



Probiotics, Zinc Re-Inforced Dextrin Compensated Reduced Glucose Hypo-Osmolar Oral Rehydration Salts

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Received on: 08-12-2017; Revised on: 11-12-2017; Accepted on: 12-12-2017

ABSTRACT

Use of standard Oral Rehydration Salts in diarrheal diseases has been limited by its inability to reduce severity or duration of diarrhea. Proposed formula has dextrin replacing 70% glucose, a reduced osmolarity (206 mosmol/L), with zinc and probiotics, the last three being therapeutically effective. Starch in rice grains of Madathawalu species was partially hydrolyzed to dextrin at 130°C for 3.5 hours. Comparative glucose release studies with rice flour and dextrinized rice flour and electrolyte contents were determined where Na⁺, K⁺ and Zn²⁺ was found distributed within ± 4% of the target British Pharmacopeia specifications. The product was stable for three months with no effect to flow properties of the powder and color.

Keywords: Polysaccharide ORS, Dextrin, Probiotics, Zinc, Osmolarity

INTRODUCTION

Diarrheal disease has become one of the leading causes of worldwide morbidity and mortality, especially in children as it causes loss of body fluids with electrolytes which leads to severe dehydration, electrolyte imbalance, shock and even death [1]. Three or more loose stools in the last 24 hours are defined as diarrhea according to the World Health Organization (WHO) definition [2].

Diarrhea can be mainly classified as acute and chronic according to the duration of symptoms and as osmotic, secretory and motility diarrhea according to the pathological disorders [3]. Treatment options for diarrhea mainly drive towards symptom management and correction of underlying causes. The major concern with diarrhea is dehydration, regardless of the cause of the diarrhea. Therefore oral rehydration is an important aspect in the prevention of

dehydration. Oral rehydration can be achieved by the intake of fluids that contain specific quantities of electrolytes and glucose and should be started with the onset of diarrhea [3].

Oral Rehydration Salts (ORS) was first discovered and used in 1969 and was approved, recommended, and distributed by United Nations Children's Fund (UNICEF) and WHO as a treatment option for the clinical dehydration throughout the world. In 1984, another formulation containing trisodium citrate instead of sodium bicarbonate was developed with an improved stability in hot and humid climates. For more than 20 years, WHO and UNICEF have recommended this single formulation of ORS to prevent or to treat dehydration from diarrhea regardless of the cause or age group affected [4].

Sodium and potassium present in the formula are needed to replace the body losses of these essential ions during diarrhea and vomiting. Glucose facilitates the absorption of sodium and water on a 1:1 molar basis in the small intestine. Citrate corrects the acidosis that occurs as a result of diarrhea and dehydration [4].

Over the past few decades the composition of ORS has been subjected to few changes with a better efficiency. In May 2004, WHO and the UNICEF released a joint statement stating that zinc (Zn) and low osmolarity ORS were critical for the reduction of diarrheal mortality [5]. In the same year, WHO introduced an improved ORS formula with low osmolarity and reduced content of glucose and sodium chloride as compared with the formula introduced in 2003 [6,7].

This reduced stool output or stool volume by about 25%, vomiting by almost 30% and need for unscheduled IV therapy by more than 30% when compared to the original ORS solution and resulted in less hospitalization, less risk of hospital acquired infections, less disruption of breastfeeding, decreased use of needles and less cost.

The substitution of glucose monomer in the ORS with glucose polymers is advancement in ORS and has been evaluated as the better method to reduce both the volume and duration of diarrhea. The aim of this is to release glucose slowly into the gut and improve the absorption of water and salt in the solution. Research has been carried out on corn, wheat, sorghum, potatoes, rice and other substrates. All possible solutions worked as well or better than simple sugars, but since rice had less risk of allergy research has been continued on rice.

Several different studies have been carried out using rice in the formulation of ORS since 1980s and several clinical trials had been conducted in between 1981 and 1996. Over this period feasibility and superiority of rice based ORS compared to glucose based ORS has been reported [2].

In 1985 a randomized clinical trial was carried out in 342 patients with watery diarrhea. Patients were treated with either rice or glucose based ORS. Results of the trial showed that glucose or sucrose components in ORS can be replaced by rice powder with improved results [8].

In 1993, a precooked rice based ORS (Pc. R-ORS) was developed and its effectiveness was tested through a clinical trial in International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). The outcomes of the clinical trial have revealed that Pc. R-ORS is more efficacious than glucose based ORS in respect of, shorter duration of diarrhea, less ORS solution used, less stool output and better rehydration [2]. Better recovery is attributed due to active digestion of polysaccharides unlike digestion process lying dormant in the case of glucose.

In 1999, a multinational company has invented Rice Dextrin Oral Rehydration Solution. The results of the foregoing rice dextrin ORS and glucose ORS study indicate that the rice dextrin ORS was more effective than glucose ORS in treating diarrhea. The infants fed with rice dextrin ORS had significantly lower stool output and greater water and potassium balance [9].

In 2009 Bangladesh researchers combined rice powder and salts in to one single mixture. In this method salts are absorbed in to rice by soaking the rice in a solution of salts. Then the soaked rice was dried, fried, powdered and packaged [2]. Furthermore, in a randomized, placebo-controlled trial that assessed the effectiveness and efficacy of zinc given to 792 cases of acute diarrhea in Nepalese children indicated that zinc supplementation has also reduced the duration of diarrheal episodes [10]. In addition, it has been found out that zinc supplementation benefits children with diarrhea for protein synthesis, cell growth and differentiation and intestinal transport of water and electrolytes [11]. Zinc also has potent antibacterial properties which reduces the need of antibiotic therapy in diarrhea [12].

Therefore, in this study hypo-osmolar Rice based ORS (R-

ORS) was further improved by adding zinc and probiotics into the formulation. In addition to the anhydrous glucose it contains rice which is presented in dextrinized form differing from the conventional R-ORS preparations. Colloidal silicon dioxide has also been added for pharmaceutical stability of the formulation. The new preparation contains *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, and *Lactobacillus sporogenes*. Among these *Streptococcus faecalis* (lactic acid bacteria), *Clostridium butyricum* (butyric acid bacteria), *Bacillus mesentericus* (amylolytic bacteria) are the three bacteria that act as prebiotic agents as well as probiotics. These are “Live microbial feed supplements, which beneficially affects the host by improving the host's intestinal microbial balance. *Lactobacillus sporogenes* is a probiotic which modify the balance of the intestinal micro flora, stimulating the growth and activity of beneficial organisms suppressing the potentially deleterious bacteria.

MATERIALS AND METHODS

Identification of suitable rice variety

Among brown rice a variety rich in fiber content, carbohydrate content and nutritional value was chosen according to the literature on Ayurveda system of medicine from Gampaha Wickramarachchi Teaching Hospital, Sri Lanka. Madathawalu, one of traditional rice varieties was selected as the most suitable variety for the R-ORS preparation as it contains a higher amylose content of 28.4%. It also contains small percentages of fat, crude fiber, iron, zinc, phosphorus and antioxidants [13]. The sample of rice was obtained from Bombuwela Rice Research Institute, Sri Lanka.

Dextrinization process

Appropriate temperature for the dextrinization process was identified using a systematic process. Parameters for the dextrinization was first identified using 50 g of rice sample which was purified by soaking in 50 mL of water for 15 minutes and washed five times with 50 mL water, air dried and crushed.

The resulting purified rice grains (30 g) was crushed and spread on an aluminium tray and dextrinized for 15 minutes with increasing temperature using a Bunsen burner. The temperature of the powder bed was monitored every 2½ minutes using an electric thermometer while collecting 1 g

samples for analysis. Each sample was ground using a motor and pestle to perform relevant identification tests according to the monograph on dextrin in the British Pharmacopoeia (BP) [14]. Same identification tests were carried out for untreated rice flour as well. Dextrinization temperature was identified as 130°C.

Fresh purified and crushed rice grains (50 g) were sieved using 850 mesh sieve and dextrinized at 130°C using the hot air oven in order to identify the appropriate time for the dextrinization process. Approximately 3 g samples were taken out at 30 minutes intervals up to 4 hours and identification tests were repeated [14]. Properties such as angle of repose, pouring and tapping densities were determined for the 850 mesh dextrinized rice flour samples according to British Pharmacopoeia as specified under powder flow [15].

Free glucose content in dextrinized rice flour sample was also determined [16]. Test was repeated three times for 10 g samples with 150 mesh sieved dextrinized rice flour. Two reagent blank determinations were conducted in the same manner, substituting sample filtrate with distilled water.

Determination of potential glucose content in dextrinized rice flour sample

Determination of potential total glucose content in rice flour and dextrinized rice flour samples was carried out by complete acid hydrolysis of starch [17]. Test was performed in triplicate on each of 2.5 g of 150 mesh rice flour and dextrinized rice flour samples. Total glucose content in each sample was determined [16].

The amount of dextrinized rice sample needed in the new formula was calculated so as to replace 70% of the total anhydrous glucose of the standard ORS formula (WHO 2004) with dextrinized rice flour sample while keeping 30% of the anhydrous glucose for immediate onset of action. On this basis the amount of dextrinized rice flour needed the new formula was calculated and the osmolality was determined using an osmometer. (Model-3320, Serial No-13014748B, Made in USA).

Determination of total viable aerobic count in dextrinized rice flour sample

Total viable aerobic count in dextrinized rice flour sample was determined by pour plate method given under the tests for microbial contaminations in British Pharmacopoeia [18]. Plates were incubated at 30°C for fungi and at 35°C for bacteria. Total viable aerobic count was taken after 4 days.

Glucose release studies

Glucose release studies were performed for both dextrinized rice flour sample and rice flour. The same procedure used in acid hydrolysis of starch was repeated for both dextrinized rice flour and rice flour samples [17]. Portions of 5 mL were taken at the beginning and at 30 minutes intervals during the refluxing process up to 2.5 hours. Those samples were titrated for glucose according to the method described in Food Chemicals Codex [16]. The percentage of glucose released was plotted against time.

Formulation of oral rehydration salts

New rice based ORS with zinc and probiotics was formulated to include 23.41 g of the powder in a final pack to be reconstituted with 1.0 L water.

Based on the proven safety and effectiveness of zinc supplementation as an adjunct therapy for diarrhea, the WHO has made the following recommendations. Oral zinc for 10-14 days at 20 mg per day in adults and children older than 6 months and 10 mg per day in children younger than 6 months for acute diarrheal illness. Zinc sulfate, Zinc acetate or Zinc gluconate can be used as the zinc salt [11]. Therefore 0.02 g of zinc sulfate for 1.0 L retail pack was included in the formulation.

Six R-ORS packets were prepared using geometric dilution method. Initially zinc sulfate 0.12 g was triturated with 1.20 g of potassium chloride (KCl) in a mortar. Then 3.00 g and 4.80 g of KCl was added successively to the above mixture and triturated each time. Contents of BIFILAC probiotics; 0.50 g sachet (Tablets India limited, Plot No, 3, 1st cross street, Ravichandran Nagar, Thiruvandar Koll, Puducherry, India) was first triturated with 1.50 g of anhydrous glucose. Then 9.0 g and 13.80 g of glucose were added and triturated successively. The above two mixtures were mixed together. Sodium chloride 15.60 g was mixed and triturated with 17.40 g of sodium citrate in a separate mortar and added to the above blend and was triturated. Dextrinized rice flour 73.14 g was

added to the blend and triturated. Finally, 0.41 g of colloidal silicon dioxide was added and mixed. Total final blend was divided in to six packets each weighing 23.41 g. Packets were suitably labelled.

Stability testing

Qualitative and quantitative analysis was carried out after 3 months of packaging. Physical characteristics of the Zn R-ORS powder were observed. Quantitative analysis was carried out for sodium (Na⁺), potassium (K⁺) and zinc (Zn²⁺) using atomic absorption spectrophotometer as specified under Oral Rehydration Salts monograph in the British Pharmacopoeia [19].

RESULTS AND DISCUSSION

Several experiments were performed in the laboratory to formulate and to test the quality of Zn R- ORS preparation. Brown rice bran consists of certain amount of protein, fat, minerals, vitamin B and dietary fiber. Therefore, a long grain brown rice variety was selected as it is generally more nutritious than white rice. Glycemic index is comparatively lower in brown rice than that in white rice.

A study had been performed on the effectiveness of different types of rice in relation to their ability to accelerate the recovery of diarrhea. It was evaluated in a rat model of osmotic diarrhea and has indicated that rice varieties with higher amylose content has more beneficial effects in terms of increased viscosity of stools [20]. Therefore, Madathawalu was selected as the rice variety for the preparation of Zn R-ORS as it is a red rice variety with high amylose content and freely available in the country. In the determination of appropriate temperature for dextrinization, dark blue color appeared from 120°C and upwards for the iodine test and a brown precipitate was observed with copper sulfate test at 148°C and above which however decreased at 166°C. Therefore, samples dextrinized at 120°C-166°C were taken as positively responding to the iodine test and copper sulfate test. The sample taken after 3 hours and 30 minutes was determined as the most appropriate time duration for the dextrinization process at 130°C, given the highest intensity of blue color observed for the iodine test and the thickest brown color precipitate produced for the copper sulfate test by this sample. To optimize the dextrinization process and to establish the reproducibility, a uniform particle size and a constant heat was

required. This was achieved by sieving the rice particles using 850 mesh sieve and using a hot air oven. Starch is a mixture of two polymers of glucose, amylose and amylopectin. With iodine amylose gives blue color, whereas amylopectin gives red/brown color. Combination of both colors gives dark purple color. Therefore, rice flour sample was observed as purple color in iodine test. During the dextrinization process this amylose may have hydrolyzed in to short chain amylose or linear dextrin called as amylopectin. Amylopectin may also have de-branched to form amylopectin which gives blue color with iodine solution. Prepared dextrinized rice sample gave blue color with iodine test, brown color precipitate with copper sulfate, a pH value of 7, and moisture content of 6% and produced no opalescence in chloride test specified in the dextrin monograph. Results complied with the test for the reducing sugars. Angle of repose for the dextrinized rice flour was 38.35° and the compressibility index was lying between 16-20.

Free glucose content of the dextrinized rice flour sample was determined as 0.32% and the potential glucose content was determined as 77.52%. Free glucose ends of dextrin react with copper sulfate solution in alkaline medium to reduce the blue copper (II) ion to form a brick red precipitate of copper (I)

oxide. The -CHO group of the glucose gets oxidized to -COOH group. The hydroxide ions remove the H⁺ ions and promote the forward reaction. Percentage of reducing sugar in dextrinized rice flour sample was determined by iodometric determination of excess copper (II).

The sugar solution should be slightly acidic so that no appreciable amount of the alkali of Fehling's solution will be neutralized later in the copper reduction procedure. On the other hand, it must be slightly acidic to prevent tautomeric changes and decomposition of the sugar present [16]. Sodium thiosulphate solution used in the iodometric titration was standardized to achieve higher accuracy and precision of results.

Osmolality reading for dextrinized rice flour from the osmometer was given as 14 mOsm/kg. Since non ionized molecule's osmolality becomes equal to the osmolarity, the osmolarity of dextrinized rice flour sample was taken as 14 mmol/L. As dextrin is a large, osmotically inactive molecule it shows a low osmotic value. However, when it is hydrolyzed it produces glucose which is highly osmotically active.

Therefore, final product was formulated with a further reduction in osmolarity of existing WHO formula. Osmolarity changes in WHO formula and new rice based ORS with zinc and probiotic is shown in Table 1.

Table 1: Osmolarity comparison of the two WHO formulas and the proposed formula

Ingredients	Amounts per pack (g)			Ions/ Glucose	Osmolarity mosmol/L		Proposed formula
	Before 2003	After 2004	Proposed formula		Before 2003	After 2004	
Anhydrous glucose	20	13.5	4.05	Glucose	111	75	22.5
NaCl	3.5	2.6	2.6	Na ⁺	90	75	75
KCl	1.5	1.5	1.5	K ⁺	20	20	20
				Cl ⁻	80	65	65
Na citrate	2.9	2.9	2.9	Citrate	10	10	10
Dextrinized rice flour	-	-	12.19	Dextrinized rice flour	-	-	14
Zn sulfate	-	-	0.02	Zinc	-	-	0.12
				Sulfate	-	-	0.12
Colloidal SiO ₂	-	-	0.07				
Probiotics	-	-	0.08*				
Total	27.90	20.50	23.41		311	245	206.74

*Streptococcus faecalis 5 million, Clostridium butyricum 0.3 million, Bacillus mesentericus JPC 0.16 million, Lactobacillus sporogenes 8.33 million.

British Pharmacopeia specification for total viable aerobic count of oral formulations should not be more than 10^4 bacteria

and not more than 10^2 fungi per gram or per milliliter. Experimental results obtained from the study are as in Table 2.

Table 2: Number of fungal and bacterial colonies in dextrinized rice and rice flour sample

Sample	Number of Fungi colonies	Number of Bacterial colonies
Dextrinized rice flour sample in Sabouraud dextrose agar- plate I	5	Nil
Dextrinized rice flour sample in Sabouraud dextrose agar- plate II	3	Nil
Rice flour sample in Sabouraud dextrose agar	Field full	Field full
Peptone solution in Sabouraud dextrose agar	2	Nil
Sabouraud dextrose agar Only	Nil	Nil
Dextrinized rice flour sample in Casein soya bean digest agar – Plate I	38	119
Dextrinized rice flour sample in Casein soya bean digest agar-Plate II	11	87
Rice flour sample in Casein soya bean digest agar	Field full	Field full
Peptone solution in Casein soya bean digest agar	Nil	Nil
Casein soya bean digest agar only	Nil	Nil

Therefore, dextrinized rice flour sample was found to be microbiologically acceptable [18].

The number of bacteria and fungi present in dextrinized rice flour sample was much lower than untreated rice flour sample, because the dextrinization process reduces the moisture content of the rice flour sample to 6% thereby reducing the microbial attacks. Further it destroys all the heat labile microorganisms that are present in the sample following heating at 130⁰C for 3 hours and 30 minutes.

Determination of total viable aerobic count was carried out under conditions design to avoid accidental contaminations of the product to be examined. The precautions taken to avoid contaminations were designed not to affect any organisms which are actually present and revealed in the test.

Glucose release study results for rice flour sample and dextrinized rice flour sample are shown in Figure 1.

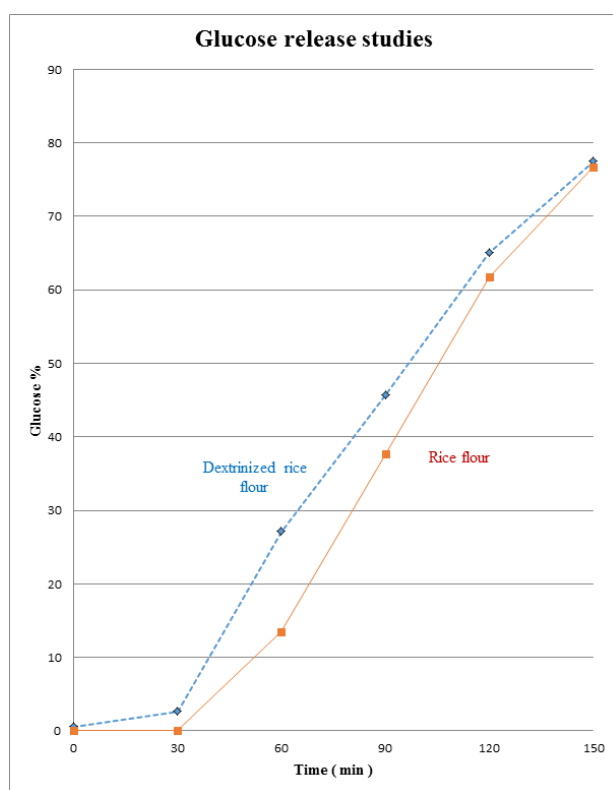


Figure 1: Plot of time vs. glucose % release in dextrinized rice and rice flour

Dextrin and starch have the general formula, $-\text{[C}_x(\text{H}_2\text{O})_y\text{]}_n$ ($y = x-1$), in which glucose units are joined to one another usually head-to-tail, but dextrin has a smaller and less complex molecule than starch. This leads to the faster release of glucose

during the hydrolysis of dextrin when compared to rice flour even though the total glucose amount present in both dextrinized rice flour and rice flour was equal in a given weight. According to Figure 1 at the end of one hour glucose released from dextrinized rice flour is twice as that of rice flour. This would favor faster rate of rehydration sustaining a low osmolarity at the same time.

Starch hydrolysis was done in-vitro conditions using an acid, on the assumption that the same results would be obtained *in vivo* with enzyme hydrolysis. The *in vitro* studies carried out on dextrinized rice flour and rice flour has also indicated that higher levels of glucose are produced from dextrinized rice flour than rice flour during digestion by both amylase and maltase. Results have further demonstrated that glucose produced during maltase digestion of rice dextrin but not rice flour indicated that free glucose would be continued to be available in rice dextrin but not from rice flour during periods of pancreatic amylase insufficiency [9].

As dextrin release glucose slowly, rehydration process of the patient is expected to occur slowly. To retain the immediate rehydration process of the new formulation, anhydrous glucose and dextrinized rice flour were added in a 30: 70 ratio. Rice flour was preferred over rice starch since the former is expected to contain a more diverse composition of nutrients.

Reconstitution of the Zn R-ORS product results in a suspension with cloudiness due to the presence of insoluble particles of the rice grain. Clarity of the solution can be improved by further reduction of the particle size of dextrinized rice flour.

Content of sodium, potassium, and zinc was presented within required BP limits (90.0% - 110.0%) with a $\pm 4\%$ variation indicating homogenous mixing of ingredients [19]. Therefore chloride content of the preparation also can be assumed to be complied as it is supplied only by sodium chloride and potassium chloride.

R-ORS is already available in India and few other countries. Zinc syrups containing zinc sulfate or zinc acetate are available based on its bacteriostatic and physiological co-enzyme activity. This attempt was made to incorporate probiotics consisting of four microbes *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus*, and *Lactobacillus sporogenes*. This is to reinforce antibacterial activity of the commensals that are common in gastrointestinal

tract. The product was found to be stable with regard to light brown color and caramel odor of the finished product. No clumping has formed.

The results of the quantitative analysis of concentrations of sufficiently diluted reconstituted solutions of Na^+ , K^+ and Zn^{2+} are shown in Table 3.

Table 3: Atomic absorption test results of Na^+ , K^+ and Zn^{2+} in the Zn, R-ORS preparation

Ion	Expected Concentration (ppm)	Concentration of the test sample (ppm)	Equivalent percentage of label claim
Na^+	4.9	5.001	101.45
K^+	5.2	5.42	104.23
Zn^{2+}	0.523	0.507	96.88

According to the British Pharmacopeia specifications content of sodium, potassium and zinc should be 90.0 to 110.0% of the requisite amount indicating that this product complies with British Pharmacopeia specifications.

CONCLUSION

According to the BP specifications powders with an angle of repose between 36° - 40° and a compressibility index between 16-20 has a fair flow property. The regular WHO ORS was successfully improved in this study by in cooperating additional ingredients such as zinc and probiotics which are also used as adjunct therapy in the treatment of diarrhea.

With a series of trials, it was possible to convert rice flour in to dextrin of acceptable pharmacopoeia grade except for heavy metals and sulfated ash. These two parameters may not comply in the presence of iron and zinc in high concentrations in rice dextrin. Identification tests were established with marginal color variations between rice flour and dextrinized rice flour sample which is now established after the trials. Free reducing sugars in dextrin were successfully determined and the microbial counts were found to be within limits.

The electrolyte levels were accurate to $\pm 4\%$ against $\pm 10\%$ allowed. However probiotic contents, glucose, dextrin and other tracer electrolytes have to be determined in a future study. The product was found to be stable for three months with free-flowing powder. The beneficial effect of the new product has to be established following well designed clinical trials at a future date.

ACKNOWLEDGEMENT

We extend our thanks to Dr. (Mrs) Kamani Jayakodi, Director, Gampaha Wickramarachchi Ayurvedic Teaching Hospital, Mrs. Y. J. P. K. Mithrasena, the Deputy Director of Bombuwela Rice Research Institute for their generous support and to the Department of Chemistry, University of Colombo for carrying out atomic absorption spectrophotometric analysis. We also thank the academic staff and the technical staff of the B. Pharm Degree Program of the University of Sri Jayewardenepura, Sri Lanka, for their contributions.

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