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*Full Length Research Paper*

# Quality attributes of homemade tomato sauce stored at different temperatures

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**Tomato (*Lycopersicon esculentum*) is processed into different products including tomato sauce. The aim of this study was to determine the quality attributes of homemade tomato sauce during storage at 6 and 30°C for 8 weeks. At 4 weeks, there were no significant changes in sensory attributes during storage at both 6 and 30°C. There were significant differences (P<0.05) of sensory attributes after 6 weeks. Sauce stored at 30°C had higher microbial load than at 6°C. Both sensory mean scores and pH were inversely related to microbial growth. Therefore, the shelf-life of homemade tomato sauce could be increased at low temperature (6°C).**

**Key words:** Homemade tomato sauce, storage temperatures, sensory, attributes.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important agricultural products among fresh vegetables in most countries in the world. It is widely distributed in Asia, Europe, North and South America, and North Africa. Tomato belongs to the Solanaceae family. Tomatoes are consumed widely throughout the world in different forms such as canned whole tomatoes, tomato juice, tomato sauce, tomato paste and ketchup sauce (Wasim and Singh, 2015; Anthon and Barrett, 2010; Hossain et al., 2011). Among crops, tomato (*L. esculentum*), with a total production of around 160 million tons per year, is the second most important source of nourishment (after potatoes) for the world's population (FAOSTAT, 2015). Its consumption has increased in the last few years with the commercialization of several processed products such as sauces, juices, soups and purees. It has been estimated that 35% of raw tomatoes are consumed as sauces, 18% as tomato paste, 17% as canned tomatoes, 15% are transformed into juices and 15% into ketchup (Canene-Adams et al., 2005; Raiola et al., 2014).

Tomato consumption has recently been demonstrated to possess health benefits (Levy and Sharoni, 2004; Hsu et al., 2008; Eunmi et al., 2012), because of its rich content of bioactive phytonutrients such as lycopene, β-carotene, vitamins E and C, phenolics, organic acids

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and flavonoids (Kaur et al., 2002; Periago and Garcia-Alonso, 2009; Kalogeropoulos et al., 2012; Trivedi and Patel, 2015). Consumption of tomatoes and tomato sauce was associated with a reduced risk of developing digestive tract and prostate cancers (Campbell et al., 2004). Tomatoes are also one of the main parts of the Mediterranean diet which has been associated with a low mortality from cardiovascular troubles. Because tomatoes constitute the almost exclusive source of lycopene, this pigment could be one of the active agents of this protection (Helyes et al., 2009; Shi et al., 2009).

Tomato is a relatively short duration crop and gives a high yield, it is economically attractive and the area under cultivation is increasing daily, but prone to spoilage due to high water content (AVRDC, 1992). To provide diverse tomato products, drying and making of tomato pulp remain the most commonly used processing and preservation method practised by smallholder farmers (AVRDC, 1992). Solar drying in particular is a widely practised form of preserving fruits and vegetables because it is cheap and no energy cost is incurred (Ayua et al., 2017). However, dried products may require reconstitution, which may be inconvenient to consumers. As an alternative solution to drying, refrigeration and holding tomatoes at room temperatures either in their fresh forms or as processed tomato sauces are widely done (Raci et al., 2014).

Tomato sauce is made from tomato concentrate containing 8 to 24% tomato solids (excluding seeds and peels), and usually containing flavorings such as salt, onion or garlic powder, herbs and flavorings (Featherstone, 2015). To have economic impacts, tomato sauce must retain quality attributes. However, quality changes have been reported during storage of tomato products (Wasim and Singh, 2015). Some of the factors that have been reported to influence the quality of tomato sauce include storage temperature and preservatives added during tomato processing (Khan et al 2011; Hossain et al., 2011). Some studies have reported that tomato products stored at ambient temperatures are prone to rapid spoilage than those refrigerated or frozen (Safdar et al., 2010; Hossain et al., 2011; Wasim and Singh, 2015; Lavelli and Torresani, 2011). These studies observed increased rate of enzymatic reactions, increased microbial growth or activities and decreased consumer acceptance in tomato products stored at ambient temperature. Another significant factor that influences the quality of tomato sauce is lycopene (Martinez-Hernandez et al., 2016). Lycopene, a pigment responsible for the red colour of tomatoes and their products can be degraded by processing conditions such as lengthened durations of heating, high temperatures and exposure to oxygen (Shi et al., 1999). These authors reported that lycopene can be isomerised to its *cis* form during processing and storage subsequently leading to significant losses in the color of tomato sauce. Oxidation of ascorbic acid, enzymatic and Maillard reactions have also been reported to influence color loss in tomato products and these impacted the overall acceptability of tomato products (Wasim and Singh, 2015; Charles et al., 2005). However, results are inconsistent, as some authors have reported an increase in extraction efficiency in lycopene when tomatoes were processed then stored (Re et al., 2009). These discrepancies could be attributed to differences in genetic composition, temperature of storage, and the different heat treatments applied during tomato products processing. As such, unanimous conclusions on the effect of various storage conditions on quality of tomato sauce have not been concluded. Therefore, this study aimed to evaluate quality attributes of homemade-tomato sauce stored at 6 and 30°C.

## MATERIALS AND METHODS

### Preparation of homemade tomato sauce

The tomato sauce was prepared as described by Khan et al. (2011) with modifications. One kilogram of sorted ripe tomatoes bought from Lilongwe market, Malawi, was washed then blanched at 80°C for 15 to 20 min after which they were cooled using cold water, peeled and the resultant product was strained through a sieve to remove the seeds. A table spoon each of oil, ginger, cloves garlic, thyme leaves and corn starch were added to the resultant paste and mixed well in a blender. The mixture was then heated for 4 to 5 min at 100°C and then sugar and salt were each added. The mixture was further left to boil at the same temperature for 25 to 30 min with stirring. The product (sauce) obtained was further cooked at a lower temperature until a desirable thickness was obtained. Finally, corn starch was added to enhance thickening.

### Tomato sauce packaging and storage

The prepared tomato sauce was packaged in 500 ml-bottles. Prior to packaging, the bottles were pasteurized at 100°C for 15 min. Sodium benzoate (0.01%) was added to the sauce (Heinz, 2013). Finally, the bottles were cooled and stored at two different temperatures, 6 and 30°C.

### Sensory evaluation

The widely used 5-point hedonic scale for evaluating sensory characteristics such as color, taste, flavor and texture was used (De Groote et al., 2014). Panelists were asked to evaluate tomato sauce at each testing time session on the 5 point scale (5 = Like extremely; 4 = Like very much; 3 = Like moderately; 2 = Neither like nor dislike; 1 = Dislike). In order to determine how much stored tomato sauce (stored at 6 and 30°C) deviated from fresh sauce (control) in terms of sensory attributes, a fresh tomato sauce was made during each sensory evaluation session. Separately, same panelists were asked to give point difference between fresh sauce and stored sauce (6 and 30°C) on 0-5 scale (0 = no difference to 5 = very different). The differences between the stored sauce and the fresh sauce were used in determining the paired mean differences. Water was also availed to each panelist to clean their palates before testing the next sample.

### Study participants

Panelists were recruited through advertisement and those that met the recruitment criteria were involved. The inclusion criteria included people who consume or use tomato sauce in their homes or places

**Table 1.** Mean sensory scores for quality attributes of tomato sauce stored at 30 and 6°C for 8 weeks.

| Storage periods (weeks) | Temperature (°C) | Color | Taste | Flavor | Texture |
|---|---|---|---|---|---|
| 2 | 30 | 4.08 ± 0.67[a] | 4.02 ± 0.41[a] | 4.58 ± 0.52[a] | 4.42 ± 0.39[a] |
| | 6 | 4.50 ± 0.67[a] | 4.72 ± 0.46[a] | 4.67 ± 0.49[a] | 4.51 ± 0.57[a] |
| 4 | 30 | 3.58 ± 0.51[a] | 3.58 ± 0.51[a] | 3.75 ± 0.62[a] | 3.61 ± 0.51[a] |
| | 6 | 4.00 ± 0.74[a] | 4.00 ± 0.74[a] | 4.08 ± 0.67[a] | 4.19 ± 0.39[a] |
| 6 | 30 | 3.25 ± 0.75[a] | 3.00 ± 0.74[a] | 2.75 ± 0.62[a] | 2.66 ± 0.49[a] |
| | 6 | 3.75 ± 0.62[a] | 3.75 ± 0.64[b] | 3.75 ± 0.84[b] | 3.33 ± 0.19[b] |
| 8 | 30 | 2.00 ± 0.73[b] | 1.75 ± 0.75[b] | 1.50 ± 0.52[b] | 1.75 ± 0.62[b] |
| | 6 | 3.58 ± 0.79[a] | 3.08 ± 0.79[a] | 2.75 ± 0.63[a] | 2.75 ± 0.67[a] |

Results shown as means ±SD, *n* = 52 means with different letters within column and per storage time are significantly different p<0.05 Tukey's test.

of work and were at least 20 years of age. Fifty two panelists were used at each testing session and they were blinded such that they had no idea on how the sauce was made or stored. Panelists signed a consent form before participation. All panelists were briefed before testing sessions in order to enable them understand the terminologies and procedures used during sensory evaluation.

#### Measurement of sauce pH

The pH meter (HI2210 Benchtop, Hanna Instruments, Inc., USA) was used to measure pH of the samples and was done soon after preparing the sauce to know the initial pH and during each successive testing interval.

#### Evaluation of yeast and molds in tomato sauce

Yeast and molds were assessed using malt extract agar using the standard spread plate technique (Thomas et al., 2015). Malt extract agar was chosen because of its specificity to supporting growth of yeast and molds, the main microbes growing in acidic tomato sauce (Azam-Ali et al., 2003). The plates were covered, inverted, taped shut and put at room temperature (25°C) for 3 to 4 days. After 4 days, the colonies were enumerated using a colony counter.

#### Statistical analysis

Data was analyzed using SPSS version 17 (SPSS Inc., USA) to generate means, paired mean difference and standard deviations for the quality attributes. T-test was used to determine significant differences between means and paired mean differences. Results were reported as means and standard deviations. Differences were significant when the p-value < 0.05.

## RESULTS AND DISCUSSION

### Sensory evaluation

During the first 4 weeks of storage, there were no significant differences (p>0.05) in color, taste, flavor and texture between sauce storage at 6 and 30°C. Nonetheless, tomato sauce stored at 30°C was lower for most sensory attributes (Table 1). At 6 and 8 weeks, significant differences (p<0.05) in color, taste, flavor and texture were noticed in sauce stored at 6 and 30°C. This suggested that sensory attributes of homemade tomato sauce can remain stable up to four weeks after which deterioration in the attributes begin. Color remained relatively stable from 2 to 6 weeks at 6°C (4.50±0.67 to 3.75±0.62) and 30°C (4.08±0.67 to 3.25±0.75), while flavor exhibited the highest loss (p<0.05) with more deterioration at 30°C (67%) compared to 6°C (41%) within the same period. Taste and texture deteriorated faster at 30 than at 6°C. This indicated that the change of sensory characteristics could have been due to increased microbial colonization (Table 3) and chemical reactions taking place in the sauce. Therefore, temperature is very critical for management of sensory qualities in stored tomato sauce. These results are different from those of Khan et al. (2011) who found insignificant differences in sensory attributes in tomato sauce stored at 32 to 38°C within the first 8 weeks. Unlike results in this study in which only sodium benzoate was used, their highest counts were $0.35 \times 10^4$ CFU/ml perhaps due to a large array of hurdles they used such as vinegar, sodium metabisulphite or sodium benzoate. The rapid changes in sensory attributes during storage at 30°C could also be due to increased metabolic activities of the microorganisms present in the food (Heinz, 2013) which speeds up the loss of sensory quality. At 8 weeks, the average score for color and taste for sauce stored at 30°C were 2.00±0.73 and 1.75±0.75, respectively, while that of flavor (1.50±0.52) and texture (1.75±0.62) also scored very low (Table 1). Flavor and color changes have been previously reported to be the key parameters used to assess quality changes of stored tomato paste products (Eckerle et al., 1984).

After 8 weeks, more than 60% quality characteristics were lost at 30°C compared with 45% quality loss at 6°C (Figures 2 and 3). This implies that shelf-life of

**Table 2.** Paired mean differences for color, taste, flavor and texture between 6 and 30°C and fresh sauce (control) at different storage time.

| Weeks/Color | INC vs. Fresh | p-value | REF vs. Fresh | p-value | INC vs. REF | p-value |
|---|---|---|---|---|---|---|
| 2 | 0.9167±0.229 | 0.002 | 1.1667±0.207 | 0.000 | 0.2500±0.329 | 0.463 |
| 4 | 3.1667±0.271 | 0.000 | 2.4167±0.260 | 0.000 | -0.7500±0.351 | 0.056 |
| 6 | 3.6000±0.195 | 0.000 | 3.3333±0.225 | 0.000 | -0.1667±0.207 | 0.438 |
| 8 | 3.3333±0.142 | 0.000 | 3.3333±0.142 | 0.000 | 0.0000±0.174 | 1.000 |
| **Week/Taste** | **INC vs. Fresh** | **p-value** | **REF vs. Fresh** | **p-value** | **INC vs. REF** | **p-value** |
| 2 | 1.4167±0.499 | 0.016 | 0.6667±0.449 | 0.166 | -0.7500±0.524 | 0.180 |
| 4 | 2.6667±0.284 | 0.000 | 2.1667±0.271 | 0.000 | -0.5000±0.379 | 0.214 |
| 6 | 3.8333±0.112 | 0.000 | 3.3333±0.188 | 0.000 | -0.5000±0.151 | 0.070 |
| 8 | 3.3333±0.188 | 0.000 | 3.2500±0.218 | 0.000 | -0.0833±0.149 | 0.586 |
| **Week/Flavor** | **INC vs. Fresh** | **p-value** | **REF vs. Fresh** | **p-value** | **INC vs. REF** | **p-value** |
| 2 | 1.6667±0.541 | 0.010 | 0.8330±0.313 | 0.795 | -1.5833±0.417 | 0.003 |
| 4 | 2.9167±0.336 | 0.000 | 1.5000±0.337 | 0.010 | -1.4167±0.358 | 0.020 |
| 6 | 2.9167±0.149 | 0.000 | 3.0833±0.229 | 0.000 | 0.1667±0.207 | 0.438 |
| 8 | 3.6667±0.142 | 0.000 | 3.0000±0.174 | 0.000 | -0.6667±0.188 | 0.005 |
| **Week/Texture** | **INC vs. Fresh** | **p-value** | **REF vs. Fresh** | **p-value** | **INC vs. REF** | **p-value** |
| 2 | 0.9167±0.434 | 0.059 | 0.8333±0.297 | 0.017 | -0.8330±0.260 | 0.754 |
| 4 | 3.0000±0.348 | 0.000 | 2.8333±0.271 | 0.000 | -0.1660±0.423 | 0.701 |
| 6 | 2.9167±0.149 | 0.000 | 3.0833±0.229 | 0.000 | 0.1667±0.207 | 0.804 |
| 8 | 3.3333±0.188 | 0.000 | 3.1667±0.207 | 0.000 | -0.1667±0.112 | 0.166 |

INC: Incubated sauce (30°C), REF: refrigerated sauce (6°C), Fresh: fresh sauce (Control).

**Table 3.** Microbial population (Yeast and Molds) in the tomato sauce during the storage at different conditions.

| Storage period (weeks) | Refrigeration (6°C) | Incubation (30°C) |
|---|---|---|
| | CFU/ml | |
| 2 | $9.8 \times 10^2$ | $9.8 \times 10^2$ |
| 4 | $2.1 \times 10^3$ | $3.2 \times 10^4$ |
| 6 | $3.9 \times 10^4$ | $1.51 \times 10^5$ |
| 8 | $6.2 \times 10^4$ | $1.14 \times 10^5$ |

homemade tomato sauce can be increased at low temperatures especially at 6°C.

In order to understand how freshly made tomato sauce (control) compared with stored ones, paired mean differences between the three sauces were calculated and results are shown in Table 2. There were significant differences in color between fresh sauce and stored at 30°C after 2 weeks of storage ($p = 0.002$). The differences remained significant for the rest of storage period for all attributes. Similarly, sauce stored at 6°C was significantly different in color and texture from fresh sauce during whole storage time ($p<0.001$). However, taste ($p = 0.166$) and flavor (p-values = 0.795) were not different for the first two weeks of storage period. Interestingly, the difference in color, taste and texture for sauce stored at 6 and 30°C were insignificant throughout the storage period ($p>0.05$) (Table 2). However, flavor was significantly different between sauce stored at 6 and 30°C during the storage period ($p <0.05$) except after 6 weeks when the difference was non-significant ($p = 0.438$).

At low temperature, microbial growth was slowed down. The 6°C that was used in this study could not possibly decrease microbial growth significantly as psychrotrophs can still grow at this temperature (King and Cheetan, 2012). However, the difference in temperature seemed to contribute to the difference in microbial population with sauce stored at 6°C having lower counts than that stored

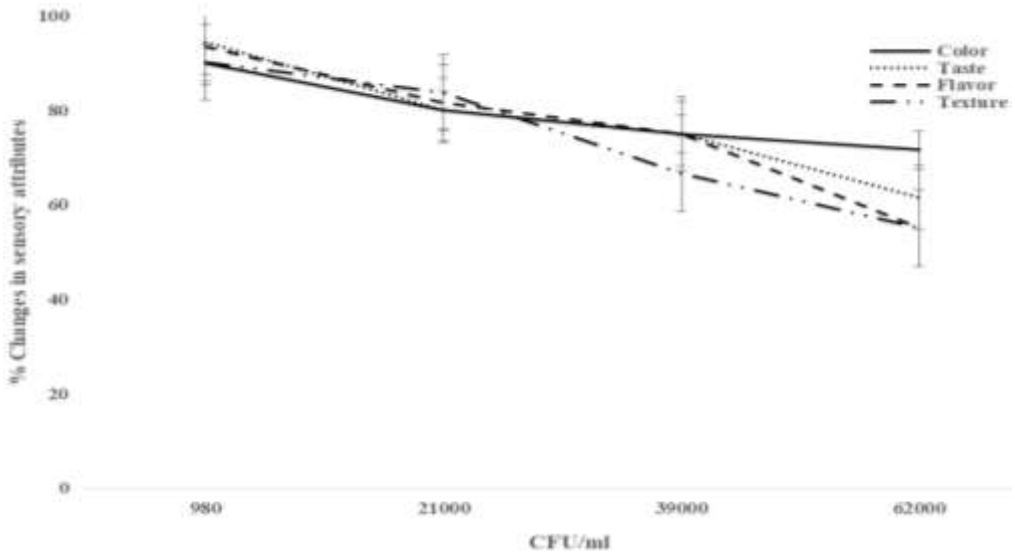


**Figure 1.** Relationship between yeast and molds count and percentage change in sensory quality attributes at 6°C.

at 30°C. Chilled foods stored at temperatures near 0 to 6°C have been reported to have longer shelf-life because of slower growth of psychrotrophs leading to delayed onset of spoilage (King and Cheethan, 2012). In fresh fruits and vegetables, low temperature does not necessarily stop enzymatic and non-enzymatic chemical reactions but instead slows their rates thereby leading to increased shelf-life in products (Kitinoja and Kader, 2002).

The quality loss of sensory attributes during 8 weeks storage appeared a linear relationship (Figure 3). There was strong negative linear relationship between sensory attributes and storage time with coefficient of determination ($R^2$) for color, taste, flavor and texture being 0.9576, 0.9663, 0.9697 and 0.9923, respectively, for sauce stored at 30°C. The same trend was observed in sauce stored at 6°C where the $R^2$ for color, taste, flavor and texture were 0.9539, 0.9775, 0.9576 and 0.9783, respectively. For all the attributes the coefficient of *x* (slope) was higher for sauce stored at 30°C ranging from -0.3415 to -0.4415 than at 6°C which ranged from -0.1795 to -0.2840. This indicates that the rate of quality loss was higher at $30^0$C than that at 6°C perhaps due higher microbial population in sauce stored at 30°C (Table 3). Microbial spoilage results in drastic effects on food quality as they can produce odor and gases as they ferment the food particles (Stewart and Amerine, 2012). Therefore, it is not surprising that after 8 weeks, sauce flavor stored at 30°C had lowest score than other attributes. In particular, spoilage in tomato sauce is mainly attributed to yeasts and molds as they can survive in acidic conditions (Azam-Ali et al., 2003) and the fact that both sauces had a pH less than 4.5 it is more likely that yeasts and molds were the most prevalent microorganism.

**Yeast and mold counts and pH**

The second measure that was employed to assess quality changes of tomato sauce was by counts of yeasts and molds. Yeasts and molds were greater in sauce stored at 30 than 6°C in all cases except after 2 weeks. At 8 weeks, sauce stored at 30°C yeast and molds count was $1.14 \times 10^5$ CFU/ml while at 6°C was $6.2 \times 10^4$ CFU/ml (Table 3). Slight changes in pH were also observed with time, 4.01 at zero time to 3.74 after 8 weeks. The pH of the homemade tomato sauce ranged from 4.01 to 3.74, which falls within reported optimum pH for controlling growth of microorganisms in tomato sauce (Garcia and Barret, 2006). These findings suggested that sodium benzoate can be used to maintain the pH of tomato sauce where microbial growth can be controlled (Khan et al., 2011). It has been previously reported that benzoic acid was useful ingredient for microbial growth inhibition in tomato pastes in a 40 weeks storage study (Khan et al., 2011).

Deeper understanding of quality changes was sought by plotting percentage change in sensory attributes against microbial counts. There was an inverse relationship between microbial population and sensory qualities. The inverse association was more pronounced in sauce stored at 30°C than at 6°C (Figures 1 and 2). Table 3 shows that 30°C was more favorable for microbial growth than 6°C as indicated by having higher microbial count at 30°C than at 6°C after 8 weeks. As such, a storage temperature is crucial for the storage of sauces which may have high water activity usually

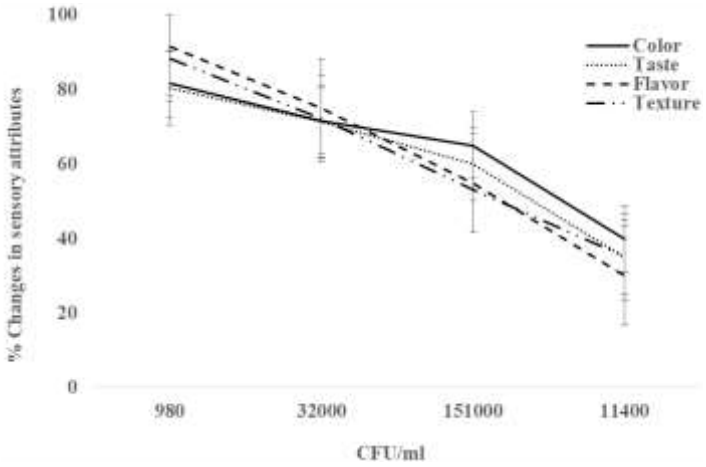


**Figure 2.** Relationship between yeast and molds count and percentage changes in sensory quality attributes at 30°C.

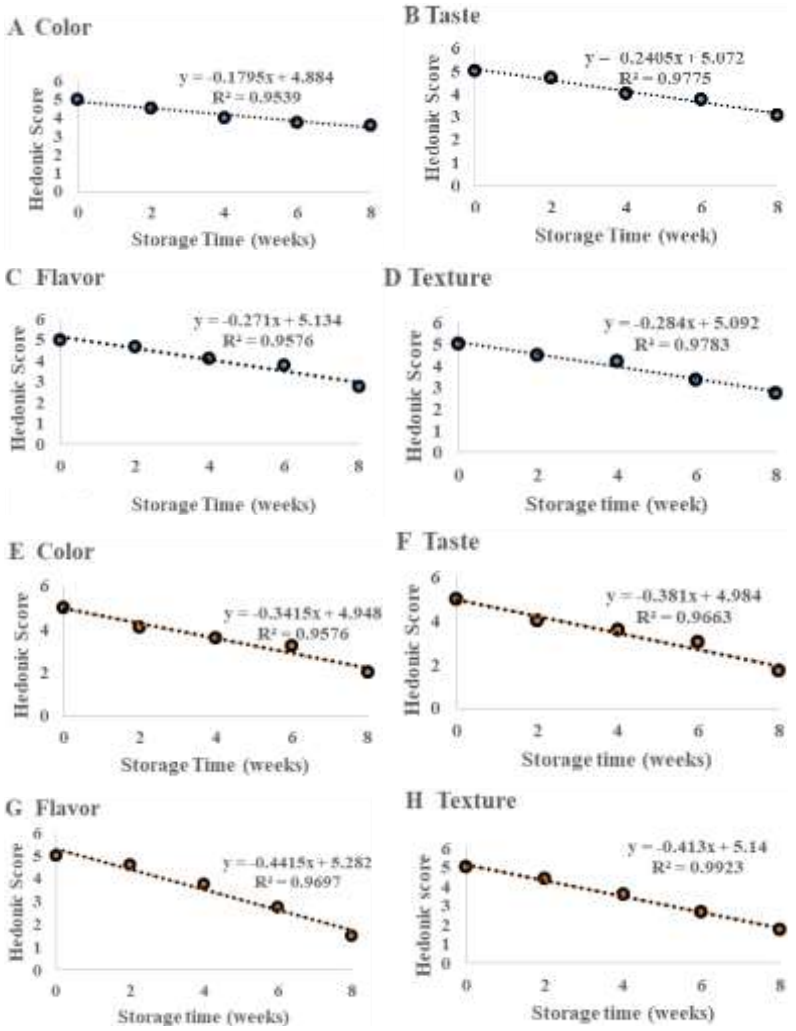


**Figure 3.** The linear regression lines between sensory mean score and storage time for source stored at 6°C (A, B, C, D) and 30°C (E, F, G, H) stored for 8 weeks.

between 0.93 and 0.98 (Fernandez et al., 1993) and therefore prone to microbial storage.

## Conclusion

Sensory characteristics of stored homemade tomato sauce are dependent on storage temperature and microbial loads. Tomato sauce that was stored at 30°C had higher microbial load and lost its sensory qualities faster than that stored at 6°C. Tomato sauce stored at 6°C had consistently higher sensory scores than ones at 30°C. These findings suggest that shelf-life of homemade tomato sauce can be improved through storage at refrigeration temperature.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Quality of porridge from sub-Saharan Africa evaluated using instrumental techniques and descriptive sensory lexicon. Part 2: Thin porridge

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**Thin porridge is a popular nourishment drink for adults and complementary food for children in sub-Saharan Africa. It is made from straight (unblended) or composite flours of maize, sorghum, finger millet and cassava in neutral or chemically-acidified media, or after spontaneous fermentation of the flours. The objective of this study was to determine the impact of type of composite flour and pH on the sensory quality of thin porridges. Instrumental methods and modified quantitative descriptive analysis were used to identify the main sensory attributes of thin porridges made from different composite flours in neutral or acidic media or after spontaneous fermentation. The results of the study indicated that irrespective of the pH, cereal-based composite flours had higher onset pasting temperatures; and lower peak, breakdown, final and setback viscosities than cassava-cereal flours. Thin porridges formulated from cereal-based composite flours tended to have lower firmness, consistencies, cohesiveness and indices of viscosity than those made from cassava-cereal flours. The colour of thin porridges depends on the botanical origin of the composite flours, their ratios and whether the pH was adjusted using citric acid or by spontaneous fermentation. Principal component analysis identified three major principal components (PCs) that accounted for 83.7% of the total variance in the sensory attribute data. The principal component scores indicated that the location of the thin porridges on each of the three scales corresponded with cassava aroma (PC1), finger millet/maize aroma (PC2), and colour and fermented aroma (PC3). This study has shown that thin porridges with different sensory profiles can be produced in sub-Saharan Africa for different population groups.**

**Key words:** Colour, texture, thin porridge, quantitative descriptive analysis.

## INTRODUCTION

Thin porridge is an important breakfast and refreshment drink for adults (Oi and Kitabatake, 2003), complementary food for children (Kikafunda et al., 2006; Oi and Kitabatake, 2003; Onyango, 2003) and a source of nourishment for the sick and invalid in sub-Saharan Africa (Wanjala et al., 2016). Thin porridge is prepared from tropical cereal and root crops, such as maize (*Zea mays*), finger millet (*Eleusine coracana*), pearl millet

(*Pennisetum glaucum*), sorghum (*Sorghum bicolor*) and cassava (*Manihot esculenta*) (Wanjala et al., 2016; Taylor and Emmambux, 2008). Thin porridge is prepared by stirring flour (10 to 20% w/v) in boiling water for a few minutes to obtain viscous slurry (Taylor & Emmambux, 2008). The transformation of flour into thin porridge is associated with irreversible physical modification of starch in excess water. This process involves loss of starch lamellar structure as it gelatinizes followed by formation of complex fractal structures during pasting and retrogradation (Doutch et al., 2012).

Thin porridge is made from unfermented, fermented or chemically-acidified slurries. Unfermented thin porridge, which is prepared by cooking the flour in tap water, includes *uji* in East Africa, *edi* in Uganda, *isidudu* in South Africa and *kunu* in Nigeria (Murty and Kumar, 1995). Fermented thin porridge, which is prepared from spontaneously fermented slurry, includes *uji* in East Africa, *Obushera* in Uganda, *nasha* in Sudan, *ogi* (*kamu* or *akamu*) in Nigeria, *koko* in Ghana, and *imbila* in South Africa (Mukisa et al., 2010; Murty and Kumar, 1995; Obilana, 1982). The slurry used to make thin porridge may be fermented before (Mukisa et al., 2010; Onyango et al. 2004) or after cooking it (Mugula et al., 2003; Kitabatake et al., 2003). Chemically-soured thin porridge is prepared by adding plant extracts, such as lemon (*Citrus limon*) juice extract, tamarind pulp (*Tamarindus indica*) or the shoot of the camel foot plant (*Piliostigma thonningii*) to the slurry during cooking (Wanjala et al., 2016). Chemical-souring can also be achieved by adding pure citric (Wanjala et al., 2016) or lactic acid to the flour (Novellie, 1982).

The texture, flavour and colour of thin porridges are important sensory attributes that affect consumer preferences and acceptance of the products. The texture of thin porridge is described by attributes such as stiffness, stickiness, cohesiveness and coarseness (Kebakile, 2008). Aboubacar et al. (1999) reported that stickiness in the mouth and cohesiveness are the most important textural attributes of thin porridge. Thin porridge with acceptable sensory texture has homogenous distribution of gelatinized starch granules, a free-flowing creamy consistency and smooth texture (Obilana, 1982). When the porridge is drank or eaten with a spoon, it disperses readily in the mouth before it is swallowed. Consumer perception of the texture of thin porridge is influenced by the botanical origin of the flour (Kebakile, 2008), processing technique (Onyango, 2014) and solids concentration (Carvalho et al., 2014; Ojijo and Shimoni, 2004).

Taste and aroma are also important sensory attributes of thin porridge. Thin porridge made from plain flours has a starchy taste and aroma (Wanjala et al., 2016). Sugar and milk are frequently added to thin porridge in order to improve its taste (Murty & Kumar, 1995). Fermented thin porridges are more popular than their unfermented counterparts because the process of fermentation gives the product a complex sour taste, which is due to lactic acid and other flavour and aroma compounds produced by lactic acid bacteria (Mukisa et al., 2016; Mugula et al., 2003; Muyanja et al., 2003). By contrast, thin porridge soured with pure organic acids has a sharp, 'clean' sourness devoid of any taste overtones (Novellie, 1982).

Colour is the first contact point of a food for the consumer even before it enters the mouth (Wu and Sun, 2013). Colour has a close association with quality factors such as desirability, however, when the colour of a food product changes consumers' reactions to the product are likely to be affected (Wu and Sun, 2013). The colour of thin porridge is dependent on the colour of the flour used to prepare it (Aboubacar et al., 1999; Obilana, 1982). Sorghum and millet-based porridges or their composites with maize or cassava are generally light- to dark-brown in colour, with a tinge of redness (Wanjala et al., 2016; Mukisa et al., 2010).

The choice of composite flours used in the current study to prepare thin porridges was derived from the results of a field study done in western Kenya in 2016 (Wanjala et al., 2016). The objective of the current study was to utilize modified quantitative descriptive analysis and instrumental techniques to evaluate the impact of type of composite flour and pH on the sensory quality of thin porridges. The pH of the flours was adjusted with the aid of normal tap water, spontaneous fermentation or citric acid.

## MATERIALS AND METHODS

### Preparation of composite flours and slurries

Maize (*Zea mays*) and cassava (*Manihot esculenta* Crantz) flour were purchased in Busia County, Kenya. Finger millet (*Eleusine coracana* (L) Geartn) variety P224 and sorghum (*Sorghum bicolor* (L.) Moench variety IESV 24029-SH were donated by ICRISAT (Alupe Research Station, Busia, Kenya). The grains were cleaned to remove foreign substances and milled in a hammer mill fitted with 800 µm sieve to obtain whole-milled flours. Four types of composite flours (cassava: finger millet, 90:10; cassava: finger millet, 30:70; finger millet: maize, 90:10; and maize: sorghum, 75:25) were prepared, packed in moisture-proof zip-lock polythene bags and

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stored at 10°C prior to use. Neutral slurries (pH range: 6.34-6.52) were prepared by mixing the composite flours with distilled water. Food-grade anhydrous citric acid (2 g/1,000 ml) was used to prepare acidic slurries (pH range 3.88 to 4.22). Fermented slurries were prepared by adding 200 g composite flour to 200 ml distilled water and incubating the mixture at 25°C for 24 h. After 24 h, the fermented slurry was added to fresh slurry (400 g flour and 400 ml water) and incubated at 25 °C for 24 h. The pH of the fermented slurries ranged between 3.92 and 4.48. The fermented slurries were dried in the oven at 50 °C to about 11% moisture content.

**Pasting properties of composite flours**

Pasting properties of the composite flours were measured using a Brabender Viscograph-E (Brabender GmbH & Co. KG, Duisburg, Germany) at 85 rpm and 700 cmg torque. Neutral, acidic or spontaneously fermented slurries made up of 40 g flour (adjusted to 14% moisture content) and 420 ml distilled water was added into the Viscograph-E canister. The canister was put in the Viscograph-E heating chamber and the mixing spindles attached. The slurry was heated from 30 °C and temperature increased at 1.5°C/min up to 93°C. The temperature of the slurry was held at 93°C for 15 min before it was decreased at 1.5°C/min up to 30°C and subsequently held at this temperature for 15 min. The resistance to stirring was recorded as viscosity in Brabender Units (BU). The pasting temperature (°C), peak viscosity, time to peak viscosity (min), breakdown viscosity (peak viscosity minus trough viscosity) and setback viscosity (cold paste viscosity minus trough viscosity) were determined from the viscograph.

**Objective evaluation of the texture of thin porridge**

Thin porridge was made by mixing 40 g composite flour with 150 ml tap water to make a slurry. Separately, 200 ml water was brought to boil in a stainless steel pot on an electric cooker set at 150°C. The cold slurry was added to the boiling water and stirred continuously for 5 min, using a flat wooden ladle, to avoid formation of lumps. The porridge was boiled for a further 2 min without intervention. After cooking it was cooled to 26±1°C before pouring 80 g into a 50 mm diameter A/BE back extrusion container (Stable Micro Systems, Surrey, UK). Back extrusion force was measured using TA-XT*plus* Texture Analyzer (Stable Micro Systems, Surrey, UK) at the following settings: 50 kg load cell; height calibration: 50 mm; disc diameter: 45 mm; pre-test speed: 1 mm/s; test speed: 1 mm/s; trigger force: 0.05 N; post-test speed: 10 mm/s; data acquisition rate: 200 pps. When a surface trigger force (that is, point at which the disc's lower surface was in full contact with the product) of 10 g was attained the disc proceeded to penetrate the porridge to a depth of 30 mm after which it returned to its original position. Firmness (maximum positive force), consistency (area of the positive region of the curve), cohesiveness (maximum negative force) and work of cohesion or index of viscosity (area of the negative region of the curve) were calculated using EXPONENT Texture Analysis software version 6.1.5.0 (Stable Micro Systems, Surrey, UK).

**Objective evaluation of the colour of thin porridge**

Thin porridges were prepared as described earlier and subsequently dried in a laboratory incubator (Memmert GmbH + Co. KG, Schwabach, Germany) at 70°C to about 10% moisture content. The dehydrated thin porridge was milled using a MRK hummer mill (Mitamura Riken Kogyo Inc., Tokyo, Japan). A Konica Minolta Chroma Meter CR-200 (Minolta Co. Ltd., Osaka, Japan) operating at D65 standard illuminant and observer 2º was used to evaluate the colour of dehydrated thin porridges. The sample (20 g) was put in a clean Petri dish and covered. The equipment was calibrated using the standard white tile provided with the equipment. CIE-LAB-System colour values of light (L* = 100) to dark (L* = 0); red (+a*) to green (-a*); and yellow (+b*) to blue (-b*) were recorded for each sample.

**Descriptive sensory evaluation of thin porridge**

Thin porridges were prepared as described earlier using:

(1) 80 g composite flour and 900 ml water
(2) 80 g fermented composite flour and 900 ml water; and
(3) 80 g composite flour and 900 ml citric acid solution.

After cooking, the thin porridge was cooled to 30°C and served in white plastic cups. Sugar was not added to the thin porridge because in preliminary studies it was found to mask the aroma of the porridge. Eight students from local universities were recruited to undertake descriptive sensory evaluation of the thin porridges. They were given a consent form to sign, listing ingredients in the products and possible allergens. The study was done in a well-ventilated laboratory at 25±1°C. Since sensory booths were not available, the panellists were spaced 2 m apart to avoid interaction. The panellists were trained for 10 sessions with each session lasting 2 h. The first five sessions consisted of attribute generation, whereby the panellists were asked to list all the sensory attributes present in the thin porridges, which were served in random order. The panel generated 15 descriptive terms (Table 1). The next five sessions involved identifying references (Table 1) that fit the sensory attributes of thin porridges and rating them on 100 mm unstructured line scales for intensity. During product evaluation, panellists were served with 50 g of thin porridge in 120 ml white plastic cups labeled with three-digit codes. The samples were served monadically in random order with a 5 min break between each sample. Panellists rinsed their mouth with mineral water before testing each sample and in between the tests. All attributes of a specific sample were evaluated before the next sample was served. Panel sessions were repeated until all samples were scored in triplicate.

**Experimental design and statistical analysis**

The instrumental experiments were set-up as a 4x3 factorial combination in a randomized complete block design. The treatment combinations consisted of four types of composite flours and three treatment methods (neutral, chemically-acidified and spontaneously fermented). Each treatment was conducted in triplicate and the results reported as mean ± standard deviation. The data were analysed using a two-way factorial analysis and further analysis done using a one-way factorial analysis. The sensory evaluation data was analysed using PCA in a covariance matrix with the product in rows and the mean panellists and replication scores for the 15 sensory attributes in columns. All data were analysed with Minitab Release 14 (Minitab Inc., Pennsylvania, USA).

**Table 1.** Descriptive sensory lexicon developed by the sensory panel to evaluate the quality of thin porridge.

| Attribute | Description | Reference and rating scale |
|---|---|---|
| **Appearance** | | |
| Colour | Perception of colour ranging from white to dark brown | Cassava starch (10% w/v) stirred in hot water = 0 (white)<br>[a]Baker's dark compound chocolate = 10 (dark brown) |
| White specks | Quantity of white specks observed on the surface of porridge in a white plastic cup | 0 = No white specks<br>10 = Many white specks |
| Brown and dark specks | Quantity of brown and dark specks in porridge when it is lifted with the back of a white teaspoon | Cassava starch (30% w/v) stirred in hot water = 0 (no dark specks)<br>[b]Indian hemp hair and scalp treatment oil = 7 (many dark specks) |
| Gloss | Perception of a shiny appearance on the surface of porridge when light is directed on it | [c]Brookside farm fresh milk (fat content 3%) = 3 (slightly glossy)<br>Pure glycerin for cosmetic application = 10 (very glossy) |
| **Aroma** | | |
| Cassava aroma | Aroma characteristic of cassava flour in hot water | Cassava flour (30% w/v) stirred in hot water = 10 (very intense) |
| Finger millet aroma | Aroma characteristic of finger millet flour in hot water | Whole-milled finger millet flour (30% w/v) stirred in hot water = 10 (very intense) |
| Maize aroma | Aroma characteristic of maize flour in hot water | Whole-milled maize flour (30% w/v) stirred in hot water = 10 (very intense) |
| Sorghum aroma | Aroma characteristic of sorghum flour in hot water | Whole-milled sorghum flour (30% w/v) stirred in hot water = 10 (very intense) |
| Fermented aroma | Intensity of aroma associated with fermented and cooked cereal porridge | Unfermented and cooked finger millet porridge = 0 (no aroma)<br>Fermented and cooked finger millet porridge = 10 (intense fermented aroma) |
| **Taste** | | |
| Sour taste | Intensity of sour taste associated with fermented milk | [b]Brookside farm fresh milk (fat content 3%) = 0 (not sour)<br>[d]Bio yoghurt natural (fat content 3%) = 5<br>Whole-milled maize porridge (10% w/v) cooked in citric acid solution 1% w/v = 10 |
| **Texture** | | |
| Viscosity | Resistance to flow when the porridge is stirred with a teaspoon once in a clockwise direction | [b]Brookside farm fresh milk (fat content 3%) = 1 (thin)<br>Honey = 10 (thick) |
| Coarseness | Degree to which particles are perceived in the mouth during mastication | Honey = 0 (not perceived)<br>Fresh pressed, unsieved carrot juice = 10 (intensely perceived) |
| Adhesiveness | Degree to which porridge adheres to the palate when it is manipulated by the tongue | Water melon = 0 (not adhesive)<br>[e]American Garden U. S. creamy peanut butter = 10 (very adhesive) |
| **After swallow** | | |
| Sour aftertaste | Perception of lingering sourness in the mouth after mastication and swallowing | 0 = No after taste<br>10= Strong after taste |
| Residual particles | Perception of particles in the mouth after swallowing porridge | Water melon = 0 (no residual particles)<br>Fresh pressed, unsieved carrot juice = 10 (many residual particles) |

[a]PT Gandum Mas Kencana, Tangerang, Indonesia; [b]Dynamix Trading Ltd., London , Britain; [c]Brookside Dairy Ltd., Ruiru, Kenya; [d]Bio Food Products Ltd., Nairobi, Kenya; [e]American Garden Co. New York, USA.

## RESULTS AND DISCUSSION

### Pasting proprieties of composite flours

The pasting properties of the composite flours in neutral or acidic media or after spontaneous fermentation are shown in Table 2. Cereal-based slurries (finger millet-maize, 90:10; and maize-sorghum, 75:25) tended to have higher onset pasting temperatures but lower peak, breakdown, final and setback viscosities than cassava-cereal slurries (cassava-finger millet, 90:10; and cassava-finger millet, 30:70). The cassava-cereal slurry with a high cassava content (that is, cassava-finger millet, 90:10) had higher onset pasting temperature but lower peak, breakdown, final and setback viscosities than that with a lower cassava content (that is, cassava-finger millet, 30:70). Among the cereal-based slurries, maize-sorghum slurry (75:25) had higher onset pasting temperature but lower peak, breakdown, final and setback viscosities than the finger millet-maize slurry (90:10).

Starch is the main structure- and texture-forming constituent of cereal- and cassava-based foods (Delcour et al., 2010; Moorthy, 2002). The starch content and relative proportions of amylose and amylopectin polymers in starch granules influence their pasting behaviour (Biliaderis, 2009; Colonna and Buleon, 2010). The viscous nature of gelatinized starch is due to suspended swollen starch granules dispersed in a macromolecular solution created by amylose polymers (Alloncle and Doublier, 1991). Cereal flours have lower starch contents but higher amylose contents than cassava flour (Eckoff and Watson, 2009; Breuninger et al., 2009). In addition, the high lipid content of cereal flours enables them to form more amylose-lipid complexes than cassava flour, which has a low lipid content (Colonna and Buleon, 2010). As a result of the preceding factors, cassava starch has a lower gelatinization temperature but higher peak, breakdown, final and setback viscosities than cereal starches. Consequently, when cassava flour is blended with cereal flours, the pasting behaviour of the composite flour is a reflection of the relative amounts of the cereal and cassava flours in the blends.

The time to peak viscosity of the composite flours ranged between 42 to 44°C except for spontaneously fermented cassava-finger millet (30:70) and maize-sorghum (75:25) slurries where it was about 50°C. The time taken by starch to reach peak viscosity in a viscograph is a reliable indicator of the amount of energy required to produce rapidly digestible starch. Slurries that require more time to reach peak viscosity consume more energy than those that require less time (Bolade et al., 2009). Also, the time taken by starch to reach peak viscosity affects the texture of thin porridge. Slurries that require more time to reach peak viscosity have lower rates of water absorption and swelling of starch granules and consequently have lower hot paste viscosities than those with higher rates of water absorption and swelling of starch granules (Ragaee and Abdel-Aal, 2006).

The viscosity of starch slurry begins to decline in the viscograph after reaching peak viscosity because the solubilised starch polymers reorient themselves in the direction of the shearing force. In addition, temperature- and shear-induced destruction of swollen starch granules also contribute to the decrease in viscosity after the peak viscosity has been attained (Delcour and Hoseney, 2010; Ragaee and Abdel-Aal, 2006). Slurries with low breakdown viscosities are better able to withstand temperature and shear-induced destruction of starch granules than slurries with high breakdown viscosities (Bressiani et al., 2017; Ragaee and Abdel-Aal, 2006). During the cooling phase, starch molecules begin to re-associate leading to formation of a gel structure with higher viscosity than the hot-paste slurry. The paste viscosity increases due to decreased energy in the system, which allows re-association of leached amylose molecules with each other and with gelatinized starch granules (Delcour and Hoseney, 2010; Ragaee and Abdel-Aal, 2006).

Two-factor analysis of variance showed that the interaction effect between the type of composite flour and pH was significant ($p < 0.05$) for onset pasting temperature; and the peak, breakdown, final and setback viscosities. The simple main effect of pH was significant ($p < 0.05$) for all pasting properties except for the setback viscosity of the maize-sorghum slurry (75:25) (Table 2). In comparison with the neutral slurries, spontaneous fermentation increased ($p < 0.05$) the pasting temperature and decreased ($p < 0.05$) the breakdown and setback viscosities of all slurries. On the other hand, citric acid increased the pasting temperature of all slurries, except cassava-finger millet (90:10) slurry. It also increased the peak, breakdown and final viscosities of all slurries, except the final viscosity of the cassava-finger millet (90:10) slurry. Pure organic acids (Bertolini et al., 2000; Haros et al., 2004) and organic acids produced during lactic acid fermentation (Yang and Tao, 2008) hydrolyse starch granules internally causing them to lose the ability to absorb water and swell resulting in products with low (thin) viscosity. This effect was not clearly evident in our results probably due to the minimal impact of the weak acids on the starch granules in the composite flours.

### Objective evaluation of the texture of thin porridge

The texture of thin porridge was measured using the back-extrusion method in a Texture Analyzer (Stable Micro Systems, Surrey, UK). The back-extrusion method is recommended for evaluating the texture of viscous

**Table 2.** Pasting properties of composite flours segregated by pH.

| pH | PT (°C) | PV (BU) | Time PV (min) | BV (BU) | FV (BU) | SV (BU) |
|---|---|---|---|---|---|---|
| **Cassava-finger millet (90:10)** | | | | | | |
| Neutral[1] (6.52) | **66.9±0.3$^{b}$** | 751±24$^{b}$ | 44.3±0.2$^{b}$ | 135±11$^{b}$ | 1016±14$^{c}$ | 433±4$^{c}$ |
| Citric acid[2] (4.08) | 65.9±0.0$^{a}$ | 825±8$^{c}$ | 43.9±0.2$^{b}$ | 250±6$^{c}$ | 937±9$^{b}$ | 387±6$^{b}$ |
| Spontaneously fermented[3] (4.48) | 78.8±0.1$^{c}$ | 552±1$^{a}$ | 42.8±0.1$^{a}$ | 109±3$^{a}$ | 582±1$^{a}$ | 155±2$^{a}$ |
| **Cassava-finger millet (30:70)** | | | | | | |
| Neutral (6.34) | 76.8±0.1$^{a}$ | 361±6$^{ab}$ | 43.4±0.0$^{a}$ | 71±4$^{b}$ | 483±6$^{a}$ | 202±5$^{b}$ |
| Citric acid (4.18) | 78.9±0.7$^{b}$ | 402±11$^{b}$ | 43.3±0.1$^{a}$ | 82±8$^{b}$ | 553±2$^{b}$ | 241±0$^{c}$ |
| Spontaneously fermented (4.42) | 87.5±0.1$^{c}$ | 350±16$^{a}$ | 48.6±0.2$^{b}$ | 12±1$^{a}$ | 442±14$^{a}$ | 112±1$^{a}$ |
| **Finger millet-maize (90:10)** | | | | | | |
| Neutral (6.36) | 78.9±0.2$^{a}$ | 133±1$^{a}$ | 42.1±0.1$^{a}$ | 39±1$^{b}$ | 217±0$^{a}$ | 127±1$^{b}$ |
| Citric acid (4.22) | 84.4±0.1$^{b}$ | 221±0$^{c}$ | 42.9±0.0$^{b}$ | 58±0$^{c}$ | 335±1$^{c}$ | 175±1$^{c}$ |
| Spontaneously fermented (4.24) | 86.7±0.1$^{c}$ | 192±1$^{b}$ | 43.3±0.2$^{b}$ | 18±1$^{a}$ | 289±1$^{b}$ | 117±1$^{a}$ |
| **Maize-sorghum (75:25)** | | | | | | |
| Neutral (6.51) | 82.8±0.1$^{a}$ | 65±9$^{a}$ | 41.6±0.5$^{a}$ | 5±0$^{b}$ | 137±14$^{a}$ | 91±8 |
| Citric acid (3.88) | 85.6±0.1$^{b}$ | 82±0$^{a}$ | 42.5±0.0$^{a}$ | 8±2$^{b}$ | 172±2$^{ab}$ | 104±1 |
| Spontaneously fermented (3.92) | 86.9±0.1$^{c}$ | 114±6$^{b}$ | 50.0±0.8$^{b}$ | 0±0$^{a}$ | 224±18$^{b}$ | 114±7 |

PT - pasting temperature; PV - peak viscosity; Time PV - time to peak viscosity; BV - breakdown viscosity; FV - final viscosity; SV - setback viscosity; BU – Brabender Units.

[1]Neutral slurry (10% w/v) was prepared using distilled water; [2]Chemically-acidified slurry (10% w/v) was prepared using anhydrous citric acid solution (2 g/1000 ml); [3]Spontaneously fermented and dried slurry (10% w/v) was reconstituted in distilled water.

Values with the same superscript letters in the same column for each type of composite flour are not significantly different at $p < 0.05$. Data sets without superscript letters for each type of composite flour are not significantly different at $p < 0.05$.

foods with a paste-like consistency and suspended particles (Carvalho et al., 2014; Gujral and Sodhi, 2002). The firmness, consistency, cohesiveness and index of viscosity of the thin porridges ranged from 0.73 to 3.54 N, -19.89 to -95.64 N·s, -1.09 to -7.13 N and -2.34 to -8.98 N·s, respectively (Table 3). Thin porridges made from cereal-based composite flours (that is, finger millet-maize and maize-sorghum) tended to have lower firmness, consistencies, cohesiveness and indices of viscosity than similar products made from cassava-cereal flours. Cassava-based thin porridge with high cassava content (that is, cassava-finger millet, 90:10) had higher firmness, consistency, cohesiveness and index of viscosity than that with lower cassava content (that is, cassava-finger millet, 30:70).

Generally, the consistency of cooked starch slurries increases sharply with increasing amount of flour from about 10% w/v (Carvalho et al., 2014). This is due to the high volume occupied by the swollen starch granules and the leached amylose polymers (Carvalho et al., 2014; Carvalho et al., 2013). Nonetheless, cassava-based thin porridges still tend to be thicker than cereal-based thin porridges because of the higher starch content and lower amounts of extraneous substances, such as fats and proteins in cassava as compared to cereal flours (Juliano, 1999).

Two-factor analysis of variance showed that the interaction effect between the type of composite flour and pH was significant ($p < 0.05$) for firmness, consistency, cohesiveness and index of viscosity. The simple main effect of pH had no significant effect ($p > 0.05$) on the firmness, consistency, cohesiveness and index of viscosity of the maize-sorghum porridge (75:25, Table 3). The simple main effect of pH showed that firmness, consistency, cohesiveness and index of viscosity of thin cassava-finger millet (90:10), cassava-finger millet (30:70) and finger millet-maize (90:10) porridges treated with citric acid tended to be higher than for the neutral or spontaneously fermented porridges. The higher acidity of thin porridges treated with citric acid as compared to the neutral or spontaneously fermented thin porridges (Table 3) may be responsible for the observed differences in the textures of the porridges. The authors postulated that citric acid freed the starch granules from any interfering

**Table 3.** Texture of thin porridge segregated by pH.

| pH | Firmness (N) | Consistency (N·s) | Cohesiveness (N) | Index of viscosity (N·s) |
|---|---|---|---|---|
| **Cassava: finger millet (90:10)** | | | | |
| Neutral[1] (6.52) | 2.33±0.11[a] | 64.24±2.66[a] | -4.28±0.19[a] | -5.11±1.28[b] |
| Citric acid[2] (4.08) | 3.54±0.76[b] | 95.64±20.94[b] | -7.13±1.10[b] | -8.98±4.02[b] |
| Spontaneously fermented[3] (4.48) | 2.50±0.49[a] | 66.78±11.23[a] | -5.24±1.10[a] | -4.35±1.97[a] |
| **Cassava: finger millet (30:70)** | | | | |
| Neutral (6.34) | 0.73±0.11[b] | 19.89±3.34[a] | -1.28±0.28[a] | -2.87±0.62 |
| Citric acid (4.18) | 1.65±0.07[a] | 44.74±1.30[b] | -3.44±0.17[b] | -4.38±1.27 |
| Spontaneously fermented (4.42) | 1.61±0.24[a] | 44.65±6.51[b] | -3.09±0.58[b] | -3.69±1.10 |
| **Finger millet: maize (90:10)** | | | | |
| Neutral (6.36) | 0.90±0.18 | 24.86±5.14 | -1.43±0.29[a] | -3.07±0.51[a] |
| Citric acid (4.22) | 1.25±0.08 | 34.00±2.05 | -2.18±0.15[b] | -4.45±0.30[b] |
| Spontaneously fermented (4.24) | 1.20±0.33 | 33.01±9.57 | -2.12±0.61[b] | -4.05±0.73[b] |
| **Maize: sorghum (75:25)** | | | | |
| Neutral (6.51) | 0.94±0.22 | 24.74±5.96 | -1.26±0.30 | -2.65±0.57 |
| Citric acid (3.88) | 0.89±0.31 | 23.21±8.00 | -1.09±0.40 | -2.34±0.84 |
| Spontaneously fermented (3.92) | 1.02±0.12 | 27.24±3.47 | -1.54±0.16 | -3.28±0.32 |

[1]Neutral slurry (10% w/v) was prepared using distilled water; [2]Chemically-acidified slurry (10% w/v) was prepared using anhydrous citric acid solution (2 g/1000 ml); [3]Spontaneously fermented and dried slurry (10% w/v) was reconstituted in distilled water..
Values with the same superscript letters in the same column for each type of composite flour are not significantly different at $p < 0.05$. Data sets without superscript letters for each type of composite flour are not significantly different at $p < 0.05$.

matrices, thus enabling them to swell more readily and form thicker pastes.

## Objective evaluation of the colour of thin porridge

The lightness, redness and yellowness of thin porridges prepared in neutral or acidic media or from spontaneously fermented composite flours ranged between 53.1-68.8, 4.4-9.3 and 8.4-15.7, respectively (Table 4). Two-factor analysis of variance showed that the interaction effect between the type of composite flour and pH was significant ($p < 0.05$) for lightness, redness and yellowness. Acid-treated thin cassava-finger millet (90:10) porridge was the least dark sample (that is, it had the highest L* and b* values), which implies that addition of citric acid to the cassava-rich sample actually made the porridge lighter in colour. By contrast, neutral finger millet-maize (90:10) porridge was the darkest sample (that is, it had the lowest L* and b* values). This could have been due to the high content of coloured finger millet and low content of white maize in this thin porridge. Pigmented grains are rich in phenolic acids which stain porridges with a dark colour during cooking (Anyango et al., 2011; Kebakile, 2008). Comparison of thin porridges made from cereal-based composite flours showed that irrespective of the method of pH adjustment, thin maize-sorghum (75:25) porridges were lighter, yellower and redder than the finger millet-maize (90:10) porridges. In conclusion, these results show that the colour of thin porridge was influenced by the botanical origin of theflours, their ratios and the method of acidification.

## Descriptive sensory evaluation of thin porridge

Principal component analysis was used to evaluate the mean panellist and replication scores of the 15 sensory attributes identified in thin porridges (Table 1). The first three PCs accounted for 83.7% of the total variance (Table 5).

Loadings with absolute values greater than 0.354 (marked with an asterisk) represented a strong influence on the sensory character of the thin porridges. The first PC accounted for 32.9% of the variance in the sensory attribute data and separated the thin porridges on the basis of the botanical origin of the flour (Table 5). Thin porridges made from cereal-based composite flours were situated on the right side of the PCA plot whereas those containing cassava were on the left side, except for the

**Table 4.** Colour of thin porridge segregated by pH.

| pH | Colour | | |
|---|---|---|---|
| | L* | a* | b* |
| **Cassava: finger millet (90:10)** | | | |
| Neutral[1] (6.52) | 64.6±0.1[b] | 4.7±0.1[a] | 13.1±0.1[b] |
| Citric acid[2] (4.08) | 68.8±0.6[c] | 6.1±0.1[b] | 15.7±0.4[c] |
| Spontaneously fermented[3] (4.48) | 57.4±0.3[a] | 6.2±0.3[b] | 8.7±0.2[a] |
| **Cassava: finger millet (30:70)** | | | |
| Neutral (6.34) | 57.2±0.3[a] | 6.3±0.1[b] | 9.1±0.2[a] |
| Citric acid (4.18) | 58.1±0.7[a] | 7.4±0.3[c] | 9.8±0.4[a] |
| Spontaneously fermented (4.42) | 65.8±0.9[b] | 4.4±0.1[a] | 13.1±0.3[b] |
| **Finger millet: maize (90:10)** | | | |
| Neutral (6.36) | 53.1±0.4[a] | 6.4±0.2[a] | 7.8±0.2[a] |
| Citric acid (4.22) | 53.8±0.7[a] | 7.8±0.1[b] | 9.1±0.1[c] |
| Spontaneously fermented (4.24) | 57.2±0.4[b] | 6.4±0.1[a] | 8.4±0.3[b] |
| **Maize: sorghum (75:25)** | | | |
| Neutral (6.51) | 56.2±0.3[a] | 6.9±0.1[a] | 9.3±0.3[a] |
| Citric acid (3.88) | 62.2±0.6[c] | 9.3±0.1[c] | 14.2±0.4[c] |
| Spontaneously fermented (3.92) | 59.6±0.3[b] | 7.7±0.2[b] | 10.9±0.3[b] |

L*: lightness; a*: redness; b*: yellowness.
[1]Neutral slurry (10% w/v) was prepared using distilled water; [2]Chemically-acidified slurry (10% w/v) was prepared using anhydrous citric acid solution (2 g/1000 ml); [3]Spontaneously fermented and dried slurry (10% w/v) was prepared using distilled water.
Values with the same superscript letters in the same column for each type of composite flour are not significantly different at $p < 0.05$. Data sets without superscript letters for each type of composite flour are not significantly different at $p < 0.05$.

**Table 5.** Principal component factor loadings for thin porridge attributes.

| Attribute | PC1 | PC2 | PC3 |
|---|---|---|---|
| Colour | 0.196 | -0.327 | -0.354* |
| White specks | 0.163 | 0.217 | 0.196 |
| Dark specks | 0.088 | -0.261 | -0.238 |
| Gloss | -0.213 | -0.168 | 0.100 |
| Cassava aroma | -0.393* | -0.221 | 0.380 |
| Finger millet aroma | 0.347 | -0.496* | -0.212 |
| Maize aroma | 0.297 | 0.406* | 0.230 |
| Sorghum aroma | 0.021 | 0.070 | 0.011 |
| Fermented aroma | -0.289 | 0.326 | -0.672* |
| Sour taste | -0.315 | 0.252 | -0.248 |
| Viscosity | -0.264 | -0.182 | 0.073 |
| Coarseness | 0.342 | 0.183 | -0.050 |
| Adhesiveness | -0.126 | -0.073 | 0.003 |
| Sour aftertaste | -0.165 | 0.125 | -0.064 |
| Residual particles | 0.325 | 0.163 | -0.039 |
| Variance % | 32.9 | 27.4 | 23.4 |
| Cumulative variance % | 32.9 | 60.3 | 83.7 |

*Loadings with absolute values greater than 0.354.

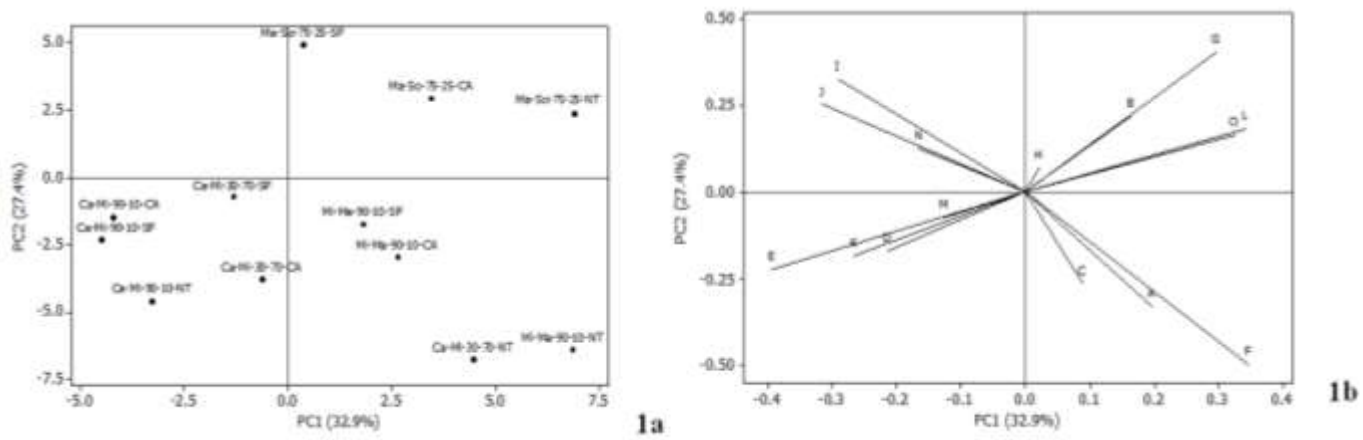


**Figure 1.** Principal component analysis of thin porridge. (1a) Plot of the first two principal component scores of composite flours used to prepare thin porridge. (1b) Plot of the first two principal component loading vectors of sensory attributes of thin porridge. Ca: cassava; So: sorghum; Mi: finger millet; Ma: maize; SF: spontaneously fermented; CA: chemically acidified; NT: neutral. The numbers refer to the ratios of the flours used. A: colour; B: white specks; C: dark specks; D: gloss; E: cassava aroma; F: finger millet aroma; G: maize aroma; H: sorghum aroma; I: fermented aroma; J: sour taste; K: viscosity; L: coarseness; M: adhesiveness; N: sour aftertaste; O: residual particles.

neutral cassava-finger millet (30:70) porridge (Figure 1a). Cassava-containing thin porridges were characterized by a strong cassava aroma (loading value -0.393) in addition to being more viscous and glossy than the cereal-based thin porridges (Figure 1b). Cereal-based thin porridges were characterized by finger millet or maize aroma, coarse mouthfeel, and residual particles in the mouth after swallowing.

Unblended flours are largely unsuitable for making thin porridges. Thin porridge made from cassava flour has a starchy taste and flavour, and jelly-like consistency, whereas cereal-based thin porridges have a bland taste and rough mouthfeel (Wanjala et al., 2016). These undesirable sensory attributes of thin porridges can be mitigated by blending cassava with cereal flours in appropriate ratios. Cassava imparts a smooth texture to thin porridges and decreases the grittiness caused by the cereal endosperm and bran particles. By contrast, cereal flours decrease the viscosity of thin porridges that contain cassava flour (Wanjala et al., 2016).

The second PC accounted for 27.4% of the variance in the sensory attribute data (Table 5). It separated the thin porridges on the basis of maize aroma (loading value 0.406) and finger millet aroma (loading value -0.496). Thin porridges located in the upper part of the plot were characterized by a strong maize aroma due to the high concentration of maize in the composite flours (Figure 1a). The thin maize-sorghum porridges were also characterized by presence of many white specks, coarse mouthfeel and residual particles in the mouth after swallowing (Figure 1b). By contrast, the thin maize-finger millet (90:10) porridges, which were located in the lower part of the plot, were dark in colour and had many dark specks (Figure 1b). Cereal grains each have their characteristic flavour profiles and precursors, which intensify further during processing due to process-induced changes in grain biopolymers and flavour-active compounds (Heiniö, 2003). The flavour of cereal grain products originate from the inherent volatile compounds, such as aldehydes, ketones and alcohols; and non-volatile compounds such as phenolic compounds, amino acids, small peptides, fatty acids and sugars (Heiniö et al., 2016).

The third PC accounted for 23.4% of the variance in the sensory attribute data (Table 5). It separated the thin porridges on the basis of the fermented aroma (loading value -0.672) and colour (loading value -0.354). The aroma of spontaneously fermented thin porridges is due to the production of lactic acid and minor products of bacterial metabolism during fermentation (Mugula et al., 2003; Muyanja et al., 2003; Mukisa et al., 2016). As shown in the section on instrumental analysis of colour, the colour of thin porridges was influenced by the botanical origin of the flour, their ratios and the method of acidification (Table 4).

Small loadings (that is, values close to zero) are a source of valuable information in the interpretation of PCA data because they indicate that the PC is not related to those variables (Lawless and Heymann, 2010). Thus, the low loading value for sorghum aroma across all PCs (Table 5) is in agreement with the low content of sorghum in the thin maize-sorghum (75:25) porridge. Sorghum

aroma was hardly detectable in the neutral, chemically-acidified or spontaneously fermented thin porridges made from maize-sorghum (75:25) flours.

## Conclusion

Thin porridge is an important refreshment drink for millions of people in sub-Saharan Africa. In addition, it is an important complementary food and source of nourishment for the sick and the elderly. Instrumental and sensory methods are both useful in identifying the sensory attributes of thin porridge. Instrumental tests showed that the pH and type of composite flour affected the firmness, cohesiveness, consistency, index of viscosity and colour of thin porridges. The sensory attributes identified were influenced by the blending ratios of the flours and pH of their slurries. Aroma and colour were identified as the most important sensory attributes of thin porridge.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Two withanolides from *Withania somnifera* (solanaceae) and activity of methanolic extracts against fungal and bacterial pathogens that affects food crops

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**Methanolic extract obtained from the dried leaves of *Withania Somnifera* was subjected to column chromatography leading to the isolation of two withanolides. Their structures were elucidated using 1D and 2D NMR spectroscopy. The isolated withanolides were determined to be 5α, 17β-dihydroxy-6α, 7β-epoxy-1-oxo-witha-2, 24-dienolide (1) and 4, 5, 6, 15-tetrahydroxy-1-oxo-witha-7-enolide (2). The bioassay of the methanolic extracts showed activity against the fungal pathogens, *Fusarium moniliforme*, *Fusarium graminearum*, *Colletotrichum lindemuthianum* and *Pythium* spp. The extract was also active against bacterial pathogens, *Xanthomonas campestris* pv. *phaseoli* and *Pseudomonas syringae* pv. *phaseolicola.* The withanolides did not show any bioactivity indicating that they are either active only synergistically with other compounds within the extracts or there are other active compounds in the extract.**

**Key words:** *Withania somnifera*, withanolides, methanolic extract, antifungal, antibacterial.

## INTRODUCTION

*Withania somnifera*, known commonly as ashwagandha (in India), Indian ginseng, poison gooseberry or winter cherry (English), is a plant in the genus solanaceae or nightshade family. This species is a short shrub growing 1 to 2 m tall. It is the most common and widespread species in the genus and occurs naturally, mainly in the drier regions, from the Mediterranean through tropical Africa to South Africa. It is grown in India and elsewhere as a medicinal crop plant, mainly for its fleshy roots and is the main herb used in Ayurvedic medicine (Rani et al., 2012).It occurs naturally in East and Central Africa. The major chemical compounds reported from *W. somnifera* are withanolides (Misra et al., 2007). These are structurally diverse steroidal compounds with an ergosterol skeleton, whereby C-22 and C-26 are oxidized to form a δ-lactone. Other main chemical constituents are alkaloids and flavanoids, (Bashir et al., 2013). Numerous studies have been done and many compounds are isolated from the leaves, roots and berries of *W. somnifera*, withanolides, notably withaferin A, 27-hydroxy

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withanone, 17-hydroxy withaferin A, 17-hydroxy-27-deoxy withaferin A, withanolide D, 27-hydroxy withanolide B, withanolide A, withanone, 27-deoxywithaferin A, L-asperaginaseetc Mirjalili et al., 2009; Oza et al., 2010 Singh et al. 2010). The presence of the many chemical constituents have been confirmed through phamacognistic and phytochemical analysis of this plant (Rani et al., 2012).

There is increasing infestation of diseases on food crops caused by fungal and bacterial pathogenss. It is estimated that 10% of food production is lost to diseases every year (Strange and Scott, 2005). The chemical means of control used has brought more harm than good. Reseachers are on the look for better and more safe ways to control these diseases. In this study, the activity of the methanolic extracts of *W. somnifera* against fungal pathogens; *Fusarium moniliforme*, *Fusarium graminearum*, *Colletotrichum lindemuthianum* and *Pythium* spp., bacterial pathogens; *Xanthomonas campestris* pv. *phaseoli* and *Pseudomonas syringae* pv. *phaseolicola* was investigated. Isolation of two withanolides from the leaves of *W. somnifera* is also reported. The structures were ascertained by comparing the spectral data with those reported in the literature. Elucidation was done by spectroscopic methods and chemical transformations, 1D and 2D NMR was used to determine the structures, which are discussed in this paper.

## MATERIALS AND METHODS

The leaves of *W. Somnifera* were collected at the Botanical garden of Egerton University. The taxonomical identification was done at the department of Biological Sciences where the voucher specimen was deposited. The dried leaves were dried under shade and ground into a fine powder using a grinding mill (Thomas-Wiley mill model 4). About 500 g of the ground sample was soaked with about 700 ml of methanol for 72 h and filtered through activated charcoal. The dried methanol extract was subjected to thin layer chromatography (TLC) analysis to determine the optimum solvent system which was found to be ethyl acetate and hexane in the ratio of 7:3. Purification was done through column chromatography with this solvent system.

### Preparation of the sample for bioassays

From the dry methanol crude extracts, the following concentrations were prepared in dimethylsulfoxide (DMSO) 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml; the different concentrations were used for antibacterial tests. For compounds 1 and 2, 40 mg/ml was prepared. Sensitivity discs (6 mm) were used. The discs were sterilized by autoclaving at 121°C for 15 min.

### Screening for antifungal activity

The extracts were screened for antifungal activities. Paper disc diffusion inhibition test was used to screen for antifungal activity of the methanolic crude extracts. One millilitre of fungal suspension (approximately 106 spores) was uniformly spread on sterile SDA media in Petri dishes (khan et al., 2008). A concentration of 100 mg/ml sample was used. Sterilized paper discs were impregnated with 10 µl of the prepared concentration and placed at the centre of the inoculated culture plates. The experiments were done in triplicates. The plates were then incubated at 25°C.

The inhibition zones were clearly visible on the third day after the incubation and measurement of the diameters of inhibition zones was done and the average of the three measurements taken. Nystatin discs were used as reference standard (positive control) while the negative control was DMSO. The fungi that were used include *Fusarium moniliforme*, *Fusarium graminearum, pyThium ultimum* and *Colletotrichum lindemuthianum.* The diameters of the inhibition zones are shown in Table 1.

**Table 1.** Antifungal activity of methanol crude extracts from *W. somnifera* (100 mg/ml).

| Test sample/organism | FG | FM | PU | CL |
|---|---|---|---|---|
| *Withania somnifera* | 11.5 | 8.0 | 7.3 | 12.0 |
| Compound 1 | 6.0 | 6.0 | 6.0 | 6.0 |
| Compound 2 | 6.0 | 6.0 | 6.0 | 6.0 |
| Nystatin | 29.6 | 21.0 | 13.0 | 10.6 |
| DMSO(control) | 6.0 | 6.0 | 6.0 | 6.0 |

FG-*fusariumgraminearum*, FM- *fusarium moniliforme*, PU- *pythium ultimum*, CL- C*olletroticum lindermuthianum,* inhibition zones inclusive of 6mm discs. Measurements are in mm.

### Test for antibacterial activity

*Xanthomonas campestris* pv. *Phaseoli* and *P. syringae* pv. *phaseolicola* were isolated from infected beans and identified through various tests and molecular assays at the Biological Sciences Laboratory of Egerton University. The pathogens were then sub cultured in nutrient broth. The bacterial suspension (10 µl) was spread over 90 mm Petri dishes containing Mueller Hinton agar using a sterile cotton swab (Yeo and Livermore, 1994).Then, 6 mm diameter sterile discs were impregnated with 10 µl of the methanolic extract and placed at the centre of the inoculated Agar Petri dishes. They were allowed to diffuse for 5 min and the plates were then kept in an incubator at 37°C for 24 h, the experiment was done in triplicate and an average was taken. The antibacterial activity was evaluated by measuring the zones of growth inhibition surrounding the discs in millimetre with a ruler (Wahi et al., 2011). Chloramphenicol discs (30 µg) which is a broad spectrum antibiotic, was used for comparison as a positive control and DMSO as a negative control.

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration in this report is defined as the minimum concentration of the extract that can inhibit the growth of bacteria. The MIC for the extract was determined by dilution of the extract using a double fold serial dilution (Sule and Agbabiaka, 2008). The initial concentration of 200 mg/ml (stock solution) was prepared by measuring 400 mg of the methanol extract and dissolving in 2 ml of DMSO. By diluting 1 ml of 200 mg/ml (stock solution) to 2 ml using DMSO, 100 mg/ml concentration was obtained. From 100 mg/ml (stock solution), 1 ml was diluted to 2 ml to make a concentration of 50 mg/ml. The above process was repeated several times to obtain the other concentrations, 25, 12.5, 6.25 and 3.15 mg/ml. Then 6 mm diameter sterile discs were impregnated with 10 µl of the different concentrations and placed at

**Table 2.** Inhibition zones for antibacterial activity of methanol leaves extract of *W. somnifera* (mm).

| S/N | Concentration mg/ml | *Pseudomonas syringae* pv. *phaseolicola* | *Xanthomonas campestris* pv. *phaseoli* |
|---|---|---|---|
| 1 | 200 | 13 | 13 |
| 2 | 100 | 8.0 | 12.0 |
| 3 | 50 | 7.0 | 7.0 |
| 4 | 25 | 7.0 | 7.0 |
| 5 | 12.5 | 7.0 | No inhibition |
| 6 | 6.25 | No inhibition | No inhibition |
| 7 | 3.125 | No inhibition | No inhibition |
| 8 | Chloramphenicol | 30 | 30 |

DMSO- No activity, inhibition zones inclusive of 6 mm discs.

the centre of the inoculated Agar Petri dishes. After allowing diffusion for five minutes, the plates were incubated at 37°C for 24 h. The experiment was done in triplicates and the average measurements taken as shown in Table 2.

#### Isolation and purification by column chromatography

The column was packed by slurry method using silica gel 60 0.6-0.2 mm (70-230 mesh ASTM), Supplied by Indo-lab suppliers. Approximately 2 g of the dry crude extracts was weighed and mixed with 2 g of silica gel. Using a pestle and motor, the extracts and silica gel, were ground into fine dark brown powder. The finely ground sample was then introduced into the column and eluted with the solvent system established through TLC analysis, (7:3 ethyl acetate, hexane). Fractions of equal volumes were collected and TLC analysis of each fraction was done. Fractions with the same TLC patterns were pooled together and concentrated. After extensive column purification, two compounds were isolated and analysed by NMR spectroscopy. The NMR data are shown in Tables 3 and 4 and the NMR spectrum is shown in Appendices 1 to 9.

## RESULTS AND DISCUSSION

The extract showed significant inhibition on all the tested fungal pathogens. The highest activity was observed against *C. Lindermuthianun (*12 mm) as compared to the reference standard; Nystatin which registered an inhibition zone of 10.6 mm (Table 1). The isolated compounds 1 and 2 did not show any bioactivity, indicating that they are either active only synergistically with other compounds within the extracts or there are other active compounds in the extract.

In Table 2, it is evident that methanolic extracts of *W. somnifera* showed significant inhibition on the growth of the bacterial pathogens even at lower concentrations. The activity of the extract increased with increasing concentrations. The inhibition zones were visible even after 72 h of incubation for bacterial and hence the serial dilution was done to establish the minimum inhibitory concentrations (MIC). The MIC for *P. syringae* pv. *Phaseolicola* was found to be 12.5 and 25 mg/ml for *X. Campestris* pv. *phaseoli*.

### Structure elucidation

Two compounds were isolated from *W. somnifera* (1 and 2) whose structures have steroidal lactones skeleton. Compound 1(figure1) was obtained as a crystalline solid after running the column on crude methanol extract using ethyl acetate and hexane in the ratio 7:3, respectively. The HRMS spectrum showed [M] $^{+}$atm/z 470.6028 which corresponds to the molecular formula $C_{28}H_{38}O_6$. The 1HNMR spectrum revealed presence of five methyl signals of withanolides at δ= 0.89 (s), 1.21 (s), 1.07 (d), 1.91 (s), 1.97 (s) for H-18, H-19, H-21, H-27 and H-28, respectively.

The $^{13}$C NMR spectra of compound 1 supported the presence of 28 carbon resonances. Which include five methyl, six methylene, nine methyne and eight quaternary carbons. Downfield signals at δ= 203.1, s for C-1; 129.0, d for C-2; 139.7, d for C-3 were indicative of α, β-unsaturated ketone. The signals for E ring (α, β-unsaturated lactone at δ=167.2for C-26; δ=78.5 for C-22; δ=150.5 for C-24 and δ=121.3 for C-25) imply that C-26 and C-22 have oxidized to form the usual E ring of a withasteroid. The singlet at δ=73.1 for C-5 along with the doublets at δ=56.3 for C-6 and δ=57.2 for C-7 corresponded to the hydroxyl and epoxide groups, respectively, matching with the iso-withanone isolated from the berries of *W. somnifera*, (Lal et al., 2006).$^{13}$C NMR was also indicative of the presence of another hydroxyl at the quaternary carbon C-17.

With further correlations through HSQC spectrum, there were correlations between C-2 and a proton absorbing at δ5.88, while C-3 was attached to a proton resonating at δ 6.62. C-4 was observed to correlate with proton resonating at δ 2.55, while C-6 and C-7 correlated with protons absorbing at δ 3.08 and δ 3.35, respectively. The proton resonating at δ 1.81, δ 1.58, δ 2.86 and δ 2.50 correlated with C-8, C-9, C-11, and C-12, respectively. There was a correlation between proton absorbing at δ 2.05 δ 1.95 and 2.58 with C-14, C-15 and C-16, respectively. C-20 and C-21 correlated with proton at δ 2.36 and δ 1.07, while C-22 correlated with a proton

**Table 3.** NMR data of compound 1 in CDl3.

| Carbon | $^{13}C(\delta)$ | HSQC(δ) | APT | COSY | HMBC |
|---|---|---|---|---|---|
| 1 | 203.1 | - | C | - | - |
| 2 | 129. | 5.8 | $CH_2$ | 3 | 3,4 |
| 3 | 139.7 | 6.62 | $CH_2$ | 2 | 2,4 |
| 4 | 36.8 | 2.55 | CH | 3 | 2,3,5,10, |
| 5 | 73.3 | - | C | - | - |
| 6 | 56.3 | 3.08 | CH | 7 | 5, 8,10, |
| 7 | 57.2 | 3.35 | CH | 6,8 | 8,9 |
| 8 | 36.0 | 1.81 | CH | 7,9,14 | 9,14 |
| 9 | 35.2 | 1.58 | CH | 8,11 | 8,10,11 |
| 10 | 51.0 | - | C | - | - |
| 11 | 21.6 | 2.86 | $CH_2$ | - | 9,12 |
| 12 | 32.8 | 2.50 | $CH_2$ | - | 11,13 |
| 13 | 48.7 | - | C | - | - |
| 14 | 45.9 | 2.05 | CH | - | 8,9,13,15 |
| 15 | 22.9 | 1.94,1.40 | $CH_2$ | 16 | 14,16 |
| 16 | 36.6 | 2.58 | $CH_2$ | 15 | 14,15,17,20 |
| 17 | 84.7 | - | C | - | - |
| 18 | 15.1 | 0.89 | $CH_3$ | - | 12,13,1417 |
| 19 | 14.7 | 1.21 | $CH_3$ | - | 1,5,9,10 |
| 20 | 42.9 | 2.36 | CH | 21 | 17,21,22 |
| 21 | 9.5 | 1.07 | $CH_3$ | 20 | 17,20,22, |
| 22 | 78.7 | 4.64 | CH | 20,23 | 23 |
| 23 | 32.5 | 1.67 | $CH_2$ | 22 | 20,22,24 |
| 24 | 150.5 | - | C | - | - |
| 25 | 121.4 | - | C | - | - |
| 26 | 167.2 | - | C | - | - |
| 27 | 12.4 | 1.91 | $CH_3$ | - | 24,25,26 |
| 28 | 20.5 | 1.97 | $CH_3$ | - | 23,24,25 |

**Table 4.** NMR data of compound 2 in CDl3.

| Carbon | $^{13}C(\delta)$ | $^{1}H(\delta)$ | APT | COSY | HMBC |
|---|---|---|---|---|---|
| 1 | 211.4 | - | C | - | - |
| 2 | 37.4 | 1.8,1.67 | $CH_2$ | 3 | 3,4 |
| 3 | 25.9 | 1.67 | $CH_2$ | 2 | 2,4 |
| 4 | 70.9 | 3.8 | CH | - | 5,10 |
| 5 | 73.3 | - | C | - | - |
| 6 | 77.4 | 3.21 | CH | 7 | 5,7 |
| 7 | 130 | 3.57 | CH | | 5,6 |
| 8 | 136 | C | C | | - |
| 9 | 52.7 | 2.53 | CH | | 5,7,8,10,11 |
| 10 | 47.7 | - | C | | - |
| 11 | 25.4 | 1.67 | $CH_2$ | | 9,12 |
| 12 | 32.4 | 1.37 | C | | 11,13 |
| 13 | 49.6 | - | C | | - |
| 14 | 51.0 | 3.53 | CH | 15 | 13,15 |
| 15 | 84.3 | 4.05 | $CH_2$ | 14 | 13,14,16,17 |
| 16 | 26.4 | 2.45, 2.16 | $CH_2$ | 17 | 14,17,20 |
| 17 | 48.4 | 2.11 | CH | 16 | 13,16,18,21 |

**Table 4.** Contd.

| | | | | |
|---|---|---|---|---|
| 18 | 11.9 | 0.77 | $CH_3$ | 12,13,14,17 |
| 19 | 16.2 | 0.94 | $CH_3$ | 1,5,9,10 |
| 20 | 41.1 | 1.4 | CH | 17,20,21 |
| 21 | 16.7 | 0.72 | $CH_3$ | 17,20,22, |
| 22 | 81.2 | 3.97 | CH | 17,21.23, |
| 23 | 28.3 | 1.7,1.1 | $CH_2$ | 20,22,24,25 |
| 24 | 29.3 | 1.2 | CH | 23,25,26 |
| 25 | 40.8 | - | C | - |
| 26 | 182.2 | - | C | - |
| 27 | 16.3 | 0.94 | $CH_3$ | 24,25,26 |
| 28 | 17.0 | 1.36 | $CH_3$ | 23,24,25 |

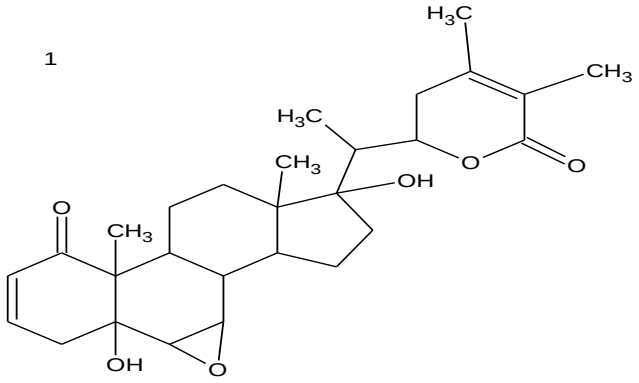


**Figure 1**. Structure of compound 1.

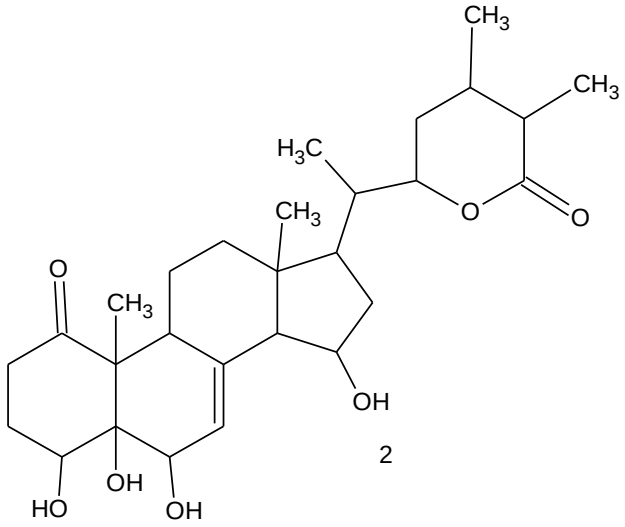


**Figure 2.** Structure of compound 2.

at δ4.64. Also, C-23 correlated with proton at δ1.67, while C-27 and C-28 correlated with protons resonating at δ 1.91 and δ 1.07, respectively.

The proton-proton COSY correlation for compound 1 was determined. From the COSY spectrum, the proton H-2 absorbing at δ5.88 showed correlations with proton at carbon 3 (δ 6.62), also H-3 absorbing at δ 6.62 showed correlation with proton at carbon 4 (δ 2.55). Protons 6 (δ 3.08) had only one cosy correlation with proton 7 (δ3.35) but 6 and 7 showed cosy correlation with each other. Proton 7 showed two cosy correlations, with 6 (δ 3.08) and 8 (δ 1.81). Furthermore, proton 20 (δ 2.36) showed two cosy correlations with proton 21 (δ 1.07) and proton 22 (δ 4.64).

The proton-carbon correlation (HMBC) of compound 1 showed that, C-3 and C-4 with protons absorbing at δ 5.88 and δ 6.62 showed correlations. Proton absorbing at δ 2.55(H-4) showed correlations with C-2,C-3, C-5 and C-10, while a proton absorbing at δ 3.08 (H-6) showed correlations with C-8, C-5 and C-10. C-8 and C-9 correlated with a proton resonating at δ 3.35 (H-7) while proton absorbing at (δ 1.58) showed correlations with C-8 and C-10. H-11 at δ 2.86 correlated with C-9 and C-12 and correlations was also observed between proton at δ 2.50 (H-12) with C-11 and C-13. H-14 absorbing at δ 2.05 showed correlation with C-8, C-9, C-13 and C-15. While a proton absorbing at δ 1.95 (H-15) showed correlations with C-14 and C-16. H-16 absorbing at δ 2.58 correlated with C-14, C-15, C-17 and C-20. H-20 at δ2.58 showed corrections with C-17, C-21 and C-22, while another correlation was observed at H-22 (δ4.64) with C-23. A proton at H-23 absorbing at δ 2.50 correlated with C-20, C-22 and C-24. The methyl proton absorbing at δ 1.91 correlated with C-24, C-25 and C-26, while proton absorbing at δ 1.97 (H-28) correlated with C-24, C-25 and C-26. The NMR data for compound 1 are shown in Table 3.

Compound 2(figure 2) was obtained as a crystalline solid after running the column on crude methanol extract using ethyl acetate and hexane in the ratio of 7:3, respectively. The 1HNMR spectrum revealed presence of five methyl signals of withanolides at δ= 0.77, 1.13, 0.72, 1.36,0.94for H-18,H-19,H-21,H-27and H-28, respectively.

The $^{13}C$ NMR and DEPT spectra of compound 2 supported the presence of 28 carbon resonances with the mass formula of 490.699 and molecular formula $C_{28}H_{48}O_6$, which include five methyl, six methylene, nine methyne and eight quaternary carbons. Compound 2 was similar to 1 with the withanolides skeleton structure. The difference occurred in the orientation of the functional groups. At C-4, C-6 and C-15 are hydroxyl groups and a double bond between C-7 and C-8. Between C-24 and C-25, usual double bond for withanolides was missing. This was evidenced through C-26 absorbing at δ 182. The HSQC, COSY and DEPT were determined and are shown on Table 4.

## Conclusions

The compounds isolated from *W. somnifera,* 5α, 17β-dihydroxy-6α,7β-epoxy-1-oxo-witha-2,24-dienolide(1) was isolated for the first time from the leaves, since it was first isolated from the berries of the plant (Lal et al., 2006). We also report for the first time, the isolation of 4, 5, 6, 15 tetrahydroxy-1-oxo-witha-7-enolide (2) from the leaves of *W. somnifera,* and the bioassays of the crude methanolic extract against the fungal pathogens which revealed that, the extract was more active against C. *Lindermuthianum (*12 mm) than the standard nystatin which is registered as an inhibition zone of 10.6 mm. The extract showed significant activities against all the test pathogens. These results could justify the extensive use of *W. somnifera* for treatment of various ailments. The results also confirm reports of other researchers (Bashir et al., 2013; Baskaran and Velus, 2012; Singariya et al., 2012). The isolated compounds 1 and 2 did not show any activity on the tested pathogens.

## Conflict of interest

The authors declare there is no conflict of interest.

## ACKNOWLEDGEMENTS

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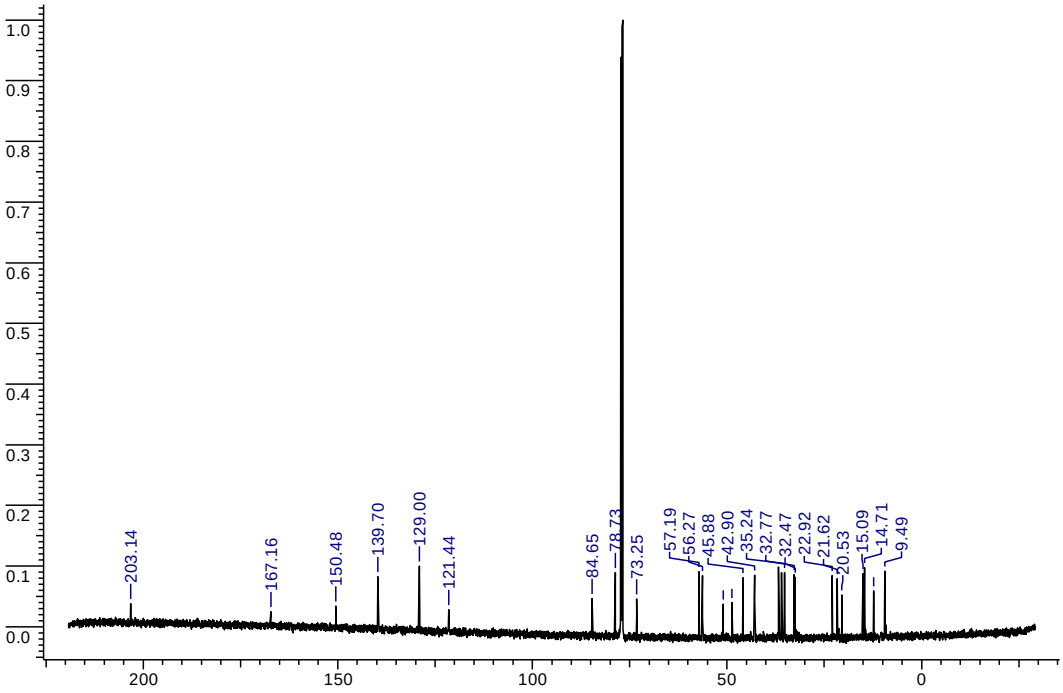
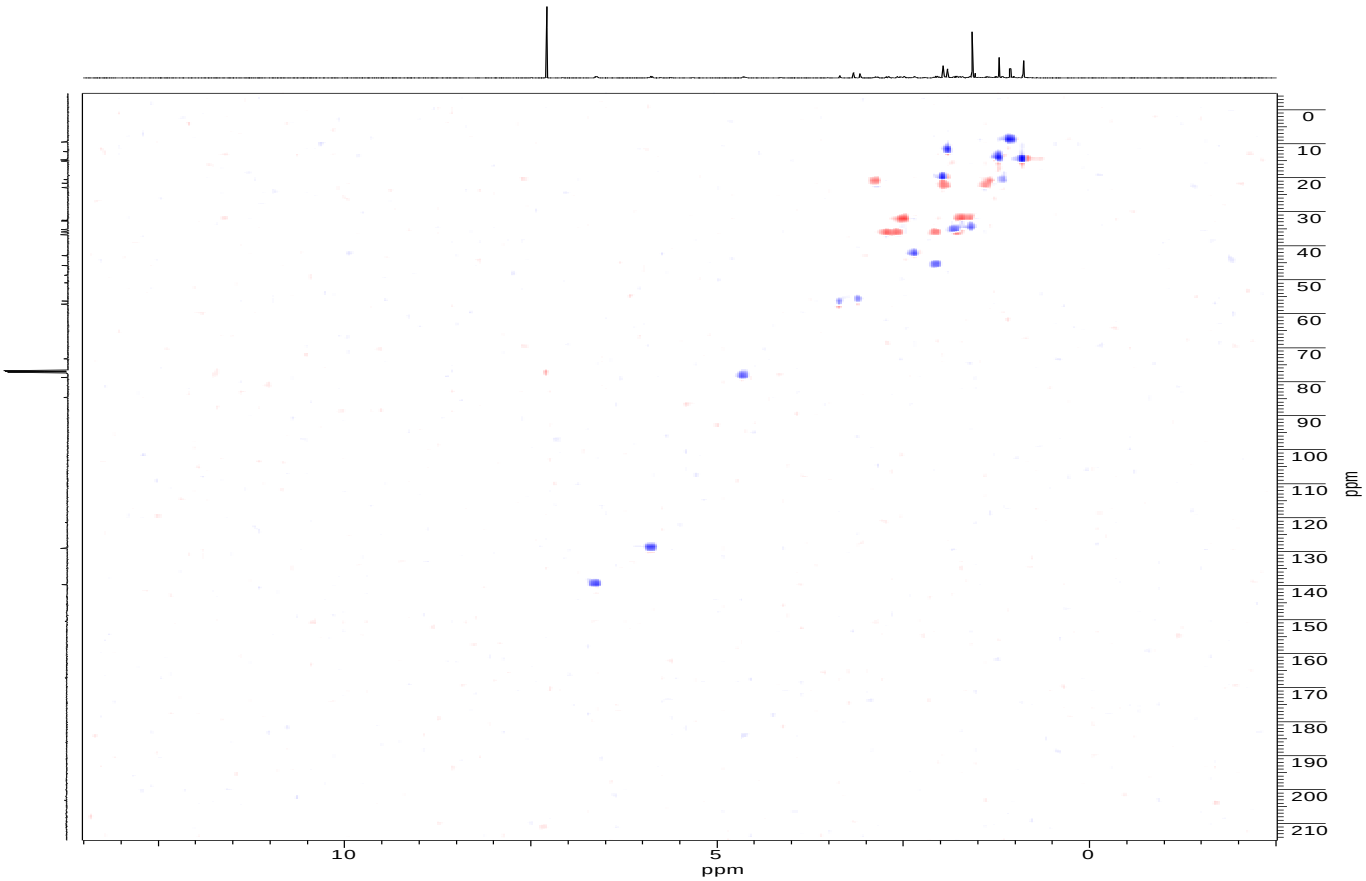
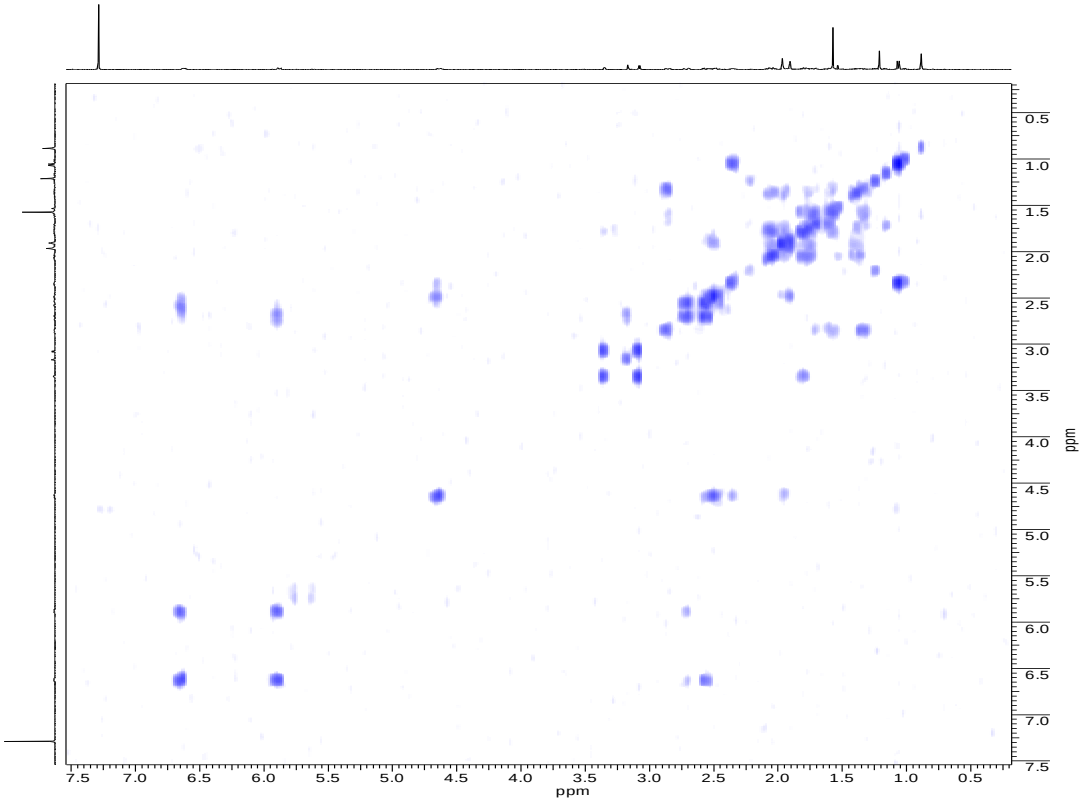


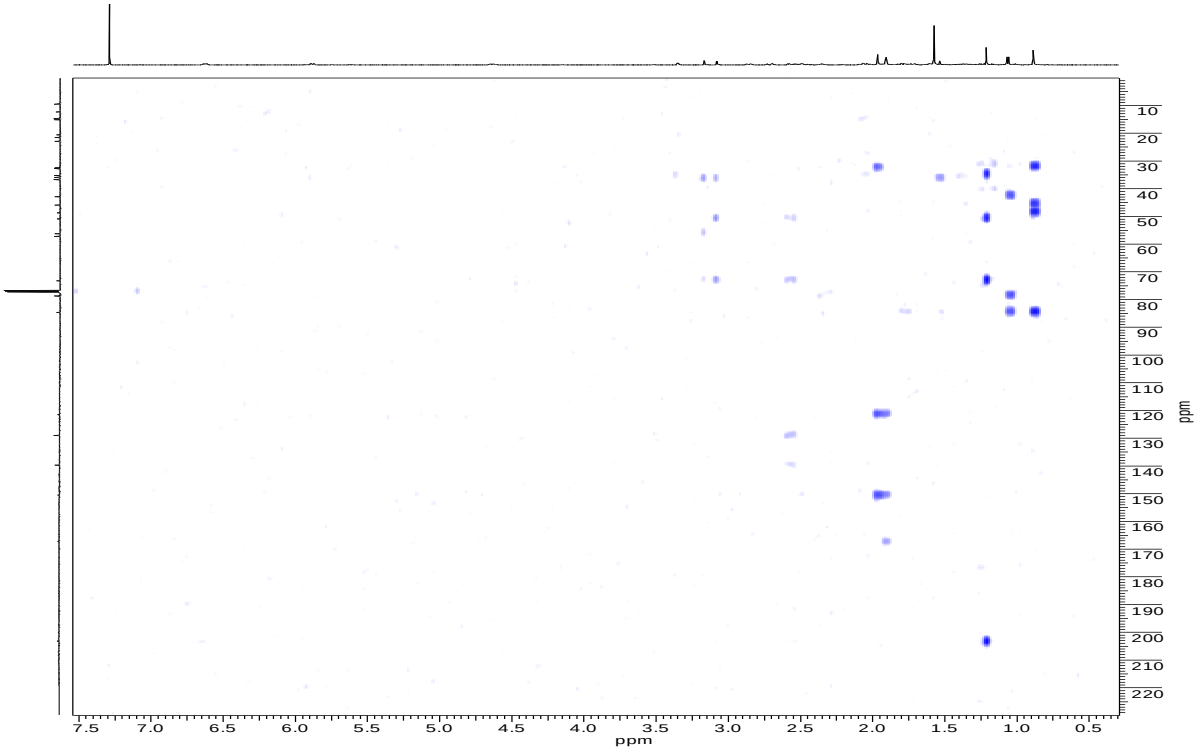

**Appendix 1.** $^{13}C$ NMR of compound 2.



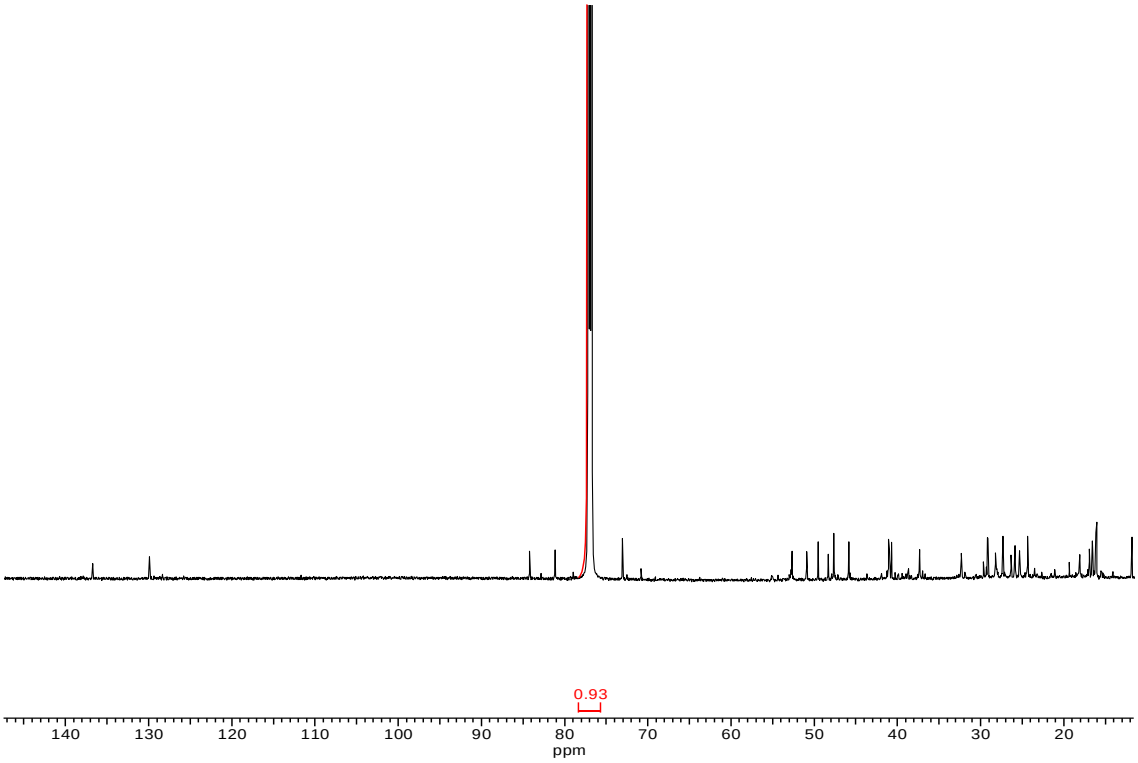

**Appendix 2.** HSQC spectrum of compound 1.



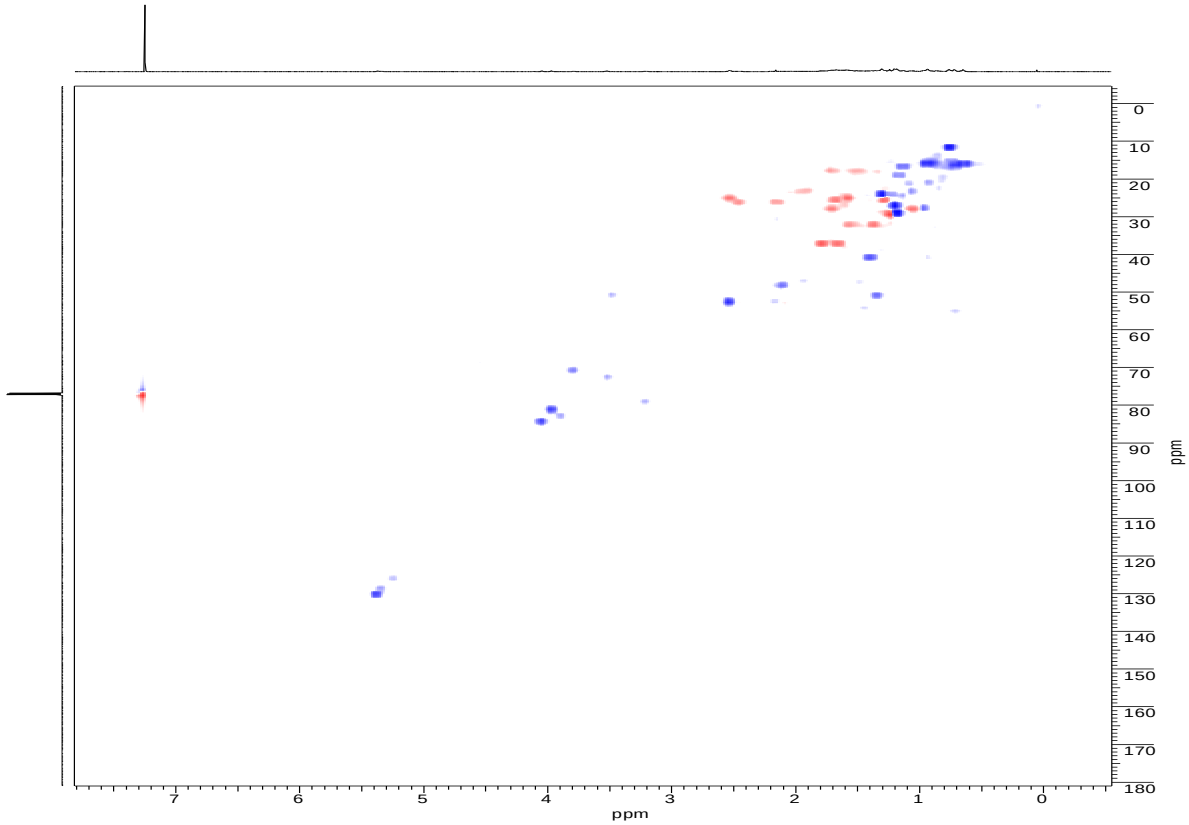

**Appendix 3.** COSY Spectrum of compound 1.



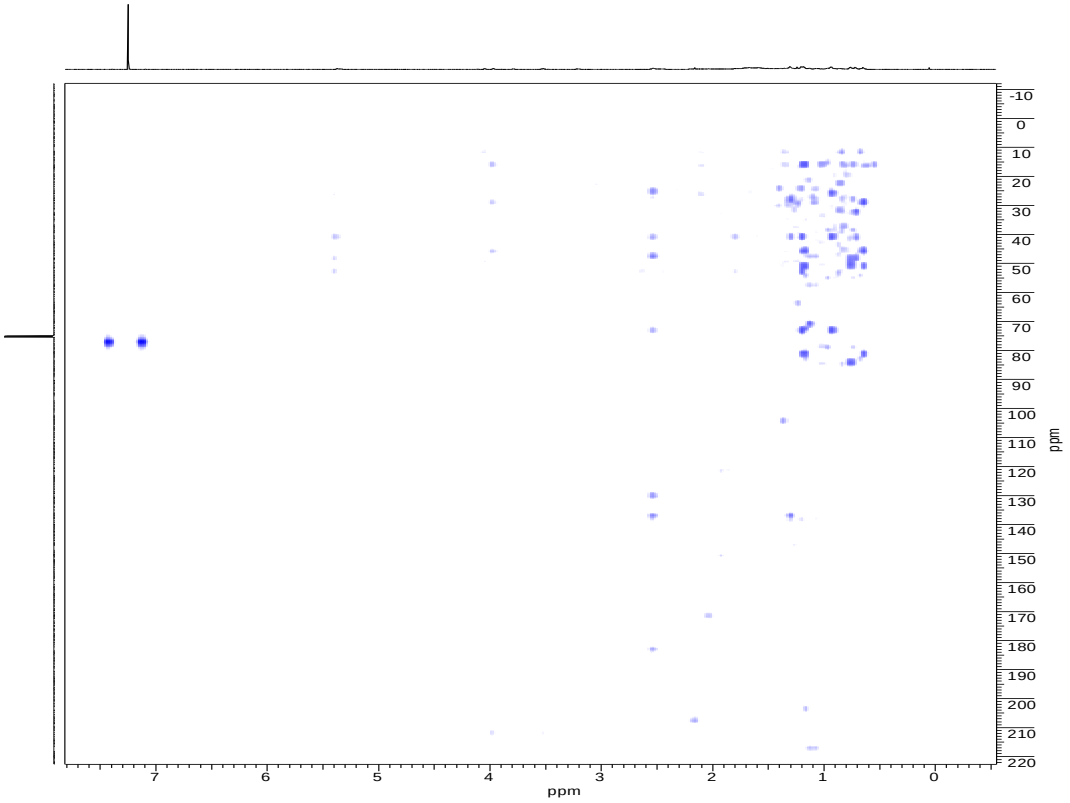

**Appendix 4.** HMBC spectrum of compound 1.



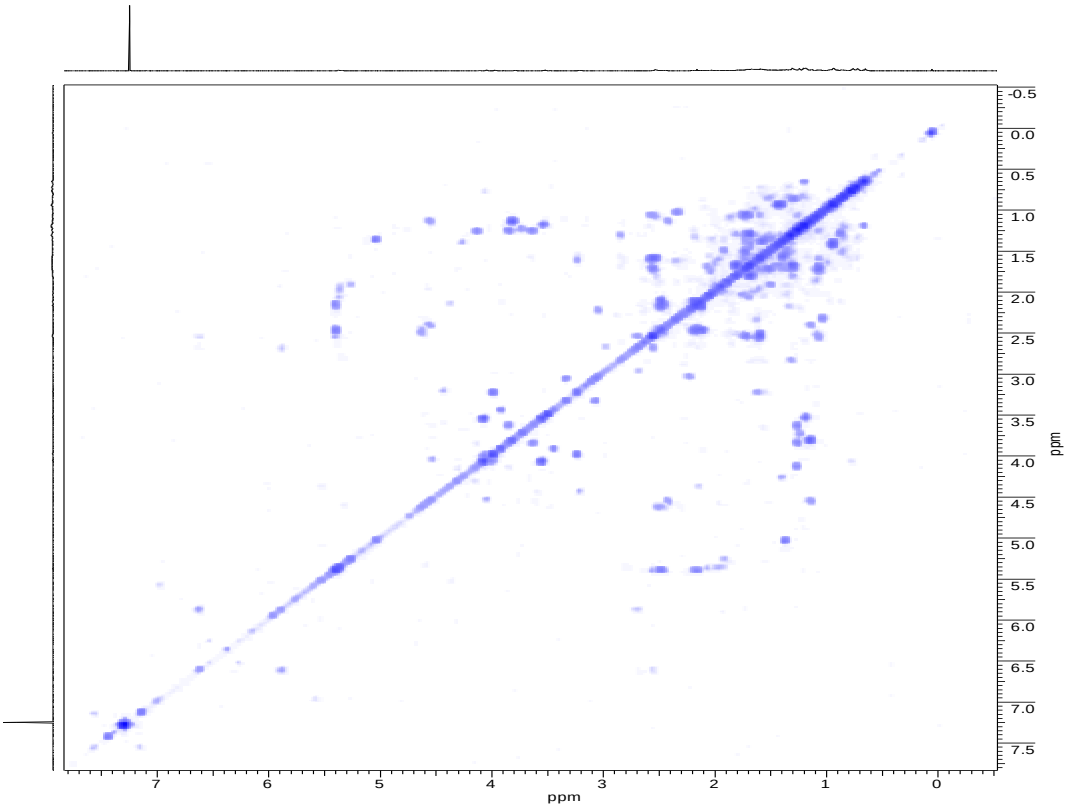

**Appendix 5.** $^{13}C$ NMR of compound 2**.**



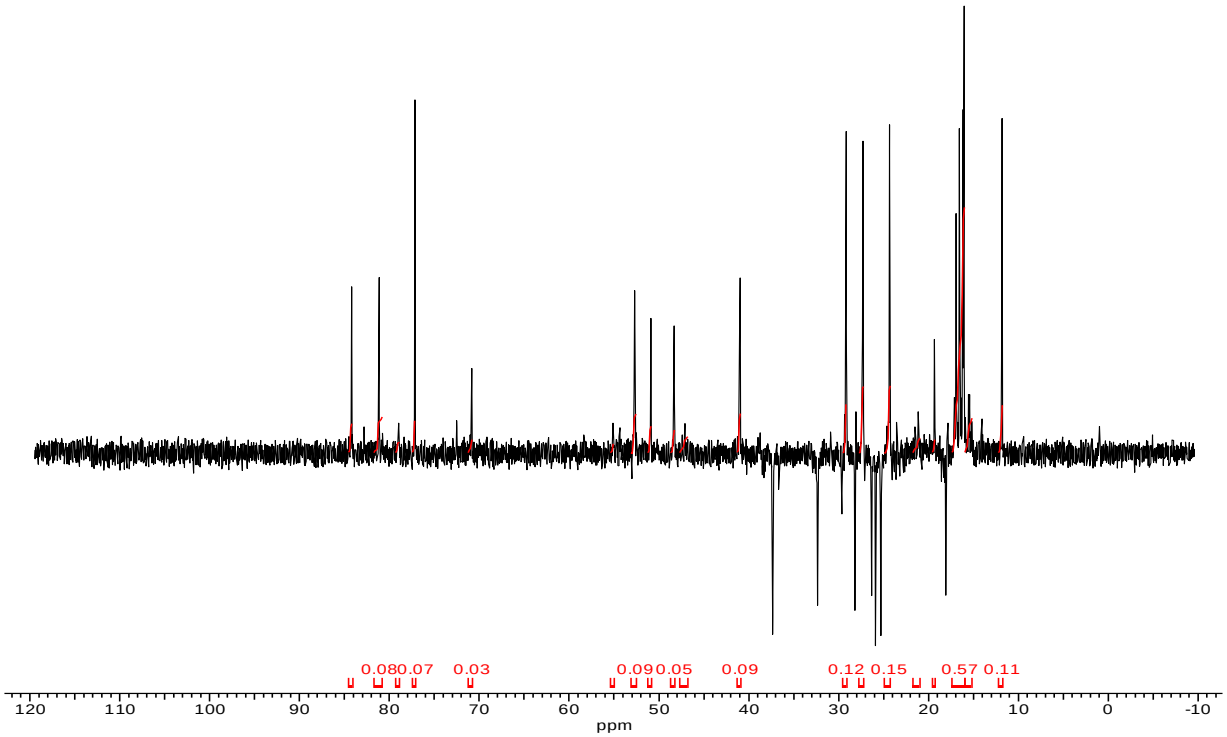

**Appendix 6.** HSQC Spectrum of compound 2.




**Appendix 7.** HMBC spectrum of compound 2.




**Appendix 8.** COSY Spectrum of compound 2.




**Appendix 9.** DEPT spectrum of compound 2.

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