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Enzymatic Synthesis, Detection and Quantification of Short Chain Fructooligosaccharides and Inulooligosaccharides for Potential Pharmaceutical Applications

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ABSTRACT

The industrial production of short chain fructooligosaccharides (FOS) and inulooligosaccharides (IOS) is expanding rapidly due to the biotechnological and pharmaceutical importance of these compounds. These compounds are concisely termed prebiotics and have biofunctional properties and health benefits if consumed in recommended dosages. In this study, prebiotics were produced enzymatically from sucrose elongation and via enzymatic hydrolysis of inulin by exoinulinases and endoinulinases acting alone or synergistically. Optimization for inulin hydrolysis and sucrose elongation was determined. The production of FOS and IOS of varying degrees of polymerization (DP) was optimized by the central composite design (CCD) and response surface methodology (RSM). The FOS produced were quantified and maximal GF2, GF3 and GF4 were 211.09 mM, 156.06 mM and 43.99mM respectively at pH 5.6 and 60 ^{o}C. Maximal IOS produced were quantified and F3, F4 and F5 were 70.3 mM, 38.8 mM and 12.43 mM respectively at pH 6.0 and 60 ^{o}C. The detection and quantification of oligosaccharides of specific chain length from inulin hydrolysis and sucrose elongation were determined by high performance liquid chromatography with refractive index (HPLC-RI) detection, thin layer chromatography (TLC) and mass spectrometry with electrospray ionization (MS-ESI). FOS and IOS were ionized and detected in the positive ion mode using 1 mM LiCl, $[M + Li]^{+}$. The FOS produced by the action of fructosyltransferases were monosaccharides (*m/z* = 187.98), disaccharides (*m/z*= 349.19), 1-kestose (GF2; *m/z* = 511.25), nystose (GF3; *m/z* = 673.27) and fructofuranosyl nystose (GF4; *m/z* = 835.45). IOS produced were identified as fructose (F; *m/z* = 187.01), inulobiose (F2; *m/z* = 349.22) inulotrioses (F3; *m/z* = 511.23), inulotetraoses (F4; *m/z* = 673.29) and inulopentaoses (F5; *m/z* = 835.22). These saccharide products have potential pharmaceutical applications as prebiotics.

Key Words: Fructooligosaccharide; Inulooligosaccharide; Endoinulinase; Exoinulinase; Fructosyltransferase

INTRODUCTION

The industrial production of short chain FOS and IOS is expanding rapidly due to the biotechnological and pharmaceutical importance of these compounds. These compounds are concisely termed prebiotics and have biofunctional properties and health benefits if consumed in recommended dosages. Prebiotics are produced enzymatically from sucrose elongation and via enzymatic hydrolysis of inulin by exoinulinases and endoinulinases acting alone or synergistically. Exoinulinases act by cleaving the non-reducing β-(2, 1) end of inulin releasing

fructose while endoinulinases act on the internal linkages randomly to release short chain IOS. Fructosyltransferases act by cleaving a sucrose molecule and then transferring the liberated fructose molecule to an acceptor molecule such as sucrose or another oligosaccharide to elongate the short chain FOS. IOS and FOS are reported to have vital pharmaceutical applications and health benefits in humans. The biofunctionalities, applications and health benefits of short chain IOS, especially the pentamers (IOS_5) and hexamers (IOS_6), in human nutrition have been well documented and include immune system activation, resistance to infections, synthesis of B-complex vitamins, enhanced mineral absorption from the gut, lowering of serum cholesterol, phospholipids and triglycerides, preventing carcinogenic tumours and suppress prevalence of diarrhea (Mutanda *et al*., 2014: 894; Nemukula *et al*., 2009: 2040; Mutanda *et al*., 2008: 362).

Analytical techniques for the accurate qualitative and quantitative analysis of oligosaccharides are rare because of the diversity of carbohydrates occurring in nature (Guignard *et al*., 2005: 137). Chromatographic methods, in particular HPLC, have led to rapid and accurate determination of FOS and IOS and play a central role for the analysis and characterisation of carbohydrates (Prapulla *et al*., 2000: 299; Farine *et al*., 2001: 300; Bruggink *et al*., 2005: 104; Sangeetha *et al*., 2005: 442). Pure authentic compounds are needed for the determination of the exact response factors in several quantitation methods and it is technically difficult to separate and quantitate inulin or oligofructose because commercial standards are not available in sufficient quantities (Guignard *et al*., 2005: 138; Sangeetha *et al*., 2005: 450; Ronkart *et al*., 2007: 82). Moreover, the wide range of functional groups (hydroxyl, amino, acetamino, phosphate) add to the chemical diversity and consequently complicate the choice of stationary and mobile phases for high resolution with HPLC (Guignard *et al*., 2005: 137). Photometric detection of carbohydrates is problematic and insensitive because of the lack of natural chromophores or fluorophores and therefore requires derivatisation for analysis by fluorescence and gas chromatography (Guignard *et al*., 2005: 137). Modern methods for the analysis of carbohydrates include gas chromatography (GC), HPLC, and capillary electrophoresis (CE) or by a combination of techniques which include nuclear magnetic resonance (NMR) and gas chromatography - mass spectrometry with electron impact ionisation (GC-MS-EI), *inter alia* (Montilla *et al*., 2006: 453; Fernandez, 2007: 797). The aim of this research was to evaluate the efficacy of different analytical methods such as HPLC, TLC, and MS-ESI for detecting and quantifying IOS and FOS produced enzymatically for potential pharmaceutical applications.

MATERIALS AND METHODS

Production of IOS and FOS

The IOS and FOS were produced from enzymatic reactions using crude fungal inulinases and fructosyltransferase cocktails as previously reported (Nemukula *et al*., 2009: 2041). The IOS and FOS were produced under optimal conditions using the CCD and RSM optimization strategy as previously described (Mutanda *et al*., 2008: 363). FOS and IOS were produced from fructosyltransferase elongation of sucrose and inulinase hydrolysis of inulin respectively from 3 experimental trials. The hydrolysis of inulin was accomplished by exoinulinases and endoinulinases acting alone or synergistically.

Determination of Inulinase and Fructosyltransferase Activities

Endoinulinase activity was measured by the modified procedure of Mutanda *et al*., (2008: 363) by determining the increase in reducing power liberated from random hydrolysis of inulin chains (5 %; w/v) as the model substrate. Reducing power was estimated by the 3, 5-

Dinitrosalicylic acid (DNS) method as previously described (Mutanda *et al*., 2008: 363). Exoinulinase activity was measured by modification of the procedure by Jing *et al.*, (2003: 110) using pure non-hydrolysed commercial chicory inulin as a model substrate. Exoinulinase activity was determined by measuring the amount of fructose liberated from inulin hydrolysis using a Fructose Assay Kit (Product code FA-20), as recommended by the manufacturer (Sigma-Aldrich, St Louis). One inulinase unit was defined as the amount of enzyme catalysing the liberation of 1 μmole of fructose per minute under the experimental conditions. Fructosyltransferase activity was measured according to a modified procedure of Ghazi *et al*., (2005: 20) using sucrose as the substrate. Fructosyltransferase activity was determined based on the amount of glucose liberated after the reaction. Glucose released was measured by the Glucose (HK) Assay Kit (Product Code GAHK-20) as recommended by the manufacturer (Sigma-Aldrich). One unit of fructosyltransferase was defined as the amount of enzyme required to release 1 μmole of glucose per minute under the conditions of the experiment.

Analytical Procedures

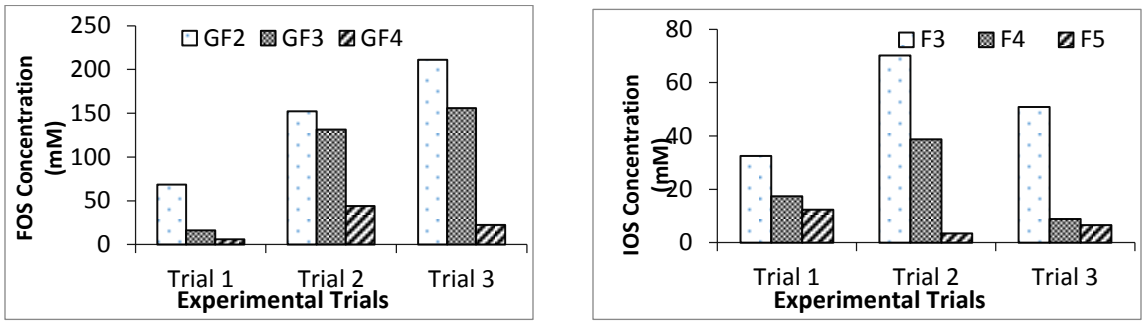
Qualitative determination of IOS and FOS produced in the reaction mixture was performed by TLC. An aliquot (5 μl) of the reaction mixture was spotted on the TLC plate and then developed with a solvent system of butanol / acetic acid / water (5 : 4 : 1) (v / v / v). The plate was allowed to dry and then sprayed with *p*-anisaldehyde spray reagent. The plate was then dried in an oven at 110 ^{o}C for 15 minutes to visualize the saccharide spots. IOS and FOS produced were identified and quantified using HPLC coupled to a refractive index (RI) detector using a Prevail™ Carbohydrate ES HPLC column. The retention times of the individual FOS and IOS were compared with those of standards for identification. MS-ESI with a quadrupole ion trap mass analyser was used to ascertain the degree of polymerisation of IOS and FOS produced in the reaction mixture. The IOS and FOS samples were prepared by ionising the sugars with 1 mM LiCl to form FOS/IOS lithium adducts. The FOS and IOS samples were directly infused into the ion source using the syringe pump and mass spectra were recorded. The response was monitored in the positive ion mode $[M + Li]^{+}$ (100 to 1 000 *m/z)* which gave enhanced response factor. The IOS were identified using the mass to charge ratios of available authentic FOS standards.

RESULTS AND DISCUSSION

Production of IOS and FOS

Conditions for the optimal production of FOS and IOS were established and maximal concentrations were produced under batch conditions using RSM and CCD for 3 trial experimental runs. Figure 1[A] shows the maximal concentrations of FOS for three trials. Trial 1 (4 h incubation, 60 ^{o}C, 20 U/ml enzyme dosage, 400 g/L sucrose, pH 5.6); trial 2 (4 h incubation , 60 ^{o}C, 50 U/ml enzyme dosage, 600g/L sucrose, pH 5.6 and in trial 3, the DP3 was produced (16 h incubation , 100 U/ml enzyme dosage, 400 g/L sucrose), DP4 was produced (16 h incubation, 150 U/ml enzyme dosage, 600 g/L sucrose), DP5 was produced (24 h incubation 150 U/ml enzyme dosage, 600 g/L sucrose), pH and temperature were kept constant at 5.6 and 60 ^{o}C respectively. Figure 1 [B] shows the maximal concentrations of IOS produced using RSM and CCD for 3 trial runs. Trial 1 (8 h incubation, 60 ^{o}C, 60 U/ml enzyme dosage, 100 g/L inulin, pH 6.0); trial 2, (48 h incubation, 60 ^{o}C, 60 U/ml enzyme dosage, and 150 g/L inulin, pH 6.0) and trial 3 (3 h incubation, 60 ^{o}C, 60 U/ml enzyme dosage, 150 g/L inulin, pH 6.0). The FOS produced were quantified and maximal GF2, GF3 and GF4 were 211.09 mM, 156.06 mM and

43.99 mM respectively at pH 5.6 and 60 ^{o}C. Maximal IOS produced were quantified and F3, F4 and F5 were 70.3 mM, 38.8 mM and 12.43 mM at pH 6.0 and 60 ^{o}C.



[A] **[B]**

Figure 1 Maximum concentrations of FOS [A] and IOS [B] produced using RSM and CCD for 3 trial runs.

The detection and quantification of oligosaccharides of specific chain length from inulin hydrolysis and sucrose elongation were determined by HPLC-RI detection, TLC and MS-ESI. FOS and IOS were ionized and detected in the positive ion mode using 1 mM LiCl, $[M + Li]^{+}$. IOS produced were identified as fructose (F; *m/z* = 187.01), inulobiose (F2; *m/z* = 349.22) inulotrioses (F3; *m/z* = 511.23), inulotetraoses (F4; *m/z* = 673.29) and inulopentaoses (F5; *m/z* = 835.22) (Figure 2 [A] and [B]). These were further confirmed by HPLC (Figure 2 [C]and TLC (Figure 2 [D]. The FOS produced by the action of fructosyltransferases were monosaccharides (*m/z* = 187.98), disaccharides (*m/z*= 349.19), 1-kestose (GF2; *m/z* = 511.25), nystose (GF3; *m/z* = 673.27) and fructofuranosyl nystose (GF4; *m/z* = 835.45) (Figure 3 [A] and [B]). The FOS produced were confirmed by HPLC (Figure 3 [C] and TLC (Figure 3 [D]. One of the most important prerequisites for the development of a successful biotechnological process for the production of FOS and IOS is a stable biocatalyst (Catana *et al*., 2007: 261). The development of a full enzymatic process thus requires the operational optimization of the 3 key enzymes used in this study, endoinulinase, exoinulinase and fructosyltransferase. These enzymes are highly stable and have biotechnological and industrial applications for the synthesis of novel sugars and fructose for pharmaceutical applications. As a consequence, the majority of industrial processes are now using fungal fructosyltransferases and inulinases for the conversion of sucrose or inulin substrates to the FOS and IOS intermediates. The chemical approach for the production of oligosaccharides is not attractive because of problems such as colour formation in the final product and also formation of difructose anhydrides with no sweetening properties (Singh *et al*., 2007: 2518).

Oligosaccharides are important due to their prebiotic properties as they are selectively fermented by Bifidobacteria. Due to the health benefits of these compounds, the emphasis of this research was on the optimization of batch enzymatic production of oligosaccharides using simple raw materials such as inulin and sucrose as substrates. The formation of oligosaccharides require a planned optimization strategy to cut costs and time since the one-factor-at-a-time approach has some inherent shortcomings such as being tedious and the requirement of a large number of experimental runs.

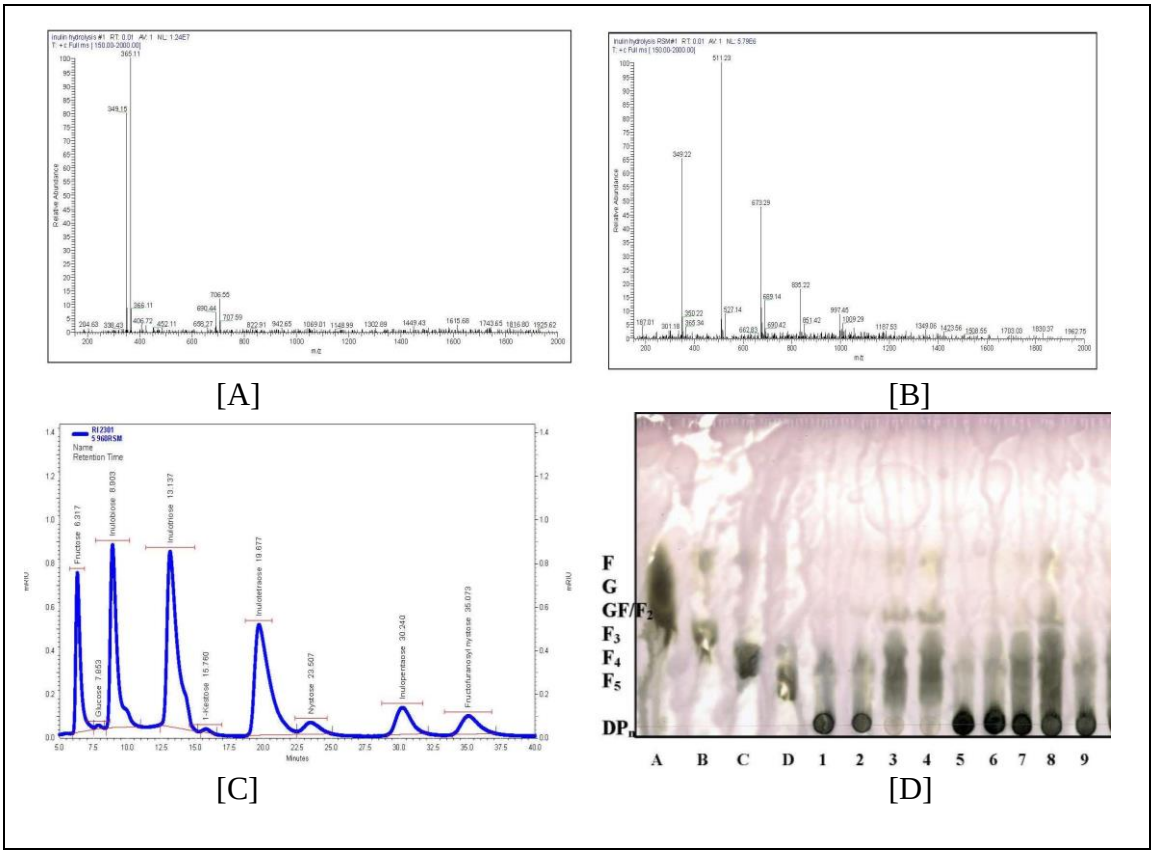


Figure 2 [A] MS-ESI of the reaction mixture at the start of the reaction during inulin hydrolysis. The m/z ratio of 349.15 corresponds to inulobiose $[M + Li]^+$. [B] MS-ESI of the reaction mixture of inulin hydrolysis at the end of the reaction. [C] HPLC trace of the IOS reaction products [D] TLC trace showing IOS intermediates of the 3 experimental runs for varying incubation periods from 1 to 9 h compared to authentic standards.

RSM has proved to be the most beneficial optimization technique in this research in which multivariate factors were simultaneously varied and therefore minimizing the number of experimental runs. The full potential of this experimental approach can be hampered by the fact that RSM is only approximate, and if not properly planned, may not give the optimal response. The use of neural networks in combination with RSM provides a promising alternative to the currently available optimization strategies (Bas and Boyaci, 2007: 846). Structural determination and identification of inulin and its hydrolysis products presents a major limitation because of its polydispersity. The polydispersity of inulin was therefore a particular challenge to be considered in this research and hampers the accurate determination of the stoichiometry of the reaction products. IOS standards such as inulotriose (F3), inulotetraose (F4), inulopentaose (F5) are not commercially available at present so glucose-linked standards are used and these can introduce some inaccuracies because the retention times of GFn and Fn are different (Ronkart *et al*., 2007: 82). Identification of these compounds is generally based on the elution order since the longer chain IOS and/or FOS elutes later. The separation of Fn and GFn can be a problem by using HPLC procedures, therefore in order to avoid errors in quantitative determination of oligosaccharides in food products, proper method development and validation should be done (Ronkart *et al*., 2007: 82).

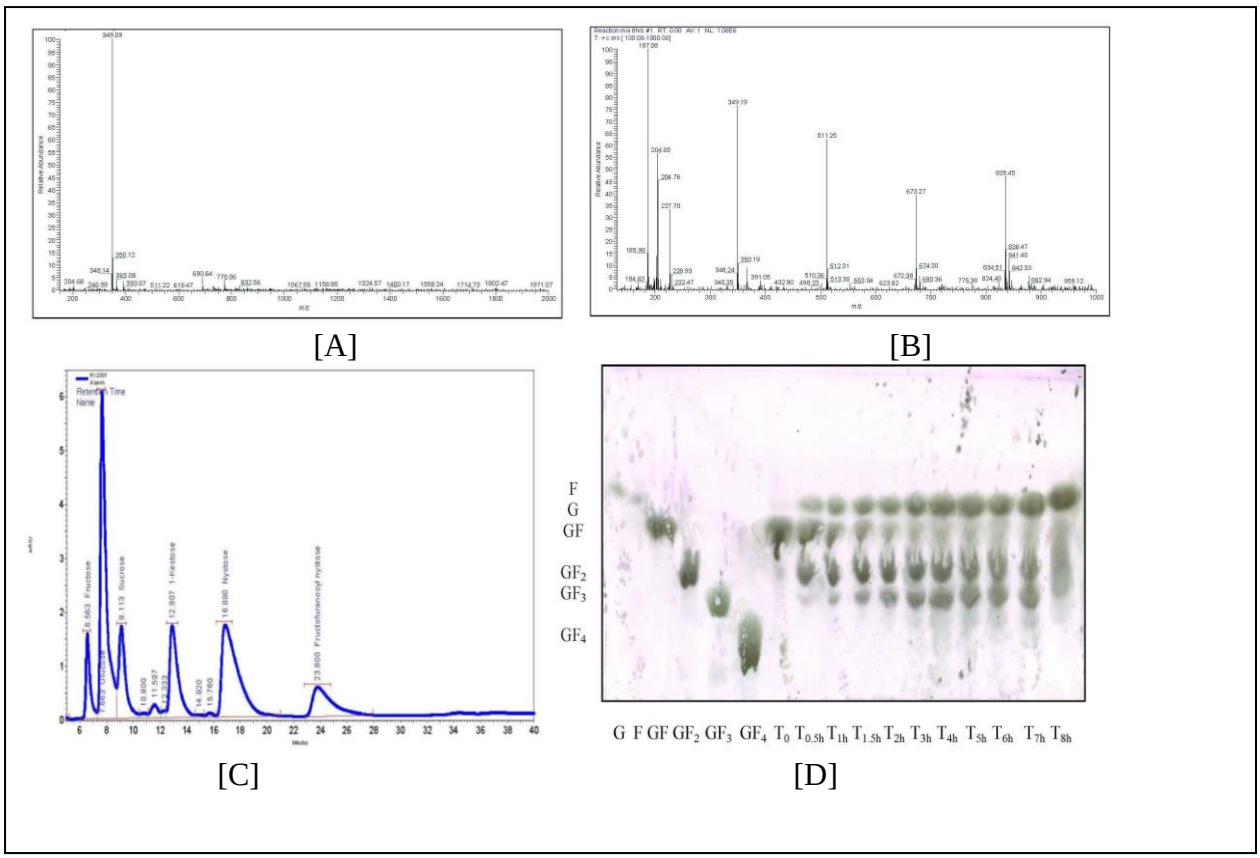


Figure 3 [A] MS-ESI of the reaction mixture at the start of the reaction during FOS synthesis. The *m/z* ratio of 349.09 corresponds to sucrose $[M + Li]^+$. [B] MS-ESI of the reaction mixture of FOS synthesis at the end of the reaction. [C] HPLC trace of the FOS reaction products [D] TLC trace showing FOS intermediates of the 3 experimental runs for varying incubation periods from 0 to 8h compared to authentic standards.

The elution of higher IOS/FOS from HPLC columns is technically difficult since they adsorb and get retained on the column and therefore are not resolved. The use of MS-ESI is reasonably sensitive though sugars are not ionized and therefore need derivatisation by forming metal ion sugar adducts. This analytical technique was applied in this research and gave confirmatory information on the identities of the enzymatic reaction products obtained (Figure 2 and 3). Other researchers have used HAEPAC-PAD for the analysis of oligosaccharides (Ronkart *et al*., 2007: 82). Peak areas on the chromatograms do not supply information about the concentrations of the oligosaccharides using this technique. The reason for this is that the response factors of the pulsed electrochemical detector vary significantly with the various oligomers, with lowest responses occurring with oligosaccharides with a high DP (Ronkart *et al*., 2007: 82).

HPLC is a method of choice for the quantification and identification of sugars from a wide range of sources. This method uses a suitable column for the separation of sugars and the main advantage is that it is convenient and direct (Medeiros and Simoneit, 2007: 272). Polar-bonded-phase and resin-based HPLC columns are commonly used for the analysis of FOS and IOS of different degrees of polymerisation and different glycosidic linkages (Prapulla *et al*.,

2000: 299; Farine *et al*., 2001: 300; Sangeetha *et al*., 2005: 450). The former columns are efficient and carbohydrates elute in order of increasing chain length in contrast to the latter type of columns where the sugar components elute in order of decreasing molecular weight (Prapulla *et al*., 2000: 299; Sangeetha *et al.*, 2005: 450). A number of detection methods are available for HPLC analysis of sugars, and these include, refractive index (RI) detection, PAD, evaporative light-scattering detection (ELSD) and mass spectrometry (MS). The concentration detection limits of RI and PAD are 0.1 parts per million (ppm) and 0.01 parts per billion (ppb) respectively but, the major drawback is that these conventional detectors cannot determine the chemical structure of the carbohydrates; hence the use of MS.

CONCLUSION

In conclusion, the present study has provided an in-depth analysis of the enzymology of the production of IOS and FOS using simple substrates such as inulin and sucrose. An understanding of the intrinsic enzyme mechanisms for the hydrolysis of inulin as well as the transfructosylation of sucrose will open new vistas in complex polysaccharide research in general for potential pharmaceutical applications. It has been demonstrated in this study that IOS and FOS production can be enhanced by optimizing reaction conditions in order to get high yield of intermediates. To date no optimization strategy has been performed for the production of oligosaccharides using RSM and it would be of interest to establish if the optimal conditions found under laboratory-scale RSM applies on a larger scale at industrial level. Using the knowledge gathered in this study it would be possible to optimise the conditions for the maximal production of IOS and FOS and further investigate potential pharmaceutical applications of these saccharides.

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Technological Innovation in Nigeria: Using Open Source Electronic Health Recording Systems to Improve Health Management

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Abstract

This article critiques the quality of clinical care received in Nigeria, while advocating for a new means of intervention through an improved educational and management infrastructure. This new infrastructure will be fostered by the use of Open Source Electronic Health Recording Systems (EHRS). As the viability of this method is explored, examples will be drawn from Open Source Software (OSS) Practice Fusion, which will lead to the construction of an algorithm for an OSS EHRS that is more applicable and can be easily implemented in the developing world.

Key Words: Healthcare, Open Source Electronic Health Record System, Quality Clinical Care

Introduction

Nigeria is one of Africa's most populace countries, filled with a multiplicity of individuals. Housing over 300 ethnic groups and 170 languages, Nigeria has become a powerhouse in the African continent; however, much of this success has yet to show forth in its citizens. After 51 years of independence, Nigeria is still an adolescent country, with most of its population living in underdeveloped regions. Despite signing their first constitution in 1999 (Abdulraheem et al., 2011), and the formation of a centralized government, with thirty six state governments and a federal capital territory, ethnic and religious rivalry still threaten this country's foundation (Smith, 2012).

Despite the ethnic uproar, Nigeria has managed to be counted amongst the important members of the Oil Producing and Exporting Countries (OPEC). Before oil became its largest domestic product, the economy was fueled by agriculture, which included coca, groundnut and palm oil. "Since the production of commercial quantities of crude oil in 1970, its dependence on oil as its sole foreign currency has grown to the extent that diversifying the economy has proven troublesome (Smith, 2012)."

The need for intervention in Nigeria's health system is paramount for the steady growth of this country. The World Health Organization (WHO) has deemed Nigeria's primary health system "uncoordinated" and "unsustainable". Despite having joined the International Push for Primary Health in 1979, Nigeria's primary health standards and efficacy have since deteriorated. Out of the 191 countries ranked in the year 2000, by the WHO, Nigeria ranked 187, which is reflective of its low budget allocation and expenditure for health. This resource poor country finds itself plagued with inadequately trained staff and undereducated personnel.

Strides have been made to change the face of Nigeria's health system. The first was the decentralization of control, and the second was the division of stewardship and financing into 3 tiers(federal, state and local). Even with this organized appearance, great levels of inadequacy still exist. The prominence of cardiovascular disease, hypertension, chronic kidney disease, diabetes, hepatitis A B & E, thyroid fever, malaria, dengue fever, yellow fever and lassa fever, are at an all-time high. Nigeria has one of the highest HIV/AIDS rates amongst its population; still, health is the least touched area for the federal government (Abdulraheem et al., 2011).

According to the World Health Organization, Nigeria is unable to uphold international standards of health, despite housing Africa's largest population.

The public and private sector owe much of this discrepancy to the lack of coordination and resource distribution between them (Dutta et al., 2009). Data tracking between them has also proven ineffective and delivery points for goods and services lack, especially in regions outside of the major cities, where the disease burden is greatest. This lack is not a result of a shortage in graduates, but rather the lack of an efficient and sustainable infrastructure.

Program Needs

A vast majority of Nigeria's health care facilities still rely on paper based medical records. This has not only, stunted effective clinical care, but also nationwide data collection, as well as global collaborations. Nigeria's health infrastructure needs a means of maintaining an integrated history on individual medical and health status, as well as day to day management needs, at a low cost to the country and other partner organizations. To accomplish this, an open source electronic health recording system should be used. This will allow for a large volume of information to be collected, at minimal cost. We have partnered with Millennium Compassionate Care, a primary health facility in Imo state Nigeria, and they have agreed to allow us test the efficacy of this proposal, using their clinic. The duration of this test is 5 years and this system will allow for constant monitoring, both in Nigeria and the United states. It is important to note that constant electricity and internet connection cannot be guaranteed, thus paper based documentation will be used alongside the OSS EHR, to ensure functionality of the facility during times of power outage.

Software of Choice

Practice Fusion was chosen due to its ease of implementation and applicability in vast areas of clinical settings. Founded in 2005, it is presently the largest cloud based electronic health recording system in the United States. It works to connect doctors, patients and laboratories. Though based in the United States, it offers a plethora of features that can be easily applied to practices across the globe. To effectively deploy this new health recording system in Nigeria, first, it will tested in one clinic, and then gradually phase to neighboring facilities and hospitals, fostering a national network.

Research into the benefits of EHR's in the third world is limited, thus it is necessary that this proposal is transitioned into an experiment. For the next 5 years, the results of implementation will be recorded and compiled, then used as concrete data for legislative action.

Methodology

Practice Fusion was chosen as the software of choice after a series of tests for adaptability in differing clinical settings, user friendliness and navigation, congruency with a wide variety of users and role specification, ease of data management and reporting, collaborations, data portability and training/ training support. Each criterion was tested extensively with simulated patient information. The results of these tests served as a platform for an algorithm for a more applicable open source electronic health recording system.

Results

Adaptability in Differing Clinical Settings

The clinic we are collaborating with has a pharmacy, imaging center, lab, training center and billing center. Practice Fusion offers a simple platform that can be used across the board. Accounts were established for each of these settings and the needs of each department were accommodated by the software. This system covers the basic necessitates of any health record system:

- Access to patient demographic information and family history
- Access to physical assessments
- Access to new and past test results and immunizations
- Access to Nursing care plan and treatment history
- Medication recording
- SOAP Notes
- Basic order entries
- Scheduling
- Data storage and reporting
- Portable data

User Friendliness and Navigation

The user is met with bold letters and color coordinated icons, which, not only give this system a welcoming appearance, but also serves to appease reservations that the system is difficult to use. Many opponents have claimed that EHR's are cumbersome, impractical and technically complex (Baillie et al., 2013) but Practice Fusion's simple layout attests that technical novices can use it with relative ease. Since it will be implemented in a rural setting, in conjunction with nurses of limited technological exposure, it is crucial that this system appear to the staff as something they can easily master. Other user friendly features include spell check with data entry and bold labels to locate vital information. The formatting of the system is simple enough that advanced computational skills is not needed. With and Open source system, it does not require the use of database maintenance personnel, unlike the Harvard school of Public Health and AIDS Prevention Initiative in Nigeria, PEPFAR Program. This program used FileMaker Pro in the implementation of their EMR system, but required personnel with advanced knowledge of relational databases in order to modify it from facility to facility.

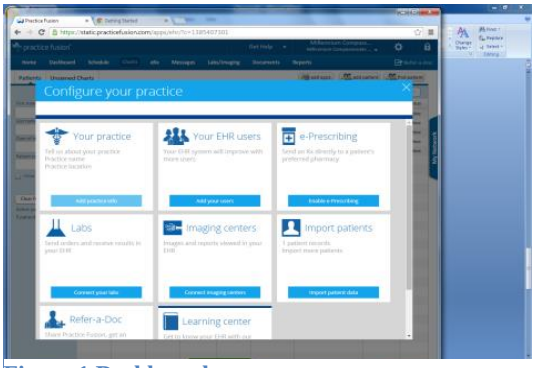

Figure 1 Dashboard

The large navigation bar (figure one), promotes easy navigation from tab to tab. After exiting the dashboard, all tabs are located on one screen. This makes it very easy to locate specific information about a patient, rather than a paper based system that would require shuffling between rooms and rustling through files. It also conveniently lists recently seen patients, an asset for physicians and nurses looking to quickly locate a patient, rather than enter the patient search tab.

Congruency with a wide variety of users and role specification

A wide variety of users are accommodated given the simplicity of this software's formatting, even those who are accustomed to paper based documentation. Once the employee data has been added, it allows for role specification by the system administrator, which limits the accessibility of some features. In doing so, roles are explicitly defined for monitoring and reporting purposes.

Ease of data management

It is imperative that patient entry (figure two) is analyzed extensively when it comes to data management. This tab is not located on the opening screen dashboard; it is featured on the very next page. Before any patient can be imputed, the user must perform a system search to prevent data duplication. This section may prove more difficult to learn than others, since it requires a greater attention to detail. As the search list is being generated, the user must go through each result and determine if it matches the patient originally entered. Misunderstanding of this may lead to data duplication and mistakes. A more effective OSS will perform data matches with all the information given and only list those who match all three categories, rather than listing matches for each category.

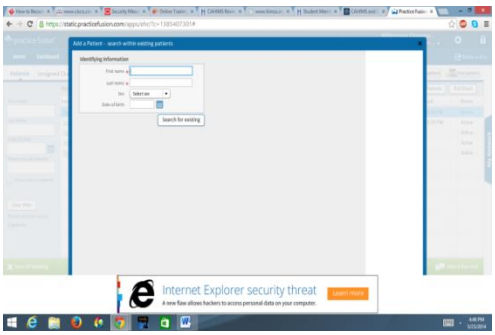

Figure 2 Patient Search Screen

Unlike the patient search screen, the patient data input screen (figure three) can be navigated with relative ease. Sections are labeled "Identifying information", "contact", "address", "demographics" and "next of kin". This layout allows for onsite imputation, as the patient is being admitted, or the data can be inputted at a later time by the data entry clerk. The bolded labels make it easy to locate vital information within a short amount of time.

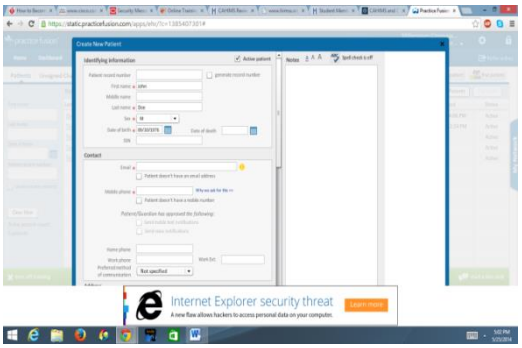

Figure 3 Patient Data Input Screen

The patient profile contains tabs (figure four) which make locating and assessing specific information simple. The tab categories include basic information, Insurance information, profile settings, patient summary, doctor history, prescription history, allergies, etc. On the right hand side of the patient profile is the "patient action" portal, which gives directives as to what action the user wants to take with this particular patient. These include ordering lab tests, printing patient records, creating clinical records, sending referrals and exporting patient records. Physicians will no longer need to flip through numerous pages to perform these actions. To look for another patient, the user can easily navigate to the search patient icon (highlighted in yellow)

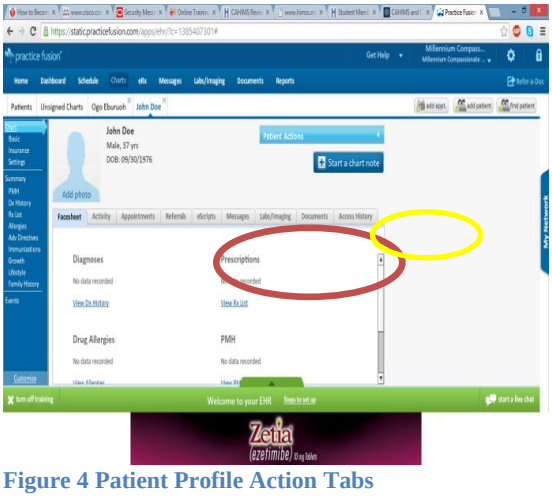

Figure 4 Patient Profile Action Tabs

Collaborations and Data Portability

If used within the United States, Practice Fusion allows neighboring facilities to connect and transmit data, but once out of the Unites States, this

feature is no longer available. To overcome this, the department feature will be used. These departments are not required to be within the same geographical location; therefore, Millennium Compassionate care can collaborate with local pharmacies and include them as departments under their clinic's main account. The new department will automatically integrate their information and allow for a network to be created. Another pivotal benefit of this feature is that community centers no longer need to have all their medical equipment within one facility. With Practice Fusion, partnerships can be established between facilities that have specialized equipment, then patients can be referred to them. Also, a center can be allocated specifically for labs and imaging, then the results can be uploaded and transmitted where necessary. Additionally, patient records can still be maintained within the hard drive of the facility's computer by simply backing up the patient information into an excel spread sheet.

Training/ Training support

If this system will be implemented in the third world, it must offer training support. For this software, technical support is not offered outside of the United States, making it imperative that whoever takes this software to another country, must first assign two individuals to serve as technical support for that region. These individuals must be well trained in computation, the technical aspects of a computer system and the software. One person will remain in the United States for data management and monitoring, while the other will work in the third world region, where they will answer any questions about the system, as well as train all new employees.

Already, Practice Fusion includes training videos, which give an overview of many of the basic capabilities of the software. These videos are short, but offer detailed information on the subject matter. This will be combined with in class training and practical demonstrations, to ensure that staffs have an adequate understanding of the system uses and their responsibilities. There will be a training center that will be open for the duration this system is in use.

Algorithm for more applicable OSS EHR

Problem: Enter Patient Data in OSS EHR for Nigeria

1. Login with appropriate credentials

   These credentials will include Student, Nursing Assistant, Physician Assistant, Physician, Pharmacist, lab coordinator, Lab technician, Data Entry Clerk, Receptionist or Other.
2. Select language

   5 main languages will be included in first prototype – English, Igbo, Yoruba, Hausa, and French.
3. Select "Add New Patient"
4. Enter Patient Search Criteria: First name, Last name and Birth-date
5. If patient information matches search results, pull file, check referring doctor and verify demographics are current
6. If patient information does not match search results, click "enter new information"
7. Enter corresponding patient information

   This must include profession, village of origin, current medications, previous physician and physician location.

Problem: Print Data Reports from OSS EHR for Nigeria

1. Login with appropriate credentials

2. Select language
3. Enter "reports" tab
4. Print corresponding reports
    a. Productivity reports
    b. Disease tracking
    c. Patient satisfaction
    d. Patient flow
    e. Medication reports
    f. Drug interaction reports
    g. Training Center Comprehension Exam Reports
5. Export reports to Excel sheets and send to Data collaboration Centers

Problem: Establish New Partner Organization in OSS EHR for Nigeria

1. Login with appropriate credentials
2. Select language
3. Enter "New Network" tab
4. Enter facility name, address, email and professional information ie. Physician name and specialization
5. Send request to collaborate
6. If request is approved, click "complete collaboration" to sync data

The 5 keys to the usefulness of this algorithm include:
(1) A simple interface that allows for easy learning and adaptability
(2) The ability to establish local and global connections to promote referrals, collaborations and access to current global health information and trends
(3) Incorporation of numerous languages, mainly, English, Igbo, Yoruba, Hausa and French,
(4) A comprehensive reporting system that allows for easy internal and external data tracking
(5) Self-contained training material with limitless access to staff and trainees

Discussion

The algorithm was created taking into account the areas where Practice Fusion failed, but were crucial areas for a health system in a developing country. Amongst these are local and global connections. Granted Practice Fusion allows for the creation of departments, if this system is to be used on a large-scale, facilities need to be independent of each other and have separate accounts for security. Secondly, the language barrier must be reduced. Nigeria houses over 170 languages, therefore, it cannot be assumed that all health workers will be well versed in English. Also, adequate training support must be present if there is to be longevity for this system.

In addition, Open Source Learning Content Management Systems such as NTER (National Training and Education Resource) will be employed for longer, more detailed training videos, lectures, assignments and assessments. NTER is an open source suite of browser based software solutions, made to assist institutions in deploying and managing educational and training courses for audiences of any type. It securely shares information across institutions as well as to the public. Unlike Practice Fusion, NTER can be installed and managed in the United States, with directives sent to trainers oversees. Creation of teaching materials will be done in the

United States by an experience Health informatics professional. NTER will also be advertised to other public healthcare providers as a means of offering training to their staff, and with time, it is hoped, Nigeria will incorporate this low cost teaching system.

Conclusion

Practice Fusion has many applications in Nigeria, and may be one of the few inexpensive resources available to this struggling country. Using OSS EHR's will allow physicians to view patient files in real time as well as receive alerts on patients who need adherence counseling. The information is more legible, more accessible, more accurate and easily retrieved. It also removes the complexity of data management, so doctors can spend more time caring for patients, and less time searching for files. Doctors can easily manipulate patient accounts and make adjustments where necessary, and administrators can easily perform quality control audits and report to the national census bureau with ease. In light of present global health issues, it is imperative that resources such as these are continuously sought and utilized to improve the health of developing nations.

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A Complementary Gynaecologist for Financially Challenged Pregnant Women

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Abstract

Pregnant women undergo a number of physiological and emotional changes which impact negatively on their health. The women exhibit different effects of these as every body system adapts uniquely to ensure a healthy foetal environment. Appropriate management of these changes is important for a successful gestation period. In Africa indigenous medicine in the form of herbal, mineral and animal extracts is usually used to supplement the western medicine. Some cultures are known to go through gestation relying only on this form of medicine with successful healthy deliveries. In Zimbabwe use of traditional medicine has been formalised through the establishment of a governing body, the Zimbabwe National Healers Association (ZINATHA). In this paper we discuss an online knowledge-based expert system designed to offer gynaecological consultancy services to expecting women. A knowledge base of pregnancy related symptoms and their naturally occurring associated remedies as recommended by traditional healers, the elders in the society and from review of literature is designed. This is used to respond to online queries posed by the expecting women providing appropriate advice on either to use traditional remedies or to consult a conventional gynaecologist where necessary.

Key words: Indigenous medicine, Knowledge-based expert system, gestation, maternal health, low-cost gynaecologist, Zimbabwe

Introduction

Gestation is the process of carrying a baby in the womb between conception and birth. The gestation period is divided into 3 phases known as trimesters. During pregnancy, a woman's body undergoes significant physiological changes to support the needs of the developing foetus and prepare the pregnant woman for childbirth and lactation.

In today's world, computers have become a vital component of every aspect of life. The medical field has not been spared as most medical procedures have become computer supported. Expert systems in particular have been widely used in the medical field to diagnose health problems and recommend remedies accordingly. A knowledge-based decision support system is an expert system capable of undertaking intelligent tasks in a specific domain that is normally performed by highly skilled people (Miresco and Pomerol, 1995). Accurate knowledge of a patient's medical problem is critical for clinical decision making, quality measurement, research and clinical decision support. In recent years knowledge-based decision support systems for healthcare have been developed to support clinical decision making. One such example is that of a knowledge-based decision support system for healthcare diagnosis and advisory that performs

deep diagnoses for pregnant Asian women on diabetes and heart related diseases. The knowledge-based decision support system provides an efficient way to take care of the patient's health, to promote the human quality of life and to provide disease monitoring and control to alleviate or to reduce the medical condition at stake (SMC, 2011).

Pregnant women in general undergo a number of significant physiological and emotional changes which at times impact negatively on their health. These changes are a natural phenomenon of pregnancy and a better understanding of these by the women helps them cope with the changes. Each woman is affected differently during pregnancy. Under normal circumstances, virtually every system in the pregnant woman's body adapts as pregnancy progresses to ensure a healthy foetal environment and support dynamic and complex maternal physiologic needs. Normally, the uterus weighs sixty (60) grams and is as large as a chicken egg. By the end of a pregnancy it weighs 1 kilogram and contains a baby, a placenta and more than a quart of water. As the uterus grows it presses against the woman's abdominal organs. The uterus presses against the bladder, stomach and lungs, the arteries, veins and nerves and stretches the abdominal skin. This results in frequent urination, heartburn, congestion in the veins, difficulty in breathing and other conditions that pass after birth as the uterus returns to its pre-pregnancy size (Robinson, 2012)**.** Recent studies, have also shown that up to 20% of women suffer from mood or anxiety disorders during the gestation and postpartum periods. Understanding these changes and effects on the various body systems helps relieve the burden during pregnancy and reduces anxiety and unnecessary tensions (Fink, 2013).

During the first gestation period, many pregnant women use the Internet to search for pregnancy-related information to supplement the scanty information they are given by their health care professionals (Adolfsso and Jansson , 2012). Lack of knowledge of processes in their bodies and ignorance of how to manage these symptoms, results in women making unwarranted visits to the gynaecologist. The consequence is an increase in their gestation period bills unnecessarily. In trying to cut down on these expenses, more and more women have turned to the use of traditional medicines. In Zimbabwe, a study by (Mureyi D. D et al, 2012) shows that at least fifty two (52) percent of the study sample had used at least one traditional medicine during the third trimester of their most recent pregnancy to induce labour, avoid perineal tearing and improve the safety of their delivery process.

In this research, we seek to package this indigenous knowledge so as to supplement the gynaecologist's advice to women by employing a knowledge based decision support system to classify physiological changes in pregnant women. We classify these according to those that require the conventional doctor's immediate attention and those that can be handled from home using naturally occurring remedies and traditional medicine as suggested from the data gathered in this study.

Data Collection

Interviews were carried out with health care providers, traditional midwives, traditional healers, pregnant women and the elderly in the society. Interviews were chosen to gain relevant data because they produce high quality information due to the personal touch during the one on one session. The persons interviewed were selected based on their roles in pregnancy management. From health care providers we were interested in knowing the various complaints presented by the women during pregnancy as well as knowing whether they acknowledged the use of naturally occurring remedies. Health Care Providers also gave us insight into the line of questioning used with pregnant women when they come for consultation. In our interviews we gathered that not all physiological changes symptoms require the doctor's immediate attention though it is important to mention these during the scheduled check-up visits. Some of the most common physiological changes that women can handle themselves include dizziness, fatigue, sleep problems and nausea.

Interviewing the pregnant women and those that had had children before, was done to find out the challenges that pregnant women face when it comes to acquiring knowledge concerning the physiological changes and how they handle them. We were also interested in knowing how the women dealt with their challenges, and whether they knew of any naturally occurring remedies for the symptoms. Further we asked the women if they had used any remedies to manage any pregnant related symptoms.

To concretise the information gathered, researchers also reviewed literature to understand the role of gynaecologists and to identify any documented traditional medicines that were prescribed for pregnancy related issues. Documents reviewed include journals (medical and pharmacy), textbooks, existing gynaecological documents and procedure descriptions. We were careful not to study out-dated materials as this could render our sources invalid.

The collected data shows that naturally occurring remedies play a vital role in pregnancy management. Most women interviewed professed to have used some traditional means to manage their pregnancy. However among the younger women these remedies were not popular, in most instances because they were unknown. This gives the researchers the urge to design and develop a system that will help to preserve this wealth of indigenous knowledge, at the same time minimising expenses on pregnancy related consultations.

System Design and Implementation

In conventional medicine, once a woman discovers that she is pregnant she visits a Health Care Provider to register her pregnancy in preparation for delivery. She is then given dates and times that she is expected to visit the doctor depending on her trimester. During the first trimester, she visits the doctor after every four weeks and after every two weeks during the second trimester. During the third trimester, she visits the doctor once every week. Whenever she has any health concern in between visits, she contacts the doctor or visits the hospital for consultation. Some of the symptoms she experiences can be handled at home without necessarily calling the doctor so as to cut down on the costs associated with each consultation.

We implemented a web based Gestation Monitoring system that accepts information by prompting the user to select a physiological change they want to know about. Thereafter the user is asked a number of questions based on the gynaecologist line of reasoning in order to reach a diagnosis and offer suggestions on how to handle the physiological change. For issues requiring rare naturally occurring remedies, a vernacular name (Ndebele and Shona) is also provided and a

picture to minimise identification errors. Further the system can be prompted to give an explanation for the suggestions so as to guard against errors associated with data entry.

The pregnancy monitoring system is designed and deployed as a web based application. Expertise2go i**s** used as a shell as it is a web-enabled expert system shell. It supports diagnostics assistance and job aids. It has free expert system building tools which support delivery of expert systems as Java applets and Android applications. XAMPP is used as a cross-platform web server solution stack package, consisting mainly of the Apache Hypertext transfer protocol (HTTP) Server, MySQL database, and interpreters for scripts written in Hypertxet pre-processor (PHP) and Perl programming languages. PHP is a server-side, Hypertext Markup Language (HTML)-embedded scripting language that is used to create dynamic web pages. It is available for most operating systems and web servers. It can access most common databases, predominantly MySQL. PHP may be run as a separate program or compiled as a module for use with a web server. Apache is generally recognised as one of the world's most popular HTTP servers. The Apache Web server provides a full range of web server features, including Common Gateway Interface (CGI), Secure Socket Layer (SSL) and virtual domains with support for plug-in modules for extensibility(Mitchell, 2014). MySQL as a database system is ideal for both small and large applications**.** It is very fast, reliable and easy to use**.** It supports standard Structured query Language (SQL) and compiles on a number of platforms, and thus it supports the various technologies used by the women to access the system.

System Architecture

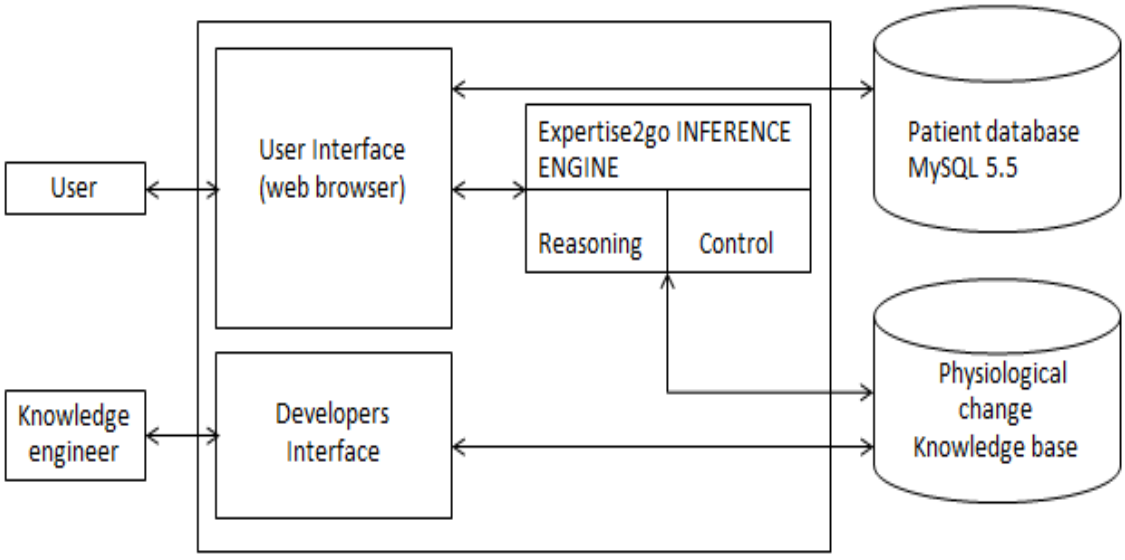

Figure 1: Gestation monitoring system architecture

The architecture in figure 1 separates the conceptual designs into the client, web and business tiers. The Client tier constitutes the user interfaces that interact with the user. The Web tier is responsible for generating and rendering components that are viewed by the user in the

client tier including the inference of information from the knowledge base. The business tier contains the database. The business tier feeds data to the web tier.

Patients and the Health care provider interact with the system through a graphical user interface designed using PHP. Figure 2 shows a screen shot of the welcome screen that the user sees when they go to the website. The interface has pictures of a pregnant woman that gives the women a sense of belonging. The main menu screen has general information of the physiological changes taking place in pregnant women. To consult the system the user clicks on a physiological change that they are experiencing. From that point a number of questions related to the change are then asked as shown in figure 3. The system then uses the supplied responses to deduce and recommend a remedy to the user.

Figure 2: Gestation Monitoring welcome screen

To ensure that the woman's concerns are handled accordingly, they are also prompted to indicate their trimester. This helps in the recommendations to be given as some medications may not be taken in certain trimesters.

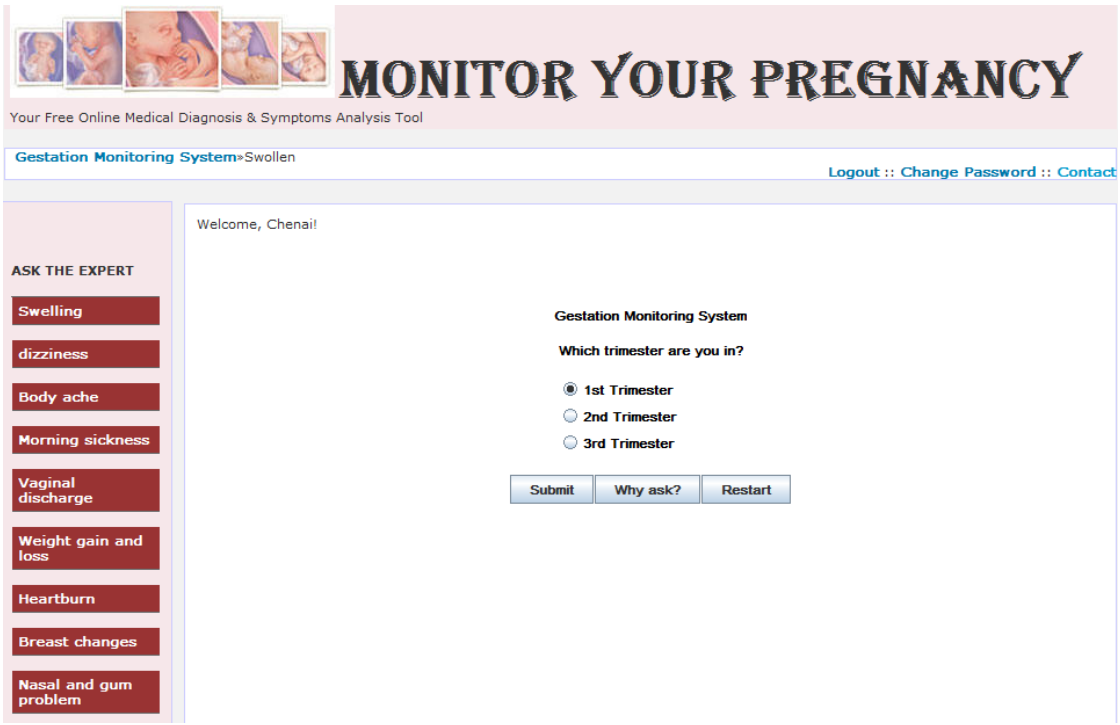

Figure 3: Consultation in progress

The woman is also given an option to restart the consultation if she requires making some changes to the information supplied. The consultation has a side bar menu with a list of physiological changes for easy navigation. The user clicks on the option that addresses their symptoms, and is given the opportunity to send the doctor an SMS in the event that the expert advice is to visit a doctor. The list of physiological changes addressed is shown in figure 4.

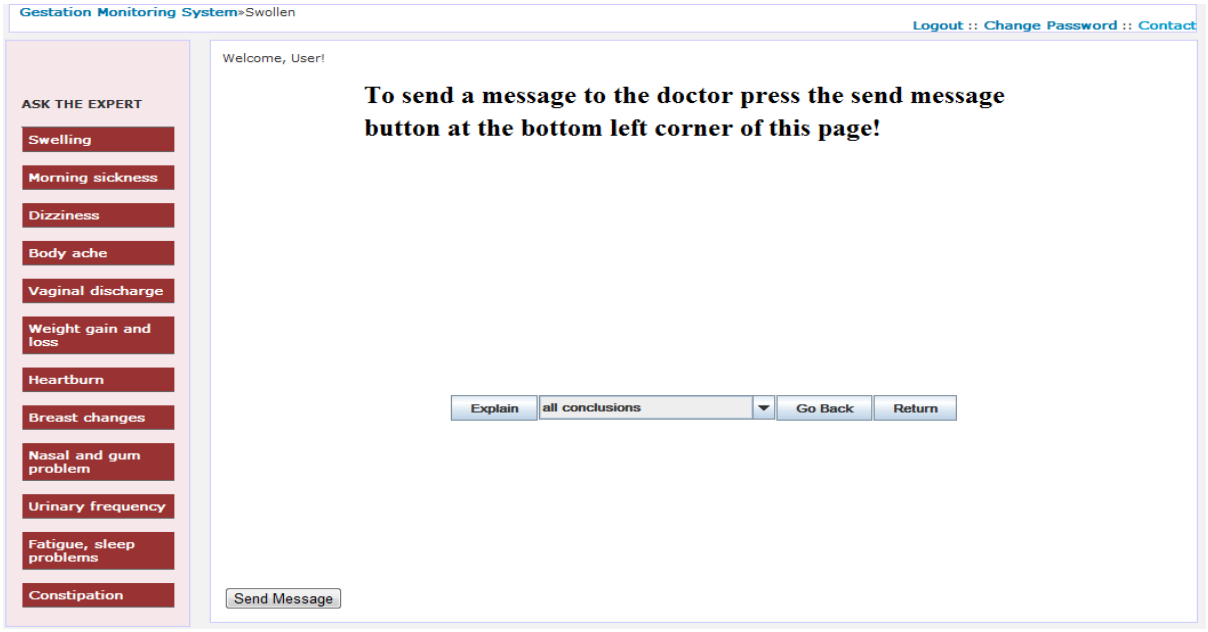

Figure 4 Consultation menu with option to send the doctor an SMS

For a patient to fully utilize the benefits of the system, they need to be registered with a gynaecologist that subscribes to the system, so that in the event that they need a specialist doctor's attention, an e mail is automatically generated to their doctor to alert their doctor and make a booking on their behalf.

The Low Cost Facility

On average, specialist doctor's visits in Zimbabwe cost US$50. If a woman does not have any complications in between visits, then she spends 3 * US$50 for the once a month visits for the first trimester (giving US$150), 2 * US$50 * 3 (giving US$300) for the twice a month visits for the second trimester, and 4*US$50 * 3 (giving US$600) for the weekly visits for the third trimester. This gives a total expense of US$1 050 just for the normal visits. If however the woman has two unexpected symptoms coming between visits in the first trimester, and visited the doctor for these, that would raise her expenses by $100. Any unscheduled visit would cost the woman US$50 more.

On the other hand if the woman manages to use the consultation system successfully, gestation Health Care Provider's bill will be fixed at US$1 050 with minimal extras for some naturally occurring remedies that can be obtained from the market for urban dwellers. This only comes in for cases where the traditional remedy may not be found in the woman's yard or locality. Most of the remedies are freely obtainable for those in a rural set up, thus providing a low cost supplementary gynaecologist.

Conclusion

The pregnancy monitoring system provides a convenient, readily available and accessible gynaecologist that can be accessed any time of the day. This reduces unnecessary visits to the doctor by the women and lowers their medical expenses during pregnancy. It also leaves the specialist doctor to deal with more pressing issues, making him more accessible for such. Further this low cost facility serves as an indigenous knowledge preservation tool, ensuring that indigenous knowledge on pregnancy related medicines is passed from one generation to the other. Our recommendation is that in future this knowledge based system be expanded to incorporate other medicines from other cultures, besides Ndebele and Shona which were used in this study. This will make this low cost facility accessible to the rural community which in developing countries is normally the poorest of the society. Also making this system a mobile application would ensure its accessibility to many pregnant women as most people now have access to a smart phone. The application can be made available through a server so that anyone could download it. This would further reduce the expenses associated with pregnancy related consultations.

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