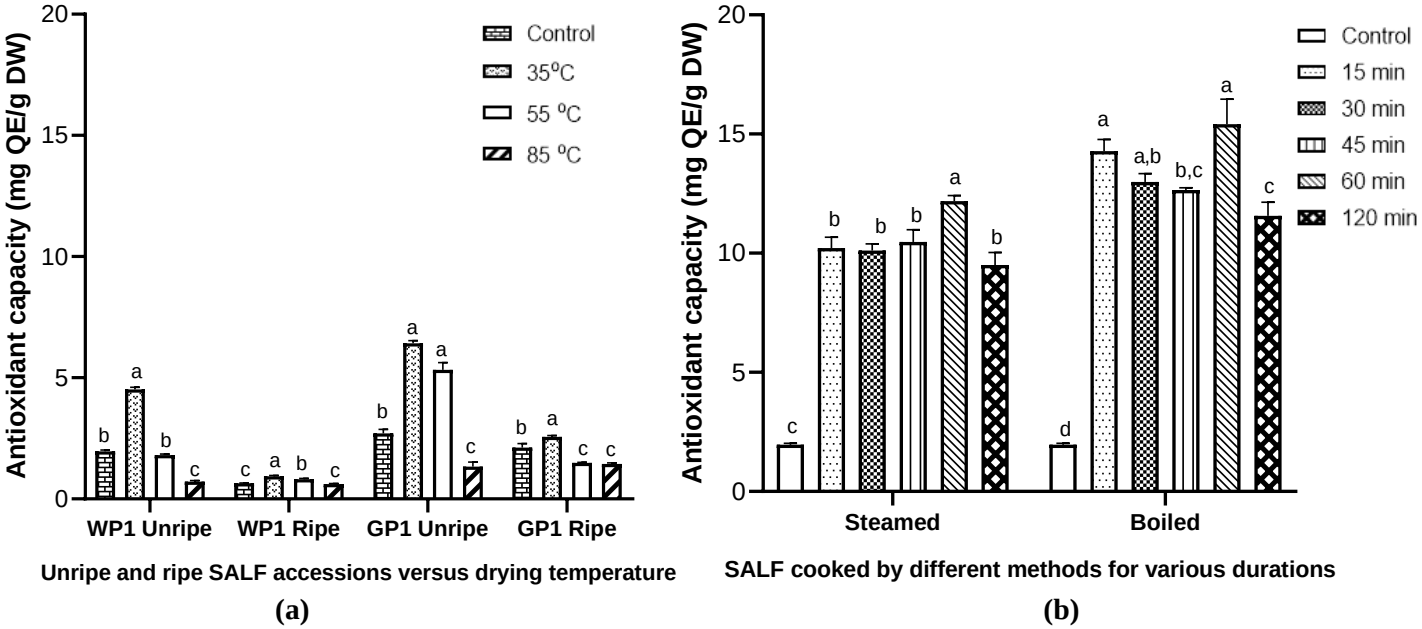


Figure 14. Effect of thermal treatments on the total antioxidant capacity of SALF. Total antioxidant capacity of (a) unripe and ripe SALF accessions (WP1 and GP1), dried at either 35, 55, or 85 °C, or remained undried (control), and (b) unripe SALF accession WP1 that was either steamed or boiled for 15, 30, 45, 60 and 120 min or remained uncooked (control). QE = Quercetin equivalent, DW = dry weight. Bars on the graphs show the mean ± SEM of two independent experiments measured in triplicates. Different letters on the bar graphs represent significant differences per sample or treatment.

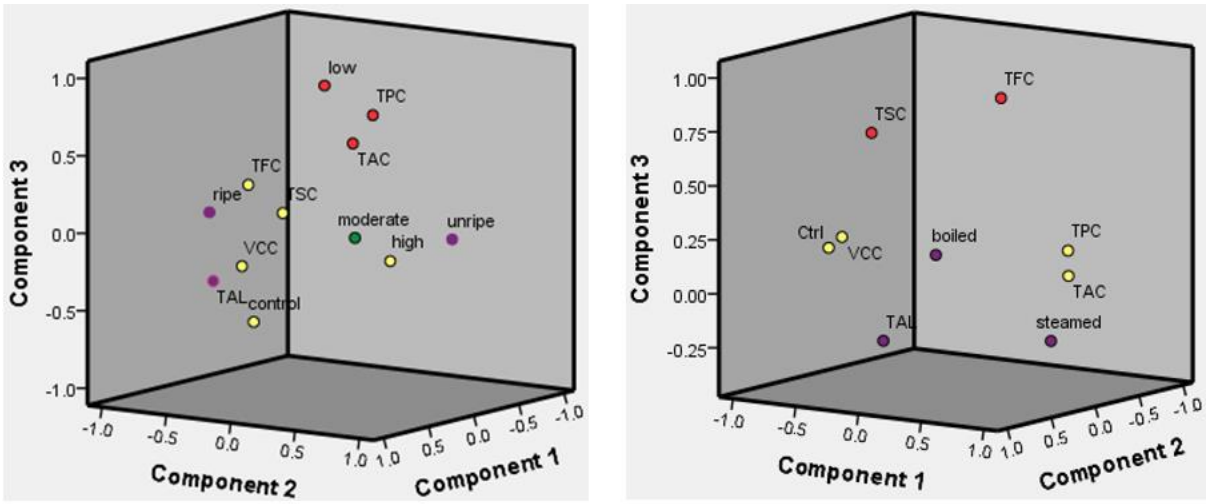
Steaming and boiling unripe WP1 SALF significantly increased its TAC (Figure 14b), with the highest amounts at 60 min for steaming and 15 and 60 min for boiling. The trend for TAC in the boiled and steamed SALF was also similar to that of TPC. Overall, boiling resulted in significantly higher TAC in SALF than steaming.

3.3.4.2. The relationship between thermal treatments, BCC, and TAC

3.3.4.2.1. Drying data

The correlation analysis of the drying data showed that TAC had significant positive correlations with TPC, TFC, and TSC (r = 0.901, 0.658, 0.504, respectively), and a negative correlation with alkaloids (r = -0.487), at $p < 0.01$. The correlation between TPC and antioxidant activity (AA) of SALF in this study was strong and higher than reported for *S. melongena* cultivars (r = 0.52) (Okmen et al., 2009) and *S. melongena* accessions (r = 0.44). The positive correlation between TSC and antioxidant activity of SALF in this study is agreeable with findings by Elekofehinti, Kamdem, Kade, Adanlawo, et al. (2013), who demonstrated the antioxidant activity (FRSC) of SALF saponins. The strong positive correlation between TPC and TAC suggests that TAC in the dried SALF was mostly contributed by TPC, and hence, the low TPC at 85 °C and ripe stage samples may have resulted

in their low TAC. Thus, the negative correlation between TAL and TAC of SALF was likely influenced by the low TPC in the samples with high TAL. The PCA for the drying data derived four components (Figure 15a), which cumulatively explained 86.5% of the data variance.



a) b)

Figure 15. Components formed from principal component analysis based on the drying and cooking data. Components plot from the principal component analysis of the (a) drying and b) cooking data. TPC = total phenolic content, TFC = total flavonoid content, TAL = total alkaloid content, TSC = total saponin content, VCC = Vitamin C content, TAC = total antioxidant capacity, low = low temperature (35 ^{o}C), moderate = moderate temperature (55 ^{o}C), high = high temperature (85 ^{o}C), control = undried, Ctrl = uncooked, yellow dots = component 1, purple dots = component 2, red dots = component 3 and green dots = component 4. Drying data components 1 = TFC, TSC, VCC, control, and high temp; 2 = ripe, TAL, unripe; and 3 = low temp, TPC, and TAC; and 4 = moderate temp. Cooking data components 1= VCC, control, TPC and TAC; 2 = TAL, steamed, and boiled; 3 = TFC and TSC.

Components 1, 2, 3, and 4 explained 28.1, 24.6, 21.6, and 14.2 %, respectively, of the data variance. Component 1 showed that VCC, TFC, and TSC were highest in fresh/raw SALF, and they were reduced by high drying temperature (85 ^{o}C). Component 2 indicated that alkaloids were higher in ripe than unripe SALF. Further, the third component showed that drying SALF at low temperature (35 ^{o}C) increased the TPC and TAC, and TAC strongly correlated with TPC. Component 4 only constituted of moderate temperature (55 ^{o}C).

3.3.4.2.2. Cooking data

The cooking data correlation analysis showed that TAC had significant positive correlations at $p < 0.01$, with TPC and TFC (r = 0.963 and 0.285, respectively) as in the drying data, and a negative correlation with vitamin C (r = -0.398). Contrastingly, Ilahy et al. (2010) reported that the AA of tomato cultivars negatively correlated with TPC (r = -0.14) and positively correlated with vitamin C (r = 0.60). The strong positive correlation between TPC and TAC implied that TPC contributed the most to the TAC of SALF in cooking data. Further, the negative

correlation between VCC and TAC may show VCC pro-oxidant properties that are more prevalent in the presence of iron and copper (Arrigoni & De Tullio, 2002; Davey et al., 2000; Pehlivan, 2017), which are reportedly present in SALF (Dan et al., 2014; Oyeyemi et al., 2015).

Subjecting the cooking data to PCA derived three components, which cumulatively explained the 86.4% variance of the data. Components 1, 2, and 3 explained 39.4, 28.2, and 18.7%, respectively, of the variance in the data. Component 1 indicated that TPC strongly correlated with TAC and that uncooked SALF had the least TPC, TAC, and highest VCC. Component 2 illustrated that TAL was reduced by steaming but increased by boiling. The third component showed an association between TFC and TSC.

3.3.4.3. Effect of the independent variables (drying temperature, ripeness stage, cooking method, and cooking duration) and their interactions on the BCC and TAC of SALF

3.3.4.3.1. Drying data

The variables that included accession, drying temperature, and ripeness, as well as their interactions, had significant effects on the BCC and TAC of SALF (Table 11).

Table 11. Effect sizes of variables (accession, ripeness stage, and drying temperature) on the bioactive compounds content and antioxidant capacity of SALF

Chemical composition	Variables			Interaction (*) between variables				Adjusted r^2
	Accession	RS	Temp	Accession *RS	Accession *temp	RS* temp	Accession* RS*temp	
TFC	0.96	ns	0.94	ns	0.15	0.79	0.30	0.97
TPC	0.90	0.89	0.94	ns	0.36	0.76	0.27	0.97
TSC	0.89	0.18	0.51	ns	0.23	0.28	0.38	0.91
TAL	0.12	0.81	0.72	0.11	0.35	0.59	0.62	0.89
VCC	0.46	ns	0.80	ns	ns	0.13	ns	0.81
TAC	0.91	0.91	0.92	0.32	ns	0.79	0.54	0.97

Partial eta squared was carried out to assess the effect sizes of the variables. All the displayed effect sizes of the variables and variable interactions in the table were significant at $p < 0.05$. ns = not significant, TFC = total flavonoid content, TPC = total phenolic content, TSC = total saponin content, TAL = total alkaloid content, VCC = vitamin C content, TAC = total antioxidant capacity, RS = ripeness stage, Temp = temperature, * = interaction

Accession and the drying temperature significantly affected all the BCC and TAC of SALF, while the ripeness stage significantly affected the TAC, TPC, TSC, and TAL. The interaction

of all the three variables accession, ripeness stage, and drying temperature was significant in TAC and all the BCC except VCC. All the models had high adjusted r^2 (0.81 - 0.97), which showed that they may explain more than 80% variance of the BCC and TAC of SALF.

3.3.4.3.2. Cooking data

The variables cooking method and cooking duration and their interaction (cooking method*cooking duration) had significant effects on the BCC and TAC of SALF (Table 12).

Table 12. Cooking method and duration effect sizes on the bioactive compounds content and antioxidant capacity of SALF

Chemical composition	Variables		Interaction of variables	Adjusted r^2
	Method	Duration	Method*duration	
TFC	0.21	0.45	ns	0.46
TPC	0.52	0.60	0.35	0.80
TSC	0.45	0.27	0.22	0.64
TAL	0.88	0.95	0.65	0.96
VCC	0.33	0.21	0.26	0.57
TAC	0.67	0.40	0.19	0.79

Partial eta squared was carried out to assess the effect sizes of the variables. All the displayed effect sizes of the variables and variable interactions in the table were significant at $p < 0.05$. ns = not significant, TFC = total flavonoid content, TPC = total phenolic content, TSC = total saponin content, TAL = total alkaloid content, VCC = vitamin C content, TAC = total antioxidant capacity, * = interaction.

The partial eta squared analysis showed that both cooking method and cooking duration had statistically significant effects on all the BCC and TAC. The effect of the cooking method on the TAC and BCC except for TFC was significantly influenced by the cooking duration. The models ranged from good to very good, with adjusted r^2 ranging from 0.46 to 0.96, suggesting that they may explain 46 to 96 % variance of BCC and TAC of unripe SALF.

3.3.5. Conclusion

The study has shown that thermal treatments impact the BCC and TAC of SALF. The BCC and TAC of SALF were significantly affected by the drying temperature, accession, ripeness stage, cooking method, and duration. The VCC was reduced by all thermal treatments. Drying at 35 ^{o}C showed the highest TPC and TAC increment and retained the highest VCC and TFC, while drying at 85 ^{o}C produced the least contents. Both boiling and steaming greatly increased the TPC and TAC, with higher values in boiled than steamed SALF. Variably, TFC was

reduced by drying and not by cooking, except for the recorded increment in SALF boiled for 15 min. Concurrently, TAL was reduced by steaming and drying but increased by boiling. However, prolonged cooking time (120 min) resulted in the highest TAL for both methods. Saponins were reduced by the thermal treatments, except boiling for 15 or 60 min, which did not affect the content. Overall, boiling resulted in the highest TPC and AA, compared to the control, drying, and steaming. The findings may inform consumers on the thermal parameters that would result in the increment or highest retention of the bioactive compounds of interest for potential health benefits. The results may also inform the industry on the processing parameters that would yield higher extraction of bioactive compounds for the manufacture of supplements.

3.4. *Solanum anguivi* Lam. fruit preparations counteract the negative effects of a high-sugar diet on the glucose metabolism in *Drosophila melanogaster* [5]

Abstract

Solanum anguivi Lam. fruits (SALF) are traditionally consumed as a remedy for type 2 diabetes mellitus (T2DM). However, data regarding the potential antidiabetic effect of SALF and its underlying mechanisms are scarce. Apparently, the fruit fly and mammal energy metabolisms are comparable, including the secretion of insulin-like peptides. Thus, *D. melanogaster* were fed a high-sugar diet (HSD) to induce a T2DM-like phenotype and subsequently exposed to HSD supplemented with SALF. Consequently, the flies were analyzed for various biomarkers in relation to energy metabolism. The HSD-induced glucose levels were significantly lowered in flies exposed to HSD supplemented with SALF. In addition, flies exposed to SALF-supplemented HSD exhibited a better survival in comparison to HSD-fed counterparts. Other energy metabolism parameters such as triglyceride levels, weights, and fitness were not affected by SALF supplementation. This was also true for the expression levels of the insulin-like peptides 3 and 6 as well as for spargel, the *Drosophila* homolog of PPARγ co-activator 1α and a central player in mitochondrial biogenesis. Overall, the present study shows that SALF significantly lowered the HSD-induced glucose levels while biomarkers of the energy metabolism were unaffected.

Keywords: *Drosophila melanogaster*, glucose metabolism, bioactive compounds, *Solanum anguivi*, diabetes mellitus

[5] This content has been published in the *Food and Function* Journal. Nakitto, A. M. S., Rudloff, S., Borsch, C., and Wagner, A. E., 2021, *Solanum anguivi* Lam. fruit preparations counteract the negative effects of a high-sugar diet on the glucose metabolism in *Drosophila melanogaster*. *Food Funct.* https://doi.org/10.1039/D1FO01363G.

3.4.1. Introduction

Non-communicable diseases (NCD), including cardiovascular diseases and type 2 diabetes mellitus (T2DM), are a fundamental developmental and socioeconomic issue, striking both the rich and the poor (WHO, 2020). The social burden associated with NCD includes prolonged disability, diminished resources within families, and reduced productivity, consequently putting tremendous demands on the health systems (WHO & World Economic Forum, 2011). In humans, T2DM is characterized by two fundamental defects, impaired insulin action (insulin

resistance) in skeletal muscle, liver, and adipocytes and impaired secretory function of the pancreatic islets (Forbes & Cooper, 2013). It represents a multifactorial disease not only involving dysfunctional insulin secretion and insulin resistance but also lifestyle factors, such as overweight, obesity, and a lack of exercise (Ghosh & Collier, 2012; Kaku, 2010; Ozougwu et al., 2013). In addition, the advanced age of an individual is a probable risk factor to develop T2DM (J. D. Pereira et al., 2021). In many African countries, plant components such as the fruits of *Solanum anguivi* Lam. (SALF) are not only commonly consumed as vegetables but are traditionally used in the treatment of T2DM (Elekofehinti, 2015; Elekofehinti, Kamdem, Bolingon, et al., 2013). *Solanum anguivi* Lam. belongs to the family *Solanaceae* and the genus *Solanum* L (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013). Previous studies reported the presence of bioactive compounds including phenolics, flavonoids, saponins, alkaloids, and steroids in *Solanum anguivi* Lam. fruit, which possibly explains its suggested antidiabetic effects (Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015). The present study aimed to investigate the potential effects of SALF on energy metabolism using the fruit fly *D. melanogaster* as a model organism. It has been documented that the energy metabolism of *D. melanogaster* and mammals is seemingly comparable (D. Chen et al., 2021; Morris et al., 2012). Previous studies further indicated that the intake of a high-sugar diet (HSD) results in hyperglycemia, fat accumulation, insulin resistance, and protein glycation as well as a reduced life span in *D. melanogaster*, reflecting a kind of a T2DM phenotype (Musselman et al., 2011; Rovenko et al., 2015; van Dam et al., 2020). Therefore, flies in the present study were exposed to HSD with and without SALF, and various biomarkers reflecting the flies' energy metabolism were determined, including glucose and triglyceride levels as well as the expression of associated genes.

3.4.2. Material and methods

3.4.2.1. *Solanum anguivi* Lam. fruit preparation

Unripe SALF accession WP1 (Figure 1) were obtained from Nabiyagi village in Mukono district (Uganda). The results in section 3.3 (Figure 14) of this thesis revealed that boiled SALF had a higher total antioxidant capacity than uncooked SALF and, therefore, SALF in the present study was boiled in water for 15 minutes as described by Ahmed & Ali (2013). The boiled SALF were sliced into four pieces each and then air-dried (B. Master, Tauro, Camisano Vicentino, Italy) at 35 °C for 16 hours. Two dried SALF samples were obtained from two independent batches. The dried SALF samples were then milled (Wonder Mill, Pocatello,

Idaho, USA) at the coarseness setting for fine flour. The SALF powder was transferred into sealed plastic bottles and stored at -20 °C until further use.

3.4.2.2. Extraction and quantification of total bioactive compounds content and antioxidant capacity of SALF.

The bioactive compounds in SALF powder were extracted according to Kim et al. (2003), with slight modifications (0.2 g/ 20 ml 80% methanol w/v, three times, 10 min each). The obtained extracts were pooled together for the subsequent BCC analyses. The methanolic extracts were analyzed for total phenolics, flavonoids, saponins, alkaloids, and vitamin C as well as AA by spectrophotometry (Q. D. Do et al., 2014; D. Kim et al., 2003; Turkmen et al., 2006). The total phenolic content (TPC) was determined by applying the Folin-Ciocalteu method Singleton et al. (1998), calculated from a gallic acid standard curve and expressed as gallic acid equivalent (GAE). The total flavonoid content (TFC) was determined according to the method described by Kumar et al. (2012), calculated from the quercetin standard curve and expressed as quercetin equivalent (QE). The total saponin content (TSC) was determined by applying the method of Hiai et al. (1976). The TSC was then calculated from the diosgenin standard curve and expressed as diosgenin equivalent (DE). The vitamin C content (VCC) was determined according to the method of Omaye et al. (1979), calculated from the ascorbic acid standard curve and expressed as ascorbic acid equivalent (AAE). The total antioxidant capacity (TAC) was analyzed by applying the method described by Brand-Williams et al. (1995). The TAC was subsequently estimated from the quercetin standard curve and expressed as quercetin equivalent (QE). The absorbances were measured using a UV-VIS Photometer (UV Mini-1240, Shimadzu, Duisburg, Germany) with 80% methanol as blanks. All quantities were expressed on a dry weight (DW) basis.

3.4.2.3. Quantification of phenolic compounds by HPLC

To quantify the phenolics, dried and milled SALF samples (0.5 g) were extracted using methanol (5 ml) as described by Elekofehinti, Kamdem, Bolingon, et al. (2013), for 30 min under constant shaking (IKA Vibrax VXR, IKA-Labortechnik, Staufen i.Br., Germany). The rest of the method is as described in section 3.1.2.6 of this thesis.

3.4.2.4. Fly strains and husbandry

The female *D. melanogaster* wild-type strain w^{1118} (Bloomington *Drosophila* Stock Center, Indiana, USA; #5905) was used in all experiments. Flies were kept in a humidified (60%) and

temperature-controlled (25 °C) incubator (Memmert, HPP400, Buechenbach, Germany), with a 12 h/12 h light-dark cycle on standard fly medium (CT) according to Wagner et al. (2015). Age-matched flies were obtained from synchronized eggs as described by Linford et al. (2013). with slight modifications. Once emerged, 1-day old adult flies were transferred to fresh CT medium and allowed to mate for two days before the sexes were separated. Three day-old flies were then separated according to their sex, and female flies were transferred into vials containing the corresponding experimental medium. In this study, medium consisting of 10% sucrose (Carl Roth, Karlsruhe, Germany), 10% inactive yeast (Genesee via Kisker), and 2% agar (Apex via Kisker, Steinfurt, Germany) was used as a control medium [SY10 (Linford et al., 2013)]. To the medium, 1.5% tegosept (Apex via Kisker), and 0.3% propionic acid (Carl Roth) were added as preservatives. To induce a T2DM-like phenotype in the flies, they were exposed to a high sugar diet (HSD), i.e., the SY10 medium with an increased sucrose content (30%). To investigate whether SALF affects the survival, weight, fitness, glucose, and triglyceride contents and the gene expression of insulin-like peptides and PPARγ-Co-activator 1α (PGC-1α) fly homolog, spargel, in HSD-fed flies, HSD was supplemented with various amounts of SALF.

3.4.2.5. Gustatory assay

To exclude differences in food intake between control flies and those fed with SALF, a gustatory assay was carried out. Five different concentrations of SALF (0.0, 1.0, 2.5, 5.0, and 10 mg/ml) in the SY10 medium were analyzed for their effects on food intake in *D. melanogaster*. Age-matched flies were transferred to the SALF-supplemented diets with a transfer to fresh medium-containing vials every 2-3 days until experimental day 10. On day 10, the flies were transferred into empty vials covered with feeder caps that contained the same experimental diets stained with 0.2% sulforhodamine B for nine h (Shell et al., 2018). Then, 20 flies were homogenized in 250 µl PBS containing 1% Triton-X-100 (Sigma-Aldrich, Taufkirchen, Germany) in a Tissue Lyser (Qiagen, Tissuelyser II, Hilden, Germany) at a frequency of 1/s = 25 for 2 min. Homogenates were centrifuged (Hettich, Universal 32R, Tuttlingen, Germany) at 5,000 x g for 5 min, and 50 µl per sample was transferred onto a 96 well plate. The fluorescence (Fluoroscan Ascent FL, Thermo Electron Corporation Helsinki, Finland) was detected at excitation 535 nm and emission 590 nm, and the results were used to estimate the food intake of the flies. As none of the tested SALF-concentrations affected the food intake, the two highest SALF concentrations (5 mg/ml and 10 mg/ml) were used in the proceeding experiments.

3.4.2.6. Experimental design

A T2DM-like phenotype was induced by feeding the female flies on the HSD for ten days. Thereafter, the flies were either kept on the HSD or switched to HSD supplemented with 5 mg SALF (HSD+SALF5) or HSD with 10 mg SALF (HSD+SALF10) until day 24 of the experiment. The study duration was based on preliminary studies which showed a low percentage of male flies survived upon HSD feeding by day 24, and yet samples needed to be obtained for analysis. Notable, SY10-fed flies served as controls. Age-matched female flies were randomly selected and sorted into medium-containing vials. Each treatment was performed in triplicate with 25 flies per vial. Flies were transferred to vials with freshly prepared medium every 2-3 days. The whole experiment was repeated four times.

3.4.2.7. Survival

Survival of w^{1118} flies was documented over the entire 24 day study period. The number of dead flies per vial was recorded every 2-3 days when flies were transferred to vials with fresh medium.

3.4.2.8. Climbing assay

At the end of each experiment, the fitness of the flies was assessed based on their climbing ability according to the protocol of Madabattula et al. (2015). Briefly, flies were anaesthetized using CO_2 9 h prior to the start of the climbing assay purposely to standardize the number of flies in the different treatment groups. Immediately before the start of the climbing experiment, the counted flies were transferred into a glass cylinder where a target line was marked at 5 cm from the bottom. Then, the flies were quickly tapped to the bottom three times, and their climbing due to natural geotaxis was video-recorded for 2 min. (Madabattula et al., 2015). The climbing ability of the flies was calculated as the percentage of flies having crossed the target line after 2 min.

3.4.2.9. Weighing of female w^{1118} flies

On day 24, flies were transferred into a pre-weighed empty vial, and the mean weight of a single fly was calculated by dividing the total fly weights by the number of flies per vial.

3.4.2.10. Determination of glucose, triglycerides, and protein in whole fly lysates

The w^{1118} flies fed on different experimental diets were analyzed for their content of glucose, triglycerides, and protein. Five flies per vial and treatment were transferred into a 2 ml

Eppendorf tube and homogenized in 250 µl PBS containing Triton-X-100 (1 % v/v) in a Tissue Lyser at a frequency of 1/s = 25 for 2 x 3 min. Then, the fly lysates were centrifuged (Hettich, Universal 32R, Tuttlingen, Germany) at 5,000 x g, 4 °C for 10 min. The supernatant was transferred into fresh 0.5 ml tubes and either used immediately or stored at - 80 °C until further use. To prevent interference of proteins in the glucose assay, samples were heated at 70 °C for 5 min, immediately cooled down and centrifuged at 16,000 x g, 4 °C for 10 min. Supernatants were used immediately for analysis or stored at -80 °C until further use. The samples were diluted in the homogenization buffer and analyzed for glucose, triglyceride, and protein levels using the Glucose GOD-PAP assay, the Triglyceride GPO-PAP assay (both from DIALAB, Wiener Neudorf, Austria), and the Roti-Quant® universal kit (Carl Roth), respectively. All assays were performed according to the manufacturers' instructions. Triglyceride and glucose levels were normalized to protein levels. The analyses were performed in duplicates of the three replicates per treatment and from all four independent experiments. Absorbance was measured in a plate reader (Digi Scan 400, Asys Hitech GmbH, Seekirchen am Wallersee, Austria).

3.4.2.11. Real Time-PCR analysis

Total RNA from whole flies was extracted by using TriFast reagent (Peqlab Biotechnologie, Erlangen, Germany) according to the manufacturer's instructions. Before RNA isolation, seven flies per sample were homogenized in a Tissue Lyser at a frequency of 1/s = 25 for 2 x 3 min and RNA concentrations and purity were determined in a spectrophotometer (UV Mini-1240, Shimadzu, Duisburg, Germany). The following primers for *D. melanogaster* genes were designed using the open-source software primer3 and purchased from Eurofins (Ebersberg, Germany): *Drosophila* insulin-like peptide 3 (*dIlp3*), *dIlp6*, spargel (*Srl*), and ribosomal protein l32 (*rpl32*) (Table 13). Gene expression levels were analyzed by two-step real-time PCR. DNA synthesis was performed by using OligodT primer (Promega, Mannheim, Germany), RNase inhibitor Ribolock (Promega), dNTPs (Fisher Scientific, Schwerte, Germany), and revert Aid H Minus Reverse Transcriptase plus reaction buffer (both Promega) in a thermocycler (Biometra, Göttingen, Germany). For real-time PCR, a PerfeCTa SYBR green SuperMix (Quantabio, Beverly, MA, USA) was applied, and samples were detected in a 7500 Real-Time PCR System (Applied Biosystems, Darmstadt, Germany). Relative mRNA quantification was performed by applying the ΔΔCt method. *rpl32* served as the housekeeping gene.

Table 13. Primer sequences (*D. melanogaster*) used for real-time PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
dIlp 3	ATCCTTATGATCGGCGGTGT	GTTCACGGGGTCCAAAGTTC
dIlp 6	TGGCGATGTATTTCCCAACAG	CCTTCACTATCCTTTGCAGTACT
Srl	CTCTTGGAGTCCGAGATCCGCAA	GGGACCGCGAGCTGATGGTT
rpl32	GGCAAGCTTCAAGATGACCA	GTTCGATCCGTAACCGATGT

dIlp3 – *Drosophila* insulin-like peptide 3; *dIlp6* – *Drosophila* insulin-like peptide 6; *Srl* – spargel, *rpl32* – ribosomal protein l32

3.4.3. Statistical analysis

Statistical tests were performed using Prism 8 (Version 8.4.3; GraphPad Software, LLC, San Diego, CA, USA) and SPSS (Version 21, IBM, Armonk, NY, USA). Data are presented as means ± SEM, except otherwise indicated. The data were proven for normality of distribution (Kolmogorov-Smirnov or Shapiro-Wilk) and homogeneity of variances (Brown-Forsythe or Bartlett's) and were subjected to one-way ANOVA followed by a *posthoc* test (Tukey's). Not normally distributed data were transformed (log10). In the case of heterogeneous variances, Brown-Forsythe-Welch ANOVA was performed, followed by Dunnet's T3 multiple comparison test. Significance was accepted at $p < 0.05$. Fly survival was calculated using a Kaplan-Meier approach and applying the log-rank test to determine significant differences.

3.4.4. Results

3.4.4.1. The bioactive compounds content of SALF methanolic extracts was corroborated by the HPLC analysis

The analysis of the methanolic SALF extract revealed high total phenolic and saponin contents and high antioxidant capacity (Table 14).

Table 14. Bioactive compounds content and total antioxidant capacity of methanolic extracts from boiled and dried *Solanum anguivi* Lam. fruit

VCC (mg AAE/g)	TPC (mg GAE/g)	TFC (mg QE/g)	TSC (mg DE/g)	TAC (mg QE/g)
1.84±0.25	26.01±0.83	2.16±0.01	15.8±1.18	15.3±0.10

Values are the mean ± SEM of two independent experiments measured in triplicates. Results refer to the corresponding dry weights of SALF (in g). VCC = Vitamin C content, AAE = ascorbic acid equivalent, TPC = total phenolic content, GAE = gallic acid equivalent, TFC = total flavonoid content, QE = quercetin equivalent, TSC = total saponin content, DE = diosgenin equivalent, TAC = total antioxidant capacity.

Therefore, the methanolic SALF extract was further characterized for the presence of phenolic compounds by HPLC analysis (Table 15). The highest level was found for chlorogenic acid, which potentially contributes the most to the high TAC detected in the examined SALF extract.

Table 15. Phenolic acids and flavonoids quantification of methanolic extracts from boiled and dried *Solanum anguivi* Lam. fruit

Compounds	Gallic acid	Chlorogenic acid	Caffeic acid	Rutin	Quercetin
Weight (µg/g)	35.59±0.98	4865±63.19	77.67±9.06	40.77±0.41	11.31±0.25

Values represent the mean ± SEM from two independent experiments analyzed in duplicates. Results refer to the corresponding dry weights of SALF (in g).

3.4.4.2. Both the food intake and the fly weights were not affected by supplementation with SALF

The gustatory assay showed no significant difference in food intake among all tested SALF concentrations (Figure 16a). Therefore, the two highest SALF concentrations (5 and 10 mg/ml) were selected for further experiments. By analyzing the weights of flies receiving either a control diet, HSD or HSD+SALF5 or HSD+SALF10, no significant differences between the different treatments were observed (Figure 16b).

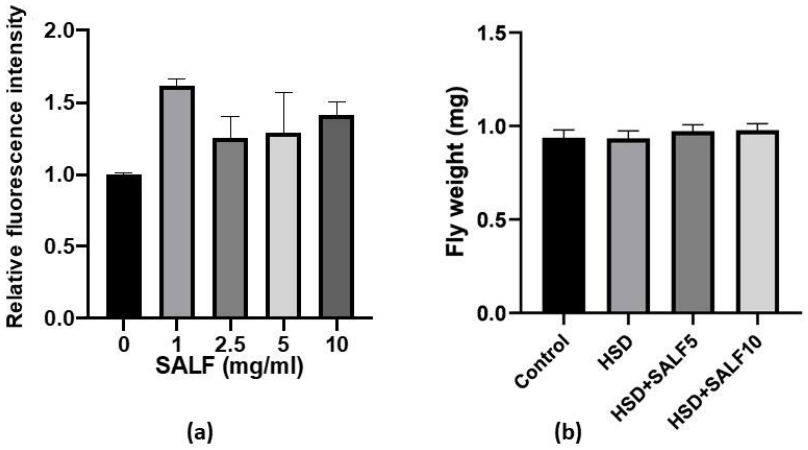


Figure 16. Food intake and weights of female w^{1118} *Drosophila melanogaster* receiving increasing amounts of dietary SALF. (a) Food intake. Flies were fed on SY10 with 0, 1.0, 2.5, 5.0 or 10 mg SALF/ml for 10 days. On day 10, flies were transferred into empty vials covered with feeder caps containing the experimental diets supplemented with 0.2% sulforhodamine B. After 9 h, 20 flies per treatment were homogenized, and fluorescence was measured. The relative fluorescence intensity of the fly homogenates represents the amount of ingested food. Bars show the mean ± SEM of three biological replicates from 20 flies, and each referred to the corresponding fly weights (n = 60). The food intake of the flies did not differ among the treatments. (b) Weights following the treatment with either control, HSD, HSD+5 mg *Solanum anguivi* fruit (HSD+SALF5) or HSD+10 mg SALF (HSD+SALF10) on day 24 of the experiment. Bars show the mean ± SEM of three biological replicates with up to 25 flies each from four independent experiments (n = 12) experiments (n = 225). The weights

of flies exposed to HSD were not significantly affected in comparison with the control-fed. Additionally, the supplementation with SALF did not significantly affect the fly weights.

3.4.4.3. A high sugar diet alone or supplemented with increasing concentrations of SALF did not affect the fitness of w^{1118} flies

Following the treatment with SALF-supplemented HSD (HSD+SALF5, HSD+SALF10), no statistically significant changes in the climbing abilities of the flies were detected. This indicates that the fly's fitness that is reflected by the climbing ability was not affected by HSD alone and SALF supplemented HSD (Figure 17a).

3.4.4.4. *Solanum anguivi* Lam. fruit supplemented HSD significantly increased the survival of w^{1118} flies

The w^{1118} flies were reared on a control diet or a HSD for ten days. Then, flies were either maintained on these diets or switched to SALF-supplemented HSDs (HSD+SALF5, HSD+SALF10) until experimental day 24.

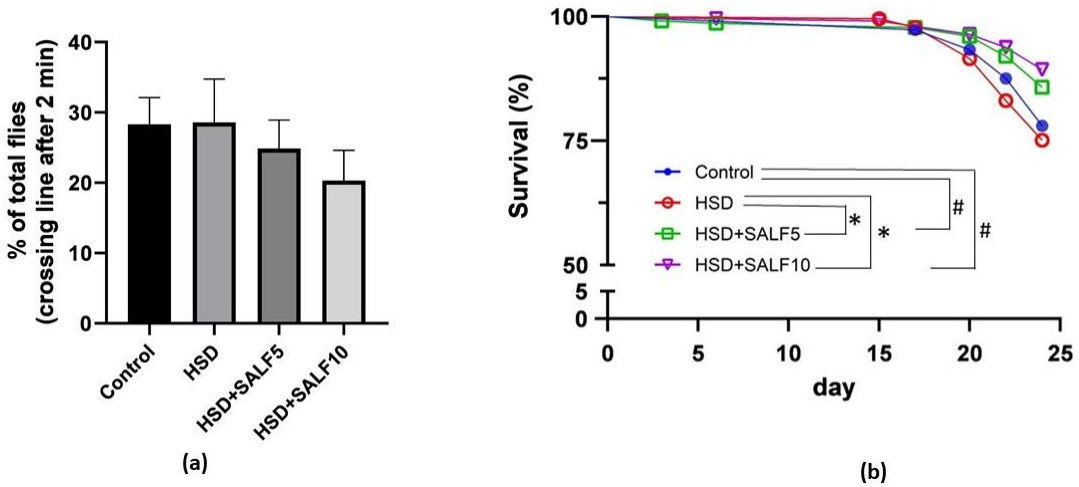


Figure 17. Climbing ability and survival rates of female w^{1118} *Drosophila melanogaster* receiving increasing amounts of dietary SALF. (a) Climbing ability. Flies were fed on a control or high sugar diet (HSD) for 10 days. Thereafter, they were either kept on a control diet or a HSD without or with 5 mg *Solanum anguivi* fruit (HSD+SALF5) or 10 mg SALF (HSD+SALF10)/ml until day 24. Subsequently, flies were subjected to a climbing assay to assess their fitness. Bars show the mean ± SEM of the percentage of flies that crossed the 5 cm line of a glass cylinder after 2 min. Experiments were performed in biological triplicates from four independent experiments (n = 12). (b) Survival rates of female w^{1118} flies fed on a control diet or HSD for 10 days. In the next step, flies were further kept on a control diet and HSD or switched to HSD+SALF5 or HSD+SALF10 until day 24. Values represent the mean of three biological replicates with 25 flies each from three independent experiments (n = 225). # indicates significant differences ($p < 0.05$) to the control group, * indicates significant differences ($p < 0.05$) to the HSD group calculated by the log-rank test.

There was no significant difference in survival of flies exposed to HSD compared to flies fed on a control diet. Supplementation of the HSD with SALF, however, significantly increased

the flies' survival. Evidently, when flies were exposed to HSD+SALF5 and HSD+SALF10, their survival was significantly increased compared to flies receiving HSD without SALF supplementation (Figure 17b).

3.4.4.5. SALF supplemented HSD significantly lowered the glucose levels while the triglyceride levels were not affected in w^{1118} flies

The w^{1118} flies were fed a control diet or a HSD for ten days. Afterwards, flies were either kept on these diets or switched to SALF-supplemented HSD (HSD+SALF5, HSD+SALF10) until day 24 of the experiment. Compared to flies fed on the control diet, HSD feeding caused a significant increase in the glucose levels from 624±37.5 µg/mg protein to 879±76.5 µg/mg protein (Figure 18a). By exposing flies to HSD+SALF10, the HSD-mediated glucose increase was significantly lowered to 656±49.8 µg/mg protein, which closely reflects the glucose levels of flies on the control diet. The lower SALF-concentration (HSD+SALF5) did not affect the HSD-induced glucose levels indicative of a potential dose-dependent effect. Contrary, the triglyceride levels were significantly unaffected in *D. melanogaster* that were exposed to either HSD, HSD+SALF5, or HSD+SALF10 in comparison to the control-fed (Figure 18b).

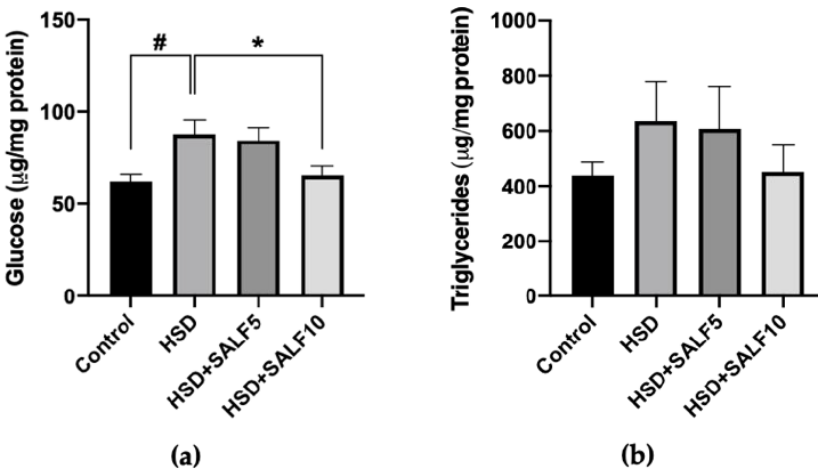


Figure 18. Glucose and triglyceride levels of female w^{1118} *Drosophila melanogaster* receiving increasing amounts of dietary SALF. Flies were fed on a control or high sugar diet (HSD) for 10 days. Following, the flies were either kept on a control diet, and HSD or HSD supplemented with 5 mg (HSD+SALF5) or 10 mg SALF (HSD+SALF10)/ml until day 24. Bars show the mean ± SEM of three biological replicates with homogenates from 5 flies per replicate and four independent experiments referred to the corresponding protein levels (n = 12). # indicates a significant difference of HSD-fed flies compared to control-fed flies ($p < 0.05$), and * indicates significant difference to HSD treated flies ($p < 0.05$) calculated by performing ANOVA followed by Tukey's post hoc test.

3.4.4.6. SALF supplemented HSD did not impair the expression of genes involved in energy metabolism

Female w^{1118} flies were exposed either to a control diet or a HSD for ten days. Next, flies were either kept on these diets or were switched to HSD+SALF5 and HSD+SALF10, respectively, until experimental day 24. Compared to control flies, HSD-fed flies showed a significant decrease in *Srl* (Figure 19c) mRNA levels while the expression levels of *dIlp3* and *dIlp6* (Figure 19a) were not affected. However, the expression of *dIlp6* (Figure 19b) was significantly increased in *D. melanogaster* exposed to HSD+SALF5, but it was not affected for HSD+SALF10. The mRNA levels for *dIlp3* and *Srl* remained unchanged for HSD+SALF5 and HSD+SALF10, in comparison to HSD-treated flies and the control (Figure 19a and c).

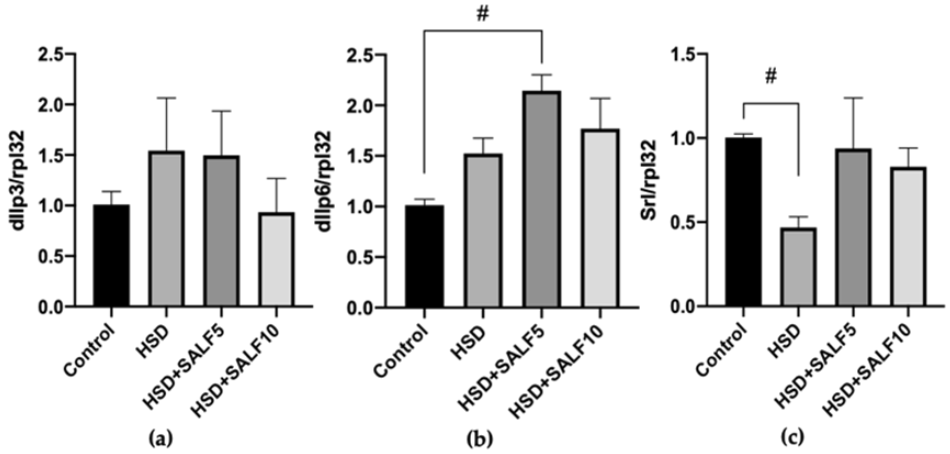


Figure 19. Relative mRNA expression of *dIlp3*, *dIlp6*, and *Srl* of female w^{1118} *Drosophila melanogaster* receiving increasing amounts of dietary SALF. (a) = *dIlp3,* (b) = *dIlp6,* and (c) = *Srl*. Flies were fed on a control or high sugar diet (HSD) for 10 days. Thereafter, the flies were either kept on a control diet, and HSD or HSD supplemented with 5 mg *Solanum anguivi* fruit (HSD+SALF5) or 10 mg SALF (HSD+SALF10)/ml until day 24. mRNA levels were determined in three biological replicates of 7 flies per replicate and three to four independent experiments (n = 9-12). Bars show the mean ± SEM. # indicates significant differences compared to control-fed flies ($p < 0.05$). The Brown-Forsythe-Welch ANOVA followed by Dunnet's T3 multiple comparison tests showed no significant difference in mRNA levels of *dIlp3, dIlp6,* and *Srl* flies exposed to HSD+SALF5 and HSD+SALF10 in comparison to HSD treated flies ($p < 0.05$).

3.4.5. Discussion

In humans, T2DM is characterized by a combination of various pathologies, including insulin resistance and dysfunctional insulin secretion, consequently leading to relative insulin deficiency and hyperglycemia (Kahn & Flier, 2000). The *D. melanogaster* can be used as a model organism in T2DM research as it is well documented that it develops a T2DM-like phenotype including hyperglycemia, insulin resistance, increased levels of triglycerides, and

fatty acids, as well as hyperinsulinemia (Musselman et al., 2011; Pasco & Léopold, 2012). In addition, a high number of genes, including those of the insulin/insulin-like growth factor signalling pathway, are conserved between fruit flies and humans. Invariably, *D. melanogaster* represents a valid model system to investigate potential treatments of T2DM (Lushchak et al., 2014). SALF is commonly consumed as vegetables in African countries and is traditionally believed to lower the risk of T2DM (Elekofehinti et al., 2012). The bioactive compounds present in SALF confer the hypothesized antidiabetic attributes of this fruit (Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015). However, there is a dearth of information on the potential therapeutic effect of dietary (whole) SALF on a T2DM-like phenotype in any model organism. Nevertheless, numerous studies have documented the glucose-lowering potential of different bioactive compounds in *D. melanogaster*, including epigallocatechin gallate (Wagner et al., 2015), gallic and tannic acid (Oboh et al., 2019) as well as extracts from the medicinal plants *Syzygium cumini* and *Bauhinia forficata* (Ecker et al., 2017) and radish sprouts (Baenas et al., 2016).

Upon exposure of the w^{1118} flies to HSD, a significant increase of their glucose levels compared to flies on a control diet was observed (Figure 18a), indicating that the flies in the present study developed hyperglycemia, thus reflecting a diabetic phenotype. Although glucose levels significantly increased with exposure to HSD, the triglyceride levels remained unchanged (Figure 18b). This contrasts results from Musselman et al. (2011), who also detected high glucose levels in Canton-S flies after HSD feeding but also high triglyceride levels. Similar results were obtained by de Aquino Silva et al. (2021) in adult *D. melanogaster* (Harwich strain), where HSD-feeding for ten days yielded significantly higher glucose and triglyceride levels. The observed inconsistencies noted in the present results compared to those given by Musselman et al. (2011) may be a methodological variation. The latter performed their triglyceride analysis in extracts of larvae instead of adult flies. Although de Aquino Silva et al. (2021) also used adult flies for their analysis, their data resulted from homogenates prepared from flies without heads, which potentially contained lower amounts of chitin and higher amounts of fat, and thus may have overestimated the effect on both, the glucose and the triglyceride levels.

Potentially too, the different fly genotypes contribute to differences in glucose and triglyceride levels as carbohydrate levels in the hemolymph as well as the total triglyceride levels differ due to the genetic variability (Reed et al., 2010). Additionally, the effects of HSD on the weight

development of fruit flies are not consistent in various studies. Compared to the present study where the weights of the flies exposed to HSD were not affected, other studies revealed either significantly lower larval (Musselman et al., 2011, 2019) or adult weights (Kahn & Flier, 2000) or significantly higher adult weights (Rovenko et al., 2014). These results indicate a highly variable impact of HSD on fly weights.

With regard to an antidiabetic property in terms of a glucose-lowering effect, supplementation of the HSD with 10 mg SALF/ml was found to significantly reduce the glucose levels in the flies, whereas 5 mg SALF was not effective (Figure 18a). A dose-dependency of SALF-extracted saponin-fractions was also reported by Elekofehinti, Kamdem, Kade, Adanlawo, et al. (2013). The authors detected a dose-dependent increase of the free radical scavenging activities *in vitro* and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the kidney and the heart of diabetic rats *in vivo*. The data from another study of the same group (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013) are consistent with this study's findings in the fruit fly. The researchers also detected significantly lower blood glucose levels in diabetic rats following oral treatment with the saponin fraction of SALF. Besides the saponins, the high amounts of phenolic compounds may also be responsible for the observed glucose-lowering effect in SALF-supplemented HSD-fed flies in this study. In particular, the relatively high levels of chlorogenic acid detected in the administered SALF (Table 15) may play an essential role in the observed glucose-lowering effect. This assumption collaborates with findings by Espejel-Nava et al. (2018) and Yin et al. (2018), who exposed diabetic mice to phenolic fractions of *Catharanthus roseus* L. stems and a methanolic extract of *Gynura divaricate*, respectively. Both plants are traditionally used in treating diabetes mellitus and contain high amounts of chlorogenic acid, possibly the main compound mediating the antidiabetic effect in these animals (Espejel-Nava et al., 2018; X. L. Yin et al., 2018).

Glucose has been associated with negative effects on age-dependent processes in different organisms (Kassi & Papavassiliou, 2008). In the model organism *Caenorhabditis elegans*, the high availability of glucose decreased the lifespan while glucose restriction significantly increased the worm's lifespan (Schulz et al., 2007). Similar effects have been documented in *D. melanogaster*, where high levels of dietary sugar significantly decreased the lifespan compared to flies receiving a diet with low levels of sugar (Y. Bai et al., 2018; Na et al., 2013). Although no significant differences in the survival between control-fed and HSD-fed flies were detected, flies exposed to a HSD supplemented with SALF (HSD+SALF5 or HSD+SALF10),

unlike their counterparts on a non-SALF supplemented diet, showed a significant increase in survival (Figure 17b). The enhanced survival may have been mediated by the phenolics present in SALF, as prolonged survival in *D. melanogaster* flies fed on hawthorn extract, which also contains chlorogenic acid and quercetin, was observed by Zhang et al. (2014). During the aging process, an impairment of the energy homeostasis occurs; thus, prompting speculation that SALF confers its protective impact partly through an effect on the fly's energy metabolism.

Although the gene expression of the *Drosophila* homolog of the PPARγ co-activator 1 α (PGC1α), *Srl*, a central player in insulin receptor signalling, energy metabolism, and mitochondrial biogenesis (Merzetti & Staveley, 2015), was decreased in the HSD-fed flies in the present study, it was unaltered in flies exposed to HSD+SALF (Figure 4c). This result is supported by the flies' fitness levels that were unchanged following either HSD, HSD+SALF5, or HSD+SALF10 treatment (Figure 17a). Various studies in fruit flies have linked an up-regulation of *Srl* mRNA levels with different health outcome variables, including improved locomotor activity, obesity, and gut integrity (Diop et al., 2015; Rera et al., 2011; Staats, Rimbach, et al., 2018; Wagner et al., 2015). Similar to results in this study, M. Do et al. (2018) reported that the weights of mice fed with high-glucose or high-fructose diets were not significantly different from those fed with a normal diet. Furthermore, being overweight has been associated with diminished fitness in general (Millard-Stafford et al., 2013), suggesting that similar fitness levels in the flies in this study may have resulted from the unaffected weights. In addition, *dIlp*s that are homologous to mammalian insulin are also central players in the fly's energy metabolism (Rajan & Perrimon, 2012). Besides *Srl*, the mRNA levels of *dIlp3* and *dIlp6* were analyzed showing a significant increase of mRNA levels of *dIlp6* in HSD+SALF5-exposed flies compared to the corresponding control flies, while no effect was exhibited in flies exposed to HSD and HSD+SALF10 (Figure 19b). In *D. melanogaster* flies, the overexpression of *dIlp6* has been shown to extend lifespan (H. Bai et al., 2012). Despite, the insignificant differences noted between *dIlp6* levels in HSD+SALF5 and HSD-exposed flies, elevated levels in the former compared to the control alludes to a possible mechanism through which HSD+SALF5 increased survival.

Limitations in the present study likely contributed to the only weak effect SALF imposed on the HSD-induced pathologies in the *D. melanogaster* w^{1118} strain. This includes the application of a non-standardized SALF preparation and the use of extracts from whole flies for analysis. Future experiments should, therefore, focus on the hemolymph glucose levels and gene

expression levels in single organs. In particular, measuring the *dIlp* expression in the brain where they are produced and released may be beneficial and would consequently provide more precise information on the potential SALF-mediated improvement of the fly's glucose metabolism.

3.4.6. Conclusion

The results show for the first time that whole SALF significantly decreased the HSD-induced glucose levels in *D. melanogaster*. These glucose-lowering attributes of SALF were not mediated through an up-regulation of central genes of the fly's energy metabolism. As energy metabolism is closely related to oxidative stress, protective effects may be mediated through an increase of endogenous antioxidants such as SOD and CAT. Therefore, future studies on the antidiabetic effects of SALF in HSD-exposed flies should also focus on its potential antioxidant effects.

3.5. A supplementation with dried *Solanum anguivi* Lam. fruit counteracted the decreased survival of *Drosophila melanogaster* exposed to a high-sugar diet [6]

Abstract

Type 2 diabetes mellitus (T2DM) is one of the main contributors to the great rise in the rate of non-communicable diseases in both developing and developed countries. *Solanum anguivi* Lam. fruit (SALF) has been traditionally recommended as a remedy for T2DM. However, it is currently not known whether these fruits have the potential to prevent the development of T2DM. This study sought to determine the potential of dietary SALF in the prevention of T2DM by using the fruit fly *D. melanogaster* as a model organism. By rearing flies on a high-sugar diet (HSD), they develop a phenotype resembling to some extent, a mammalian-like T2DM pathology. Flies were exposed either to HSD or HSD supplemented with 5 or 10 mg/ml of dried SALF to assess whether SALF affects the T2DM phenotype. Although the glucose and triglyceride levels of flies exposed to HSD+SALF in this study were not altered compared to the HSD-fed flies, supplementation with SALF significantly increased the survival of both male and female flies compared to HSD-fed flies.

Keywords**:** *Drosophila melanogaster*, survival, plant bioactives, *Solanum anguivi*, diabetes mellitus

[6] This content will be submitted to the *Food and Nutrition* Journal.

3.5.1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia, insulin resistance, and β-cell dysfunction (Chang et al., 2013; Rolo & Palmeira, 2006). The prevalence of T2DM is rising especially in low- and middle-income countries (WHO, 2021), contributing to the increasing rate of non-communicable diseases in developing countries (Chaudhury et al., 2017). Although various drugs have been approved for the treatment of T2DM, there is still a great demand for new medications due to the often limited efficacy and side effects of the common drugs (Chang et al., 2013). Strategies to prevent the development of T2DM have been reported, which include increased physical activity and healthy eating habits such as high consumption of fruits and vegetables (Salas-Salvadó et al., 2011; Wu et al., 2014). For medicinal fruits and vegetables, effects on insulin action, insulin production, or both have been suggested (Chang et al., 2013), which are attributed to the presence of bioactive compounds such as polyphenols (De Bem et al., 2018; Domínguez Avila

et al., 2017).

Solanum anguivi Lam. fruits (SALF) extracts have been suggested to ameliorate T2DM symptoms due to possession of hypoglycemic, hypolipidemic, and antioxidant properties (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011). A study in mice showed that these therapeutic effects refer to the saponins being present in SALF (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), while also phenolics, flavonoids, alkaloids, ascorbic acid, and steroids in SALF potentially contribute to these effects (Elekofehinti, Kamdem, Bolingon, et al., 2013; Oyeyemi et al., 2015). *Solanum anguivi* Lam. belongs to the family *Solanaceae* and the genus *Solanum* L (United States Department of Agriculture, 2020) and is endemic in Africa, Asia, and Australia (Bukenya, 1993; Jayanthy et al., 2016). In addition to the demonstrated therapeutic effects of SALF extracts in diabetic mice (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011), the findings in section 3.4 of this thesis documented a glucose-lowering effect of dietary SALF in *D. melanogaster* pre-exposed to a HSD. The present study aimed to investigate the potential of dietary SALF to prevent a T2DM-like phenotype in the fruit fly *D. melanogaster*. The fruit fly's energy metabolism is similar to humans (D. Chen et al., 2021; Musselman et al., 2011; van Dam et al., 2020) as the genes for the insulin/insulin-like growth factor signalling pathway are evolutionarily conserved, suggesting *D. melanogaster* as a suitable model system for T2DM research (Lushchak et al., 2014). Although it has been reported that sex affects the pathogenesis of numerous diseases, including diabetes (Chella Krishnan et al., 2018; Tramunt et al., 2020), there is a scarcity of data on whether sex affects the outcome of fruit and vegetable consumption on T2DM development. To investigate whether a SALF-supplementation sex-specifically prevents a T2DM-like phenotype, female and male *D. melanogaster* have been exposed to SALF-supplemented HSD as a sex-specific response on HSD has already been reported in the flies (Chandegra et al., 2017).

3.5.2. Material and methods

3.5.2.1. *Solanum anguivi* Lam. fruit collection and preparation

The unripe SALF (accession WP1, Figure 1) were obtained from Nabiyagi village in Mukono district (Uganda). Fruits with undamaged pericarps were boiled in water as described by Ahmed & Ali (2013) for 15 min at 100 ^{o}C. The boiled SALF was then sliced into four pieces each, and those with pests were discarded. The remaining cut fruits were air-dried (B. Master, Tauro, Camisano Vicentino, Italy) at 35 °C for 16 hours. The dried fruits were then milled (Wonder Mill, Pocatello, Idaho, USA) at the coarseness setting for fine flour. Two dried SALF

samples were obtained from two independent batches. The SALF powder was transferred into sealed plastic bottles and stored at -20 °C until further use.

3.5.2.2. Aqueous extraction and quantification of the BCC and antioxidant capacity of SALF

The bioactive compounds content (BCC) of the SALF powders were extracted as described by Kim et al. (2003), with slight modifications. Double-deionized water (20 ml) was added to SALF powder (0.2 g), and the extraction was carried out three times on the same sample, 10 min each. The obtained aqueous extracts were pooled together and subsequently analyzed for total contents of phenolics, flavonoids, saponins, vitamin C, and antioxidant capacity. The method described by Singleton et al. (1998) was followed for the determination of total phenolic content (TPC). The TPC was then calculated from a gallic acid standard curve and expressed as gallic acid equivalent (GAE). The total flavonoid content (TFC) was determined as described by Kumar et al. (2012), computed from the quercetin standard curve, and expressed as quercetin equivalent (QE). The method by Hiai et al. (1976) was applied to determine the total saponin content (TSC), which was further computed from the diosgenin standard curve, and expressed as diosgenin equivalent (DE). The vitamin C content (VCC) was determined according to the method of Omaye et al. (1979), calculated from the ascorbic acid standard curve, and expressed as ascorbic acid equivalent (AAE). The total antioxidant capacity (TAC) was analyzed by applying the method described by Brand-Williams et al. (1995), estimated from the quercetin standard curve and expressed as QE. The absorbances were measured using a UV-VIS Photometer (UV Mini-1240, Shimadzu, Duisburg, Germany) with deionized water as blanks. All quantities were expressed on a dry weight (DW) basis.

3.5.2.3. Fly strains and husbandry

The male and female *D. melanogaster* wild-type strain w^{1118} (Bloomington *Drosophila* Stock Center, Indiana, USA; #5905) were used in the experiments. Flies were kept in a humidified (60%) and temperature-controlled (25 °C) incubator (Memmert, HPP400, Buechenbach, Germany), with a 12 h/12 h light-dark cycle on standard fly medium (CT) according to Wagner et al. (2015). Age-matched flies were obtained from synchronized eggs as described by Linford et al. (2013) with slight modifications. Three day-old flies were then separated according to their sex and then transferred into vials containing the corresponding experimental medium. In this study, medium consisting of 10% sucrose (Carl Roth, Karlsruhe, Germany), 10% inactive yeast (Genesee via Kisker), and 2% agar (Apex via Kisker, Steinfurt, Germany) were used as

the control diet [SY10 (Linford et al., 2013)]. Tegosept (Apex via Kisker) (1.5%) and propionic acid (Carl Roth) (0.3%) were added as preservatives to the medium. The flies were exposed to a high sugar diet (HSD), i.e., the SY10 medium with an increased sucrose content (30%), to induce a T2DM-like phenotype. To investigate whether SALF affects survival, weight, climbing ability, glucose, protein and triglyceride contents, HSD was supplemented with either 5 or 10 mg/ml SALF. These SALF concentrations in HSD were safe and did not affect the food intake of the flies, as documented in section 3.4.

3.5.2.4. Experimental design

A T2DM-like phenotype was induced by feeding the flies on the HSD for 24 days. To investigate the potential of SALF to prevent a T2DM-like phenotype, HSD was supplemented with SALF concentrations that were safe and had no effect on the food intake of the flies (section 3.4 of this thesis), that is, 5 mg SALF (HSD+SALF5) or 10 mg SALF (HSD+SALF10) for 24 days. SY10-fed flies served as controls (also fed for 24 days). To investigate the influence of sex on the *D. melanogaster* responses to the treatments, age-matched male and female flies were randomly selected and separately sorted into medium-containing vials. Each treatment was performed in triplicate with 25 flies per vial. Flies were transferred to vials with freshly prepared medium every 2-3 days. The whole experiment was repeated four times.

3.5.2.5. Survival

The survival of male and female w^{1118} flies was documented over the whole study period of 24 days. The number of dead flies per vial was recorded every 2-3 days when flies were transferred to vials with fresh medium.

3.5.2.6. Climbing assay

At the end of each experiment, the climbing ability was carried out as described by Madabattula et al. (2015) to measure the fitness of the flies. The climbing ability was calculated as the percentage of flies having crossed the target line after 2 min.

3.5.2.7. Weights of the w^{1118} flies

The weights of the flies were taken at the end of the study (on day 24). The flies were transferred into a pre-weighed empty vial and weighed. The mean weight of a single fly was calculated by dividing the total fly weights by the number of flies per vial.

3.5.2.8. Determination of glucose, triglycerides, and protein in whole fly lysates

The w^{1118} male and female flies fed on different experimental diets were analyzed for their content of glucose, triglycerides, and protein. Whole-body extracts were obtained from flies for each treatment as described in section 3.4.2.10 of this thesis. The samples were analyzed for glucose, triglyceride, and protein levels using the Glucose GOD-PAP assay, the Triglyceride GPO-PAP assay (both from DIALAB, Wiener Neudorf, Austria), and the Roti-Quant® universal kit (Carl Roth), respectively, according to the manufacturers' instructions. The protein levels were used for the normalization of the triglyceride and glucose levels. The analyses were carried out in duplicates of the three replicates per treatment and from all four independent experiments. The absorbances were measured in a plate reader (Digi Scan 400, Asys Hitech GmbH, Seekirchen am Wallersee, Austria).

3.5.3. Statistical analysis

The statistical analyses were performed using Prism 8 (Version 8.4.3; GraphPad Software, LLC, San Diego, CA, USA) and SPSS (Version 21, IBM, Armonk, NY, USA). Data are presented as means ± SEM, except otherwise indicated. The data were proven for normality of distribution (Kolmogorov-Smirnov or Shapiro-Wilk) and homogeneity of variances (Brown-Forsythe or Bartlett's) and were analyzed by applying one-way ANOVA followed by a *posthoc* test (Tukey's). The data that was not normally distributed was transformed using log10. The significance was accepted at $p < 0.05$. Fly survival was calculated using a Kaplan-Meier approach and applying the log-rank test to determine significant differences**.**

3.5.4. Results

3.5.4.1. Bioactive compounds content and total antioxidant capacity of SALF aqueous extract

The results showed that the water extract of boiled and then dried SALF contained substantial amounts of phenolics, flavonoids and saponins (Table 16).

Table 16. Bioactive compounds content and total antioxidant capacity of aqueous extracts from boiled and dried *Solanum anguivi Lam.* fruit

VCC (mg AAE/g)	TPC (mg GAE/g)	TFC (mg QE/g)	TSC (mg DE/g)	TAC (mg QE/g)
0.45±0.2	30.91±0.42	3.12±0.07	6.93±0.41	17.22±3.11

Values are the mean ± SEM of two independent experiments measured in triplicates. Results refer to the corresponding dry weights of SALF (in g). VCC = Vitamin C content, AAE = ascorbic acid equivalent, TPC = total phenolic content, GAE = gallic acid equivalent, TFC = total flavonoid content, QE = quercetin equivalent, TSC = total saponin content, DE = diosgenin equivalent, TAC = total antioxidant capacity.

3.5.4.2. A high sugar diet alone or supplemented with increasing concentrations of SALF did not affect the weights and climbing ability of w^{1118} flies

The weights (Figures 20a and b) and climbing ability (Figure 20c) of male and female flies receiving either a control diet, HSD or HSD+SALF5, or HSD+SALF10 did not differ significantly.

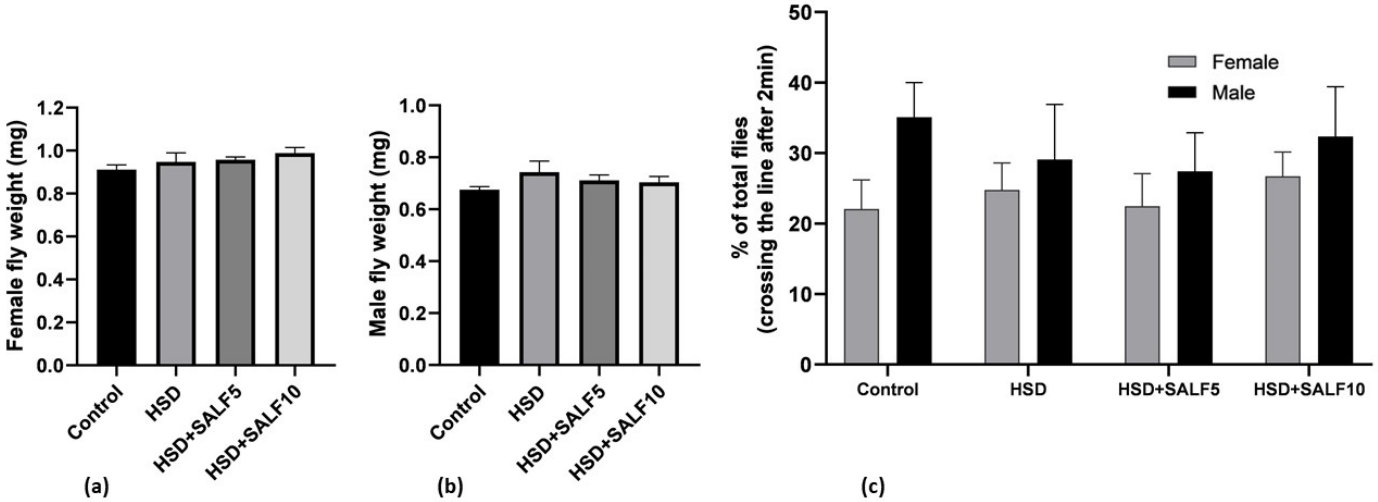


Figure 20. Weights and climbing ability of female and male w^{1118} *Drosophila melanogaster* fed on receiving increasing amounts of SALF via the diet. The flies were fed on a control diet, HSD, or HSD supplemented with *Solanum anguivi* Lam. fruit concentration of 5 mg (HSD+SALF5) or 10 mg (HSD+SALF10)/ml until day 24. Subsequently, the weights of (a) female (b) and male flies, as well as the (c) the climbing ability of the male and female flies, were determined. Bars show the mean ± SEM of weights (Figures a and b) and the percentage of flies that crossed the 5 cm line of a glass cylinder after 2 min (Figure c). Experiments were performed in biological triplicates from four independent experiments (n = 12). The weights and fitness of flies exposed to the four treatments did not differ significantly.

3.5.4.3. Supplementation with SALF significantly increased the survival of both male and female w^{1118} flies reared on HSD

The w^{1118} flies were reared on a control diet, HSD or SALF-supplemented HSDs

(HSD+SALF5, or HSD+SALF10) until experimental day 24. Female flies fed on either HSD+SALF5 or HSD+SALF10 had significantly higher survival than flies fed on HSD only (Figure 21a). There was, however, no significant difference in survival of flies fed on HSD supplemented with either 5 or 10 mg/ml SALF. Comparison with control flies showed that the survival of female flies exposed to HSD was not significantly different, while that for female flies exposed to HSD+SALF5 and HSD+SALF10 was significantly higher.

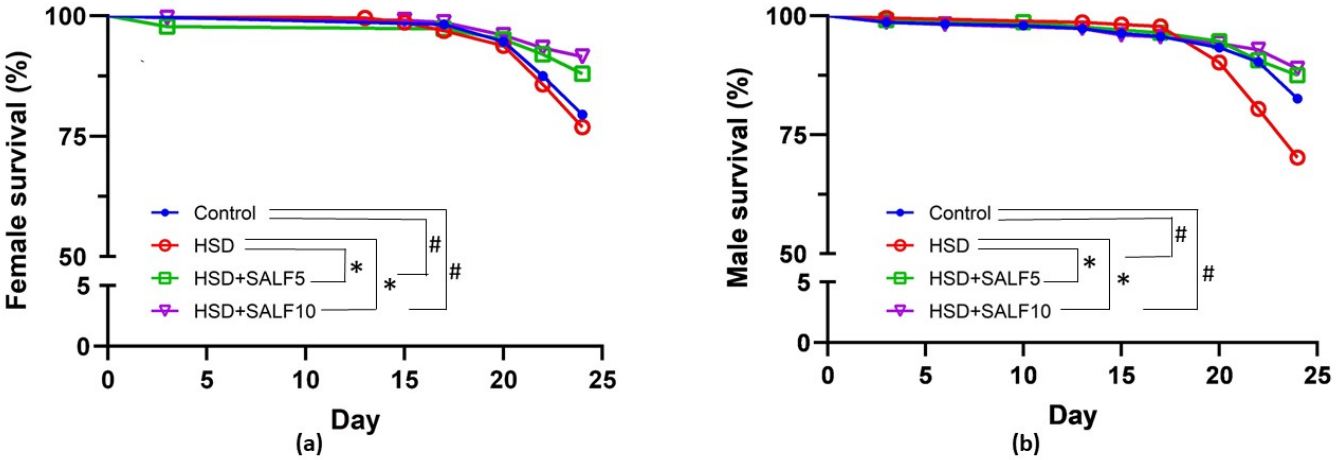


Figure 21. Survival rates of female and male w^{1118} *Drosophila melanogaster* receiving increasing amounts of dietary SALF until day 24. Flies were exposed to either a control diet, HSD, or HSD supplemented with 5 mg (HSD+SALF5) or 10 mg (HSD+SALF10)/ml SALF until day 24. Values represent the mean ± SEM of three biological replicates with 25 flies each from three independent experiments (n = 225). # indicates significant differences ($p < 0.05$) in comparison with the control group, * indicates significant differences ($p<0.05$) compared to the HSD group, calculated by the log-rank test.

The survival of male flies exposed to HSD+SALF5 and HSD+SALF10 was significantly higher compared to HSD-only flies, while the survival of male flies exposed to either HSD+SALF5 or HSD+SALF10 did not differ significantly (Figure 21b). In comparison to flies on a control diet, the survival of male flies exposed to HSD-only significantly decreased while male flies fed on HSD+SALF10 exhibited a significantly increased survival. There was no significant difference in survival between male flies on a control diet and HSD+SALF5.

3.5.4.4. SALF did not affect the glucose and triglyceride levels in HSD-fed w^{1118} flies

The w^{1118} flies were either fed a control diet, HSD, HSD+SALF5, or HSD+SALF10 until day 24. Compared to flies exposed to the control diet, HSD feeding with or without SALF supplementation did not alter glucose (Figures 22a and b) and triglyceride (Figures 22c and d) levels in both males and females.

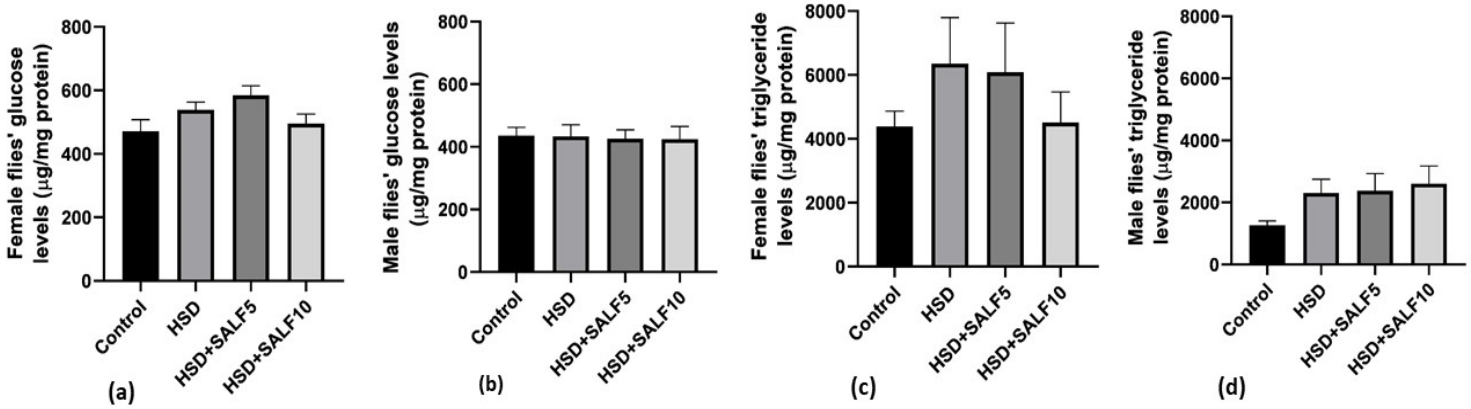


Figure 22. Glucose and triglyceride levels of female and male w^{1118} *Drosophila melanogaster* receiving increasing amounts SALF-supplemented HSD. Glucose levels of female (a) and male (b) flies and triglyceride levels of female (c) and male (d) flies reared on either a control diet, HSD, HSD+SALF5, or HSD+SALF10 until day 24. Bars show the mean ± SEM of three replicates from four independent experiments referred to the corresponding protein levels (n = 12). Glucose and triglyceride levels of male and female flies exposed to the 4 treatments did not differ significantly (analyzed using ANOVA followed by Tukey's *post hoc* test).

3.5.5. Discussion

Chronic over-nutrition has been shown to cause chronic hyperglycemia that can gradually induce insulin resistance and insulin secretion impairment, which are characteristics of T2DM in humans (Yan, 2014). The fruit fly, *D. melanogaster,* is a versatile and potent model used in recent years for studies in nutrition, metabolism, and metabolic disorders, including T2DM. The *D. melanogaster* insulin signalling has been demonstrated as highly conserved, both structurally and functionally, in molecular and genetic studies (Murillo-Maldonado & Riesgo-Escovar, 2017). The development of a T2DM-like phenotype upon exposure to HSD has been demonstrated in *D. melanogaster*, including; hyperglycemia, insulin resistance, hyperinsulinemia, and elevated triglycerides and fatty acids levels (Musselman et al., 2011; van Dam et al., 2020), making it a suitable model for nutrition-related diabetes research.

Commonly consumed as a vegetable, SALF has been traditionally believed to reduce the risk of T2DM (Denton & Nwangburuka, 2011; Elekofehinti, Kamdem, Bolingon, et al., 2013). The therapeutic effects of SALF extracts have been reported in diabetic mice (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011), while results in section 3.4 of this thesis documented the therapeutic effects of dietary (whole) SALF in *D. melanogaster* exposed to HSD. The therapeutic effects were suggested to be due to the bioactive compounds present in SALF, such as saponins (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013) and phenolics. However, data on the potential of dietary SALF to prevent the pathogenesis of T2DM-like

phenotype in any model organism is scarce.

Compared to the present study where the weights of the flies exposed to HSD were not affected (Figure 20a and b), other studies have reported significantly lower larval (Musselman et al., 2011, 2019) and adult weights (Kahn & Flier, 2000), respectively, or a significant increase in weight of adult flies exposed to HSD (Rovenko et al., 2014) indicating a high variability of the effect of HSD on fly weights. Weight gain and overweight have been linked to impaired overall fitness (Millard-Stafford et al., 2013), which is defined as climbing ability in the present study. The fitness of the male and female flies exposed to the four experimental treatments was also not altered (Figure 20c), which may result from the unaffected weights upon exposure to the four treatments. These findings are similar to results obtained in section 3.4 (Figures 16b and 17a) of this thesis.

One of the detrimental consequences of metabolic dysfunctions, including T2DM in humans, is increased premature mortality compared to healthy subjects (WHO, 2021). The association of glucose with negative effects on age-dependent processes has been reported in various model organisms, including *Caenorhabditis elegans* (Schulz et al., 2007) and *D. melanogaster* (Y. Bai et al., 2018; Lushchak et al., 2014; Na et al., 2013; van Dam et al., 2020), where the lifespan significantly decreased due to the exposure of high levels of dietary sugar. A study by van Dam et al. (2020), however, demonstrated that the observed decrease in survival of HSD-exposed *D. melanogaster* was not related to insulin resistance and/or hyperglycemia but rather resulted from dehydration induced by a high intake of sugar. The findings by van Dam et al. (2020) were based on female flies exposed to dietary sugar concentrations of 20% and 5% for HSD and control diet, respectively, which were lower than concentrations to which females in the present study were exposed (30% and 10% sugar in HSD and control diet, respectively). In the present study, the survival of the female flies on a control diet on day 24 was not significantly different from those fed a HSD (Figure 21a). In contrast, van Dam et al., (2020) further reported a significantly lower survival female flies fed on HSD with a similar sucrose concentration as the present study (30% sucrose) as compared to female flies on a control diet (5% sucrose diet), suggesting that the survival of the HSD-fed female flies in the present study was not linked to dehydration. The observed differences may result from the differences in the genotype (Dahomey versus w^{1118}), and the diet composition as an interaction between diet and genotype has been shown to significantly affect the survival of *D. melanogaster* (Reed et al., 2010). The significantly increased survival of female flies exposed to HSD+SALF5 and HSD+SALF10

compared to HSD alone (Figure 21a) may therefore not be linked to a prevention of dehydration. The results obtained for survival of female flies are similar to those in section 3.4 (Figure 17b).

Male flies exposed to HSD, however, exhibited a decreased survival compared to control-fed flies (Figure 21b). The tolerance of female flies towards HSD suggests sexually dimorphic effects on the survival of flies in the present study, as similarly reported by Chandegra et al. (2017). Interestingly, male flies exposed to HSD+SALF5 and HSD+SALF10 were significantly protected from the HSD-mediated decrease in survival (Figure 21b), demonstrating that the SALF induced survival was not sex-specific. The expected decrease in survival of HSD-fed flies may be to some extent comparable to the observed premature death in humans with T2DM (WHO, 2016), indicating that SALF may prevent T2DM-like pathological outcomes. The protective effect of SALF may partly be due to the presence of high phenolic content (Table 16), as reported in *D. melanogaster* flies exposed to phenolic-rich hawthorn extract (Zhang et al., 2014). The protective effect of SALF may also be mediated through the prevention of oxidative stress that potentially results from HSD exposure in *D. melanogaster* (Ecker et al., 2017). As also the saponin fraction of SALF has been reported to exhibit antioxidant and antiperoxidative properties in diabetic mice through the restoration of catalase and superoxide dismutase (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013) the observed protective effects of SALF in the present study (Table 16) may have been – at least partly – mediated through the present saponins.

Exposure of *D. melanogaster* adult flies to HSD has been reported to result in elevated hemolymph glucose levels in adult flies (Na et al., 2013) and larvae (Musselman et al., 2011). The glucose levels of both male and female flies exposed to HSD in the present study did not differ from the control-fed flies (Figure 22). These differences may be because the glucose levels in the present study were analyzed in whole-body extracts of the flies while Na et al., 2013 detected the glucose levels in the hemolymph. Similar to the present findings, Morris et al. (2012) reported that the glucose levels in whole-body extracts of HSD-fed (containing 30% sucrose) *D. melanogaster* were unaffected. However, Morris et al. (2012) observed that the insulin activity in HSD-fed flies decreased, reflecting an insulin-resistant phenotype. As in humans, chronic hyperglycemia has been suggested to result in insulin resistance (Giri et al., 2018). The HSD-fed flies in the study conducted by Morris et al. (2012) may have developed a hyperglycemic state which was, however, not detectable in whole fly extracts but may have

been present by analyzing the hemolymph of the flies (Musselman et al., 2011; Na et al., 2013). This may also be true for the present study, as glucose levels were analyzed in whole fly lysates rather than the hemolymph.

In contrast to the present study where the triglyceride levels were not affected either by any treatment or by sex (Figure 22), elevated triglyceride levels have been reported in *D. melanogaster* larvae (Musselman et al., 2011) and adult flies (de Aquino Silva et al., 2021; van Dam et al., 2020). While the developmental stage of the fly potentially affects the triglyceride levels, the dietary composition of the HSD (20% sucrose) (van Dam et al. 2020) may also affect the triglyceride levels. On the other hand, de Aquino Silva et al. (2021) analyzed the homogenates prepared from flies without heads, which may have contained lower amounts of chitin and thus resulting in higher triglyceride levels in adult flies, making it difficult to compare the results with the present study.

The present study also had some limitations, including the use of extracts from whole flies for glucose and triglyceride analysis, as well as the application of a non-standardized SALF preparation. Future experiments should, therefore, focus on hemolymph glucose levels and the application of a standardized SALF preparation to investigate a potential protective effect on T2DM-like phenotype in *D. melanogaster*.

3.5.6. Conclusion

Although a hyperglycemic state, which has been connected to increased T2DM-induced mortality in humans, was not detected in the flies of this study, the exposure of male and female flies to HSD supplemented with SALF resulted in a higher survival rate on day 24 compared to HSD-fed and control diet-fed flies. This showed that supplementing a HSD with SALF protected against the HSD-mediated reduced survival, which may be due to its high contents of phenolics and saponins. However, more studies are needed to elucidate further the underlying mechanism(s) through which SALF may prevent the pathogenesis of a T2DM-like phenotype.

CHAPTER FOUR

GENERAL DISCUSSION

4.1. Discussion of materials and methods

Traditionally, SALF was believed to alleviate and treat T2DM, hence, its selection for this study. However, there is scanty scientific evidence regarding this concept.

4.1.1. Study area

In Uganda, *Solanum anguivi* Lam. reportedly occurs in various districts, including Masaka, Mpigi, Mukono, and Kampala (Stedje and Bukenya-Ziraba, 2003). Although SALF is present in Kampala, where the laboratory analyses were conducted (Makerere University), it is mainly an urban setting. Thus, the likelihood of finding various accessions of SALF in one village in Kampala would be limited. Conveniently, SALF is prevalent in Mukono district, which is near Kampala and yet less urban, hence the most preferred study area. Proximity to Makerere university was crucial to prevent postharvest deterioration of the bioactive compounds. Nabiyagi village (GPS 0.472336, 32.802484) was randomly selected from Mukono district as the study area. Subsequently, a quadrat random sampling technique was used to identify the occurrence of *Solanum anguivi* Lam. accessions. Fourteen *Solanum anguivi* Lam. accessions occurring in the study area were identified with reference to their documented (Bukenya & Carasco, 1995) phenotypic characteristics.

4.1.2. Morphological characterization

The morphological characteristics of the leaves, stems and fruits from fourteen *Solanum anguivi* L. accessions were studied (section 3.1). Morphological characterization was carried out on the same day the samples were collected from the plants to ensure an accurate analysis. Due to the absence of standardized descriptors of *Solanum anguivi* L., the morphological characteristics in this study were based on descriptors of tomatoes and eggplants (ECPGR, 2008; Menda et al., 2004; Minoia et al., 2010; Solanke & Kumar, 2013), with modifications. The modifications were based on the characteristics observed on the accessions. They included the addition of "ovoid" in the fruit shapes, fruit top (area of the scar left by the style) appearance, the average number of fruits per twig (cluster), and the description of the venations (stripes) on fruits based on quantity and type. The fruit size description was also modified based on the reported SALF diameter of 1-2 cm by Stedje & Bukenya-Ziraba (2003). The fruit sizes

were grouped as very small if less than 1 cm and large if greater than 1.8 cm.

4.1.3. Bioactive compounds analyses

The study determined the BCC and AA of SALF accessions (section 3.1), as affected by the ripeness stage (section 3.2), as well as the thermal treatments (section 3.3). The BCC and AA were also analyzed in the SALF used in the therapeutic (section 3.4) and prevention (section 3.5) of T2DM-like phenotype studies. For quantification of BCC, liquid chromatography methods such as ultra-performance liquid chromatography (UPLC) and high-performance liquid chromatography (HPLC), have been reported as the most accurate. Unlike other methods, including spectrophotometry, UPLC accurately quantifies the content of a bioactive compound and analyses individual compounds using standards instead of estimating values based on reagent reactions (Way et al., 2020). Although UPLC has a shorter analysis time and sensitivity than HPLC and is the most accurate method, both methods are very time-consuming, expensive, and impractical compared to spectrophotometer methods (Raczkowska et al., 2011; Revathi et al., 2011; Way et al., 2020). Thus, due to limited funds and access to UPLC equipment, HPLC analyses were only carried out to quantify the phenolic compounds in this study (Tables 4, 7, 10, and 15). Although spectrophotometry is less superior to liquid chromatography, the former is the most commonly employed technique based on its simplicity, accuracy, and chemical specificity (Mantri et al., 2017). Ultraviolet spectrophotometry was, therefore, used for the quantification of SALF total contents of phenolics, flavonoids, saponins, vitamin C, and AA, while gravimetry was employed for total alkaloid content. The discussion below focuses on methods other than liquid chromatography, which are commonly used for BCC analyses.

The TPC determination by the method of Singleton et al. (1998) gave very stable results. The protocol was based on the reduction of phosphotungstate–phosphomolybdate complex in Folin-Ciocalteu reagent (FCR) by phenolics, thereby resulting in a blue colour of the reaction products. This principle is similar to the method by Makkar (2003). However, a white precipitate was formed when the method by Makkar (2003) was used in this study, possibly due to the higher concentration of FCR to which sodium carbonate is added in the method. Although the filtration using a filter paper would have removed the precipitate to allow reading of the absorbances, some of the blue colour from the reaction was likely adsorbed to the filter paper, hence the inaccurate results. Additionally, the filtration step was tedious given the large number of samples in this study, as compared to the method by Singleton et al. (1998).

The TFC was determined following the $AlCl_3$ colorimetric method described by Kumar, Rajkapoor, & Perumal (2012). The basic principle is that $AlCl_3$ forms complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols (Bag et al., 2015). Furthermore, $AlCl_3$ forms acid-labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids (Bag et al., 2015). Another commonly used method, as described by Pękal & Pyrzynska (2014), also used $AlCl_3$ and other chemicals different from the method by Kumar, Rajkapoor, & Perumal (2012). Comparison of the two methods by Pękal & Pyrzynska (2014) showed that the first method by Kumar, Rajkapoor, & Perumal (2012) may be selective for flavonols (quercetin, morin, kaempferol, and rutin) and flavones luteolin while the second method by Pękal & Pyrzynska (2014) seemed specific for rutin, luteolin, and catechins. Although the methods are still the most used by researchers, they may not adequately quantify TFC as they are dependent on the flavonoid structures (Pękal & Pyrzynska, 2014). However, the former method probably offers a more accurate measure of flavonoids due to a higher number of flavonoid compounds accounted for than the latter, and it was hence the preferred method in this study. Furthermore, the former method quantifies both quercetin and rutin, which have been confirmed present in SALF (Elekofehinti, Kamdem, Bolingon, et al., 2013), while the latter may not include the quercetin content.

The TSC was determined using the vanillin-sulfuric acid assay method by Hiai, Oura, & Nakajima (1976). Basically, sulphuric acid-oxidized triterpene saponins react with vanillin, resulting in a distinctive red-purple colour (Le et al., 2018). Another method where crude saponin fractions were obtained in n-butanol also has been reported (Benyong et al., 2014; Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013). However, the method is very time-consuming and tedious (J. Zhang & Qu, 2013) and therefore unsuitable for analyzing many samples. Simultaneously, very large amounts of dried sample powders are required for the crude extraction, which were not available for most of the samples in this study. The method by Hiai, Oura, & Nakajima (1976) has been adopted by many scientists as it is fast, simple, and cheap (Le et al., 2018), which is why it was preferred for this study.

The TAL was determined by gravimetry following the Harborne (1973) method. Although gravimetry has been reported to provide very accurate results (N. Singh et al., 2013), this was a very time-consuming and tedious method. Unlike spectrophotometry, gravimetry only analyzes a single bioactive compound at a time (N. Singh et al., 2013), and yet it also requires a larger quantity of the sample. However, due to limited funds, it was not possible to obtain the

reference standard, atropine, which was required for the spectrophotometric analysis of total alkaloids.

The VCC was determined according to the method of Omaye et al. (1979). This procedure gives the sum of L-ascorbic acid and dehydroascorbic acid. Firstly, the L-ascorbic acid is oxidized to dehydroascorbic acid and diketogulonic acid by copper sulphate (Avdeeva & Gvozdev, 2020; Omaye et al., 1979). These products are then treated with 2,4-dinitrophenylhydrazine (DNPH) to form the derivative bis-2,4-dinitrophenylhydrazone (Omaye et al., 1979), whose red colour is developed when a strong acid (H_2SO_4) is added (Schaffert & Kingsley, 1955). The reaction took place in the presence of thiourea, which provided a mild reducing medium to prevent discolouration of DNPH by oxidants (Roe & Kuether, 1943). Trichloroacetic acid was used as described in the method to precipitate any protein present; however, none was formed for all the SALF extracts in this study. Noteworthy, the DTC (DNPH, thiourea, and copper sulphate) reagent was left to stand overnight and then filtered with filter paper (Schaffert & Kingsley, 1955) before use due to the presence of an emulsion, which would have interfered with the absorbances. Although Schaffert & Kingsley (1955) proposed a more rapid and simple method, it involved the use of very high temperature (100 ^{o}C) for the water bath, which may have negatively affected the heat-sensitive vitamin C. Furthermore, the use of 37 ^{o}C instead of 100 ^{o}C would avert the possible interference by ascorbic acid-2-sulphate (Omaye et al., 1979). The titrimetry has also been reported for vitamin C quantification; however, spectrophotometry is more reproducible (Al-Ani et al., 2007).

The HPLC analyses were conducted to determine the contents of selected phenolic compounds previously reported to be present in SALF by Elekofehinti, Kamdem, Bolingon, et al. (2013). The HPLC analysis was only carried out for dried samples, excluding the control and cooked SALF samples, due to the time-consuming logistics involved in shipping the extracts from Uganda to Germany, where the HPLC analyses were performed. Additionally, shipping undried SALF samples would require first freezing, which is expensive and yet may have affected the phenolic contents (Khattab et al., 2015) of the samples. Due to limited resources, only one independent sample per treatment was analyzed in sections 3.1, 3.2, and 3.3, and therefore, statistical analyses were not possible. However, the results gave an overview of how the contents of the phenolic compounds varied among the SALF accessions (section 3.1) and how they were affected during ripening (section 3.2) and by drying temperature (section 3.3).

4.1.4. Fly experiments

To investigate the SALF efficacy in T2DM prevention and management, the use of human subjects was not possible in this study due to the ethical issues and high costs involved. Additionally, plant bioactive compounds sometimes impair various body processes such as gene expression; the complexity of these interactions justifies the use of more accessible model organisms to elucidate these mechanisms (Rubio-Aliaga, 2012). The fruit fly *D. melanogaster* has been recently used by various researchers in studies related to the pathogenesis and treatment of T2DM (Y. Bai et al., 2018; Na et al., 2013; van Dam et al., 2020). Although *D. melanogaster* does not develop T2DM, its similar energy metabolism, a high number of genes conserved between fruit flies and humans, and its development of T2DM-like phenotype upon HSD-intake (Morris et al., 2012; Musselman et al., 2011; Skorupa et al., 2008) made it a suitable model for this study. The available literature relating SALF to T2DM only alluded to the therapeutic effects of its extracts in diabetic mice (Elekofehinti, Kamdem, Kade, Rocha, et al., 2013; Seble, 2011) while the potential of SALF to prevent the pathogenesis of T2DM has not been reported. Since SALF is usually consumed as a vegetable (Bukenya-Ziraba, 2004), it was crucial to investigate its potential to prevent and/ or manage T2DM-like phenotype in *D. melanogaster* using dietary (whole) SALF.

To examine whether the potential protective effects of dietary SALF-supplementation in HSD were sex-dependent, the documented sexually dimorphic responses of *D. melanogaster* upon HSD-intake (Chandegra et al., 2017) further qualified it as a suitable model. Flies fed on HSD+SALF were compared to HSD-fed and control (SY10)-fed flies to examine whether the consumption of SALF prevented (section 3.5) and/ or managed (section 3.4) T2DM-like characteristics. SALF accession WP1 was the most prevalent accession and thus selected for the fly experiments (sections 3.4 and 3.5). The concentration of SALF supplemented in HSD was determined (5 and 10 mg/ml SALF) (Figures 16a and 24a) and exposed to both male and female flies. Preliminary experiments for this study showed that upon exposure to HSD, 70% of the males died by day 24. Thus, to retain flies for the analyses (such as climbing assay, weights, body composition, and mRNA levels of genes associated with energy metabolism) after exposure to HSD, the duration of the experiments was selected as 24 days. Since glucose is excreted into the fly's hemolymph, the glucose levels may be more detectable in hemolymph as observed by Musselman et al. (2011) and van Dam et al. (2020) than whole-body extracts. Further, determining the *dIlp* levels in the organs containing the IPCs that secrete them, such as the brain and fat body for *dIlp3* and *6*, respectively (Nässel et al., 2013), may yield more

accurate results. However, due to the limited number of flies by day 24 of the fly experiments, only one extract per sample was possible, from which the glucose, triglyceride, protein, and mRNA levels of *Srl* and *dIlp3* and *6* were quantified. Thus, whole-body extracts rather than hemolymph or removal of body organs for mRNA quantification were opted for and subsequently analyzed for body composition and mRNA levels.

4.2. Discussion of the results

The protective properties of fruits and vegetables have been attributed to the presence of bioactive compounds, whose content and activity may be influenced by various factors (Grandón et al., 2013, Jansen et al., 2009, and Tolić et al., 2017). In this study, the BCC and AA of SALF varied with the accession (Tables 3, 5, 6, and 9), ripeness stage (Tables 6 and 9), the season of harvest (Tables 6 and 9 and Figures 9-14), and thermal treatments (Figures 9-14). Concurrently, the morphological characteristics of *S. anguivi* also varied with accession and may be used for the prediction of SALF accessions with similar BCC and AA (section 3.1). Accession GP1 had the highest AA in this study (sections 3.1, 3.2, and 3.3) and may thus have the highest health benefits. Although the BCC and AA of SALF were also significantly affected by ripening, this effect significantly depended on the accession. Overall, the unripe stage possessed the highest TPC, TFC, and AA, which was consistent with the findings by Abbe et al. (2019) for SALF TPC and AA, suggesting that it was the ripeness stage that would mediate the most health benefits upon consumption. Although the AA in this study, which was based on free radical scavenging capacity, is crucial for the prevention and/or management of diseases associated with oxidative stress, SALF bioactive compounds may exhibit their antioxidant potentials via other mechanisms such as iron chelation, which were not explored in this study. Hence, the ripeness stages that were rich in other BCC in this study are noteworthy. The orange stage had the highest total saponins and was also rich in flavonoids, while the red stage was rich in vitamin C (for most accessions) and alkaloids. Furthermore, the HPLC results revealed the dynamics in phenolic compounds as the fruits ripened. Specifically, the contents of chlorogenic and caffeic acids reduced, gallic acid and rutin increased, while quercetin varied among the accessions. Interestingly, a comparison with foods that are documented rich sources of the investigated BCC in this study showed that all the SALF accessions and all the ripeness stages were comparably rich in all the BCC (Tables 3 and 6).

The possibility of different effects of thermal treatments on the BCC and AA of unripe and ripe (red) SALF in section 3.3 was investigated in the two accessions that had registered the highest

and least AA (GP1 and WP1, respectively) in section 3.1. Drying led to higher TPC and AA of SALF (highest at 35 ^{o}C). However, cooking resulted in a higher TPC and AA than drying, with the highest values recorded in boiled than steamed SALF. A combination of boiling (for 15 min) and then drying (at 35 ^{o}C) further increased the AA of SALF (Table 14) compared to drying only (Figure 14). Interestingly, the AA of SALF that was boiled and then dried was similar to that of boiled-only SALF. Simultaneously, boiled and then dried SALF contained higher TPC and TFC than SALF that was boiled or dried only, and higher chlorogenic acid, caffeic acid, and rutin contents than dried-only SALF (Tables 14 and 15, and Figures 9, 10, and 14, and Table 10). Although alkaloid and saponins may have antidiabetic properties (Cao & Su, 2019; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013), their toxicity has been a concern in recent years. High alkaloid levels in some *Solanum* species have been reported as toxic (Bagheri et al., 2017). The unripe stage, which had the highest AA, also had the least alkaloid levels and may therefore be the safest ripeness stage for SALF consumption. Furthermore, when the unripe SALF was dried at 35 ^{0}C, the TAL decreased significantly, while boiling for 15 min led to significantly lower TAL than prolonged cooking time. As high levels of saponins may also be toxic (Wina et al., 2005), it is interesting to note that drying unripe SALF at 35 ^{0}C lowered its TSC levels too, and while 15 min of boiling left the contents unaltered. Further, boiling (for 15 min) and then drying (at 35 ^{0}C) unripe SALF significantly lowered it's TSC (Table 14) as compared to the boiling or drying alone (Figure 11), to possibly safer levels for consumption. Therefore, to determine the potential antidiabetic effects of dietary SALF in the prevention study (section 3.5) and therapeutic study (section 3.4), boiled (for 15 min) and then dried (at 35 ^{0}C) SALF was used.

During normal growth and in response to a hormonal or nutritional imbalance in humans, sex differences in glucose homeostasis, insulin signalling, ectopic fat accumulation, and lipid metabolism have been reported (Chella Krishnan et al., 2018; Tramunt et al., 2020). The weights and fitness of both male and female flies exposed to either HSD, HSD+SALF5, or HSD+SALF10 in the therapeutic (Figures 16b, 17a, 24b, and 25a) and prevention studies (Figure 20) were unaffected as compared to control-fed flies. However, various authors have incomparable findings regarding the HSD effect on *D. melanogaster* weight (Kahn & Flier, 2000; Musselman et al., 2011; Rovenko et al., 2014). The undetected changes in the weights and fitness in this study may partly have stemmed from the short study duration in comparison to the fly's lifespan of 60 to 90 days (Y. Sun et al., 2013), thus limiting the duration at which these outcomes would have been affected. Additionally, the unaffected weights and fitness of

the female flies exposed to HSD+SALF in the therapeutic study may be explained by their unaltered *Srl* expression (Figure 19b).

The therapeutic study revealed that the glucose levels in the females pre-exposed to HSD were elevated in comparison to those exposed to the control (Figure 18a), while no change was observed in HSD-fed males versus the control-fed ones (Figure 26a). This implied a sexually dimorphic effect of HSD regarding glucose metabolism, suggesting females rather than male flies were more likely to develop a hyperglycemic state. Compliant with these results, Beigh & Jain (2012) revealed that hyperglycemia was significantly higher in women (42%) than men (25%). Exposing the female flies having a hyperglycemic state to HSD+SALF significantly lowered their glucose levels (dose-dependently) to similar amounts as the control-fed ones. The glucose-lowering effect may be due to the inhibition of the digestive enzyme, amylase, by the high alkaloid and phenolic contents in SALF (Figure 3). Other possibly underlying mechanisms by which the SALF bioactive compounds lowered the glucose levels are summarized in Figure 23.

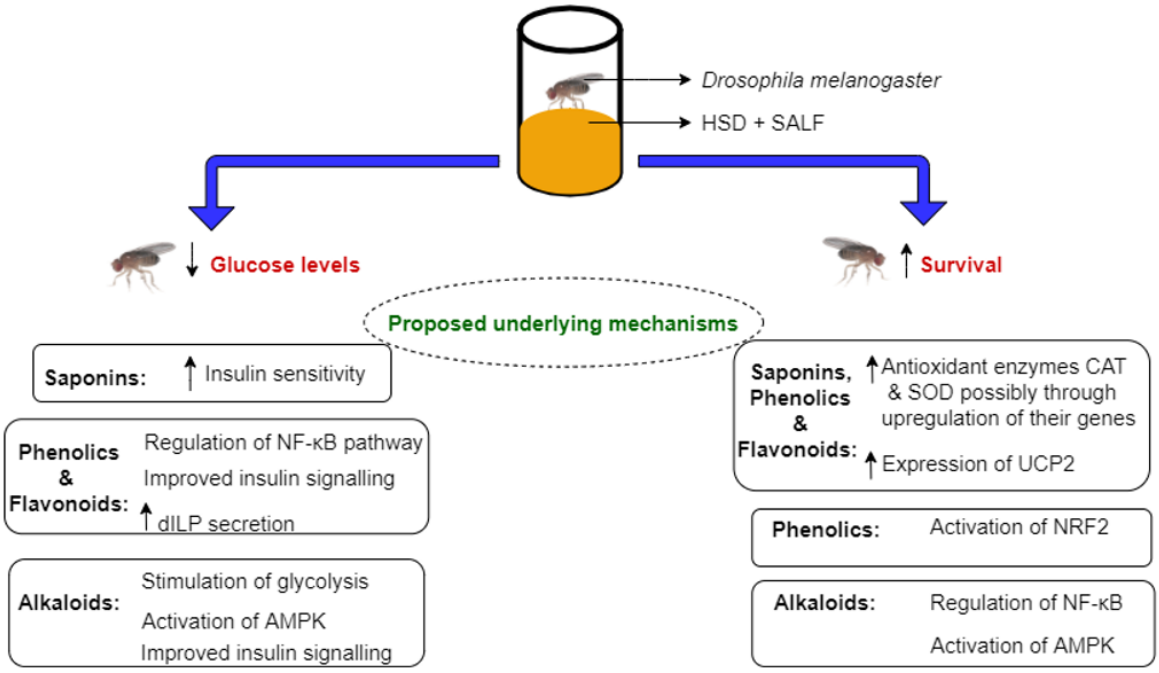


Figure 23. Proposed underlying mechanisms of glucose-lowering effect and increased survival in *D. melanogaster* by bioactive compounds in *Solanum anguivi* Lam. fruits. Consumption of HSD + SALF resulted in lowered glucose levels and/ or increased survival of the flies. The phenolics in this figure are inclusive of flavonoids. HSD= high sugar diet, SALF = *Solanum anguivi* Lam. fruit, NF-κB= nuclear factor kappa B, AMPK= adenosine monophosphate-activated protein kinase, dILP = *Drosophila* insulin-like peptide, CAT= catalase, SOD = superoxide dismutase, NRF2 = nuclear factor-erythoroid 2-related factor, UCP2 = uncoupling protein 2, ↑ = increases, ↓ = lowers, ⤵ = outcomes of exposure to HSD + SALF.

This glucose-lowering effect may partly have been stemmed from the high TSC (Table 14) in SALF, as similarly recorded in diabetic mice administered with SALF saponins (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013). The authors (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013) proposed that the saponins lower glucose levels by improving the insulin sensitivity of the animals. The high phenolic content of SALF, consisting of largely chlorogenic acid (Tables 14 and 15), may also have lowered the glucose levels, as documented for chlorogenic acid-rich extracts of *Catharanthus roseus* (Espejel-Nava et al., 2018) and *Gynura divaricata* (Yin et al., 2018). The SALF phenolics possibly reduced the glucose levels by increasing the secretion of dILP (such as dILP 2 and 5), as similarly suggested by Espejel-Nava et al. (2018). SALF quercetin (Table 15), a flavonoid, may have interacted with the nuclear factor kappa B (NF-κB) signalling pathway and also improved insulin signalling (Venkatraman, 2013), leading to the glucose-lowering effect in the female flies. Simultaneously, the SALF hypoglycemic effect may partly be attributed to its high alkaloid content (Table 12) that may have enhanced the glucose metabolism through the stimulation of glycolysis as reported for berberine (an alkaloid) (J. Yin et al., 2008). Concomitantly, SALF alkaloids may have improved the insulin-signalling pathway and also increased the glucose uptake through activation of adenosine monophosphate-activated protein kinase (AMPK), a crucial regulator of energy metabolic homeostasis, as exhibited by berberine (Cao & Su, 2019; Sirtori et al., 2017).

The glucose-lowering effect of bioactive compounds may also result from their positive effects on the expression of genes associated with T2DM-like phenotype, including those that regulate insulin-like peptides and antioxidant effect (Venkatraman, 2013). Whereas the *dIlp3* levels in females exposed to either of the four treatments in the therapeutic study were not significantly different, likely, other *dIlp*s which are also released upon glucose intake such as *dIlp2* and *5* (S. K. Kim & Rulifson, 2004; Nässel et al., 2013; Song et al., 2017) were affected. Given that levels of these *dIlp*s were not examined in this study, this justifies their future investigations. While both the therapeutic (section 3.4) and prevention (section 3.5) studies used whole body extracts for glucose analysis, the female flies in the latter had unaffected glucose levels (Figure 22a), as similarly demonstrated by Morris et al. (2012). This further showed that the use of hemolymph was best for the analysis of glucose levels in the flies. The lipid metabolism, which was assessed by quantifying the triglyceride levels, was not significantly different in the male and female flies exposed to all the treatments in both the therapeutic (Figures 18b and 25b) and prevention studies (Figures 22c, and d). This implied a non-sex-dependent effect of HSD and

SALF supplementation on *D. melanogaster* triglyceride levels. Similarly, Beigh & Jain (2012) observed no gender differences in the triglyceride levels of men and women with metabolic syndrome.

Reduced survival has been evidenced in *D. melanogaster* exposed to HSD (Musselman et al., 2011; Rovenko et al., 2015; van Dam et al., 2020), which is comparable to premature death in humans with T2DM (WHO, 2021). In both therapeutic and prevention studies, the survival of HSD-fed females was not significantly different from the control-fed ones (Figures 17b and 21a). On the other hand, the survival of HSD-fed males for both the therapeutic and prevention studies was significantly lower than the control-fed ones. This possibly resulted from defects in the insulin-signalling pathway or oxidative stress in the male HSD-fed flies (Figure 25a and 21b). The unaffected survival of HSD-fed females, unlike males in comparison to their controls in both therapeutic and prevention studies, demonstrated that the females had a higher tolerance to high levels of dietary sugars in reference to survival (irrespective of the hyperglycemic state exhibited in the therapeutic study) than males.

For the therapeutic study, the Cox proportional hazard (CPH) analysis (Table 17) showed that male control-fed flies had a significantly higher probability of survival by 52% versus the HSD-fed males while the higher probability of survival (14%) in control-fed females versus the HSD-fed ones was insignificant. The CPH analysis further showed that the probability of survival for male flies fed on HSD+SALF5 and HSD+SALF10 was significantly increased by 37.8% and 55.8%, respectively, versus the HSD-fed males. Concurrently, the probability of survival of females fed on HSD+SALF5 and HSD+SALF10 was significantly increased by 45.8% and 59.8%, respectively, versus HSD-fed ones. Regarding the prevention study, the probability of survival in male flies fed on HSD+SALF5 and HSD+SALF10 was significantly increased by 62% and 66%, respectively, versus HSD-fed males (Table 18). Likewise, the probability of survival of females fed on HSD+SALF5 and HSD+SALF10 was significantly increased by 56% and 71%, respectively, versus HSD-fed females. Male control-fed flies had a significantly higher probability of survival by 45% versus HSD-fed males, while the higher probability of survival in the control-fed female flies (8%) versus the HSD-fed ones was insignificant. These findings showed a sexually dimorphic effect of HSD on the fly's survival, as similarly observed by Chandegra et al. (2017). Although both the therapeutic and prevention studies showed higher probabilities of survival with higher SALF dosage (HSD+SALF10), survival was not significantly different at a lower dosage (HSD+SALF5) for males and

females. Interestingly, a greater probability of survival was observed when both male and female flies were exposed to SALF supplementation from day 1 (prevention study) than day 10 (therapeutic study) of the experimental feeding. Hence, the protective effect of SALF from HSD-induced reduced survival was greater when consumed early in the fly's life and before exposure to an environment that would induce T2DM-like characteristics.

A possible underlying mechanism through which SALF-supplementation increased the fly survival was the upregulation of *dIlp6* levels as shown in female flies exposed to HSD+SALF5 versus the control-fed ones in the therapeutic study (Figure 19b). Other possible underlying mechanisms through which the SALF bioactive compounds increased both male and female *D. melanogaster* survival are summarized in Figure 23. The increased survival in flies exposed to HSD+SALF could be a consequence of improved defences against processes that lead to loss of function and death (Partridge et al., 2011). The intake of HSD reportedly induces oxidative stress, which consequently reduces *D. melanogaster* survival (Ecker et al., 2017; Leonov et al., 2015). This may explain the lower survival in HSD-fed flies than those exposed to HSD+SALF. Thus, the significantly higher survival of male and female flies exposed to HSD+SALF may partially be attributed to the bioactive compounds that positively correlated with the SALF AA, as they may have prevented or reduced oxidative damage in the flies. The SALF AA correlated positively with the phenolics and flavonoids (sections 3.1, 3.2, and 3.3). The SALF phenolics possibly increased the flies' survival by increasing the endogenous antioxidant enzymes as similarly observed in *D. melanogaster* exposed to phenolic-rich hawthorn extract (Y. Zhang et al., 2014) and curcumin (a polyphenol) (Shen et al., 2013). Simultaneously, SALF AA positively correlated with saponins (sections 3.1 and 3.3), which also exhibited *in vitro* AA and *in vivo* (diabetic mice) antioxidant effects through increased antioxidant enzymes CAT and SOD (Elekofehinti, Kamdem, Kade, Adanlawo, et al., 2013; Elekofehinti, Kamdem, Kade, Rocha, et al., 2013); possibly via the upregulation of genes for CAT and SOD. Furthermore, overexpression of uncoupling protein 2 (UCP2), which reduces the drive for reactive oxidative stress (ROS) production and consequently decreases cell death (Dutra et al., 2018), may have been induced by the high flavonoid and saponin levels (Banz et al., 2007; Dembinska-Kiec et al., 2008; Venkatraman, 2013) in SALF, and thereby increased the flies' survival.

Other underlying mechanisms for the increased survival by SALF may include the activation of the nuclear factor-erythoroid 2-related factor (NRF2) *Drosophila* homologue (a redox-

sensitive leucin zipper transcription factor), and/ or the regulation of NF-κB by its flavonoids and phenolics (Shin-Hae & Kyung-Jin, 2015). The rich alkaloid content in SALF (Figure 12) possibly increased the flies' survival (Navrotskaya et al., 2012) by activation of AMPK (Cao & Su, 2019; Venkatraman, 2013) as shown in *D. melanogaster* (Sinnett & Brenman, 2016).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The morphological characteristics of *Solanum anguivi* Lam. varied among the accessions examined in this study. The BCC and AA of SALF significantly varied among the accessions and ripeness stages. Concomitantly, all the SALF accessions and ripeness stages were rich in phenolics, flavonoids, saponins, alkaloids, and vitamin C. However, the unripe stage had the highest AA, TPC, and TFC and may potentially mediate the highest nutraceutical benefits.

Thermal treatments significantly affected the BCC and AA of SALF. Although steaming has been previously suggested as a better cooking method than boiling for better nutrient retention in vegetables, boiling of SALF resulted in the highest AA than steaming or drying. Boiling SALF for 15 min is, therefore, recommended before consumption for potentially high nutraceutical benefits. For consumers who prefer to form a powder for supplementation in other foods, the drying temperature that resulted in the highest AA was 35 ^{o}C. However, boiling for 15 min before drying SALF resulted in much higher AA than dried only SALF.

The potential for dietary SALF to prevent or manage T2DM has been studied for the first time. The SALF therapeutic potential was exhibited through a glucose-lowering effect and counteracting HSD-induced reduced survival. Although a T2DM-like phenotype was not detected in the prevention study, SALF supplementation also significantly increased the survival of the flies. Additionally, the initiation of SALF supplementation early in fly's age (prevention study) resulted in stronger protection against the HSD-mediated reduced survival than observed in the therapeutic study where it was initiated on day 10 of the experiment.

The glucose-lowering effect of dietary SALF-supplementation was not mediated by the expression of *dIlp3*, which suggested that other insulin-like peptides such as *dIlp*2 and 5, which were not examined in this study, may have been affected instead. The increased survival in flies exposed to HSD+SALF was possibly associated with the increased expression of *dIlp6*. SALF-mediated increased survival of the flies was not dependent on dosage and sex.

5.2. Recommendations

All accessions investigated in this study were rich in phenolics (mostly chlorogenic acid),

flavonoids, saponins, alkaloids, and vitamin C, and are all, therefore, recommended for consumption. However, for supplementation of SALF powder into other foods, or preparation of soup, boiling SALF for 15 min before drying at low temperature (35 ^{o}C) may result in higher health benefits.

For drug development from alkaloids, the red stage of SALF is the most recommended as it contains the highest alkaloid content.

The findings of the study can be used by policy-makers to promote the production and consumption of SALF in communities, given its potential health benefits.

Future research should explore the following;

i. Standardization of morphology descriptors for *Solanum anguivi* Lam., for unified morphological characterization amongst researchers.

ii. The effect of boiling and steaming on the individual phenolic compounds, as well as the effects of ripening and thermal treatments on the individual saponin and alkaloid compounds.

iii. The antidiabetic potential of SALF at other ripeness stages as this study investigated the unripe stage. For example, alkaloids reportedly have antidiabetic effects, and the red stage of SALF had the highest total alkaloid contents.

iv. Other potential mechanisms through which SALF may prevent and manage T2DM-like characteristics. For example, the effect of SALF consumption on the mRNA levels of *dIlp* 2 and 5, the endogenous AA and pathways associated with energy metabolism, such as AMPK and NRF2.

v. Analysis of glucose levels in hemolymph where it is secreted, and gene expression analysis of *dIlps* in the single organs or tissues where they are secreted by the respective IPCs.

vi. The potential therapeutic and preventive effects of SALF in T2DM in higher organisms such as mice or rats, and then eventually humans, to elucidate its impact in humans. The amounts for consumption that are safe to result in the antidiabetic effects in humans would need to be determined.

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ANNEX

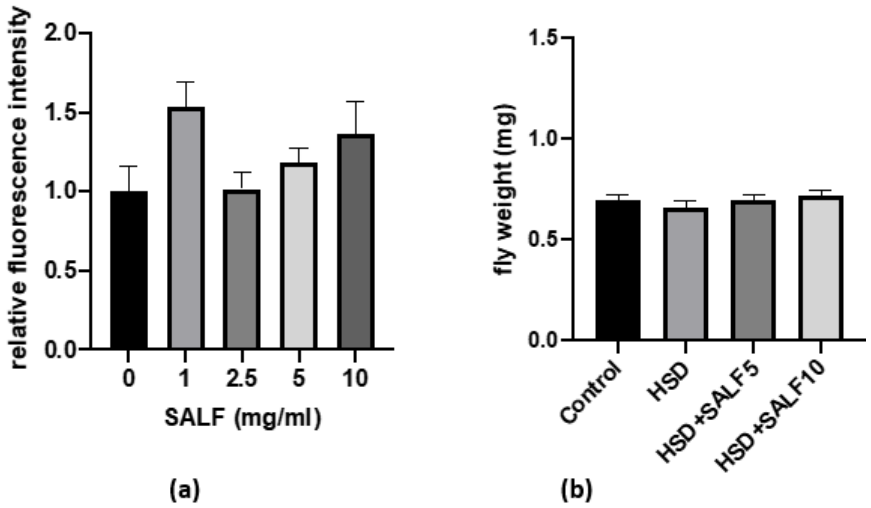

Figure 24. Food intake and weight of male w^{1118} *Drosophila melanogaster* receiving increasing amounts of SALF via the diet. (a) Food intake: Flies were fed on SY10 with 0, 1.0, 2.5, 5.0 or 10 mg SALF/ml for 10 days. On day 10, flies were transferred into empty vials covered with feeder caps containing the experimental diets supplemented with 0.2% sulforhodamine B. After 9 h, 20 flies per treatment were homogenized and fluorescence was measured. The relative fluorescence intensity of the fly homogenates represents the amount of ingested food. Bars show the mean ± SEM of three replicates from 20 flies, each referred to the corresponding fly weights (n=60). SALF supplementation had no significant effect on the food intake of flies as compared to the control ($p < 0.05$), calculated by performing ANOVA followed by Tukey's post hoc test. (b) Weight of male w^{1118} flies following the treatment with either control, HSD, HSD+5 mg *Solanum anguivi* fruit (HSD+SALF5) or HSD+10 mg SALF (HSD+SALF10) on day 24 of the experiment. Bars show the mean ± SEM of three replicates with up to 25 flies each from four independent experiments (n=12) experiments (n=225). There were no significant differences in the weights of flies exposed to the 4 treatments ($p<0.05$).

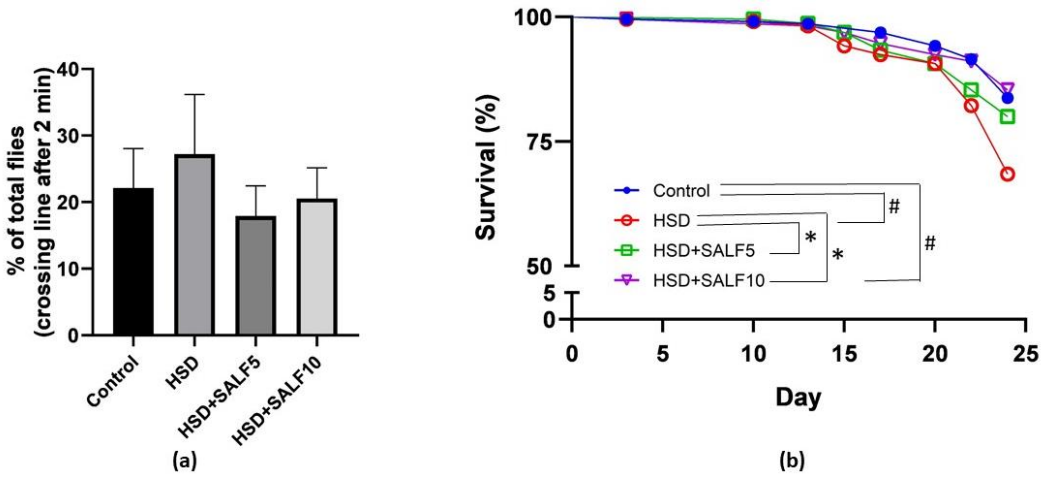

Figure 25. Climbing ability and survival of male w^{1118} *Drosophila melanogaster* receiving increasing amounts of SALF via the diet. (a) The climbing ability of male w^{1118} *Drosophila melanogaster* receiving increasing amounts of SALF via the diet. Flies were fed on a control or a high sugar diet (HSD) for 10 days. Thereafter, they were either kept on a control diet or a HSD without or with 5 mg *Solanum anguivi* fruit (HSD+SALF5) or 10 mg SALF (HSD+SALF10)/ml until day 24. Subsequently, flies were subjected to a climbing assay to assess their fitness. Bars show the mean ± SEM of the percentage of flies that crossed the 5 cm line of a glass cylinder after 2 min. Experiments were performed in triplicates from four independent experiments (n=12). (b) Survival rates of male w^{1118} flies fed on a control diet or HSD for 10 days. In the next step, flies were further kept on a control diet and HSD or switched to HSD+SALF5 or HSD+SALF10 until day 24. Values represent the mean of three replicates with 25 flies each from three independent experiments (n=225). # indicates significant differences ($p<0.05$) to the control group, * indicates significant differences ($p<0.05$) to the HSD group calculated by the log-rank test.

Table 17. Cox Proportional Hazards analysis of the survival data for the therapeutic study

Gender		B	SE	Wald	df	Sig.	Exp(B)
Male	HSD			21.344	3		
	Control	-.733	.205	12.836	1	.000	.480
	HSD+ SALF5	-.475	.191	6.226	1	.013	.622
	HSD+ SALF10	-.817	.211	15.039	1	.000	.442
Female	HSD			18.244	3		
	Control	-.151	.196	.594	1	.441	.860
	HSD+ SALF5	-.612	.222	7.634	1	.006	.542
	HSD+ SALF10	-.912	.244	13.980	1	.000	.402

Treatments were categorical variables, and HSD-only was the reference treatment. The coefficient estimate is the natural log of the hazard ratio, where a negative value indicates a decrease in death probability (increased survival).

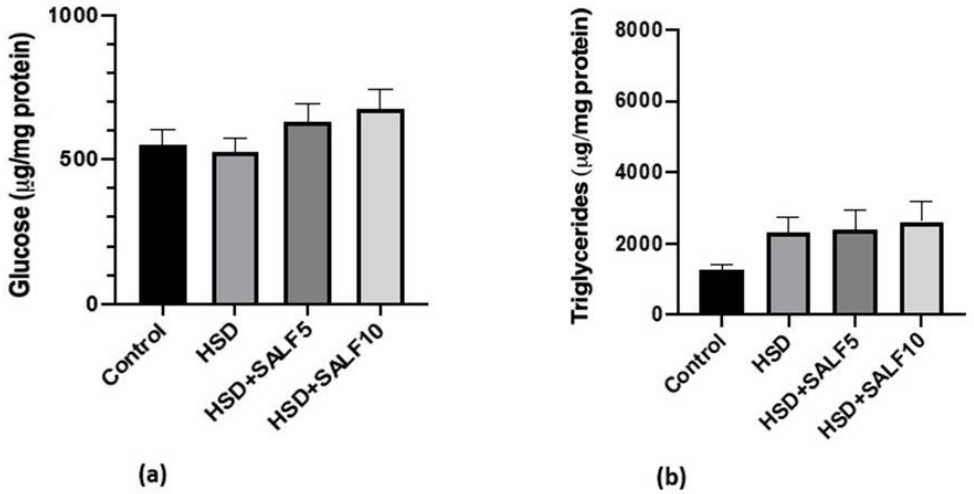

Figure 26. Glucose and triglyceride levels of male w^{1118} *Drosophila melanogaster* receiving increasing amounts of SALF via the diet. (a) Glucose and (b) triglyceride levels of male w^{1118} *Drosophila melanogaster* receiving increasing amounts of SALF via the diet. Flies were fed on the control or high sugar diet (HSD) for 10 days. Following, the flies were either kept on a control diet and HSD or HSD supplemented with 5 mg (HSD+SALF5) or 10 mg SALF (HSD+SALF10)/ml until day 24. Bars show the mean ± SEM of three replicates with homogenates from 5 flies per replicate and four independent experiments referred to the corresponding protein levels (n=12). There were no significant differences in the glucose and triglyceride levels of flies exposed to the 4 treatments ($p<0.05$) calculated by performing ANOVA followed by Tukey's *post hoc* test.

Table 18. Cox Proportional Hazards analysis of the survival data for the prevention study

Gender		B	SE	Wald	df	Sig.	Exp(B)	95.0% CI for Exp(B)	
								Lower	Upper
Male	HSD			36.090	3	.000			
	Control	-.597	.187	10.159	1	.001	.551	.381	.795
	HSD+ SALF5	-.972	.212	20.967	1	.000	.378	.249	.573
	HSD+ SALF10	-1.078	.220	23.934	1	.000	.340	.221	.524
Female	HSD			34.561	3	.000			
	Control	-.089	.178	.251	1	.616	.915	.646	1.296
	HSD+ SALF5	-.821	.220	13.914	1	.000	.440	.286	.677
	HSD+ SALF10	-1.243	.255	23.702	1	.000	.289	.175	.476

Treatments were categorical variables, and HSD-only was the reference treatment. The coefficient estimate is the natural log of the hazard ratio, where a negative value indicates a decrease in death probability (increased survival).

CONTRIBUTION REPORTS

Report on Aisha Musaazi Sebunya Nakitto's contribution in the publications and manuscripts

The following papers and manuscripts will be submitted as part of Aisha Musaazi Sebunya Nakitto's (AMSN) inaugural dissertation.

1. **Nakitto, A. M. S.**; Muyonga, J. H.; Byaruhanga, Y. B.; Wagner, A. E., 2021. *Solanum anguivi* Lam. Fruits: Their Potential Effects on Type 2 Diabetes Mellitus. *Molecules*, *26*, 2044. https://doi.org/10.3390/molecules26072044.

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Contributions
AMSN's overall contribution is estimated at 90%. AMSN's main contributions are detailed below. Percentage values of the individual parts indicate the extent to which a task was performed by AMSN.

- Conceptualization: 70%
- Literature review: 100%
- Content and structure outline: 75%
- Manuscript writing: 90%
- Developing and drawing figures: 100%

Herewith, I, declare that specific qualitative and quantitative contributions of AMSN to this article did not contradict the contributions of the rest of the co-authors and are to the best of my knowledge correct.

Giessen, 17.08.2021	ANIKA WAGNER	
Place, date	Name	Signature

2. **Nakitto, A. M. S.**, Rudloff, S., Borsch, C., and Wagner, A. E., 2021, Solanum anguivi Lam. fruit preparations counteract the negative effects of a high-sugar diet on the glucose metabolism in *Drosophila melanogaster*. *Food Funct. Accepted manuscript* https://doi.org/10.1039/D1FO01363G.

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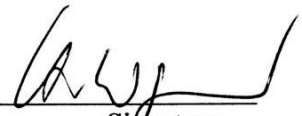
- Experimental design: 30%
- Methodology: 40%
- Sample collection and preparation: 95%
- Investigation: 95%
- Data analysis: 90%
- Figure plotting: 80%
- Manuscript writing: 50%

Herewith, I, declare that specific qualitative and quantitative contributions of AMSN to this article did not contradict the contributions of the rest of the co-authors and are to the best of my knowledge correct.

Giessen, 17.08.2021	ANIKA WAGNER	
Place, date	Name	Signature

3. **Nakitto, A. M. S.**, Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H. Influence of accession and ripeness stage on the bioactive compounds content and antioxidant activities of *Solanum anguivi* Lam. fruits. Submitted to the *International Journal of Fruit Science*.

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- Methodology: 70%
- Sample collection and preparation: 100%
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- Data analysis: 100%
- Figure plotting: 100%
- Manuscript writing: 70%

Herewith, I, declare that specific qualitative and quantitative contributions of AMSN to this article did not contradict the contributions of the rest of the co-authors and are to the best of my knowledge correct.

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Place, date	Name	Signature

4. **Nakitto, A. M. S.**, Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H. Effect of thermal treatments on the bioactive compound contents and antioxidant activity of *Solanum anguivi* Lam. fruits. **Submitted** to the *Food Science and Technology International.*

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- Methodology: 90%
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- Investigation: 95%
- Data analysis: 100%
- Figure plotting: 100%
- Manuscript writing: 70%

Herewith, I, declare that specific qualitative and quantitative contributions of AMSN to this article did not contradict the contributions of the rest of the co-authors and are to the best of my knowledge correct.

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5. **Nakitto, A. M. S.**, Rudloff, S., and Wagner, A. E. A supplementation with dried *Solanum anguivi* Lam. fruit counteracted the decreased survival of *Drosophila melanogaster* exposed to a high-sugar diet. **To be submitted** to the *Food and Nutrition* journal.

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- Investigation: 100%
- Data analysis: 100%
- Figure plotting: 90%
- Manuscript writing: 90%

Herewith, I, declare that specific qualitative and quantitative contributions of AMSN to this article did not contradict the contributions of the rest of the co-authors and are to the best of my knowledge correct.

Giessen, 17.08.2021	ANIKA WAGNER	
Place, date	Name	Signature

6. **Nakitto, A. M. S.**, Byaruhanga, Y. B., Wagner, A. E., and Muyonga, J. H. Morphological characteristics, bioactive compounds contents, and antioxidant activity of different accessions of *Solanum anguivi* Lam. Under review in the *Journal of Applied Botany and Food Quality*

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