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Comparative study on Physico-chemical characteristics of different periods of soaked minor millets flour-based diarrheal replacement fluids

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ABSTRACT

Acute diarrhoea is defined as three or more loose bowel movements in 24 hours (WHO, 1995), and is one of the principal causes of morbidity and mortality among the children. World-wide attention in the proper management of diarrheal disease in infants has led to the provision of the WHO/UNICEF glucose-based oral rehydration solution. However, this solution is only available in hospitals and health care centres which are sparsely located in most rural areas of the developing countries. Te addition of alternative is the cereal-based oral rehydration solution, which is effective in replacing the lost fluid; contribute to the nutrient intake of the patients, easily available, cheap, and familiar and more acceptable. In this present study attempt to formulate millet-based diarrheal replacement fluids and analyse the physiochemical characteristic of the fluids and correlate the foxtail and kodo millet nutrient, viscosity and Osmolality of the fluids. Among the results, there was a significant difference between unsoaked and soaked samples. The viscosity and osmolality values were reduced in soaked samples. The reduction of viscosity and Osmolality values were influenced by the soaking of millets. It was observed that, between the nutrient and viscosity of foxtail and kodo millet had a significant positive correlation in the viscosity of protein, and fat at 0.01% level. A significant positive correlation found between Osmolality and CHO at 0.01%level. Reduced Osmolality of fluids contains a minimized amount of glucose and minerals had a beneficial effect of the clinical course of diarrhoea and millet is safe for diarrheal replacement fluids. So it can be suggested that soaked millet-based replacement fluids will improve rehydration and reduce stool output due to the reduced Osmolality.

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INTRODUCTION

Diarrhea is one of the important causes of infants and young children's death in developing countries (World Health Organization) About 4 million cases of diarrhea are recorded every year with 2.2 million children's deaths under the age of five in the developing countries and a significant cause of malnutrition in children (WHO/UNICEF). World-wide attention in the proper management of diarrheal disease in infants has led to the provision of the WHO/UNICEF glucose-based oral rehydration solution. This has helped to reduce the number of deaths in the 1970s from 5 million children worldwide each year to just over 1 million by the beginning of the 21st century (World Health Organization). However, this solution is only available in hospitals and health care centres which are sparsely located in most rural areas of the developing countries. The household preparation of salt and sugar solution is inconsistent as it does not provide the essential glucose and electrolytes for optimum absorption. The addition of alternative is the cereal-based oral rehydration solution, which is effective in replacing the lost fluid; contribute to the nutrient intake of the patients, easily available, cheap, and familiar and more acceptable (Molla *et al.*, 1989; Kenya *et al.*, 1989). Several studies had used different cereal grains in the preparation of cereal-based oral rehydration solution (CBORS). These include maize (Ramadas *et al.*, 1988; Chowdhury *et al.*, 1991) rice, and mash potatoes (Molla *et al.*, 1989). Millet also one of the major cereals, which are important food ingredients for millions of people, especially those who live in hot and dry areas, hills and rainfall regions. India is one of the major millet producing countries in the world. (Stanly and Shanmugam, 2013). There are different kinds of millets like Finger millet, Barnyard millet, Foxtail millet, Pearl millet, Kodo millet, Little and Proso millet. Among the millets, foxtail millet and Kodo millets are underutilized grain grown across the world. These millet crops are grown in arid and semi-arid grains of African and Asian countries. The millets are rich in a good source of energy and fat, protein, fatty acids, vitamins, and minerals and also rich sources of phytochemicals, micronutrients, and antioxidants like phenolic acids, tannin, and flavonoids. Foxtail millet contains high dietary fiber and beta-glucan (water-soluble gum) has been attributed to improving glucose metabolism. It is gluten-free and non-acid forming, so it is soothing and easy to digest. It contains B-complex vitamins and also the vitamin A and E (Devi *et al.*, 2014) Millet has been used as a nourishing gruel or soup for pregnant and lactating women and has been used to food therapy. (Pawar and Machewad, 2006). It also contains anti-nutrients, traditional processing such as roasting, soaking, germination, and fermentation of cereal will reduce the anti-nutrients, and also improve the protein, and starch digestibility. Ramadas *et al.* (1988) indicated that the hydrolysis of starch releases glucose gradually which is absorbed rapidly and cereal ORS can be advantageously used in relatively large amounts without the risk of inducing osmotic diarrhoea. In this present study, an attempt made to formulate a foxtail and kodo millet-based diarrheal replacement fluids and to find out the impact of different periods of soaking on the Physico-chemical characteristics of the diarrheal replacement fluids.

METHODOLOGY

Selection of Samples

There are two millets namely Foxtail millet and Kodo millet were purchased from local markets in Salem, Tamil Nadu, and India. This millet was cleaned properly and stored in sealed cover. The required amount of other food-grade ingredients like sodium chloride, potassium chloride, and sodium bicarbonate, etc was collected from the local analytical laboratory of Salem city and stored.

Processing of Samples

Foxtail and Kodo millet was used as a sample to determine water absorption characteristics of millet using 4 different soaking periods. All the soaked millets will be dried and ground into powdered and packed. The raw millets were used as a control, which will be is powdered and packed in a sealed cover for further analysis.

Preparation of samples

The raw and soaked two millet flour was cooked at different concentrations in 5, 10, 15 and 20g / 100 ml of water with sodium chloride, potassium chloride, trisodium citrate dehydrate and alpha-amylase enzymes. The temperature was maintained at 90°C for 5-6mins to inactivate the enzyme. After cooling the sample at room temperature, the sample was analysed for viscosity and Osmolality.

Nutritional Composition

The nutrient compositions were determined with both unsoaked and soaked millet flour by using standard methods. Proximate compositions such as moisture, ash, energy, carbohydrate were determined by (AOAC, 2005) method. The minerals such as sodium, sodium bicarbonate, potassium, were assessed by AACC (1986) and calcium, magnesium, iron, zinc, copper, manganese were estimated by standard analytical methods. Phosphorus content was analysed by the auto-analyser method.

Anti-nutritional parameters

The unsoaked and soaked two millet flour was tested by anti-nutritional parameters of tannin and total phenol compounds. Both were estimated by (Sadasivam and Manickam, 2005).

Viscosity and Osmolality

The unsoaked and soaked foxtail and kodo millet flour was analysed by Viscosity and Osmolality. The sample was measured on the same day as prepared with enzyme. The measurements were taken at

Table 1: Mineral Composition of Soaked and unsoaked Foxtail millet flour Fluids

Mineral composition	Raw FM	Soaked periods (mins)			
		30	60	90	120
Sodium (mg/100g)	1.376 ±0.015 b**	1.463 ±0.015 c**	1.750 ±0.010 e**	1.580 ±0.010 d**	1.210 ±0.010 a**
Sodium bicarbonate(mg/100g)	2.223 ±0.015 a**	2.580 ±0.010 c**	2.776 ±0.005 e**	2.656 ±0.020 d**	2.453 ±0.083 b*
Potassium (mg/100g)	214.17 ±0.773 a**	254.73 ±3.94 c**	295.34 ±1.946 e**	265.84 ±2.06 d**	231.47 ±1.29 b**
Calcium (mg/100g)	18.153 ±0.015 a**	19.86 ±0.020 c**	20.16 ±0.015 d**	18.51 ±0.087 b*	18.26 ±0.070 NS
Magnesium (mg/100g)	130.16 ±0.015 a**	144.41 ±1.151 c**	148.85 ±0.51 d**	140.67 ±0.521 b**	139.14 ±0.051 b**
Iron (mg/100g)	2.380 ±0.010 a**	2.836 ±0.037 d**	2.970 ±0.026 e**	2.746 ±0.020 c**	2.450 ±0.010 b**
Zinc (mg/100g)	2.630 ±0.181 a**	2.983 ±0.005 d*	3.050 ±0.036 e*	2.856 ±0.02 c**	2.740 ±0.026 b**
Copper (mg/100g)	1.150 ±0.010 a**	1.350 ±0.010 c**	1.550 ±0.026 e**	1.443 ±0.045 d**	1.230 ±0.010 b**
Phosphorus (mg/100g)	284.84 ±0.55 b**	287.13 ±0.06 c**	289.20 ±0.005 d**	284.13 ±0.015 NS	280.06 ±0.035 a**
Manganese (mg/100g)	0.973 ±0.020 a*	1.16 ±0.010 b**	1.243 ±0.005 c**	1.040 ±0.010 b*	0.970 ±0.026 NS

**significant at 0.01% level, *significant at 0.05% level, NS –No Significant.
Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.01 level, where $^{a<b<c<d<}$in each column

room temperature at cP, and Osmolality was estimated at mOsm/kg.

Statistical analysis

The statistical analyses were performed using IBM SPSS Statistics 23 Software package. The analysis was done in triplicates, and results were analysed by the (one-way ANOVA) with Duncan's Post Hoc test (P<0.05) to evaluate the significant difference between the means of the study. The correlation coefficient was used to find out the interrelationship between the viscosity and nutrient parameters of the samples.

RESULTS AND DISCUSSION

Nutritional composition

Nutritional compositions of soaked and unsoaked foxtail millet results are presented in Figure 1 and Table 1. Among these results, there was a significant difference between the unsoaked and soaked samples. The sixty minutes soaked sample has the highest nutrient content when compared to another period of soaked samples. Macro and micro mineral content also increased in a soaked sample compared to the unsoaked sample. Zinc ranged from 2.63±0.18 to 3.050±0.03 there were (P<0.05) significantly varied from each other samples. Many studies have reported that foxtail millet contains minerals like manganese, phosphorus, and magnesium were significantly in a high amount than others (Gopalan *et al.*, 1987). (Hadimani and Malleshi, 1993) estimated the Ca and P contents of dehusked

Table 2: Mineral Composition of Soaked and Unsoaked Kodo Millet Flour Fluids

Mineral composition	Raw KM	Soaked periods (mins)			
		30	60	90	120
Sodium (mg/100g)	3.17 ±0.026 a**	4.156 ±0.049 c**	4.576 ±0.015 e**	4.47 ±0.015 d**	4.083 ±0.005 b**
Sodium bicarbonate (mg/100g)	1.923 ±0.073 a**	2.456 ±0.020 e**	2.49 ±0.000 d**	2.310 ±0.010 c*	2.220 ±0.020 b*
Potassium (mg/100g)	135.30 ±3.037 a**	144.89 ±0.623 b*	148.54 ±0.573 d*	146.19 ±0.09 c*	140.27 ±0.09 NS
Calcium (mg/100g)	26.11 ±0.055 a**	27.44 ±0.040 c**	27.97 ±0.026 e**	27.55 ±0.010 d**	27.03 ±0.01 b**
Magnesium (mg/100g)	138.35 ±0.358 a**	147.57 ±0.57 d**	149.21 ±0.02 e**	146.60 ±0.01 c**	144.49 ±0.42 b**
Iron (mg/100g)	2.130 ±0.026 a**	2.36 ±0.030 c**	2.463 ±0.015 d**	2.346 ±0.037 c**	2.24 ±0.045 b*
Zinc (mg/100g)	0.963 ±0.037 b**	0.983 ±0.005 NS	1.260 ±0.02 d**	1.05 ±0.010 c*	0.85 ±0.026 a*
Copper (mg/100g)	0.173± 0.020 a**	0.270 ±0.010 b**	0.376 ±0.005 d**	0.323 ±0.005 c**	0.216 ±0.02 a*
Phosphorus (mg/100g)	180.24 ±0.030 a**	188.13 ±0.026 c**	189.12 ±0.03 d**	188.53 ±0.33 c**	184.83 ±0.02 b**
Manganese (mg/100g)	0.040 ±0.010 a**	0.340 ±0.010 c**	0.410 ±0.01 e**	0.373 ±0.005 d**	0.310 ±0.01 b**

**significant at 0.01% level, *significant at 0.05% level, NS –No Significant.
Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.01 level, where $^{a<b<c<d<}$in each column

millets varied from Foxtail millet being 19.1 mg % to 280.9 mg % respectively.

Proximate and mineral compositions of soaked and unsoakedkodo millet sample results were represented in Figure 2 and Table 2. Energy and protein were (P<0.01) significantly higher in 60minutes soaked sample when compared to others. Ash content was lowest in 120 minutes soaked sample, it was ranged from 1.173±0.015to1.443±0.020g/100gm.Minerals such as potassium, magnesium, and calcium and phosphorus were rich in 60 minutes soaked millet samples. Hence all the nutrients were significantly higher between soaked and unsoaked samples. Muragod et al., 2019 stated that kodo millet is a grain of excellent nutritional quality ideal for inclusion in the daily diet for health benefits. Sudharshana et al. reported that kodo millet contains a major protein fraction of glutelin. Kodo millet had a high source of carbohydrate content (66.6g/100g) of grain, comparable to other millets. (Chandel *et al.*, 2014).

Millet contains a high amount of anti-nutrient factors such as polyphenols, tannins, and phytic acids. These anti-nutrients are reducing the vitamin and minerals solubility and bioavailability. Tannin also affects the utilization of protein and carbohydrates in humans (Balasubramanian, 2013). The anti-nutritional factor of soaked and unsoaked foxtail and kodo millet flour results were represented in Table 3. Among these results, there were no significant results between 30minutes and 60 minutes fox-

Table 3: Anti-nutritional parameter of soaked and unsoaked foxtail and kodo millet flour fluids

Variations	Raw	30minutes soaked	60minutes soaked	90minutes soaked	120minutes soaked
Foxtail millet					
Tannin (mg/100g)	2.263 ±0.02 a**	3.87 ±0.015 d**	3.963 ±0.03 e**	3.716 ±0.020 c**	3.55 ±0.010 b**
Phenolic compound (mg/100g)	7.14 ±0.09 a**	7.25 ±0.01 NS	7.73 ±0.03 c**	7.340 ±0.036 b*	7.04 ±0.02 NS
Kodo millet					
Tannin (mg/100g)	0.53 ±0.01 a**	1.06 ±0.026 c**	1.663 ±0.015 e**	1.370 ±0.010 d**	0.970 ±0.026 b**
Phenolic compound (mg/100g)	2.14 ±0.04 a**	3.846 ±0.020 c**	4.156 ±0.03 e**	3.956 ±0.035 d**	3.240 ±0.010 b**

**significant at 0.01% level,*significant at 0.05% level, NS –No Significant
Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.01 level, where $^{a<b<c<d<}$in each column

Table 4: Viscosity parameters of soaked and unsoaked foxtail millet flour based fluids

Variations/ Concentration	Raw Foxtail millet (cP)	30minutes soaked Foxtail millet (cP)	60minutes soaked Foxtail millet (cP)	90minutes soaked Foxtail millet (cP)	120minutes soaked Foxtail millet (cP)
5g	488.86±0.81^a	288.40±1.05^a	295.00±4.07^a	188.46±0.80^d	336.26±2.59^c
10g	556.13±0.66^c	488.73±1.24^c	497.80±0.78^c	177.40±0.95^c	325.20±1.57^b
15g	533.36±1.20^b	376.36±2.89^b	513.30±2.21^b	165.56±0.30^b	373.93±5.54^a
20g	595.73±40.38^d	544.70±1.83^d	555.90±0.96^d	158.83±0.47^a	337.50±23.5^d

Each value in the table are represented as Mean ± SD(n=3). Statistically significant at p < 0.01 level, where $^{a<b<c<d<}$in each column

Table 5: Viscosity parameters of soaked and unsoaked kodo millet flour based fluids

Variations/ Concentration	Raw Kodo millet (cP)	30 minutes soaked Kodo millet (cP)	60 minutes soaked Kodo millet (cP)	90 minutes soaked Kodo millet (cP)	120 minutes soaked Kodo millet (cP)
5g	263.80±6.41^a	364.90±0.87^d	276.73±2.17^c	179.30±0.60^c	224.36±0.45^a
10g	325.13±2.09^b	323.83±1.42^c	266.70±2.28^b	164.63±1.04^b	226.06±1.48^a
15g	367.20±0.70^d	287.40±2.50^b	237.00±1.38^a	166.23±0.81^b	225.30±1.41^a
20g	344.93±3.96^c	266.76±2.09^a	298.13±0.37^d	116.13±1.02^a	247.33±0.55^b

Each value in the table are represented as Mean ± SD(n=3). Statistically significant at p < 0.01 level, where $^{a<b<c<d<}$in each column

Table 6: The Osmolality of soaked and unsoaked foxtail millet flour based fluids

Conc. of the sample(g/100 ml)	Raw FM (mOsmol/kg)	Soaked Foxtail millet(mOsm/kg)			
		30 minutes	60 minutes	90 minutes	120 minutes
5g	264.0±1.0^{c}	275.00±2.00^{d}	223.66±3.05^{a}	257.66±3.05^{c}	234.00±1.00^{b}
10g	278.0±1.0^{c}	285.66±3.05^{d}	233.00±1.73 ^{a}	273.00±2.00^{c}	246.00±2.00^{b}
15g	295.6±3.0^{c}	291.33±1.52^{c}	256.66±3.78^{a}	286.00±2.64 ^{c}	276.33±4.16 ^{b}
20g	313.0±4.3^{d}	309.33±2.88^{c}	275.33±2.51^{a}	314.00±3.00^{c}	295.00±10.58^{b}

Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.05, where $^{a<b<c<d<}$in each column

Table 7: The Osmolality of soaked and unsoaked kodo millet flour based fluids

Conc. of the sample(g/100 ml)	Raw KM (mOsm/kg)	Soaked Foxtail millet(mOsm/kg)			
		30 minutes	60 minutes	90 minutes	120 minutes
5g	238.0±1.0^{a}	283.00±2.00^{d}	249.33±1.52^{b}	264.00±1.00^{c}	238.00±1.00^{a}
10g	245.66±4.1^{b}	290.66±3.51 ^{d}	266.00±1.00^{b}	278.00±1.00^{c}	245.66±4.16 ^{a}
15g	253.0±2.0^{c}	362.33±0.57 ^{d}	276.00±1.00 ^{b}	295.66±3.05 ^{c}	253.00±2.00^{a}
20g	279.3±1.5^{d}	378.00±3.00 ^{d}	314.33±3.05^{c}	309.66±1.52^{b}	279.33±1.52 ^{a}

Each value in the table are represented as Mean ± SD (n=3). Statistically significant at p < 0.05, where $^{a<b<c<d<}$in each column

Table 8: Correlation between nutrient, viscosity and Osmolality of kodo and foxtail millet

Variations	Viscosity	Osmolality
CHO	-0.961**	0.967**
Protein	0.959**	-0.976**
Fat	0.963**	-0.972**
Viscosity	1	-0.908*
Osmolality	-0.908*	1

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

tail millet soaked sample compared with raw samples. Hence kodo millet has (p<0.01)which is significantly higher in all the samples.

The viscosity of raw and soaked foxtail millet flour-based solutions result was shown in Table 4. Among the results, there were (P<0.01%) which were significantly different at concentrations. Raw samples had the highest viscose value when compared to other soaked samples.5g concentration of the solution had the lowest range comparable to other concentrations at all the variations. The peak viscosity is due to the presence of starch and protein molecules in such ingredients. Particularly starch absorbs water while cooking and forms a gelatinous mass whereas nature of protein will change and expose more hydrophilic sites that will take up more water (Mosha and Svanberg, 1983).

Mensa-Wilmot *et al.* (2001) Starch is the main component of cereals, which when soaked in water makes the enzymes active present in cereals. These enzymes hydrolysing starch are divided into amylase enzymes. Alpha amylase hydrolysing α-1.4 – glucan linkages in the starch polymers: amylase and amylopectin (Bathgate and Palmer, 1973).

The viscosity of raw and soaked kodo millet flour-

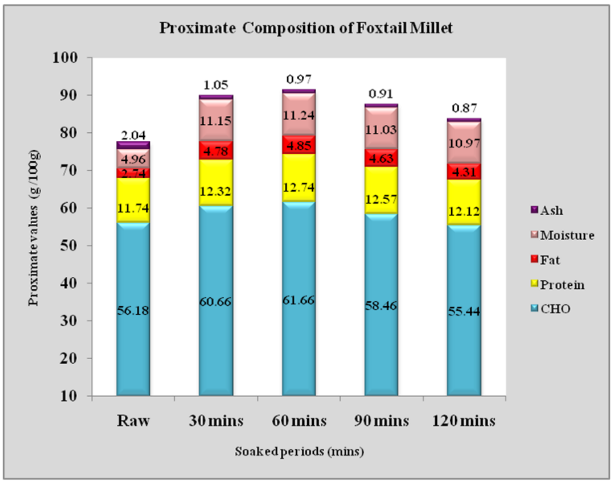

Figure 1: Proximate Composition of Soaked and unsoaked Foxtail millet flour Fluids

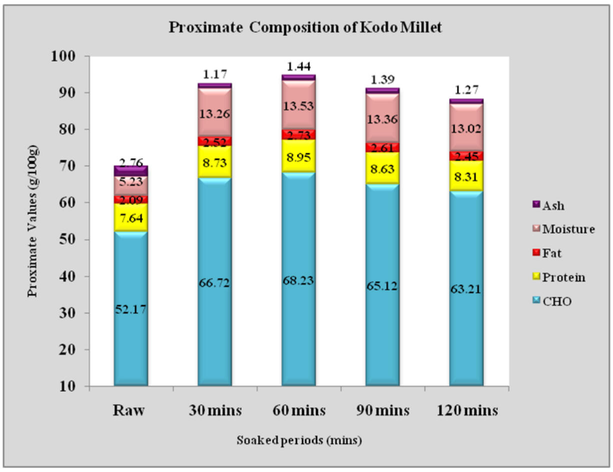

Figure 2: Proximate Composition of Soaked and Unsoaked Kodo Millet Flour Fluids

based solution results were shown in (Table 5). There was a result represent significantly (p<0.01) different between and within groups. Among the results, 60 minutes of soaked millet samples had similar values within the groups, as significantly different observed between the groups. You *et al.* (2014) reported that the amylopectin with more short-chain would result in the lowest viscosity. Some studies reported that when a soaking period and temperature increase will increase the equilibrium point of granules swelling and leaching of amylase (Hasjim *et al.*, 2013). Ultimately, amylase was converted to maltose. Amylopectin shows a more breakdown, and products released glucose, maltose, and dextrin (Bamforth and Quain, 1989). Some studies on barley (Hansen *et al.*, 1989) and wheat (Gopaldas *et al.*, 1986) reported that enzyme degrades starch granules and decrease the water holding capacity, which accordingly leads to a decrease the viscosity of gruel.

Osmolality is the ratio of solutes in a given concentration of the solvent (particles dissolved in a fluid, measured in milliosmoles/kg, mOsm/kg) (Pearson *et al.*, 2013). There was a significant difference between unsoaked and soaked samples at 5 to 20g concentration of the solutions are shown in Table 6.When the concentration was increased will increase the osmolality (Mahalanabis *et al.*, 1993). Past studies reported that, Cereal thickening agents added ready to feed mix formula impact osmolality and cross the limits of AAP safety limits. Commonly vitamin and electrolytes added solutions can remarkably increase the osmolality (Levy *et al.*, 2019).

The Osmolality of soaked and unsoakedkodo millet flour-based solution results was represented in Table 7. Among the results, significant (P<0.01) difference was found in 5g concentration at 30, 60, and 90minutes soaked samples as no significant difference was observed in 120 minutes sample. (Tomarelli, 1976) reported that the osmolality value of milk and infant formulas ranged from 225 to 660mOsm/kg H2O. Rahman et al., stated that Gruels used in a controlled trial with young children during diarrhea varied between 387 and 599mOsm/kg H2O.

The correlation of nutrient and viscosity of foxtail and kodo millet results were representing that a significant positive correlation was observed between the viscosity, protein, and fat at 0.01% level at the same time negative correlation shown between viscosity and CHO at 0.01% level (Table 8). A significant positive correlation found between Osmolality and CHO at 0.01%level. A negative correlation was shown in the viscosity and Osmolality of the sample at 0.05% level. Some studies reported that the rearrangement of starch molecules (amylose and amylopectin) to form a precipitate or a gel is called retro gradation (Bemiller *et al.*, 2008). Gunn E.Vist stated that a high concentration of carbohydrates influences the Osmolality values in the fluids. Enzymatic action is a depolymerisation of large molecular weight components of the endosperm cell walls, the storage proteins and the small starch granules. The components become soluble in water with resulting reducing viscosity (Helland *et al.*, 2002) .

CONCLUSIONS

A person with moderate to severe diarrhoea loses a significant amount of fluids and minerals quickly, therefore fluid management is necessary. Diarrheal replacement fluids should be nutrient-dense as well as have better absorption properties. Cereal based- ORS are better substitutes to WHO-ORS as they are nutritionally balanced and can be prepared at a lesser cost. The results from the study show that soaked foxtail and kodo millet has desirable properties of replacement fluids when compared to raw millets. It can be suggested that soaked millet-based replacement fluids will improve rehydration and reduce stool output due to the reduced Osmolality. Research on millets for formulating potential replacement fluids is indispensable, as it will benefit those in under-developed and developing nations to combat death due to diarrhoea.

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Conflict

The authors declare that they do not have any conflict of interest.

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