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Assessment of over time changes of moisture, cyanide and selected nutrients of stored dry leaves from cassava (*Manihot esculenta* Crantz)

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Most fresh agricultural products are quickly perishable and various methods of preservation are necessary. Cassava leaves from different types of cassava (bitter, sweet and wild) were (1) dried un-pounded and (2) dried pounded in a tunnel solar dryer, filled in high density polyethylene material, sealed and placed into opaque cartons. The packing materials were purposively chosen to limit water, oxygen and light access. The complete drying was when samples were completely brittle. The storage was done at room temperature at Sokoine University of Agriculture, Morogoro, Tanzania. The main purpose of the study was to estimate shelf life by evaluating satisfactory quality in terms of nutritional values, dryness and organoleptic parameters. Water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc were chemically analyzed at zero, three, six, nine and twelve months of storage. Dryness and organoleptic parameters were also evaluated at these different storage lengths. Processing procedure had significant effect only on water ($p=0.0358$), cyanide ($p=0.0189$) and β-carotene ($p=0.0214$) contents. Storage time affected water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc significantly ($p<.0001$). Water content increased by 6.8% and ascorbic acid decreased to zero while β-carotene, protein, minerals and cyanide showed slight decline during the storage period. The optimum storage time under the conditions was judged to be six months for nutrients and organoleptic parameters stability.

Key words: Cassava leaves, solar drying, storage time, Rwanda.

INTRODUCTION

Agriculture is a substantial food source for rural and urban populations, and also a reliable source of income through selling fresh or processed products (Legg and Tresh, 2000). However, most fresh agricultural products are usually seasonal and quickly perishable. Hunger and malnutrition can exist in spite of adequate food production because of uneven distribution, deterioration and losses of available resources. To make foods available throughout the year,

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humans have developed various methods of preservation to keep food produced in one harvest for gradual consumption until next harvest. Microorganisms and enzymes that promote spoilage in foods thrive well in foods with high moisture contents and thus drying works as a preservation method simply by reducing the water content of the products and making it unavailable for chemical reactions and growth of microorganisms (Emebu and Anyika, 2011). Dry food products can be distributed and stored at ambient temperatures and this is affordable and common system in rural world, where cooling facilities are not available. As examples, maize, rice and bean are usually dried for extending storage period. According to Mills (1989), their respective equilibrium moisture contents for a safe storage are 13.5, 13.0 and 15.0%, in controlled storage conditions of temperature (27°C) and relative humidity (70%). In rural world, storage of the dry products at uncontrolled ambient temperature and relative humidity is common, especially in developing countries, but their storing lives are not stable. Among food preservation methods by reducing water to equilibrium levels, sun drying is the simplest, inexpensive and commonly adequate for rural and poor communities. However, solar drying offers the following over sun drying: faster drying rate, greater retention of nutrients and organoleptic qualities (Eze, 2010). In addition, minimizing exposure to rain, dust and insects by solar dryers reduces contamination and biological hazards. A sensory evaluation of solar dried cassava leaves in Rwanda showed a greater retention of color, taste, aroma and texture (Umuhozariho et al., 2013). Solar drying has also some advantages over the conventional drying with respect to cost and adaptability to small scale farmers. In reality, solar dryers are promising means for tropical countries to meet their requirements as the available amount of solar energy in most cases are sufficient to cover the required heat for small dryers.

Cassava (*Manihot esculenta* Cruntz) is a staple root crop in many countries of the tropics and particularly in sub-Saharan Africa (Huzsvai and Rajkai, 2009; Legg et al., 2006). According to FAO (2013), cassava can be produced efficiently without the need for mechanization or purchased inputs, and in marginal areas with poor soils and unpredictable rainfall. In fact, ccassava is known to tolerate prolonged drought conditions and low nutrient soils (Leihner, 2002). In Rwanda, cassava is described as “classic food security crop” because it offers the advantage of a harvest even in situations of erratic rainfall and infertile soils (Mushiyimana et al., 2011). In low altitude regions of Rwanda, cassava is among main crop plants and one of the priority crops that are being promoted for economic development and poverty reduction in the agricultural sector (MINECOFIN, 2007). Achidi et al. (2005) indicate that millions of tonnes of cassava leaves are harvested and used as vegetables by many families, especially in Africa, and provide protein, vitamins and minerals (Akinwale et al., 2010; Priadi et al., 2009). They are usually utilized freshly harvested. As it applies to other vegetable products, cassava leaves price varies much according to season and market location. Leaves are available as seasonal surpluses during certain parts of the year (rainy season) and go to waste due to improper processing, pre-packaging, handling, distribution and marketing. During the peak season, vegetables in general are sold at very low prices and some are simply wasted (TCARC, 2007). This reduces income for farmers, adding to the people’s poverty.

For preservation issues in rural communities, cassava leaves are sun dried and consumed at family level during the off-season. For example in low land areas of Rwanda, where cassava is the principal crop, cassava leaves are sun dried to brittle, stored in different types of containers, without any concern about water vapor, air and light access for gradual consumption in long dry season.

However, shelf life of dry food products is for finite period, depending on the type of the product, final moisture content, packaging material and storage conditions (Boyer and Mckinney, 2009; Fellows, 2009). James and Kuipers (2003) and Thomas (2008) mentioned that optimization of storage conditions, specifically by controlling moisture, temperature, oxygen and light, is very important to postpone rotting and spoilage of food products. Therefore, containers would be not only for containing, but also for protecting the food products from outside influences, precisely from water, gases and light entry (Marsh and Bugusu, 2007).

In the present study, leaves from different varieties of *M. esculenta* (bitter, sweet and wild) were been processed by drying, using a tunnel solar dryer, and packed in opaque, water and air proof material for storage at ambient temperature. The main purpose of the study was to esti-mate shelf life by periodically evaluating satisfactory quality in terms of nutritional values, dryness, smell and appearance (color) of the improved solar dried cassava leaves product called *isombe* and *kisanvu*, respectively in Rwanda and Tanzania.

MATERIALS AND METHODS

Collection of cassava leaves

Tender cassava leaves, the first matured up to leaf position five were harvested from three different cassava varieties, named “Seruruseke” (5280), ISAR 1961 and “Igicucu” were chosen for sweet, bitter and wild, respectively. In order to minimize the effects of age, environment and soil type on chemical composition, leaves of same age were selected from similar plot at Rwanda Agricultural Board (RAB)’s field at the Karama Research Station, in Bugesera District of Eastern Province of Rwanda.

Sample preparation

Samples were collected in the field and transported in closed polyethylene bags, which were stored in a cool box containing ice.

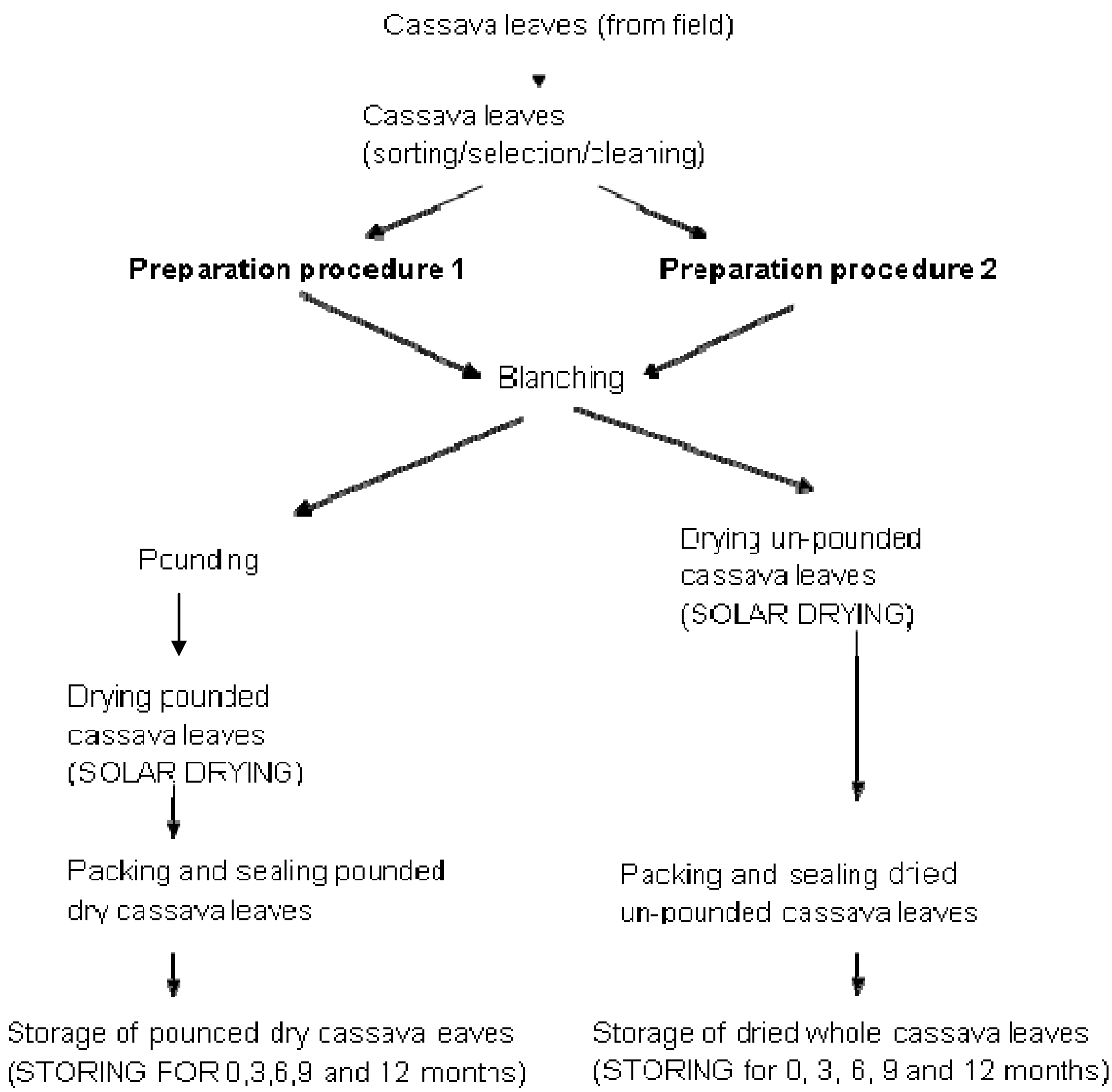

Figure 1. Flow chart of preparation procedures of cassava leaves.

Each sample was divided into two portions after blanching, first portion was dried un-pounded and for the second portion drying was done after pounding. Blanching was done by submersion in boiling water for 4-5 min, and then immediately cooled in tap water at ambient temperature as described by Kendal et al. (2010).

Two different preparation procedures were conducted, namely: (1) drying un-pounded and (2) drying pounded leaves. Pounding was done using wooden mortar and pestle, while drying was done using a tunnel solar dryer at Sokoine University of Agriculture. The products obtained by the two different preparation procedures (Figure 1) were assessed for dryness, color and smell/odor, and chemically analyzed for moisture, cyanide, protein, vitamins and minerals (Ca, Fe, K, P and Zn) at 0, 3, 6, 9, and 12 months of storage. The first four chemical analyses were conducted at Sokoine University of Agriculture laboratories, while vitamins (ascorbic acid and β-carotene) analyses were done at the Tanzania Food and Drug Authority (TFDA), in Dar-Es-Salaam. All chemical analyses were carried out in quadruple.

Drying, packing and storing

Temperatures inside the dryer were recorded at 8 a.m., noon and 8 p.m. each day, averaging 38°C. The complete drying was reached when the samples became entirely brittle. The dried samples were immediately filled in plastic bags. To avoid absorption of water, each bag was sealed in high density polyethylene (HDPT) material and placed into opaque cartons. HDPT and opaque carton were purposively chosen to limit oxygen, water and light access. Storage was done at room temperature, at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Each of the three test cassava species/varieties (bitter, sweet and wild) was treated separately.

Moisture, cyanide (HCN) and nutrients determination

Moisture content of samples was determined as outlined in AOAC (1995), official method 934.01. Cyanide (HCN) levels in the samples were determined by alkaline titrating method as described

Table 1. Effect of cassava species, processing procedures and storage time on water, cyanide and nutrients of stored dry cassava leaves.

Chemical content	Cassava type	P-value	
		Processing procedure	Storage time
Water	0.9086	0.0358*	<.0001**
HCN	0.2420	0.0189*	<.0001**
β-carotene	0.1504	0.0214*	<.0001**
Protein	0.0662	0.1704	<.0001**
Fe	0.0604	0.0639	<.0001**
Ca	0.1484	0.0797	<.0001**
P	0.1645	0.4232	<.0001**
K	0.3440	0.5625	<.0001**
Zn	0.1505	0.7478	<.0001**

*Significant effect ($p<0.05$), ** highly significant effect ($p<0.01$).

by AOAC (1995), official method 915.03B. Minerals, sample ashes and solutions were obtained respectively by official methods 965.09 and 982.23 described by AOAC (1995). Total phosphorus (P) was obtained using ascorbic acid blue color procedure and by reading the absorbance at a wavelength of 884 nm on a UNICAM 5625 UV/visible spectrometer (Okalebo et al., 1993). Calcium (Ca) and potassium (K) were measured by flame photometry, reading their absorbance at 422.7 and 766.5 nm, respectively on a Cole-Parmer instrument, Model 2655-00 Digital flame Analyzer. Iron (Fe) and zinc (Zn) were determined by reading their absorbance at 248.3 and 213.9 nm, respectively on a UNICAM 919 Atomic Absorption Spectrometer (AAS) using Hollow Cathode lamps (Okalebo et al., 1993). Crude protein content was determined by using the micro-Kjeldahl method (AOAC, 1995), official method 920.87. Vitamin C (ascorbic acid) content was determined as outlined by ISO (1984), method 6557/2. B-carotene was measured using a high performance liquid chromatography (HPLC), equipped with a photodiode array (PDA) detector fitted with a 436 nm wavelength. For sample preparation, aliquots were extracted by solvent n-hexane (Priadi et al, 2009; Tee Siong and Lam, 1992). Further extraction and clean-up was done using a dispersive Solid Phase Extraction (dSPE) technique as described in AOAC (2007), official method 2007 0.1.

Statistical analysis

Data from the chemical analysis of the samples were subjected to statistical analysis, using SAS 9.2 (SAS Institute, 2008). Longitudinal analysis techniques which account for correlation among observations over time and equally spaced measurements were used as suggested by Agresti (2007) and, Tiwari and Shukla (2011). The effect was judged significant at $p<0.05$.

RESULTS AND DISCUSSION

As revealed by Mills (1989), agricultural products change physically and chemically and need to be managed. For "dried pounded" and "dried un-pounded" cassava leaves from bitter, sweet and wild, the final moisture content was on average 4.6% and were completely brittle at packing time. As it is mentioned by James and Kuipers (2003), green vegetables contains less sugar, and thus, dryness to brittle can be considered as safe moisture content levels. In general, at brittle, water contents of green vegetables are between 4-8%, depending on the type of vegetable. Thus, dried at 4.6%, cassava leaves were at safe moisture content level or in equilibrium with a present temperature and relative humidity of the air; but, in ambient conditions, storage temperature and relative humidity were uncontrolled and could not be kept for keeping the leaves quality.

In the conditions, over time physical and chemical changes were to be evaluated because stored agricultural products are influenced by many factors that determine their keeping quality, including product condition, storage container, length of storage and type of handling (Mills, 1989). At nine and twelve months of storage, the dryness changed from brittle to pliable. The appearance did not noticeably change during the storage time of one year while the odor characteristic became more pronounced at pliable than at brittle dryness.

Results of chemical analyses of the samples (just after drying the un-pounded and pounded leaves) were statistically analyzed and effects of cassava type, processing procedure and storage period are shown in Table 1. From the table, cassava types did not have significant influence on the overtime changes of moisture, cyanide, ascorbic acid, β-carotene, protein, Iron, calcium, phosphorus, potassium and zinc contents ($p>0.05$). Processing procedure had significant effect only on moisture ($p=0.0358$), cyanide ($p=0.0189$) and β-carotene ($p=0.0214$) while storage time affected all the chemical contents significantly ($p<0.0001$). Mean concentrations of moisture, cyanide, ascorbic acid, β-carotene, protein, iron, calcium, phosphorous, potassium and zinc contents of stored un-pounded and pounded dry cassava leaves, at different period of storage (0, 3, 6, 9 and 12 months) are given in Table 2. From the table, for each cassava type an

Table 2. Mean levels of cyanide, moisture and selected nutrients of cassava leaves according to cassava type, drying procedure and storage time.

Time (month)	Dried and stored un-pounded cassava leaves										Dried and stored pounded cassava leaves									
	%	mg/kg	mg/100 g		%	mg/kg					mg/kg	%	mg/100 g		%	mg/kg				
	MC	HCN	AA	B-C	CP	Fe	Ca	P	K	Zn	HCN	MC	AA	B-C	CP	Fe	Ca	P	K	Zn
					Bitter											**Bitter**				
0	4.7	562.8	75×10^{-5}	43.7	36.8	222.5	6956.1	4590.4	15520.6	61.9	413.6	4.6	70×10^{-5}	39.1	36.6	215.2	6360.2	4454.6	14693.3	59.7
3	5.4	562.5	-	43.7	36.7	221.5	6956.1	4588.2	15518.8	60.8	413.1	5.6	-	38.7	36.2	213.9	6359.4	4453.3	14691.1	59.1
6	6.3	562.1	-	43.1	36.5	220.7	6955.3	4586.6	15515.6	58.9	412.5	6.8	-	38.2	35.9	212.2	6358.1	4451.8	14689.9	58.4
9	8.1	561.8	-	42.5	35.7	219.9	6955.1	4584.4	15513.8	56.6	412.1	8.4	-	37.8	35.5	211.3	6356.6	4450.4	14688.9	56.1
12	10.8	560.9	-	41.6	35.2	218.6	6954.5	4580.4	15511.7	55.7	411.7	11.1	-	37.1	34.9	210.4	6354.7	4448.7	14687.7	55.2
					Sweet											**Sweet**				
0	4.7	467.7	75×10^{-5}	39.8	35.2	221.8	6587.0	4212.0	14358.1	65.5	352.4	4.6	70×10^{-5}	29.1	35.1	215.3	6124.7	3957.2	12922.2	62.1
3	5.5	467.7	-	39.7	35.2	220.7	6587.0	4211.8	14356.9	64.3	352.1	5.7	-	28.4	34.7	214.2	6123.5	3955.6	12920.8	61.5
6	6.2	467. 4	-	39.1	34.8	219.6	6587.0	4209.2	14353.4	62.5	352.0	6.7	-	27.5	34.4	213.7	6122.2	3954.1	12919.4	60.9
9	7.8	467. 1	-	38.4	34.4	218.1	6586.1	4207.7	14350.1	60.7	351.8	8.4	-	26.5	33.8	212.5	6121.1	3952.7	12917. 6	59.6
12	10.5	466. 3	-	37.5	33.8	217.2	6585.2	4205.8	14348.3	59.8	351. 4	11.1	-	25.3	33.1	211.3	6119.5	3950.8	12915.7	58.6
					Wild											**Wild**				
0	4.8	873.3	90×10^{-5}	65.9	36.3	243.0	8070.2	4877.4	16157.4	73.2	684.9	4.6	80×10^{-5}	63.5	36.1	228.9	7894.8	4607.2	15765.7	69.5
3	5.8	873.1	-	65.7	36.2	242.6	8070.1	4875.7	16155.8	72.3	684.4	5.9	-	62.8	35.7	227.7	7893.9	4605.4	15763.8	68.4
6	6.5	871.8	-	65.1	36.0	241.5	8068.2	4873.5	16153.2	70.5	684.1	6.7	-	62.2	35.3	226.8	7892.5	4603.1	15761.9	67.8
9	7.8	871.4	-	64.3	35.6	240.8	8067.6	4870.7	16151.6	68.7	683.7	8.2	-	61.6	34.9	224.2	7890.4	4601.3	15759.8	67.4
12	10.5	870.7	-	63.5	36.1	238.9	8067.4	4868.8	16149.8	65.7	683.2	11.1	-	60.8	34.3	223.8	7888.9	4598.9	15757.9	65.2

Values are means of ten independent determinations in quadruple. MC= Moisture content, HCN= hydrogen cyanide, AA=ascorbic acid, β-C=β-carotene, CP=crude protein, Fe=iron, Ca=calcium, P=phosphorus, K=potassium, Zn=zinc.

processing procedure, only moisture content increased over time, while cyanide, protein, β-carotene and minerals slightly decreased as time increased. The influence of processing procedure on sample contents during storage was not surprising as some samples were pounded while others were un-pounded. Their pieces had different sizes and it is known that pounding and slicing in small pieces increase the surface available for water evaporation or absorption depending on relative humidity of the storage room (FAO, 1995). In this study, water increased with time. The increase of water during dry food storage has also been reported by Gupta et al. (2012). The increase may be attributed to water vapor absorption through packaging material. In fact, all packaging materials may be permeable to water vapor and air at a certain extent (Marsh and Bugusu, 2007; Brody et al., 2002). According to the same authors, the best barriers may have low permeability which is said to be increased by elevated and variable temperatures of storage room and a recommended storage temperature for dry foods is 21°C/70°F. However, under ambient conditions, temperatures are not controlled. In Morogoro, where the dry products were stored, the average annual temperature varies between 25 and 30°C according to altitude and season (Tanzania Minister of State, Planning and Parastatal sector Reform 1997). The temperatures are high and not stable during the year. Higher ambient temperature also accelerates oxidative degradation of foods, and the oxidative rate is promoted when the cellular integrity is destroyed (Boon et al., 2010). Besides, considerable temperature differences within container are a major driving force for moisture translocation and condensation and microorganisms and enzymes that promote spoilage in foods thrive well in foods with high moisture contents (Mills, 1989). Taga et al. (2008) noted cyanide liberation from residual linamarin after foods are processed and therefore, the noticeable increase of cyanide characteristic smell during storage period may be caused by the liberated free cyanide.

Nutrient contents deterioration of stored foods is inevitable to some extent. Among deteriorative reactions that cause food components decomposition are enzymatic and non-enzymatic oxidations. All these reactions are known to take place in the presence of oxygen, favored by water, and promoted by light (Bonilla et al., 2010; Kim et al., 2005; Gibis et al., 2011). Enzymatic reaction was excluded by blanching before drying, light was limited by opaque carton, but as reported by Brody et al. (2002), increase of water in stored dry foods through the package is inevitable. Also, despite the hermetic sealing before storing, residual oxygen is unavoidably present in the package headspace and product interstices (Kim et al., 2005), and as mentioned by Brody et al. (2002), all packaging material may be permeable, not only to water vapor, but also to air that contain oxygen. Ascorbic acid, β-carotene, protein, iron, calcium, phosphorous, potassium and zinc decreases may be attributed to the deteriorative reactions. The continuous decline of nutrient contents, especially vitamin C of dried green leaves during storage has been also reported by Negi and Roy (2001). For physical and nutritional quality, dry green vegetables may be consumed before losing its dryness and for the dried cassava leaves in this study, at six months of storage, the products were still brittle, but at nine months, the structure changed to pliable and moisture content to 8.3 and 11 percent at nine and twelve months of storage respectively. The observation was in agreement with what was reported by Boyer and Mckinney (2009) that, in general, vegetables dried until they are brittle, packed in airtight, light and moisture-proof packaging material can be stored for six months at room temperature and dry place.

Conclusion and recommendations

Storage time affected water, cyanide, ascorbic acid, β-carotene, protein, iron, phosphorus, potassium and zinc significantly ($p<0.0001$). Water content increased by 6.8% and ascorbic acid decreased to zero while β-carotene, protein, minerals and cyanide showed slight decline during the storage period. The optimum storage time under the conditions was judged to be six months for nutrients and organoleptic parameters stability. Storage at uncontrollable high and variable ambient temperatures such as these of Morogoro (25-30°C), promotes deteriorative reactions which are the main causes of nutritive and sensory quality losses of the dry foods, even when stored in opaque, water and air proof, and hermetically sealed containers.

When the adequate drying, storage and packing conditions are not combined, storage life can be very limited, less than six months. Therefore, food quality control of dry foods in rural areas, where appropriate packaging materials are not available, is recommended to ensure healthier and safe foods. At industrial level, promoting dry food staffs such as dry cassava leaf products could include water absorber use for safe and stable storage because water inevitably increase within containers.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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