IPTT

Indigenous Plant Task Team

Promoting the Sustainable Utilisation of Namibia's Indigenous Plant Resources

Promoting Indigenous Plants in Namibia:

Indigenous Green Leafy Vegetables (IGLVs)

PROCESSING AND MARKETING TRIALS
Phase 2 (2006)

FINAL REPORT

Consultancy contract
administered by the Namibian Agronomic Board (NAB)
with funds from:
MAWF / NASSP (661/003) and UPDP / IPTT

Prepared by CRIAA SA-DC
Windhoek, August 2006

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Acronyms and abbreviations

CRIAA SA-DC	Centre for Research, Information, Action in Africa: Southern Africa Development and Consulting
IGLV	Indigenous Green Leafy Vegetable
IPTT	Indigenous Plant Task Team
KAP	Katutura Artisans' Project
MAWF	Ministry of Agriculture, Water and Forestry
NAB	Namibian Agronomic Board
NASSP	National Agricultural Support Services Programme (8^{th} EDF)
NBRI	National Botanical Research Institute
RDA	Recommended daily allowance (nutritional recommendation)
UPDP	Useful Plant Development Project
VIVA	Vigorous Indigenous Vegetables from Africa (project)

1. Introduction

1.1 This report presents the final results obtained by the CRIAA SA-DC team of consultants (Roger Gamond, Saskia den Adel and Michel Mallet) in implementing Phase-2 of the Indigenous Green Leafy Vegetable (IGLV) Processing and Marketing Trials. The consultancy has been contracted by the Namibian Agronomic Board (NAB) with funds from NASSP (661/003) and IPTT/UPDP under the overall programme VIVA (Vigorous Indigenous Vegetables from Africa), co-ordinated by Patrick Hilger and the IGLV sub-committee of the Indigenous Plant Task Team (IPTT) comprising of Herta Kolberg (NBRI), Rejoice Karita-Ketji (MAWF), Christof Brock (NAB) and Pierre du Plessis (CRIAA SA-DC).

1.2 The objectives of Phase-2 were to confirm (or invalidate) some critical issues identified as a result of Phase-1 processing and marketing trials conducted in 2005. The terms of reference (see *Annex 1*) included the following:

(a) To process in Windhoek fresh leave samples of the two selected species, i.e. *Amaranthus sp.* (mainly *A. thunbergii*) commonly known as "Ekwakwa" and *Cleome gynandra* commonly known as "Ombidi", supplied by P. Hilger from Northern Namibia through overnight courier services, following three preservations methods:

- Blanching and drying
- Blanching and deep-freezing
- Blanching and sterilizing.

(b) To test consumer acceptance and rate consumer preference of the two selected species and the three preservation methods, through a panel tasting session in Katutura.

(c) To provide samples for laboratory analysis (nutritional and microbiological) from fresh leaves (reference samples) and processed/preserved products of the two species in order to:

- Assess the influence of the preservation methods on the nutritional value of the products (compared with the fresh reference samples)
- Follow the microbiological contamination (if any) in the deep-frozen and sterilized products so as to evaluate the food safety and shelf life of the products.

1.3 The main report is structured as follows:

Section 1 is this introduction.

Section 2 summarises the results obtained from the processing trials, which were carried out at KAP and CRIAA premises in Windhoek from early March to beginning of April 2006, with samples of fresh IGLVs supplied from Rundu and Tsumeb. Most of the samples were not received in very good conditions in Windhoek.

Section 3 summarises the results of the testing conducted in Katutura on 24 March 2006 with a tasting panel of 15 consumers of traditional IGLVs. Comparative tasting was organised with samples of the two species in the three processed forms.

Section 4 recapitulates the results of the nutritional and micro-biological analysis sub-contracted to a private laboratory in Windhoek over the period March to July 2006. Due to

the results of the tests on the processed samples showing early signs of microbiological deterioration, it was decided in consultation with the IGLV sub-committee to discontinue the last analyses planned for September/October.

Section 5 concludes the report and provides recommendations for future IGLV development activities.

In the annexes are presented:

- A list of **Relevant Documents** (*Annex 2*)
- The full **Technical Report** on the IGLVs processing trials, prepared by R. Gamond, dated 10 April 2006 (*Annex 3*)
- The full **Panel Test** report on the panel tasting of processed IGLVs, prepared by S. den Adel, dated 11 April 2006 (*Annex 4*)
- The results of the **Nutritional and Microbiological Analysis** contracted to Analytical Laboratory Services cc. (Silke Rügheimer, food scientist) and co-ordinated by R. Gamond and M. Mallet (*Annex 5*).

2. Main results and conclusions from the technical report on IGLV processing

2.1 Compared to the quantity of *Amaranthus* leaves (Ekwakwa) delivered (56kg), the quantity of *Cleome* leaves (Ombidi) delivered was very low (7kg) and just enough to produce samples for laboratory analysis and panel testing. The reason(s) behind this short supply of *Cleome* should be clarified by the VIVA Programme Co-ordinator and/or an alternative production strategy be pursued to avoid compromising further processing and cultivation development of this species in the future.

2.2 The condition of the fresh leaves on reception in Windhoek was generally poor due to two reasons: (i) a too long delay between harvesting and dispatch and (ii) loss of quality during transport of the leaves, which were inadequately packed. Recommendations are formulated in the Technical Report to improve the quality of supply of fresh IGLVs.

2.3 The conventional washing of fresh leaves (to thoroughly remove sand and soil particles) was difficult without using a large quantity of water. Two special "washing baskets" had to be manufactured for this purpose and proved to be appropriate.

2.4 The initial blanching (first processing) of fresh leaves was eventually carried out by wilting/braising leaves in a stainless steel pot with little water added (to minimise the loss of nutrients) after the boiling of leaves in airtight plastic bags proved ineffective.

2.5 The secondary processing (for preservation) of the blanched leaves brought the following conclusions:

- Deep-freezing is an easy and quick method
- Drying was more difficult (longer) with *Amaranthus* than with *Cleome* but seems to be an easy method provided some precautions are taken (and provided the weather is not too humid, which was not the case at the time of the trials)
- Sterilisation in glass jars proved to be technically difficult on account of the number of failed product samples (improper sterilisation mainly due to jar lids not properly airtight). Lowering the pH of the product (to prevent the development of *Clostridium botulinium*) by adding acid was discontinued as it gave a very bad taste to the product.

2.6 For a small-scale IGLV processing unit (or at household level), the main conclusion of the trials is that the two preservation methods (after proper sorting, washing and blanching of leaves) to be recommended are:

- Deep-freezing appears as the easiest and safest preservation method, provided deep-freezing facility is available
- Drying appears to be the second best preservation method provided it is carried out hygienically and in the shade.

2.7 Sterilization is not recommended for a small-scale processing unit because of the technical difficulty to obtain a properly preserved product and the risk associated with the potential development of pathogens. However, canning on a larger scale (and less artisanal level) could still be considered.

3. Main results and conclusions from the IGLV panel test report

3.1 Contrary to expectations, the ratings provided by the taste panellists showed a very clear and strong preference to Ekwakwa (*Amaranthus*) compared to Ombidi (*Cleome*). *Table 1* below highlights the ratings.

Table 1 - General Preference

	Ombidi	**Ekwakwa**
Frozen	**7%**	**93%**
Sterilised	**0%**	**100%**
Dried	**17%**	**83%**

3.2 The preference for Ekwakwa compared to Ombidi was consistent for all variables, but was more pronounced in the texture and taste, and least in the smell (see *Table 2* below).

Table 2 - Comparative preferences between Ombidi and Ekwakwa

	Taste		**Texture**		**Appearance**		**Smell**	
	Ombidi	Ekwakwa	Ombidi	Ekwakwa	Ombidi	Ekwakwa	Ombidi	Ekwakwa
Frozen	2.20	6.07	2.13	6.07	3.33	6.07	3.53	5.80
Dried	3.33	5.47	3.27	6.00	4.13	6.00	4.13	4.87
Sterilised	3.13	6.60	2.47	6.47	3.47	6.33	4.53	6.13
Average	**2.89**	**6.05**	**2.62**	**6.18**	**3.64**	**6.13**	**4.06**	**5.60**
	bad	*Very good*	*bad*	*Very good*	*average*	*Very good*	*average*	*Very good*

3.3 When combining all the variables (see *Table 3* below), it clearly appears that there are much more significant differences in preference for the type of vegetable than there are for the conservation methods. For Ekwakwa there was a slight preference for the sterilized conservation method, followed by frozen, then dried. For Ombidi the panellists slightly preferred the dried conservation method, while the frozen Ombidi was the least appreciated.

Table 3 - Comparative ratings between Ombidi and Ekwakwa

	Frozen		**Dried**		**Sterilised**	
	Ombidi	**Ekwakwa**	**Ombidi**	**Ekwakwa**	**Ombidi**	**Ekwakwa**
Taste	2.20	6.07	3.33	5.47	3.13	6.60
Appearance	3.33	6.07	4.13	6.00	3.47	6.33
Texture	2.13	6.07	3.27	6.00	2.47	6.47
Smell	3.53	5.80	4.13	4.87	4.53	6.13
Average	**2.80**	**6.00**	**3.72**	**5.59**	**3.40**	**6.38**
	bad	*very good*	*Average*	*very good*	*Bad*	*very good*

3.4 In conclusion, the panel test showed without doubts the strong preference for Ekwakwa. The sterilized Ekwakwa was highly appreciated, but also the frozen and dried Ekwakwa achieved very high scores. All three conservation methods are considered suitable for Ekwakwa (*Amaranthus*). Ombidi (*Cleome*) scored significantly less. The panellists mostly had a problem with the texture of the samples and the taste was also rated low. It is therefore recommended that special attention should be applied to Ombidi preparation (processing for preservation) to render this IGLV more palatable and appealing to consumers should further marketing development be considered for this species.

4. Main results from the laboratory analyses

Nutritional analysis

4.1 The nutritional analysis of fresh and processed leaves samples of the two species were carried out by Analytical Laboratory Services in Windhoek, apart of the vitamin A analyses sub-contracted to a South African laboratory as they could not be done in Namibia promptly and reliably. The nutritional analyses carried out consisted of the following:

- Moisture content
- Protein content (based on Nitrogen content x 6.25)
- Ash content (from which the carbohydrate content can be determined by deduction of moisture and protein contents from the total weight, ignoring the low fat content of the leaves)
- Vitamin C
- Minerals: Calcium, Iron and Phosphorus
- Pro-vitamin A (beta-carotene).

4.2 Results in raw data form are attached (*Annexes 5.1 and 5.2*). The data needs to be computed in a dry matter basis (see spreadsheet in *Annex 5.3*) to allow comparison between the different samples (which have different moisture contents). The comparison of the nutritional contents (dry matter) between the reference samples (fresh) and the three processed samples show the following:

- No significant difference (within the error margins) in the protein and ash content
- An expected drop in the vitamin C content, larger for the dried samples than the sterilised and frozen samples
- Relatively low decrease or insignificant variation in the Calcium, Iron and Phosphorus contents.

4.3 The results of the Vitamin-A (beta-carotene) content analyses carried out on the processed samples of the two species are summarised in *Table 4* below and show the following outcomes (see details in *Annex 5.4*):

- For each processing method (freezing, sterilising and drying) *Cleome* and *Amaranthus* show a similar Vitamin-A content
- For both species, drying reduces the most the Vitamin-A content (by a factor 2 to 3.5)
- Freezing and sterilising seem to maintain a similar level of Vitamin-A, although the results for *Amaranthus* show that freezing was more damaging to Vitamin-A.

Table 4 - Comparative Vitamin-A contents of processed Cleome and Amaranthus samples

	Vit. A (mg/100g) fresh weight	**Moisture content (%)**	**Vit. A (mg/100g) dry weight**
1. Cleome frozen	1.8	88%	15
2. Cleome sterilized	1.8	88%	15
3. Cleome dried	4.0	7.4%	4.3
4. Amaranthus frozen	1.4	86%	10
5. Amaranthus sterilised	2.3	88%	19
6. Amaranthus dried	5.0	3.0%	5.2

Microbiological analysis

4.4 The microbiological analyses were carried out on the deep-frozen and sterilized samples of the two species just after processing (March/April), one month later (April/May) and another month later (May).

4.5 The test results (see *Annexes 5.5 to 5.7*) showed:

- No contamination by Coliforms or *Escherichia coli*
- No or very low counts of moulds and yeasts
- Presence of Gram positive micro-organism spores (and Gram negative in one instance)

4.6 However, further tests conducted at the end of May on the sterilised IGLVs showed a positive presence of *Clostridium* spp. and *Bacillus* spp. in the *Amaranthus* sample. *Clostridium botulinium* is the micro-organism responsible for the lethal Botulism. This result shows that the sterilisation was not sufficient and the pH (5.6) not low enough to prevent bacterial development. This problem further reinforces the technical recommendation made earlier about the unsuitability of sterilisation as a processing method when performed on a small-scale and unsophisticated way.

5. Conclusions and recommendations

Conclusions

5.1 The processing and tasting trials were constrained by the quality and quantity of fresh IGLV materials supplied, particularly *Cleome* (Ombidi). The major problems behind the quality deterioration of the fresh leaves supplied were the inappropriate packaging of the materials forwarded and the delay between harvesting in the North and reception of leaves in Windhoek. In the absence of a report on the cultivation and harvesting of the IGLVs supplied, the consultants cannot comment on the conditions under which the IGLV samples were grown, harvested, sorted and dispatched (these issues were not part of the processing and marketing trials terms of reference in any case).

5.2 From the processing trials and subsequent laboratory analyses, the consultants conclude the following:

- Cleaning and washing of fresh leaves is critical to remove sand, dust and soil particles, and obtain a product of superior quality (compared to traditionally processed products). A simple and cheap device has been developed, which produces an acceptable end result. However, the use of this device is labour intensive and may only be appropriate to a small-scale production unit.

- The blanching of fresh leaves (which had to be preceded by a careful sorting of leaves) in a cooking pot with a small amount of water proved to be an acceptable method, which did not result in an exceptionally high loss of nutrients. Blanching seems an appropriate pre-processing method before leaves are preserved by drying or deep-freezing. The materials obtained are pre-cooked products, which present an additional marketing advantage.

- Comparing the three processing/preservation methods, it is concluded that:
  (i) Deep-freezing does not appear as a complicated method provided hygiene standards prior to and at packaging are met. However, preparing and marketing such products obviously require deep-freezing facility and a proper cold-chain from production to retail outlets. In such conditions, the shelf-life of deep-frozen products would be over three months.
  (ii) Drying also appears as a simple method, which seems to have an interesting advantage in terms of extended shelf-life of the products but not for Vitamins A and C preservation. However, such passive drying performed in an air ventilated and shaded place (with no direct sun) over a few days requires proper hygienic conditions to avoid contamination, and suitable climatic conditions (as much warmth and dryness as possible). If these conditions are not met (this would particularly be the case during the rainy season), alternative drying methods would have to be used, such as solar drying or oven-drying, at additional costs.
  (iii) Sterilisation trials were not conclusive and faced many technical problems: unsuitable containers (not air-tight), failed sterilisation (with microbiological development in the products) and inappropriate pH (acidity). To ensure an acceptable product with a sufficiently long shelf-life, preservation by sterilisation must be conducted skilfully with appropriate equipment and quality control. The risk of pathogen development in the products means that this preservation is not recommended for a small-scale and artisanal processing unit.

5.3 The consumer panel testing showed a clear preference for the processed *Amaranthus* (Ekwakwa) compared to the processed *Cleome* (Ombidi), as supplied from the processing/ preservation trials. However, the consultants do not conclude that further trials and promotion of *Cleome* should be discontinued. Some bias in disfavour of this species could have been introduced in the consumer testing through a low quality product (original leave samples were not high quality) and inappropriate preparation (*Cleome* is generally prepared in mixtures with other IGLVs). Nevertheless, the panel testing showed that simple processing and preservation methods can result in a product that meets consumer acceptance, at least for unmixed *Amaranthus*.

Recommendations

5.4 On account of the problems and limitations experienced during this trial phase, the following is recommended:

a) Processing/preservation trials should rather be conducted close to the IGLV cultivation and harvesting areas to minimise the problems of quality deterioration during handling, packaging and transport.

b) There could be advantages in conducting further trials in the context of a pilot IGLV processing unit or nascent small business. These advantages would be:

- A better control of the quality of IGLVs supplied
- The possibility to adapt the processing and preservation method with the quality of batches of raw materials available during the production/harvesting season
- The options to test products not only on the basis of preservation methods with unmixed materials, but also with mixed species or products in appropriated pre-cooking preparations. A range of products could then be prepared (based on recipes) and tested with different consumer market segments.

5.5 The approach advocated will not only try promoting IGLV species one by one, but developing pre-cooked preparations that have a chance to appeal to consumers and are different from those already marketed by the informal sector. This pragmatic approach will rely more on the food prepartion skills and business acumen of an emerging entrepreneur (or pilot project manager) than on the ability to follow a rigorous research protocol. However, technical back-up support should still be provided for quality control, market testing, packaging and market promotion, and financial analysis.

ANNEXES

ANNEX 1

INDIGENOUS GREEN LEAFY VEGETABLES (IGLVs)
Processing and Marketing Trials

PHASE 2 brief proposal
prepared by CRIAA SA-DC, 24/01/2006 (revised 24/02/2006)

1. **Phase 2 scope:** as per ToRs prepared by P. Hilger (22/12/2005 & 9/01/2006) and following the discussion at the sub committee meeting (1/12/2005), to make a 2nd round of preservation trials, analysis and panel test with only Ekwakwa and Ombidi; to determine the shelf life of frozen and sterilized IGLVs and make an economic assessment of the processing methods.

2. **IGLVs planned availability:** end of February, very beginning of March

3. **IGLVs processing trials:** 1st half of March

4. **IGLVs planned panel tasting:** 2nd half of March

5. **Preservation trials place:** CRIAA and/or KAP premises in Windhoek.

6. **Harvesting and supply**: to be managed and ensured by the VIVA Project Coordinator, P. Hilger. We suggest that harvesters be provided with small micro perforated or woven plastic bags taken to a cold storage as soon as full and put together in bigger bags just before transport.

7. **IGLV supply specifications:** leaves only to ensure that no further sorting is needed; only washing and rinsing before processing). It would be advisable not to send more than 25 kg of IGLVs at a time and to allow 48 or 72 hours between deliveries, taking into account our limited deep freezing capacity.
   75 kg of fresh leaves per species should be enough for limited processing trials, laboratory tests and panel tasting.

8. **Preservation methods:**
   a. **blanching + sterilization** (and storage at room temperature)**,**
   b. **blanching + deep freezing**
   c. **blanching + drying** (even though the rainy season is not particularly suitable).
   Taking into account the potentially high health risk associated with micro-waving (for the ones willing to know these risks, please have a look at Internet; for example www.nexusmagazine.com/articles/microwave.html), it was decided not to proceed with these trials.
   Pasteurisation was also dropped because of the too short IGLV shelf life: from a few days at room temperature to possibly two weeks if refrigerated.
   A few samples of pureed Ombidi and Ekwakwa will also be prepared in order to assess consumer preference.

9. **Preservation and loss of nutrients:** It has to be mentioned that the selected processing methods, if carried out properly, could lead to a minimum loss of nutrients

(we plan to make blanching trials with raw leaves already packed in sealed plastic bags, so theoretically, very little loss of nutrients should be expected).
However, it has to be highlighted that nutrient losses may be very significant at consumer level depending on the method used to prepare and cook the processed IGLVs.

10. Lab tests and analysis:

- Nutritional analysis: moisture, ash, protein, Vit.A, Vit.C, Calcium, Iron and Phosphorus levels on 2 species x 4 processing methods (fresh, blanched + dried, blanched + deep frozen, blanched + sterilized (once only).

- Microbiological test: essentially total colony count + mould and yeast count on deep frozen and sterilized IGLVs to determine their safety and shelf life:

- Deep frozen IGLVs: after blanching, 2 weeks later and 2 weeks after again.
- Sterilized IGLVs: after sterilization, 2 months later and 4 months after again.

Provision has been made for a few further tests such as identification of germs/bacteria.

11. Safety of sterilized IGLVs:

The possible presence and development of *clostridium botulinum* in sterilized IGLVs remains a risk that could only be overcome by a heat treatment at more than 130ºC or by a pH lower than 4.5. Heating at more than 130ºC is usually done through pressure or pressure steam cooking. However, if this process was feasible, it would require some trials and tests (making sure that 130ºC is reached in the core of IGLVs packed in a jar is not so easy)

Lowing down the pH of pasteurised Ombidi or Ekwakwa below 4.5 is much easier to achieve (and control) by adding some low pH material: vinegar, citric or ascorbic acid…

12. Reporting:

Technical report on processing trials: around end of March 2006
Panel tasting report: around mid-April 2006
Interim report: by end of April 2006
Final report: by end of October 2006 (taking into account the last lab tests).

Available and Additional Equipment/Consumables Needed for Phase 2

Designation	Quantity	Available	Not Avail
Plastic tubs, 40-60L	**4**		**X**
3-ring gas burner	**2**		**X**
10 kg gas bottle	**1**		**X**
10 kg gas refills	**3**		**X**
20L Ss pots and leads	**2**	**CRIAA**	
Large Ss strainers or baskets	**2**	**CRIAA**	
3-5 kg electronic scale	**1**	**CRIAA**	
Plastic chopping boards	**2**	**CRIAA**	
pH strips	**1**	**CRIAA**	
Thermometer, 0-200ºC	**2**		**X**
170 and 210L deep freezers	**1+1**	**KAP**	
80µ plastic bags 200x300 and 250x350	**1000**		**X**
Organic/food grade detergent	**10 kg**		**X**

ANNEX 2

List of relevant documents

CRIAA SA-DC (2006) "Indigenous Green Leafy Vegetables Processing and Marketing Trials", NASSP Report No 020/2006, MAWF, Windhoek, July

Du Plessis P. (2004) "Indigenous Vegetables Development Proposal", NASSP Report No 005/2004, MAWRD, Windhoek, May

Du Plessis P., Gamond R., Den Adel S. & Gallardo J. (2005) "Indigenous Green Leafy Vegetables Processing and Marketing Trials", NASSP Report No 026/2005, MAWRD, Windhoek, September

Fleissner K.W.E. (2005) "Report on VIVA Activities at Manheim Research Station: January-June 2005", report to IPTT, MAWF, Tsumeb, June

Hilger P. (2004) "VIVA Inception Report", NASSP Report No 016/2004, MAWRD, Windhoek, December

Larmond E. (1982) "Laboratory Methods for Sensory Evaluation of Food", Food Research Institute, Ottawa

Lee S.K. & Kader A.A. (2000) "Preharvest and postharvest factors influencing vitamin C content of horticultural crops" in *Postharvest Biology and Technology 20 (2000)* 207-220, Elsevier

ANNEX 3

IPTT INDIGENOUS PLANT TASK TEAM
Promoting the Sustainable Utilisation of Namibia's Indigenous Plant Resources

Promoting Indigenous Plants in Namibia:

Indigenous Green Leafy Vegetables (IGLVs)

PROCESSING AND MARKETING TRIALS

Phase 2 (2006)

TECHNICAL REPORT

Consultancy contract

administered by the Namibian Agronomic Board (NAB)

with funds from:

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Prepared by Roger Gamond
& Michel Mallet (CRIAA SA-DC)
Windhoek, 10 April 2006

1. Introduction

The objective of Phase-2 of the processing and marketing trials of Indigenous Green Leafy Vegetables (IGLVs) were to analyse and panel test the two species *Amaranthus* (Ekwaka) and *Cleome* (Ombidi) to determine the shelf life of frozen and sterilized samples, and make an assessment of the processing methods.

This technical report presents the results of the first task - the processing trials - which were conducted by Roger Gamond in Windhoek.

The equipment and consumables purchased for the trials are shown in *Appendix 1*.

2. Results

2.1 Transport and deliveries of IGLVs

The transport of IGLVs was organised by Patrick Hilger (VIVA project co-ordinator) from Rundu or Tsumeb by express truck transport (Parcel Force), mainly through the night with deliveries in Windhoek the following morning. *Table 1* below shows the quantities of IGLVs received in Windhoek for each delivery (IGLVs' weights at reception before sorting and washing).

Table 1 - Records of IGLVs delivered to Windhoek for processing trials

Deliv.	Waybill		Date &Time			Bags Nº	Cleome	Amaran-	Total kg
Nº	Nº	kg	harvesting	sending	reception		(kg)	thus (kg)	received
1	**965623**	**10.00**	**?**	**9/03 - 16h45**	**10/3 - 14h30**	**107**	*****	**1.27**	
						109	*****	**1.95**	
						111		**0.63**	
						211		**1.32**	
						307		**1.39**	
						308		**1.29**	
						309		**1.24**	
						Sub total		*9.09*	*9.09*
2	**965812**	**4.40**	**?**	**15/03 - 14h50**	**16/03 - 9h02**	**No Nº**	**3.11**		
								1.03	
						Sub total	*3.11*	*1.03*	*4.14*
3	**751078**	**17.40**	**?**	**16/03 - no time**	**17/03 - 9h39**	**No Nº**	**2.96**		
								0.81	
								1.22	
								1.49	
								0.80	
								0.91	
								0.53	
								1.12	
								1.32	
								0.69	
								1.29	
								0.85	
								8.22	
								4.00	
								2.77	
								9.28	
						Sub total	*2.96*	*35.30*	*38.26*
4	**951239**	**2.44**	**17/03**	**22/03 - no time**	**23/03 - 8h30**	**No Nº**	**0.88**		
								0.55	
						Sub total	*0.88*	*0.55*	*1.43*
5	**751349**	**11**	**31/03**	**3/04 - no time**	**4/04 - 8h30**	**No Nº**		**0.56**	
								0.50	
								0.74	
								1.52	
								1.84	
								1.32	
								1.56	
								1.92	
						Sub total	***0.00***	***9.96***	***9.96***
					Total received		**6.95**	**55.93**	**62.88**

** Bags 107 and 109 were marked "Cleome" but were actually Amaranthus.*

The total volume of IGLVs delivered was close to what was expected, but the quantities of *Cleome* received were much less than *Amaranthus*.

2.2 Condition of IGLVs at reception

The long delivery time (probably around 10 hours) and the condition of transport in the trucks (bags probably piled up) had a damaging effect on the quality of IGLVs at reception. The lesson to be learned from this exercise is not to pack a too large quantity of leaves in a bag to avoid deterioration.

- **Delivery Nº 1:** 9.09 kg received from Tsumeb after about 22 hours (and several enquiries to the transporter):
  Leaves filled in brown paper bags, packed in 5 kg woven bags and finally bundled in a bigger woven bag.
  The paper bags were immediately removed from the woven bags at reception. "Warm spots" were noticed on some paper bags, especially where the bags were in contact. The temperature measured at these points was 40ºC, i.e. 10ºC higher than room temperature.
  All the IGLVs in paper bags were immediately put to cool down in a fridge.
  When the paper bags were opened for processing (one and two days later) lumps of fermented/rotten leaves were found. It is believed that leaves at these spots had started fermenting. These lumps were discarded before washing the rest of the leaves.
  The leaves were not very clean, some literally covered with a fine red soil layer (most probably the bottom leaves close to the ground after strong rains must have sprayed mud that became a sandy coat once dried).
  The largest leaves were generally yellowish and should not have been harvested.
  The two bags marked "*Cleome*" actually contained *Amaranthus* leaves (see pictures in the photographic report in *Appendix 2*).

- **Delivery Nº 2:** 4.14 kg received in Windhoek after over 18 hours. Leaves were packaged in 2 woven bags and were in a slightly better condition than the 1st delivery. The *Cleome* received was chosen as the reference batch (for samples to be tested) even though 0.25 kg (out of 3.11 kg) had to be discarded due to small lumps of fermented/rotten leaves.

- **Delivery Nº 3:** The largest delivery with 38.26 kg received after an unknown transport time (no records available). However, the deterioration was the most important (17.78 kg or 46.47% discarded).
  A thorough sorting should have reduced the losses but this delivery was received on the Friday before the Independence week-end and some CRIAA's staff had to be called for help to quickly sort out this delivery. The small bags were first sorted (because leaves were not too much damaged) and stored in a fridge. The big bags containing more damaged leaves and big lumps of fermented/rotten leaves were then opened: 3 *Amaranthus* bags were entirely discarded due to damage and a very bad (fishy) smell (it was learnt that some leaves were washed after harvesting and then stored). Surprisingly one of the biggest bag (8.22 kg) was much less damaged than the others probably because leaves were not so packed up inside.

- **Delivery Nº 4:** 1.43 kg received after an unknown transport time. The quality was fine, but having already received more than enough *Amaranthus* leaves, the 0.55 kg of this species was not processed further.

- **Delivery Nº 5:** 9.96 kg received after an unknown transport time. The quality was better than the previous deliveries (even though the leaves were delivered 4 days after harvest). But this delivery was not initially planned (only 2.5 to 3 kg of *Amaranthus* was requested to make new reference samples for analysis and tests as most of the sterilized *Amaranthus* jars had failed and there was not enough sterilized samples to conduct all the planned tests). Only 6.84 kg were processed (not enough time to process the whole delivery and the panel tasting was concluded).

2.3 Sorting of IGLVs

IGLVs were supposed to be delivered in good conditions, and sorting was neither planned nor budgeted. However, the condition of IGLVs at reception was not good enough to skip this step.

Table 2 - Summary of IGLVs delivered, sorted and processed

Delivery Nº	Bag Nº	CLEOME			AMARANTHUS			Remark
		Received	Discarded	For processing	Received	Discarded	For processing	
1	**107**				**1.27**	**0.69**	**0.58**	**Pre-trials; discarded**
	109				**1.95**	**0.73**	**1.22**	**Pre-trials; discarded**
	111				**0.63**	**0.05**	**0.58**	
	211				**1.32**	**0.25**	**1.07**	
	307				**1.39**	**0.61**	**0.78**	
	308				**1.29**	**0.37**	**0.92**	
	309				**1.24**	**0.47**	**0.77**	
Sub total 1					9.09	3.17	5.92	
2	**No Nº**	**3.11**	**0.25**	**2.86**				**Reference batch**
					1.03	**0.00**	**1.03**	
Sub total 2		3.11	0.25	2.86	1.03	0.00	1.03	
3	**No Nº**	**2.96**	**1.73**	**1.23**				
					0.81	**0.00**	**0.81**	
					1.22	**0.00**	**1.22**	
					1.49	**0.00**	**1.49**	
					0.80	**0.00**	**0.80**	
					0.91	**0.00**	**0.91**	
					0.53	**0.00**	**0.53**	
					1.12	**0.00**	**1.12**	
					1.32	**0.00**	**1.32**	
					0.69	**0.00**	**0.69**	
					1.29	**0.00**	**1.29**	
					0.85	**0.00**	**0.85**	
					8.22	**0.00**	**8.22**	**Reference batch**
					4.00	**4.00**	**0.00**	
					2.77	**2.77**	**0.00**	
					9.28	**9.28**	**0.00**	
Sub total 3		2.96	1.73	1.23	35.30	16.05	19.25	
4	**No Nº**	**0.88**	**0.00**	**0.88**				
					0.55	**0.55***	**0.00**	Not processed
Sub total 4		0.88	0.00	0.88	0.55	0.55	0.00	
5					**0.56**	**0.00**	**0.00**	Not processed
					0.50	**0.00**	**0.00**	Not processed

					0.74	**0.00**	**0.00**	Not processed
					1.52	**0.00**	**1.52**	
					1.84	**0.00**	**1.84**	
					1.32	**0.00**	**0.00**	Not processed
					1.56	**0.00**	**1.56**	**New reference batch**
					1.92	**0.00**	**1.92**	**(3.48 kg)**
Sub total 5		0.00	0.00	0.00	9.96	0.00	6.84	
Total		**6.95**	**1.98**	**4.97**	**55.93**	**19.77**	**33.04**	

Note: as later explained, some old or damaged leaves were discarded at the washing stage. The above recorded "discarded" weights take into account leaves that have been discarded at the sorting stage only. Small bags in particular were not sorted if their content looked acceptable at reception.

2.4 Washing of IGLVs

Pouring dirty leaves in a big plastic tub filled with water and stirring them manually is not an efficient way to wash IGLVs as the water becomes loaded with dirt particles and some leaves settle at the bottom of the tub. Soil particles are long to settle and as soon as the leaves are collected, water and particles are mixed again making cleaning inefficient.

It seems that the only way to properly clean the leaves is to place a small quantity in a shallow meshed basket-strainer held in such a way that the top of the basket is slightly above the water level in the tub. The leaves and water are manually stirred for 1 or 2 minutes in the basket. Soil particles fall through the mesh. This process is then repeated (same basket, same leaves) in a second bath, and most often in a third bath until the water stays clean.

A special "basket" was made for this purpose, consisting of a plastic basin (fitting well in the plastic tub), from which the bottom was removed and replaced by a 5mm perforated metal sheet (unfortunately no stainless steel perforated sheet was available and mild steel was used). The basin rim rests on the top of the tub's rim. Two such "baskets" were manufactured by the consultant.

Adding an organic detergent to the water bath was envisaged as it could help in washing away the soil particles stuck to the leaves (the recommended product "Sanigerm" was purchased but the specification sheet, received later, indicated that it was only a disinfectant). However, it was not used as it could have altered the nutritional analysis especially for phosphorus and iron (advice from Analytical Laboratory Services).

IGLVs' washing requires a lot of water. The plastic tubes used contained about 60-l of water for a top diameter of 50 cm (we could have bought smaller tubs but the decrease of the top diameter means a lesser washing capacity, and this capacity is roughly proportional to the tub capacity and its top diameter). To give an indication, a meshed basket of 30 cm diameter can only properly clean batches of 300 to 350 g of leaves. The trials showed that our self-made baskets have a washing capacity of about 1.5 kg of leaves (per batch) and that 3 consecutive washings were required to get an acceptable cleanliness. The next batch is washed first in the second bath water (the first bath water has to be discarded and once the tub is re-filled with clean water, it becomes the 3rd bath). This means that 60 litres of water are needed to wash 1.5 kg of leaves (i.e. 40-l of water per kg of leaves).

On this basis, it is recommended to include a water recycling device (a pool filter for example) in a small scale IGLVs' processing unit.

2.5 First processing of IGLVs

In order to minimize the loss of nutrients, the processing trials were conducted with raw leaves sealed in airtight 80µ plastic bags forced into boiling water for 10 minutes or more. However, out of 4 bags processed this way, 1 burst, 2 got swollen like balloons and only 1 kept its initial shape.

Another method was then tried (and adopted). It was wilting/braising the raw leaves: 1.5 to 2 kg of clean raw leaves are slowly cooked in a 20-l stainless steel pot (lid on) with no water at all for *Cleome* (the residual washing moisture is enough) and about 20% (w/w) water for *Amaranthus*. After some 25 minutes and several stirrings the leaves are well cooked. Theoretically, the nutrient losses should be minimised as no water is left in the pot after cooking.

All the preserved leaves have been preliminarily processed this way.

2.6 Second processing and preservation of IGLVs

The further processing trials performed for preserving IGLVs are listed below:

- Deep-freezing in sealed plastic bags
- Sterilisation in glass jars
- Drying in the shade and packaging in plastic bags.

Cleome and *Amaranthus* were further-processed the same way. However, it seems that *Cleome* once cooked for about 25 minutes has a much harder texture than *Amaranthus* (very smooth). For this reason, part of the *Cleome* leaves were minced in a blender with high rotation speed blades. The panel tasting should indicate which one is preferred by consumers.

a) Deep freezing: it is the easiest and quickest method (as long as the freezing capacity can cope with the processing production) as IGLVs are packaged in plastic bags that are sealed and placed in a deep freezer.
The bags could not be vacuum sealed (as the only domestic vacuum sealer we had was neither handy nor quick) but only sealed with a good impulse sealer (with some experience, it is quite easy to limit the quantity of air in the bag).

b) Sterilisation: glass jars can be immediately filled with cooked IGLVs to be sterilised. The packed leaves inside the jars were slightly covered with brine made of clean water to which a 1.7% w/w (5g for 300ml) salt was added. However, to make sure that *Clostridium botulinium* cannot survive, the pH (around 5 for both species) of the IGLVs to be sterilised has to be reduced below pH 4.5.
This can be achieved by adding citric and/or ascorbic acid. A trial was made in a small jar with citric acid added to *Amaranthus* leaves already cooked. But, this trial was discontinued because the acid gave a bad taste to the leaves.
We believe that the pH could be adequately reduced by adding tomatoes for example, without a detrimental effect on the IGLVs' taste. But this method should rather be tried by a small scale processing unit or by a further phase of the project.

c) Drying: only small quantities of both species could be dried (on a stainless steel mesh under a shed): *Cleome* because of limited availability and *Amaranthu*s because of the weather (it had rained every day at the time). *Amaranthus* was longer to dry than *Cleome* (because it

formed lumps of leaves); leaf drying had to be finished in a domestic electric dehydrator. When dried, *Amaranthus* and *Cleome* leaves were stored in plastic bags.

d) Containers:

- **Deep freezing:** we recommend industrial plastic vacuum bags, 80 microns thick. They are strong and relatively cheap. The best size for packaging 250g to 500g of cooked IGLVs is 250 x 300 mm; the price is slightly below N$0.50 per unit, but these bags are normally sold only by carton of one thousand. These bags are easily sealed with a professional impulse sealer (220 V, ± N$600 VAT included for 300mm capacity). The major problem is that they are generally not available at supermarkets.
- **Glass jars:** 2 types of jars were tested:
  - The honey jar, 215 g tare, 350 ml capacity (cylindrical with a twist-on lid)
  - The tapered mason jar, 350 g tare, 400 ml capacity

  Both types of jars have their problems and tricks:
  - Honey jars (no brand, golden lid): 6 jars out of 16 failed to properly sterilise, most probably due to lid failure (lid swelling after a few days). We recommend reputable brands with strong lids.
  - Tapered mason jars: 3 out of 10 failed to hold air tight; the rubber seal of one cracked, the lid of the second one was probably too tightly fastened (pressure inside the jar built up and damaged the lid instead of being released), and for the third one there was no apparent defect.

  It is recalled that jars must never be filled up to the top as liquid and material expand when heated to the boil. It is advised to always keep the level in the jar at least 10 to 15mm under the top of the jar. For a small difference in capacity, the Honey jar should be selected (provided that it is of good quality) as it is much smaller and lighter.

e) Details of IGLVs processed: *Table 3* below shows the quantities processed for each species and the methods of preservation.

Table 3 – IGLVs processing quantities and methods of preservation

	Date	Deep frozen g	Sterilized g	Dried g	Discarded g	Total avail. g	Remark
	11/03/2006	1'950					3 x 500g bags + 1x 450g bag
AMARANTHUS	12/03/2006	2'830					5 x 500g bags + 1 x 330g bag
	12/03/2006	2'500					5 x 500g bags
	17/03/2006	2'915					4 x 500g bags + 1 x 665g bag + 1x 250g bag
	18/03/2006	1'000					5 x 200g bags
	Ref batch		1'650		4 x 330 (4 jars)		5 x 330g cyl. jars
				1'900 (wet)			± 240g dry
	19/03/2006	3'000					6 x 500g bags
	20/03/2006	3'280					6 x 500g bags + 1 x 280g bag
			2'450		3 x 350 (3 jars)		7 x 350g jars
	21/03/2006	3'000					6 x 500g bags
	4/04/2006 *New Ref.*	800	1583		1 x 317		4 x 200g bags 5 x 317 g cyl. jars

	batch		1061	680 (wet)	(1 jar)		3 x 354 g jars
	4/04/2006	4'050					7 x 500g + 1 x 285g + 1 x 265g bags
Total Amaranthus		***25'325***	***6'744***	***2'580***	***(-2'687)***	***31'962***	
CLEOME	16/03/2006	800					4 bags 200g
	Ref batch		1'695		390 (1 jar)		3 x 200g cyl. jars + 1095g in 3 cyl. jars
				800 (wet)			± 100g dry
	19/03/2006	1'230					2 x 500g bags + 1 bag 250g
	23/03/2006		1050				3 x 350g jars
Total Cleome		***2'030***	***2'745***	***800***	***(-390)*** *(1 jar)*	***5'185***	

In theory, the weight of processed and discarded IGLVs should match the weight of IGLVs received. However, this is not always the case for some practical reasons:

- IGLVs have dried between harvesting, storage and delivery, with a significant loss of weight, difficult to accurately assess.
- During washing, some yellow or damaged leaves that have passed through sorting are discarded. As leaves are wet, weighing them would bring some error in further weight calculation; this is why, the weight of leaves discarded during washing has not been taken into account.
- When washing IGLVs, the leaves probably re-absorb at least part of the water they had lost and keep a bit of moisture from washing even if they are left to drain for 15 to 30 minutes. As a consequence, there is most probably a weight increase.
- Leaves were wilted/braised with some water added to facilitate cooking (except when the 1st processing took place immediately after washing and straining -15 to 20 minutes). It is very difficult to assess the quantity of water that has evaporated (especially during regular stirrings when the lid is removed) and the quantity that was absorbed by the leaves.

It is, therefore, not surprising to sometimes obtain a higher weight of processed IGLVs compared to the weight of the same IGLV at reception.

2.7 IGLVs available for panel tasting

In principle, all the preserved IGLVs should be used for this purpose, except the reference samples for nutritional analysis and microbiological tests. Practically, due to the short supply, all the processed *Cleome* was provided (except the reference samples). For *Amaranthus*, only 4 jars (sterilized leaves), some 2 kg of frozen leaves and 180g of dry material were used.

2.8 Nutritional and microbiological tests

The IGLV samples were provided to Analytical Laboratory Services (Windhoek) for analysis (including Vitamin content).

- The nutritional analyses consist of: ash, protein, Vit. A, Vit. C, calcium, iron and phosphorus contents
- The microbiological analyses consist of total colony count and mould/yeast count.

Reference samples of fresh *Cleome* and *Amaranthus* were quickly provided to the Lab. for nutritional analysis just after reception, one sample of *Cleome* on 16/03/2006 (delivery Nº2) and one sample of *Amaranthus* on 17/03/2006 (delivery Nº3).

Three samples of each species (sterilised, deep frozen and dried) were provided on 23/03/2006 for nutritional analysis and for the first microbiological tests.

Because most of the sterilised *Amaranthus* jar preservation failed and there was not enough left for the next round of microbiological tests, the first reference batch tests were dropped and a new reference batch was processed from delivery Nº5.

Samples from this new reference batch were delivered to the Lab. on 10/04/2006 and results are not yet available.

3. Conclusion and Recommendations

3.1 Conclusion

- Despite the generally poor condition of the leaves at reception (due to long time between harvesting and delivery), the harvesting of leaves was generally properly done: no stems or bunches of leaves, although large yellowish and hard leaves could have been removed.

- Inadequate transport of fresh IGLVs is detrimental to their quality, especially after cold storage; if a processing unit is set up, it should be as close as possible to the IGLV collection point (distance of 20-30 km maxi), and the best option would be on-site.

- As it was the case in Phase-1, the supply of *Cleome* was low (approximately 9 times less than *Amaranthus* during Phase-2). There are not clear reasons for this short supply.

- The nutritional analyses should confirm whether the new processing method followed in Phase-2 (wilting/braising) is promising. It must be noted that the simple equipment needed for this new processing method would normally be available in any household: a big pot (preferably enamelled or stainless steel) with a lid and a wooden spoon.

- In principle, sterilisation is a very simple preservation method. However, the number of jars that failed during Phase-2 indicates that it is not so easy. The jars themselves and particularly their lids might have been faulty or the jars were filled up too much.

- For a household level or for a small-scale processing unit, when a deep freezer is available, the easiest, cheapest and safest preservation method is deep-freezing. Otherwise, drying after cooking is the second best preservation method provided it is properly done (drying the material in the shade on a clean basket and protected against insects, rodents, birds and dirt).

3.2 Recommendations

- Never pack and store freshly harvested IGLVs in airtight containers and do not pack them too tight in bags because the leaves will start fermenting and rotting and will be improper for further processing.

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- IGLV bags should not be closed with metal staples (as experienced in Phase-2) as some may fall when opening the bags and get mixed with the leaves with an obvious risk for the unfortunate consumer.

- Double sterilisation of IGLVs is recommended: a first sterilisation for 40 minutes in boiling water, and a second sterilisation, 24 hours or 48 hours later, for 20 minutes, to avoid any development of pathogen spores which would have survived the first sterilisation.

- Cooking guidelines should be provided to consumers (on packaging labels for instance) in order to keep as much nutrients as possible when preparing IGLVs at home.

- The processing costs of freezing and canning of IGLVs (by a small-scale processing unit) should be analysed to also compare the two preservation methods on a financial point of view.

- The reason(s) behind the short supply of *Cleome* should be investigated to assess whether further development and processing has any future.

APPENDIX 1

PHASE-2 EQUIPMENT AND CONSUMABLES PURCHASED

Budget NASSP 661/003 (VAT included)
Budget amount: N$2'260.00

Date	Supplier & invoice Nº	Description	Amount (N$)
7/03/06	Afrox no 2065030	Handigas cylinder & 9 kg gas, hose, 3-ring gas burner (x1), regulator and hose clamps	636.77
8/03/06	Afrox no 2065194	3-ring gas burner (x1)	351.85
8/03/06	Afrox no 2065195	9 kg gas refill	110.15
8/03/06	Medlab no 110791	Thermometers Al 10-150ºC (x2)	110.00
9/03/06	Multimax no 18880	Vacuum bags: - size 200x300, 80 microns (x200) - size 250x350, 80 microns (x200)	226.90
9/03/06	Microclean no 25034	Sanigerm 5kg	82.30
9/03/06	Game no 694809	80-l plastic tubs (x4)	219.80
7/04/06	KAP no H/74	Plastic baskets for IGLV washing (x2)	522.10
		Total:	**2'259.87**

APPENDIX 2

PHOTOGRAPHIC REPORT

Discarded loose leaves (of Amaranthus) at the sorting stage

Lumps of “cooked” leaves

1st washing trials with a 30 cm basket (cap. 300-350g of leaves)

KAP designed appropriate basket (capacity 1.5-2 kg of leaves)

The basin rim just fits on the tub rim.
Leaves are slightly rubbed with water

Wilting of leaves: little or no added water

The 2 types of jars used for sterilization: left the cylindrical or “honey” jar with its twist-on lid, right the tapered mason jar with its screw ring and seal lid.

ANNEX 4

IPTT INDIGENOUS PLANT TASK TEAM
Promoting the Sustainable Utilisation of Namibia's Indigenous Plant Resources

Promoting Indigenous Plants in Namibia:

Indigenous Green Leafy Vegetables (IGLVs)

PROCESSING AND MARKETING TRIALS

Phase 2 (2006)

PANEL TEST REPORT

Consultancy contract

Administered by the Namibian Agronomic Board (NAB)

with funds from:

MAWF / NASSP (661/003) and UPDP / IPTT

Prepared by
Saskia den Adel (CRIAA SA-DC)
Windhoek, 11 April 2006

1. Introduction

The objective of Phase-2 of the processing and marketing trials of Indigenous Green Leafy Vegetables (IGLVs) were to analyse and panel test the two species *Amaranthus* (Ekwaka) and *Cleome* (Ombidi) to determine the shelf life of frozen and sterilized samples, and make an economic assessment of the processing methods. This report presents the results of the panel test which was conducted by Saskia den Adel in Windhoek.

2. Methodology

From the earlier trials *Amaranthus* (Ekwakwa) and *Cleome* (Omboga/Ombidi) were selected as the two preferred species. Cooked, sterilized and dried were selected as the preferred conservation methods. The different Indigenous Green Leafy Vegetables (IGLVs) were compared by consumers in panel test at a Katutura household on the 24th of March 2006. The panel consisted of 15 people, all Oshiwambo speaking (for details of panel testers see Appendix I). Two women prepared the IGLVs in the Owambo traditional way. Before the panel test, the panellists were asked to fill in a simple questionnaire about the usage of IGLV (Appendix II results questionnaire). The panel test consisted of 3 rounds, and in every round the panellists had to taste and rate 2 colour coded plates. The vegetables were rated on smell, taste, texture, and appearance on a scale from 1 (horrible) to 7 (excellent). The panellists were motivated to add comments to the ratings, and were asked at the end of each round which plate they liked most, and why (Appendix III rating form).

Each of the 3 rounds consisted of 2 types of vegetables with the same conservation method, and prepared in the same traditional way. The aim of this second phase panel test was to compare the two different vegetable species, rather than conservation methods. The following rounds were done:

1	Frozen Ombidi *(red plate)*	-	Frozen Ekwakwa *(green plate)*
2	Dried Ekwakwa *(red plate)*	-	Dried Ombidi *(green plate)*
3	Sterilised Ombidi *(red plate)*	-	Sterilised Ekwakwa *(green plate)*

In handbooks describing panel test methodology (i.e. E. Larmond – *Laboratory Methods for Sensory Evaluation of Food*, Food Research Institute Ottowa, 1982), it is said to be important for the validity of the research that panellists are relatively isolated, be seated on comfortable chairs, in a neutral atmosphere, with correct lighting and a constant and comfortable temperature. It is furthermore important that panellists are not hungry, not distracted, and not entering into conversations of any kind.

Controlling these factors at a Katutura household is impossible, and the panel testing was culturally adapted by keeping it as simple as possible, and by telling the panellists to:

A. Be honest, it was explained that we were not looking for compliments, but that we needed their honest opinion. It was made clear that neither the researcher nor the cooks would be offended if they did not like the dish.

B. Give their own opinion, without discussing it with other panellists

C. Merely smell and taste the IGLVs during the test. It was clarified that they would get 6 plates to taste, and that they should not be full in the middle of the test. It was also said that they could eat as much Oshifima and IGLVs as they wanted, after they completed the tests

3. Results

The results of this panel test were much more convincing than the results from the panel test done in phase I. While 86% of the respondents claimed in the questionnaire that they preferred Ombidi in pure or mixed form to Ekwakwa, the ratings of the panel test showed a very strong preference to Ekwakwa instead. The table below (more detailed in appendix IV) shows the favourite plates of the panellists for all processing methods;

GENERAL PREFERENCE		
	Ombidi	**Ekwakwa**
Frozen	**7%**	**93%**
Sterilised	**0%**	**100%**
Dried	**17%**	**83%**

The preference for Ekwakwa as compared to Ombidi was consistent for all variables, but was more pronounced in the texture and taste, and least in the smell. (See table below and appendix V-VIII)

	TASTE		**TEXTURE**		**APPEARANCE**		**SMELL**	
	Ombidi	Ekwakwa	Ombidi	Ekwakwa	Ombidi	Ekwakwa	Ombidi	Ekwakwa
Frozen	2.20	6.07	2.13	6.07	3.33	6.07	3.53	5.80
Dried	3.33	5.47	3.27	6.00	4.13	6.00	4.13	4.87
Sterilised	3.13	6.60	2.47	6.47	3.47	6.33	4.53	6.13
Average	**2.89**	**6.05**	**2.62**	**6.18**	**3.64**	**6.13**	**4.06**	**5.60**
	bad	*Very good*	*bad*	*Very good*	*average*	*Very good*	*average*	*Very good*

When combining all the variables (see table below), one can see that there are much more significant differences in preference for the type of vegetable, than there are between the conservation methods. For Ekwakwa, there was a slight preference for the sterilized conservation method, followed by frozen, then dried. For Ombidi, the panellists slightly preferred the dried conservation method, while the frozen Ombidi was the least appreciated. What is important to note is that all the processed Ekwakwa on average achieved a very high score of 5.59 to 6.38 *(very good*), while all processed Ombidi was received much worse with average scores of 2.80 to 3.72 *(bad to average)*

	FROZEN		**DRIED**		**STERILISED**	
	Ombidi	**Ekwakwa**	**Ombidi**	**Ekwakwa**	**Ombidi**	**Ekwakwa**
Taste	2.20	6.07	3.33	5.47	3.13	6.60
Appearance	3.33	6.07	4.13	6.00	3.47	6.33
Texture	2.13	6.07	3.27	6.00	2.47	6.47
Smell	3.53	5.80	4.13	4.87	4.53	6.13
AVERAGE	**2.80**	**6.00**	**3.72**	**5.59**	**3.40**	**6.38**
	bad	*very good*	*Average*	*very good*	*Bad*	*very good*

4. Conclusions and recommendations

Compared to the panel test that was done in phase I, both the conservation and preparation methods seemed to have improved significantly. There was no comment on the presence of sand in the vegetables that were tasted, and much less comments related to the preparation of the food (i.e. too salty, too much oil), which has a positive effect on the reliability of the results.

Without a doubt, the panel test showed there is a strong preference for Ekwakwa. Especially the sterilized Ekwakwa was highly appreciated, but also the frozen and dried Ekwakwa achieved very high scores. Ombidi scored significantly lower. The panellists mostly had a problem with the texture of this vegetable, but also the taste received very low ratings. It is therefore recommended that further IGLV work should be concentrating on Ekwakwa, and all three conservation methods are suitable.

APPENDIX I

IGLV-II panel test Windhoek				
Particulars of respondents				
	gender	**age**	**ethnicity**	**occupation**
1	F	31	Owambo	Assessor
2	F	28	Owambo	Domestic worker
3	F	17	Owambo	Student
4	F	23	Owambo	Caregiver
5	F	14	Owambo	Student
6	F	47	Owambo	Police officer
7	F	28	Owambo	Prison Officer
8	M	23	Owambo	Self-employed
9	M	14	Owambo	Student
10	M	38	Owambo	Businessman
11	M	39	Owambo	Carpenter
12	M	25	Owambo	Confectioner
13	M	16	Owambo	Student
14	M	27	Owambo	Unemployed
15	M	15	Owambo	Student

APPENDIX II

General usage of IGLV - II
(Results general questionnaire panel testers - Windhoek)

1. Do you eat any of the following vegetables?

• Ombidi (mixed with ekwakwa / omundjulu)	YES	15	
	NO	0	
• Ombidi (pure, not mixed)	YES	13	
	NO	2	(bitter / no good taste)
• Ekwakwa (pure, not mixed)	YES	14	
	NO	1	(taste not so good)

2. Which one do you like best?

Ombidi, mixed	9
Ombidi, pure	3
Ekwakwa	2
No response	1

3. How often do you eat them?

more than once per week	1
once per week	4
once per month	3
seldomly	6
No response	1

4. Do you eat them throughout the year or only in a particular season?

throughout the year	5	
only when they are available	10	(from Dec till Jan/Feb/Mar/Apr)
only when there is nothing else	0	

5. Where do you get these vegetables?

mostly get them from my own field	7
mostly get / buy them from relatives or friends	5
mostly buy them at the market	3

APPENDIX III

Panel testing: Frozen leafy vegetables

Name: ……

1	2	3	4	5	6	7
horrible	**very bad**	**bad**	**average**	**good**	**Very good**	**excellent**

GREEN plate:

	ranking							Comments
Appearance	1	2	3	4	5	6	7	
Smell	1	2	3	4	5	6	7	
Taste	1	2	3	4	5	6	7	
texture	1	2	3	4	5	6	7	
General description								

RED plate:

	ranking							Comments
Appearance	1	2	3	4	5	6	7	
Smell	1	2	3	4	5	6	7	
Taste	1	2	3	4	5	6	7	
texture	1	2	3	4	5	6	7	
General description								

Which of the 2 plates do you like best? [ ] GREEN plate [ ] RED plate

Why? ……
……..
……

APPENDIX IV

Results IGLV-II panel test Windhoek									
General preference									
Panellist No.		frozen Ombidi	frozen Ekwakwa		Dried Ombidi	Dried Ekwakwa		Sterilised Ombidi	Sterilised Ekwakwa
1			x			X			x
2			x			X			x
3			x		X				x
4			x			X			x
5			x			X			x
6			x			X			x
7			x		X				x
8			x			X			x
9			x			X			x
10			x			X			x
11			x			X			x
12			x			X			x
13			x			X			x
14		x				X			x
15			x			X			x
		7%	**93%**		**13%**	**87%**		**0%**	**100%**

APPENDIX V

Results IGLV-II panel test Windhoek						
Taste						
Panellist No.	frozen Omboga	frozen Ekwakwa	Dried Omboga	Dried Ekwakwa	Sterilised Omboga	Sterilised Ekwakwa
1	3	7	2	7	3	7
2	1	7	6	6	2	7
3	1	4	3	2	2	7
4	2	7	2	7	3	7
5	1	5	2	7	4	7
6	2	6	3	6	4	6
7	2	5	3	2	3	6
8	2	3	4	7	2	7
9	1	7	5	6	4	7
10	1	7	1	4	5	7
11	3	7	1	7	2	5
12	3	5	3	5	3	6
13	3	7	7	3	3	7
14	4	7	4	6	4	6
15	4	7	4	7	3	7
AVERAGE	**2.20**	**6.07**	**3.33**	**5.47**	**3.13**	**6.60**
	very bad	*very good*	*bad*	*Good*	*bad*	*Excellent*
ST DEV	**1.08**	**1.33**	**1.72**	**1.85**	**0.92**	**0.63**

APPENDIX VI

Results IGLV-II panel test Windhoek						
Appearance						
Panellist No.	frozen Omboga	frozen Ekwakwa	Dried Omboga	Dried Ekwakwa	Sterilised Omboga	Sterilised Ekwakwa
1	4	7	4	7	2	6
2	3	7	5	6	3	7
3	4	6	6	5	3	7
4	3	7	5	7	6	7
5	6	5	2	7	5	6
6	3	6	4	5	3	6
7	4	6	4	5	3	6
8	1	4	6	7	3	7
9	2	6	5	4	4	6
10	1	7	5	7	3	7
11	5	7	1	7	4	6
12	4	5	3	5	4	6
13	2	7	6	7	4	6
14	3	5	3	5	3	5
15	5	6	3	6	2	7
AVERAGE	**3.33**	**6.07**	**4.13**	**6.00**	**3.47**	**6.33**
	bad	*very good*	*average*	*very good*	*bad*	*very good*
ST DEV	**1.45**	**0.96**	**1.51**	**1.07**	**1.06**	**0.62**

APPENDIX VII

Results IGLV-II panel test Windhoek						
Texture						
Panellist No.	frozen Omboga	frozen Ekwakwa	Dried Omboga	Dried Ekwakwa	Sterilised Omboga	Sterilised Ekwakwa
1	3	7	3	7	1	7
2	1	7	4	7	1	7
3	2	5	3	6	2	7
4	4	7	5	7	3	7
5	5	6	2	7	4	7
6	2	6	3	4	3	6
7	3	6	3	5	2	5
8	1	4	3	7	1	7
9	1	5	3	5	3	7
10	1	7	2	6	3	7
11	2	7	2	7	2	6
12	2	6	2	6	3	5
13	1	5	7	5	1	7
14	1	6	3	5	4	5
15	3	7	4	6	4	7
AVERAGE	**2.13**	**6.07**	**3.27**	**6.00**	**2.47**	**6.47**
	very bad	*very good*	*bad*	*very good*	*very bad*	*very good*
ST DEV	**1.25**	**0.96**	**1.33**	**1.00**	**1.13**	**0.83**

APPENDIX VIII

Results IGLV-II panel test Windhoek						
Smell						
Panellist No.	frozen Omboga	frozen Ekwakwa	Dried Omboga	Dried Ekwakwa	Sterilised Omboga	Sterilised Ekwakwa
1	5	6	5	6	5	7
2	1	5	4	3	6	7
3	1	6	4	2	1	6
4	3	7	6	7	5	7
5	3	4	2	7	7	6
6	3	7	4	4	5	6
7	5	6	5	2	4	6
8	4	4	4	6	4	7
9	3	6	4	3	5	6
10	5	7	3	5	6	7
11	5	7	4	7	5	5
12	5	6	4	5	5	5
13	2	6	5	4	2	5
14	4	5	4	6	4	6
15	4	5	4	6	4	6
AVERAGE	**3.53**	**5.80**	**4.13**	**4.87**	**4.53**	**6.13**
	average	*good*	*average*	*good*	*good*	*very good*
ST DEV	**1.41**	**1.01**	**0.92**	**1.77**	**1.51**	**0.74**

ANNEX 5

LABORATORY ANALYSIS RESULTS

ANNEX 5.1

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 210058 email analab@mweb.com.na

Preliminary TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com

Date received: 16-Mar-06
Date required:
Date completed: 05-June-06

Attention: Michel

Your Reference: **37, 44**
Lab. Reference: **I060218**

Type of Sample(s)

Indigenous green leavy vegetables

Samples Received

Cleome received on 16/03/2006, *Amaranthus* received 17/03/2006 and a second sample of *Amaranthus* received on the 04/04/2006 replacing the first sample

Test(s) Required

Moisture content, protein content, ash content, Vitamin C, calcium, iron, phosphorus

Test Method(s) used

The determination of moisture (Ref. Official and Standardized Methods of Analysis, 1994, 3rd edition, edited by Colin Watson)
Dried to constant weight at ±100°C/±2h
Reporting: Weight % (m/m)

Determination of nitrogen (Ref. Official and Standardized Methods of Analysis, 1994, 3rd edition, edited by Colin Watson)
Digestion: concentrated sulphuric acid/1.5h, selenium tablets
Distillation
Titration with 0.100M hydrochloric acid

Determination of protein (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Calculated from nitrogen content
Reporting: Weight % (m/m) wet weight basis

Determination of ash (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Ashed to 550°C to constant weight
Reporting: Weight % (m/m) wet weight basis

Determination of calcium, iron, phosphorus (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Ash dissolved in hydrochloric acid
Calcium, iron determined by ICP
Phosphorous determined colorimetrically
Reporting: mg/100g wet weight basis

Determination of Ascorbic acid (Ref. Lebensmittel Analytik Grundzüge-Methoden-Anwendung,2nd edition, 1992, Springer Verlag, Berlin)Redox titration with 2,6-dichlorophenolindophenol (Tillmans)
Stabilising agent: 2% oxalic acid
Reporting: mg/100g wet weight basis

Results

Test / Identification	Moisture Weight %	Protein Weight % (wet)	Ash Weight % (wet)	Vitamin C mg/100g (wet)
1. *Cleome gynandra*	79	7.1	4.2	2.9
2. *Amaranthus thunbergia*	85	3.4	2.4	60

Test / Identification	Calcium mg/100g (wet)	Iron mg/100g (wet)	Phosphorus as P mg/100g (wet)
1. *Cleome gynandra*	361	6.3	182
2. *Amarnthus thunbergia*	500	7.8	75

Remark:

_____
S. Rügheimer
Section Head: Microbiology & Food Chemistry

ANNEX 5.2

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 210058 email analab@mweb.com.na

Preliminary TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com

Date received: 23-Mar-06
Date required:
Date completed: 05-June-06

Attention: Michel

Your Reference: **40, 46**
Lab. Reference: **I060234**

Type of Sample(s)

Indigenous green leavy vegetables, processed

Samples Received

Three samples received on 23/03/2006 and three samples received on 10/04/2006

Test(s) Required

Moisture content, protein content, ash content, Vitamin C, calcium, iron, phosphorus

Test Method(s) used

The determination of moisture (Ref. Official and Standardized Methods of Analysis, 1994, 3rd edition, edited by Colin Watson)
Dried to constant weight at ±100°C/±2h
Reporting: Weight % (m/m)

Determination of nitrogen (Ref. Official and Standardized Methods of Analysis, 1994, 3rd edition, edited by Colin Watson)
Digestion: concentrated sulphuric acid/1.5h, selenium tablets
Distillation
Titration with 0.100M hydrochloric acid

Determination of protein (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Calculated from nitrogen content
Reporting: Weight % (m/m) wet weight basis

Determination of ash (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Ashed to 550°C to constant weight
Reporting: Weight % (m/m) wet weight basis

Determination of calcium, iron, phosphorus (Ref. Official Methods of Analysis for the AOAC, 1970, 11th edition, Horwitz, W.)
Ash dissolved in hydrochloric acid
Calcium, iron determined by ICP
Phosphorous determined colorimetrically
Reporting: mg/100g wet weight basis

Determination of Ascorbic acid (Ref. Lebensmittel Analytik Grundzüge-Methoden-Anwendungen, 2nd edition, 1992, Springer Verlag, Berlin)
Redox titration with 2,6-dichlorophenolindophenol (Tillmans)
Stabilising agent: 2% oxalic acid
Reporting: mg/100g wet weight basis

Results

Test / Identification	Moisture Weight %	Protein Weight % (wet)	Ash Weight % (wet)	Vitamin C mg/100g (wet)
1. *Cleome*, frozen, 16/03, 200g	88	3.8	2.3	1.1
2. *Cleome*, sterilized, 16/03	88	4.3	2.6	2.6
3. *Cleome*, dried, 16/03	7.4	31	18	7.7
4. *Amaranthus*, frozen, 200g	86	3.2	2.2	4.3
5. *Amaranthus,* sterilized, 330g	88	3.0	1.9	3.4
6. *Amaranrhus*, dried, 200g	3.0	23	16	9.7

Test / Identification	Calcium mg/100g (wet)	Iron mg/100g (wet)	Phosphorus as P mg/100g (wet)
1. *Cleome*, frozen, 16/03, 200g	161	4.0	90
2. *Cleome*, sterilized, 16/03	219	4.7	102
3. *Cleome*, dried, 16/03	1127	39	767
4. *Amaranthus*, frozen, 200g	444	6.2	70
5. *Amaranthus,* sterilized, 330g	390	4.9	62
6. *Amaranrhus*, dried, 200g	2826	49	506

Remark:

_____
S. Rügheimer
Section Head: Microbiology & Food Chemistry

ANNEX 5.3

ANNEX 5.4

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 210058 email analab@mweb.com.na

TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com

Date received:
Date required:
Date completed: 19-July-06

Attention: Michel

Your Reference: **40, 46**
Lab. Reference: I060234-2

Type of Sample(s)
Indigenous green leafy vegetables, processed

Test(s) Required
ß-carotene

Test Method(s) used
HPLC
Reporting: mg/100g

Results

Test / Identification	ß-carotene mg/100g
1. *Cleome*, frozen	1.8
2. *Cleome*, sterilized	1.8
3. *Cleome*, dried	4.0
4. *Amaranthus*, frozen	1.4
5. *Amaranthus,* sterilized	2.3
6. *Amaranrhus*, dried	5.0

Remark
Samples were outsourced to SABS Commercial (Pty) Ltd.

S. Rügheimer
Section Head: Microbiology & Food Chemistry

ANNEX 5.5

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 217102 email analab@mweb.com.na

TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com.na

Date received: 23-Mar-06
Date required:
Date completed: 18-Apr-06

Attention: Michel

Your Reference: **verbal**
Lab. Reference: **I060234**

Type of Sample(s)

Indigenous green leavy vegetables, processed

Samples Received

Four samples received on 23/03/2006 and tested on 28/03/2006; two samples received on 10/04/2006 and tested on 11/04/2006

Test(s) Required

Total colony count
Mould and yeast count
Coliform: LST-BGB MPN test
E. coli: LST-MUG MPN test

Test Method(s) used

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of aerobic mesophilic organisms in foods
CFU/g
Spread plate method
Plate count agar, 35°C/48h

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of mould and yeast in foods
CFU/g
Spread plate method
Dichloran rose bengal chloramphenicol agar, room temperatue/5 days

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of coliform organisms in foods
MPN/100g
Multiple tube fermentation technique (three tubes)
Lauryl tryptose broth (presumptive), 37°C/24-48h
Brilliant green bile broth (confirmed), 37°C/24-48h
Resuscitation step in non selective broth, 37°C/4h prior to addition of selective broth

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of Escherichia coli organisms in foods
MPN/100g
Multiple tube fermentation technique (three tubes)
Lauryl tryptose broth (presumptive), 37°C/24-48h
LST-MUG broth (confirmed), 44.5°C/24-48h
Resuscitation step in non selective broth, 37°C/4h prior to addition of selective broth

Duration of Test(s)

28/03/2006-02/04/2006
11/04/2006-18/04/2006

Result

Test / Identification	Total colony count Cfu/g	Mould and yeast count Cfu/g	
		Mould	Yeast
1. *Cleome,* sterilised, initial	>50 000 000	n/d	n/d
2. *Cleome*, frozen, initial	100 estimated	n/d	n/d
3. *Amaranthus,* sterilised, 1st batch	>500 000 000	n/d	n/d
4. Amaranthus, frozen, 1st batch	900 estimated	4	n/d
5. *Amaranthus,* sterilised, 2nd batch, Initial	<100 estimated	n/d	n/d
6. Amaranthus, frozen, 2nd batch, Initial	Spreader (1000 estimated)	60	n/d

cfu/g = Colony forming units per g < = less than > = more than
n/d = not detected by the method specified

Test / Identification	Coliform group MPN/100g	E. coli MPN/100g
1. *Cleome,* sterilised, initial	n/d	n/d
2. *Cleome*, frozen, initial	n/d	n/d
3. *Amaranthus,* sterilised, 1st batch	n/d	n/d
4. Amaranthus, frozen, 1st batch	n/d	n/d
5. *Amaranthus,* sterilised, 2nd batch, Initial	n/d	n/d
6. Amaranthus, frozen, 2nd batch, Initial	n/d	n/d

MPN/100g = Most probable number per 100g; this number is based on certain probability formulas and is an estimate of the mean density of E. coli in the sample
n/d = not detected by the method specified
Spreader: spreading growth on agar (colonial morphology)

Remark

1st batch of *Amaranthus* frozen and sterilised was replaced by 2nd batch *Amaranthus* ; further analyses will be done on the 2nd batch only.

Identification of microorganisms:

Cleome, sterilized	Gram positive sporeforming rods Gram negative rods
Amaranthus, sterilized,1st batch	Gram positive sporeforming rods
Cleome, frozen	Gram positive sporeforming rods
Amaranthus, frozen, 1st batch	Gram positive sporeforming rods Mould: *Penicillium* spp.
Amaranthus, frozen, 2nd batch	Mould: *Penicillium* spp.

From the standpoint of nutrient content, vegetables are capable of supporting the growth of moulds, yeasts and bacteria and consequently, of being spoiled by any or all of these organisms. Bacteria are generally the fastest growing, so that in conditions favourable to both, bacteria will usually outgrow fungi. The higher water content of vegetables favours the growth of spoilage bacteria, and the relatively low carbohydrate and fat contents suggest that much of this water is in available form. The pH range of most vegetables is within the growth range of a large number of bacteria, therefore

bacteria are common agents of vegetable spoilage. For fresh leafy vegetables water content will generally be greater than 70% and frequently greater than 85%. Commonly protein content will not be greater than 3.5%, fat content greater than 0.5% and ash content greater than 0.84%.

Heat preservation/heat processing
With respect to food preservation there are two temperature categories in common use: pasteurisation and sterilisation.
Sterilisation. Complete destruction of viable microorganisms; this frequently means a treatment of at least 121°C of wet heat for 15 minutes.
Commercially sterile. No viable organisms can be detected by the usual cultural methods employed or the number of survivors is so low to be of no significance under the conditions of canning and storage. These products generally have a shelf life of two years or more.
Pasteurisation. A comparatively low order of heat treatment; generally at a temperature below the boiling point of water. The more general objective of pasteurisation is to extend product shelf life from a microbial and enzymatic point of view. Destruction of all disease producing microorganisms or the destruction or reduction in number of spoilage organisms
Blanching. This is a type of pasteurisation usually applied to vegetables mainly to inactivate natural food enzymes. Depending on its severity, blanching will also destroy some microorganisms

Hermetically sealed and hot processed foods are generally regarded as non-perishable. They may become perishable unde certain circumstances when an opportunity for recontamination is afforded following processing. Glass containers are hermetic provided the lids are tight; faulty closures can make them non-hermetic.

Cleome and first batch of *Amaranthus*: not sterile
Second batch of *Amaranthus*: commercially sterile
Some of the glass jars were not hermetically sealed; this may influence the shelf life
It is generally more difficult to get rid of the sporeformers; they require a high temperature treatment. They will, however, not multiple if kept at low temperature.

Preservation by freezing
Although metabolic activities off all microorganims can be stopped at freezer temperatures, frozen foods may not be kept indefinitely if the thawed product is to retain the original flavour and texture. The suggested maximum holding time for frozen foods is not based on the microbiology of such foods but on such factors as texture, flavour, tenderness, colour and overall nutritional quality upon thawing and subsequent cooking

From the strict standpoint of food preservation, freezing should not be regarded as a means of destroying foodborne microorganisms. The type of organisms that lose their viability in this state differ from strain to strain and depend on the type of freezing employed, the nature and composition of the food in question, the length of time of freezer storage and other factors, such as temperature of freezing.
Cocci and gram positive rods are generally more resistant than gram negative rods, while endospores are apparently unaffected by low temperatures.

Upon freezing following will happen to certain microorganisms:
There is a sudden mortality immediately on freezing, varying with species;
The cells that are still viable immediately after freezing die gradually when stored in the frozen state;
This decline in numbers is relatively rapid at temperatures just below the freezing point, especially about -2°C, but less so at lower temperatures, and is usually slow below -20°C

S. Rügheimer
Section Head: Microbiology and Food Chemistry

ANNEX 5.6

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 217102 email analab@mweb.com.na

TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com.na

Date received: 11-Apri-06
Date required:
Date completed: 15-May-06

Attention: Michel

Your Reference: **verbal**
Lab. Reference: **I060234/2**

Type of Sample(s)

Indigenous green leavy vegetables, processed

Samples Received

Cleome , frozen (23/03/06) tested on the 11/04/2006 and 24/04/2006 respectively
Amaranthus, frozen (10/04/2006) tested on the 24/04/2006 and 08/05/200 resepctively

Test(s) Required

Total colony count
Mould and yeast count
Coliform: LST-BGB MPN test
E. coli: LST-MUG MPN test

Test Method(s) used

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of aerobic mesophilic organisms in foods
CFU/g
Spread plate method
Plate count agar, 35°C/48h

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of mould and yeast in foods
CFU/g
Spread plate method
Dichloran rose bengal chloramphenicol agar, room temperatue/5 days

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of coliform organisms in foods
MPN/100g
Multiple tube fermentation technique (three tubes)
Lauryl tryptose broth (presumptive), 37°C/24-48h
Brilliant green bile broth (confirmed), 37°C/24-48h
Resuscitation step in non selective broth, 37°C/4h prior to addition of selective broth

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of Escherichia coli organisms in foods
MPN/100g
Multiple tube fermentation technique (three tubes)
Lauryl tryptose broth (presumptive), 37°C/24-48h
LST-MUG broth (confirmed), 44.5°C/24-48h
Resuscitation step in non selective broth, 37°C/4h prior to addition of selective broth

Duration of Test(s)

24/04/2006-02/05/2006
08/05/2006-15/05/2006

Result

Test / Identification	Total colony count Cfu/g	Mould and yeast count Cfu/g	
		Mould	Yeast
1. *Cleome*, frozen (11/04/2006)	Spreader (100 estimated)	2	2
2. *Cleome*, frozen (24/04/2006)	3 200	n/d	n/d
3. *Amaranthus,* frozen (24/04/2006)	2 500	n/d	n/d
4. Amaranthus, frozen (08/05/2006)	Spreader (600 estimated)	n/d	n/d

cfu/g = Colony forming units per g < = less than > = more than
n/d = not detected by the method specified

Test / Identification	Coliform group MPN/100g	E. coli MPN/100g
1. *Cleome,* frozen (11/04/2206)	n/d	n/d
2. Cleome, frozen (24/04/2006)	n/d	n/d
3. Amaranthus, frozen (24/04/2006)	n/d	n/d
4. Amaranthus, frozen (08/05/206)	n/d	n/d

MPN/100g = Most probable number per 100g; this number is based on certain probability formulas and is an estimate of the mean density of E. coli in the sample
n/d = not detected by the method specified
Spreader: spreading growth on agar (colonial morphology)

Remark

Identification of microorganisms:

Cleome, frozen	Gram positive sporeforming rods
Amaranthus, frozen	Gram positive sporeforming rods

_____
S. Rügheimer
Section Head: Microbiology and Food Chemistry

ANNEX 5.7

ANALYTICAL LABORATORY SERVICES

P.O. Box 2108, Windhoek, Namibia
Tel (061) 210132 Fax (061) 217102 email analab@mweb.com.na

TEST REPORT

To: CRIAA SA DC
P.O. Box 23778
Windhoek
criaawhk@iafrica.com.na

Date received: 11-Apri-06
Date required:
Date completed: 29-May-06

Attention: Michel

Your Reference: **verbal**
Lab. Reference: I060234/3

Type of Sample(s)

Indigenous green leafy vegetables, processed

Samples Received

Cleome, sterilsed (23/03/06) and tested on the 23/05/2006
Amaranthus, sterilised (10/04/2006) tested on the 23/05/2006

Test(s) Required

Total colony count
Mould and yeast count
Clostridium spp. spores and viable cells: Presence/Absence
pH

Test Method(s) used

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of aerobic mesophilic organisms in foods
CFU/g
Spread plate method
Plate count agar, 35°C/48h

Methods for the microbiological examination of foods (American Public Health Association)
Enumeration of mould and yeast in foods
CFU/g
Spread plate method
Dichloran rose bengal chloramphenicol agar, room temperatue/5 days

Methods for the microbiological examination of foods (American Public Health Association)
Examination for the presence of viable clostridia cells and spores
Presence/absence in 25g sample
Differential cultivation in differential reinforced clostridium medium, 35°C/120h
Subculturing onto reinforced clostridium egg yolk agar, 35°C/48h/aerobically and anaerobically
Confirmation: microscopic examination

Duration of Test(s)

23/05/2006-29/05/2006

Result

Test / Identification	Total colony count Cfu/g	Mould and yeast count Cfu/g	
		Mould	Yeast
1. *Cleome*, sterilised (23/05/2006)	<100 estimated	n/d	n/d
2. *Amaranthus,* sterilised (23/05/2006)	59 000 000	n/d	n/d

cfu/g = Colony forming units per g < = less than > = more than
n/d = not detected by the method specified

Test / Identification	Clostridium spp. spores and viable cells Presence/Absence in 25g	pH
1. *Cleome,* sterilised (23/05/2206)	Absent	5.6
2. *Amaranthus,* sterilised (23/05/2006)	Present	5.6

Remark

Identification of microorganisms:

Amaranthus, sterilised	Gram positive sporeforming rods; Bacillus spp. and Clostridium spp.

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S. Rügheimer
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