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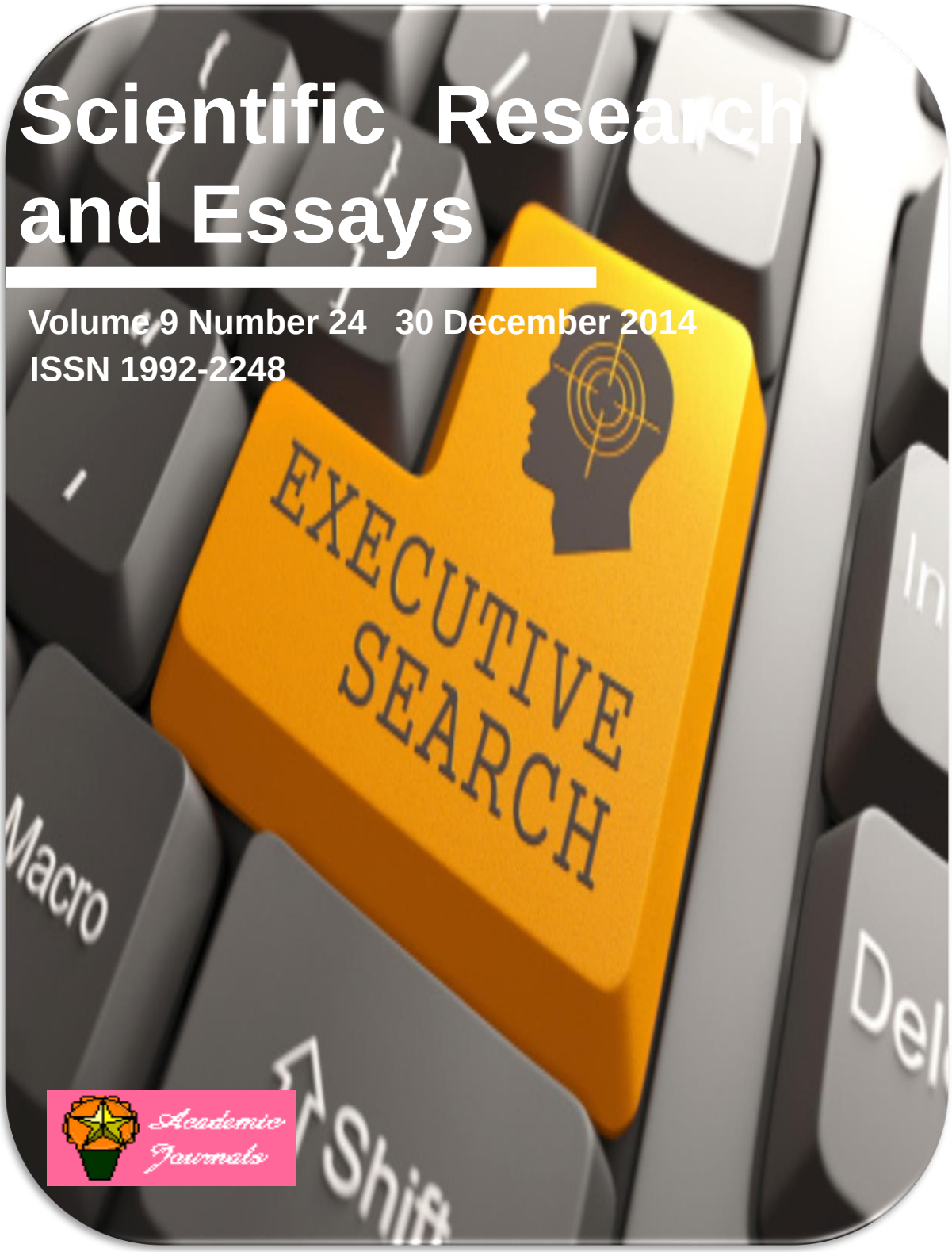

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Scientific Research and Essays

Case Report

Reiter's syndrome presenting with lumbar and leg pain as the chief complaint: A case report and literature review

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Reiter's syndrome is usually known as a clinical triad of arthritis, urethritis, and conjunctivitis. Peripheral nerve involvement in Reiter's syndrome is extremely rare. We report here on a 36-year-old man presenting to the orthopedic department with a chief complaint of lumbar and leg pain who initially was found to have lumbar intervertebral disc and was diagnosed as Reiter's syndrome 3 months later. Human leukocyte antigen (HLA)–B27 test and bacterial culture of urine were positive. Clinical symptoms were relieved after conservative treatment with celecoxib, dexamethasone and antibiotics. This case suggests that Reiter's syndrome can present as lumbar spine lesions and should be considered in the differential diagnosis of lumbar intervertebral disc.

Key words: Lumbar and leg pain, Reiter's syndrome

INTRODUCTION

Reiter's syndrome is well known by many as a seronegative spondyloarthropathy along with reactive arthritis, and since its classic form includes the triad of conjunctivitis, urethritis, and arthritis as was originally described in 1916 by Hans Reiter, cases of multiple-organ involvement in patients with classic Reiter's syndrome have been documented (Carter and Hudson, 2009; Montanaro and Bennett, 1984). It is typically identified in young male patients during their third or fourth decade of life after a urogenital or gastrointestinal infection (Howard et al., 2007; Wu and Schwartz, 2008). A significant number of patients with Reiter's syndrome also manifest their mucocutaneous symptoms, namely keratoderma blennorrhagica on the palms and soles, onychodystrophy, oral ulcers, and circinate balanitis (Edrees, 2012). These clinical features are necessary for establishing a diagnosis. However, we herein describe a case who was repeatedly misdiagnosed as lumbar disc herniation in other hospitals because of his chief complaint, and was finally diagnosed as Reiter's syndrome in our department. This paper reports a case with a view to presenting a rare clinical symptom in the diagnosis and differential diagnosis of Reiter's syndrome.

CASE REPORT

A 36-year-old previously healthy male patient who presented with a chief complaint of two months history of low back pain was admitted to our hospital. He was diagnosed as Lumbar disc herniation in other hospitals and had been treated with "fenbid capsules" to resolve

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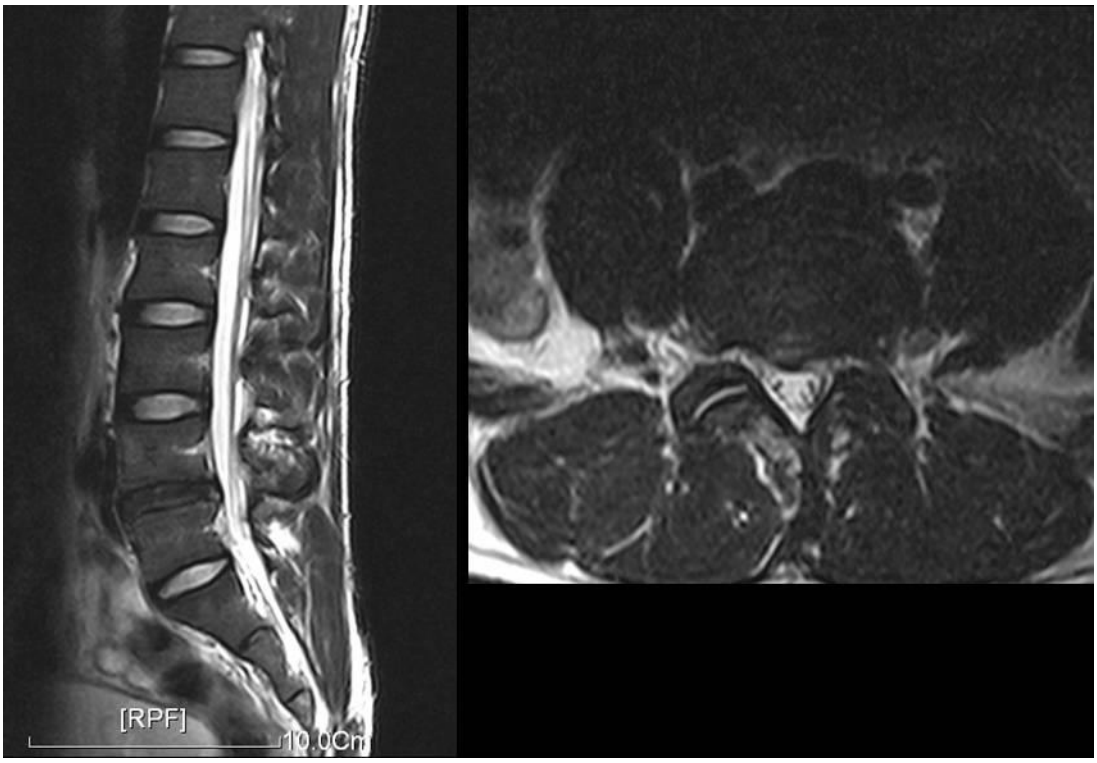

Figure 1. Sagittal (left) and axial (right) T2-weighted MRI of the lumbar spine images show a central extruded disc herniation at the L4-L5 level with inflammatory infiltration of the lumbar facet joint.

his pain symptoms, without success. Three weeks later, he started to develop left sciatica pain. On examination, he was afebrile, and his vital signs were stable. Significant positives on physical examination were positive Lasegue sign of the left leg (40°), numbness on left L5 nerve root dermatome, weak extension of the left ankle and hallux (grade 4 on a manual muscle test), lumbar magnetic resonance imaging revealed an L4–5, a central extruded disc herniation (Figure 1). His visceral, neuropsychiatric, and lymphatic examinations showed no obvious abnormalities.

When the patient arrived at our hospital, he had already been submitted to conservative treatment in accordance with the treatment of lumbar disc herniation, including fenbid (300 mg 2 times per day) and prolonged bed rest, which proved unsuccessful in relieving back pain symptoms. Later on, anti-inflammatory medication was discontinued because of stomach pain. Upon admission, in view of the unserious nature of the imaging performance of lumbar disc herniation and the parents' doubts about the effect of the surgery, we decided to continue with conservative treatment, including providing pain relief, mannitol, traction and partial massage treatment. After three days of conservative treatment, he had a sudden onset of a high fever to 39°C with urethritis symptoms, ranging from mild pain urethral discharge to dysuria with mucopurulent discharge. He was sexually active with one partner and denied any history of sexually transmitted disease. His physical examination showed swelling and tenderness of left ankle joint. Blood routine examination showed white blood cell (WBC) count 16,680 cells/cmm, hematocrit 45%. A routine urine test showed leukocyte (+++). We believed that he had urinary tract infection, and the left lower limb plaster fixation and norfloxacin injection (200 mg 2 times per day) were added for treatment. After two days of treatment, urethritis symptoms were slightly improved, however, lumbar and leg pain symptoms were lack of improvement and the fever symptom persisted. Moreover, there were small pieces erythematous papules scattered over his penis and the skin of his chest area. His eye examination showed bilateral conjunctiva congestion, but did not affect vision.

Laboratory assessment was ahead of the use of antibiotics, which revealed erythrocyte sedimentation rate (ESR) 85 mm/h, C-reactive protein (CRP) 16.2 mg/dl, uric acid 5.7 mg/dl. His antinuclear antibody (ANA), rheumatoid factor (RF), rapid plasma reagent (RPR) for syphilis, serology for human immunodeficiency virus (HIV), hepatitis B, and hepatitis C were all negative. Urethral cultures done were negative for gonococcus and positive for chlamydia just after the onset of his urinary tract. Test for human leukocyte antigen (HLA)–B27 was positive. Synovial fluid test of his left ankle joint showed

inflammatory with a total white cell count of 18,000/cmm. X-rays of the left ankle joint showed only mild joint swelling. Chest CT examinations showed bilateral small pleural effusion.

We made the diagnosis of Reiter's syndrome based on the constellation of clinical features and laboratory test results. The patient was treated with norfloxacin 200 mg twice daily as well as dexamethasone (10 mg twice daily), and then celecoxib capsules were added (200 mg twice daily). His eye lesion was treated with cortisone acetate and galtixacin eyedrops. One week after treatment, his clinical symptoms improved considerably. Lumbar and leg pain, left ankle joint swelling and pain had a significant resolution. Hallux and ankle dorsiflexion function was restored. The conjunctivitis and urinary tract irritation resolved completely. His dexamethasone was tapered (later changed to prednisone 30 mg per day) and he was maintained on oral erythrocin 500 mg four times daily and tripterygium glycosides 20 mg three times daily for 1 month. His treatment was not discontinued and his 6-month follow-up after cessation of therapy showed no recurrence of his disease.

DISCUSSION

Reiter's syndrome is a subgroup of reactive arthritis, which is historically characterized by the triad of arthritis, urethritis, and conjunctivitis; the pattern of joint disease, predominantly affecting the knees, ankles, feet, and wrists in an asymmetric manner. The joints of the lower extremities are involved more frequently than the upper extremities and symptomatic evidence of axial joint involvement has been reported in up to 50% of individuals with Reiter's syndrome (Martel et al., 1979; Killebrew et al., 1973). In the axial involvement of Reiter's syndrome, the sacroiliac joint is most commonly involved, especially early in the disease (Klecker and Weissman, 2003). Although Reiter's syndrome uncommonly affects the spine, there are several cases of Reiter's syndrome involving the cervical spine (Halla et al., 1988; Moilanen et al., 1984). This is the first report of Reiter's syndrome involving the lumbar vertebrae with lumbar and leg pain as the chief complaint.

The mechanisms and etiology of lumbar and leg pain in this case may be due to synovitis and other inflammatory lesions such as enthesopathies of lumbar facet joints occur as a consequence of infection within the genitourinary and possibly at other unidentified sites, and inflammatory aseptic joint disease has been linked with prior infection initiated by many different species of microorganisms. It is a reasonable hypothesis that such lesions result from the release of mediators- immunologic or otherwise - into the circulation at the site of the primary infection, though so far no hard evidence has emerged that microorganisms or antigens derived from them are deposited within the synovium (Keat, 1983). Microorganisms such as *Chlamydia trachomatis*, *Salmonella enteritidis*, *Shigella flexneri* etc have been detected in the joint by immunological techniques and the presence of intra-articular bacterial antigens has now been firmly established with the demonstration of bacteria, bacterial fragments, DNA, RNA, and bacterial lipopolysaccharide in joints of patients with reactive arthritis or sexually acquired reactive arthritis, although there is still some doubt as to the form in which they reach the joint and whether or not they persist (Hughes and Keat, 1994). Some authors in their study concluded that these microorganisms in the inflamed joint might be an important factor in etiopathogenesis of this disease and they are viably active, which supports the hypothesis that arthritis in Reiter's syndrome is probably of the infectious origin (Pavlica et al., 2003; Gerard et al., 1998). However, negative joint culture results from patients with reactive arthritis make it unlikely that bacteria in the joint are viable. In this patient, urethral chlamydia culture was positive. With the use of antibiotics and other drug treatment, lumbar and leg pain and other clinical symptoms has been greatly improved. Thus, it is believed that the occurrence of Reiter's syndrome in this patient was closely related to the chlamydia infection. This may provide strong etiological evidence for Reiter syndrome caused by infection.

Generally speaking, the diagnosis of Reiter's syndrome is mainly made by medical history and clinical findings, which may be difficult because only about one-third proportion of patients had the complete classic triad of arthritis, urethritis, and conjunctivitis (Rothe and Kerdel, 1991) and some of the clinical features may be developed without defining time limits until progression of the disease to a certain extent. Moreover, other specified signs and symptoms such as balanitis, mucous membrane, fever, etc which occur in Reiter's syndrome did not have higher percentages and were analyzed in relation to the presence or absence of HLA-B27 positivity (Rothe and Kerdel, 1991). The criteria for definite Reiter's syndrome which was simplified by the proposal that Reiter's syndrome consists of an episode of peripheral arthritis of more than 1-month duration occurring in association with urethritis, cervicitis, or both, showed 84.3% sensitivity and 98.2% specificity (Willkens et al., 1981). There are no definite diagnostic laboratory test for Reiter's syndrome. In addition to the increased white blood cells and erythrocyte sedimentation rate (ESR) that suggested a bacterial infection, all the laboratory investigations may be negative. Radiographic abnormalities are observed in up to 70% of patients with established Reiter's syndrome and may be characterized by asymmetrical involvement of the lower extremity and entheses, ill-defined bony erosions (Klecker and Weissman, 2003). Although the characteristic radiographic features of Reiter's syndrome are similar to other seronegative arthropathies such as psoriatic arthritis and ankylosing spondylitis, it still plays an important

role in diagnosing the disease.

Given that the patient's chief complaint can easily lead to misdiagnosis, the differential diagnosis of this disease is necessary and lumbar disc herniation should be firstly considered. Magnetic resonance imaging (MRI) is an important method of examination which played an important role in the differential diagnosis of this disease.

The patient was managed conservatively with celecoxib capsules, dexamethasone, tripterygium glycosides and antibiotics. Nonsteroidal anti-inflammatory drugs such as celecoxib capsules are considered as the mainstay of treatment and effective for alleviating the symptoms of lumbar and leg pain caused by inflammatory. Corticosteroids have powerful anti-inflammatory effect; its use can effectively reduce the amount of non-steroidal anti-inflammatory drugs required by the patient for joint symptoms, increasing their anti-inflammatory effect. There is a big controversy about the use of antibiotics in Reiter's syndrome. Some suggest that treatment with antibiotics should be prescribed pending culture results (Arora and Arora, 2005; Callen and Mahl, 1992).

Although antibiotics treatment for other bacterial triggers of Reiter's syndrome has shown little or no benefit, it has been shown that tetracycline or an analog can accelerate recovery from chlamydia induced Reiter's syndrome (Locht et al., 1993; Lauhio et al., 1991). Other drugs that may be used includes etanercept, infliximab, sulfasalazine,methotrexate, and azathioprine (Wu and Schwartz, 2008; Edrees, 2012; Hughes and Keat, 1994; Berenbaum et al., 1996; Youssef et al., 1992; Gill and Majithia, 2008).

Conclusion

Although Reiter's syndrome presenting with lumbar and leg pain as the chief complaint is extremely rare, it should be kept in mind in the differential diagnosis of cases of progressive lumbar and leg pain with urethritis and arthritis.

Conflict of Interest

The authors have not declared any conflict of interest.

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Review

Knowledge management for plant biosecurity in South Asia

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Knowledge management promotes an integrated approach to identify, capture, evaluate, retrieve and share information assets for enhanced knowledge processing. Agriculture forms the backbone of rural livelihoods and national economy in South Asia. The food security which largely depends on plant biosecurity in South Asian countries is challenged by devastations in agricultural crops caused by diseases and pests and the problem is further aggravated by the dearth of resources devoted to their management. The geographical location, climatic suitability and the diverse agricultural scenario predisposes the South Asian region to new/emerging native pests and diseases many of which have a potential to establish and cause serious economic losses. The region, with its high population and increasing trade, thus requires a proper knowledge management in biosecurity encompassing available information and programmes on regulatory, technical and infrastructural parameters at one platform. The knowledge management in plant biosecurity will help in collating and harnessing information for timely and accurate diagnosis of the pest and will be an indispensable tool for developing and implementing an early warning system and a rapid response strategy in case epidemics break out. Also, it will contribute to collate and harness the information on pests and will facilitate capturing the market for export. The South Asia countries need to come on one platform to strategize collectively in this aspect to harness the natural and knowledge resources in the region to ensure an effective plant biosecurity in the region. For this, the countries/organizations need to align their strategies with the knowledge base of the country by keeping pace with technology and using regional bodies as a platform to achieve the objectives.

Key words: Knowledge management, food security, biosecurity.

INTRODUCTION

Knowledge is universally known as the fundamental basis of survival and competition from time immemorial. In organizational context, knowledge management is concept in which an enterprise gathers, organizes, shares, and analyzes its knowledge in terms of resources, documents and skills. It is the process through which organizations generate value from their intellectual and knowledge based assets. Organizational competitive advantages ultimately rest on its knowledge resources as it is difficult to copy. To successfully bring change through

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knowledge management practices, organizations need to align their strategies with the current knowledge base of the people and such initiatives need to be further reinforced by process changes keeping pace with technology.

Reflecting its mandate and competencies, the Food and Agriculture Organization of the United Nations (FAO) plays a leading role in normative work and technical assistance, at the both the national and international levels to support the implementation of a biosecurity approach (ftp://ftp.fao.org/docrep/fao/010/a1140e/a1140e01.pdf) and the member countries owing to the complexity of the parameters that needs to be considered to ensure biosecurity. Biosecurity is a strategic and integrated approach that encompasses a policy and regulatory framework to analyze and manage risks associated with food safety, plant and animal health and the environment. It covers the introduction of plant and animal pests and diseases, and zoonoses; the introduction and management of invasive alien species and genotypes; and the introduction and release of genetically modified organisms (GMOs) and their products (http://www.fao.org). Thus, biosecurity is a holistic approach to the sustainability of agriculture, food safety, and the protection of the environment, including biodiversity. It includes biosafety, a term used to describe policies and procedures adopted to ensure environmentally safe application of modern biotechnology including products derived from the use of recombinant DNA technology.

South Asia is the southern region of the Asian continent, which comprises the sub-Himalayan countries and, for some authorities, also includes the adjoining countries to the west and the east. According to the United Nations geographical region classification, Southern Asia comprises the countries of Afghanistan, Bangladesh, Bhutan, India, Iran, Maldives, Nepal, Pakistan, and Sri Lanka. South Asia is home to well over one fifth of the world's population, making it both the most populous and the most densely populated geographical region in the world. The South Asian Association for Regional Cooperation (SAARC) is an economic cooperation organization in the region.

Agriculture forms the backbone of rural livelihoods and national economy in South Asia. Rapid growth of agriculture to meet the food security has also created multitude of problems in the production systems. Any adversity in the form of a natural or deliberate outbreak of a pest or disease can inflict severe losses to the farming community and can jeopardize the national economy. The geographical location of South Asia and the diverse agricultural scenario predisposes it to alien as well as new/emerging native pests and diseases of crops. The mealy bug problem in cotton, viruses in legumes and horticultural crops, *Parthenium* menace have highlighted the bottlenecks in the security of farming systems.

Besides, the external threats through imports of agricultural commodities, bioterrorism and from rare emerging and new diseases of plants have also to be tackled for ensuring agricultural security (Khetarpal and Gupta, 2007). The liberalization of global trade in agriculture since 1995 after the establishment of World Trade Organization (WTO) has thus brought in many challenges apart from opening up new avenues for growth and diversification of agriculture.

Many of the SAARC nations like, Afghanistan, Bangladesh, Bhutan, India, Pakistan and Nepal have contiguous land boundaries and as plant pests do not recognize geographical boundaries, the free movement of agricultural products in trade and research material has opened new routes for entry of plant pests through import of seeds, planting material, plants and their products. The geographical location, climatic suitability and the diverse agricultural scenario predisposes the South Asian region to new/emerging native pests and diseases many of which have a potential to establish and cause serious economic losses. It has been estimated that globally a total of *32.2* % of losses takes place due to various pests (Oerke, 2006). In contrast, weeds are estimated to cause a 30% loss in potential crop production in India, worth about US$ 90 billion per year in reduced crop yields (Singh, 1996). Approximately 45% of the weeds in USA pastures are alien species, which account for a loss of about US$1 billion in pasture production per year (Pimentel et al., 2001). The documented information on crop losses in South Asia is unfortunately very scanty though billions of dollars are expected to be lost in the region. It is estimated that invasive alien species alone in crops and pastures in the USA, United Kingdom, South Africa, India and Brazil result in economic losses of US$ 94.92 billion per annum (Pimentel et al., 2001).

The present article is a policy paper intended to highlight status of pests and plant protection in South Asia that impinges upon biosecurity and the status of regional cooperation that exists. It then discusses the various areas of knowledge development and sharing and proposes a knowledge management strategy that can be a part of policy guidelines to ensure plant biosecurity in the region.

PESTS AND PLANT PROTECTION IN SOUTH ASIA

The major crops of the region viz., rice, wheat, maize, cassava, tea, coffee, banana, coconut, papaya and mango are attacked by a number of pests threatening food security and also the production of high value agricultural products for domestic consumption and exports.

Brown plant hopper of rice, fruit borer, stem borer, hispine beetle, fruit fly, red palm weevil, locust, corn borer, coconut mite, coconut wilt, banana viruses, wooly aphid, rusts and bunts of cereal crops, e.t.c. are only

some of the examples of economically important pests and diseases of South Asia.

The papaya mealy bug, *Paracoccus marginatus* in papaya and cassava mealy bug *Phenacoccus manihoti* of cassava are other fast spreading emerging pests (Muniappan et al., 2008).

There are certain destructive pests of important agricultural and horticultural crops of the region which fortunately have limited or restricted distribution in the region viz, coconut cadang cadang viroid, banana bunchy top virus, San jose scale, potato cyst nematode, tea stem canker, sunflower downy mildew, coffee berry borer, phloem necrosis virus in tea and pine wood nematode. The coconut mite which has been introduced from Africa to Sri Lanka and southern India and causing serious economic losses. This pest has potential to spread to other coconut countries in Asia where 85% of the world's coconuts are grown (Khetarpal and Dashora, 2011).

It is also important to note that several pests of great economic significance are yet not reported from the region on important commercial and food security crops. This includes South American leaf blight of rubber (*Microcyclus ulei*), South American fruit fly (*Anastrepha fraterculus*), Mexican cotton boll weevil (*Anthonomus grandis*), Ergot of maize (*Claviceps gigantean*), rice yellow mottle virus and maize streak virus (Khetarpal and Gupta, 2007). South American leaf blight of rubber is one of the most economically important pest not yet occurring in Asia, particularly Southeast Asia where natural rubber is playing a prime role in the economy of the country (Khetarpal and Dashora, 2011). Also, the effective quarantine processing (including germplasm) greatly contribute in limiting the transboundary spread of pests (Khetarpal, 2004).

The countries in South Asia have different level of economic and political growth, varying agroecosystem and also different level of infrastructure, expertise and regulatory system to manage the losses caused by local and exotic pests. Countries need the capacity and knowledge management systems to carry out pest risk analysis using International Standards of Phytosanitary Measures of International Plant Protection Convention (IPPC) that are framed under the purview of SPS Agreement of WTO. Hence, there are many levels of challenges in implementation and execution of disease management programmes.

REGIONAL COOPERATION

It is imperative to have regional cooperation and coordination, information sharing about new diseases and pests in the area, and the successful management strategy to reduce the spread and adverse impact of local and trans-boundary pests and diseases. The countries need to be proactive in having an early warning mechanism, a well organized Integrated Pest Management programme, domestic quarantine programmes for pests with restricted distribution, and should develop regional and national pest diagnostic network and promote interdisciplinary research and development for ensuring biosecurity and conserving biodiversity.

The IPPC with secretariat at FAO has a strong presence in each of the FAO regions, and the regional office for Asia-Pacific provides the secretariat for the Asia and Pacific Plant Protection Commission (APPPC) which was established in 1956 and has 24 countries including those from South Asia. The commission works with National Plant Protection Organizations (NPPOs) to review the state of plant protection in the region and is actively participating in development of international and regional standards for phytosanitary measures. It also coordinates a regional response to plant protection issues including transboundary pests. The commission is active in the process of capacity building in the implementation of international and regional phytosanitary standards and in promoting information exchange among its members and other countries in the region. FAO's Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) provides support to governments in all of these areas.

AREAS OF KNOWLEDGE DEVELOPMENT AND SHARING ON PLANT PROTECTION IN SOUTH ASIA

Regulatory and legislative

Till date, we do not have a South Asian regional policy that may highlight the importance of plant protection and quarantine for integration/harmonization of existing practices and regulatory mechanisms among countries. The system, as it exists, generally lack a mechanism and scope for networking that can promote research consortia among the existing institutions in the public, private, academic and civil society sectors engaged in monitoring, biosafety testing, quarantine and other programmes. The important areas yet not addressed include harmonization of plant quarantine regulations in the region in line with the norms of European Union (EU) countries framed by European and Mediterranean Plant Protection Organisation (EPPO) to categorize pests as A1 (not known to occur in EU) and A2 (known to occur in specific countries of EU) to facilitate trade of the region with other countries outside the region and trade among the countries of the region without compromising with quarantine requirements. List of prioritized pests of national concern are generally not available and also legislative requirements for transboundary movement of transgenic material, cell cultures, pathogens, beneficial insects and biocontrol agents has yet to be in place.

Existing lists of quarantine pests also needs to be reviewed as risks from pests change over a period of time and with the emergence of new pests (Singh et al., 2013).

There is no mechanism to jointly deliberate and review the policy issues related to Sanitary and Phytosanitary (SPS) agreement of WTO, Cartagena Protocol and invasive alien species of Convention on Biological Diversity (CBD) in international fora to better protect the common interests of the region. It would be worthwhile developing common quarantine regulations according to the norms prescribed by IPPC wherein lists of common pests present in all the countries could be prepared enabling the waiver of additional declarations in the issue of import permit. Besides, development of emergency and rapid response teams to deal with epidemic/pandemic situations arising in any member country of the region can be put into place.

Technical and researchable

The South Asian countries have yet to come forward to develop pest risk analysis with joint efforts for few common crops/pests of quarantine significance to the region. For this purpose, each country would be required to take up survey for the occurrence and distribution of pests of the common crops of the region and share the information. Strengthening of domestic quarantine programmes for pests with limited distribution for "exclusion" and "containment" of new and emerging strains of pests within the countries have to check their intra-national movement as well as transboundary movement in case of countries with contiguous land borders. Countries with contiguous borders have yet not developed programmes for eradication/declaration of pest free areas (PFA) for pests of major concern in exports from the region for continuous detection, eradication, monitoring and review.

Also, majority of the countries do not have a robust survey and surveillance programme for monitoring major pests including invasive weeds by using modern tools like geographic information system (GIS) and remote sensing for e-surveillance and simulation models for early warning within the region to check for movement of pests (such as Ug99 race of wheat rust). Besides, surveillance for natural enemies and mitigation options to deal with epidemics/pandemics of pests within the region also needs to be taken up. Development of user-friendly diagnostic kits and molecular/digital identification for common threatening pests and invasive weeds with limited distribution within the region for their effective containment has not been addressed on priority. The countries also need to develop collaborative projects/programmes in the area of taxonomy, etiology of unknown/emerging pests to deal with SPS issues in trade etc., particularly in view of scarcity of scientific and financial resources in the region.

Capacity building, extension and awareness

Capacity building needs to commensurate with the need assessment of the member countries for establishing infrastructure at the very basic level. Awareness generation is being made on the necessity to reach the farmers with actionable information through human intervention and also through use of mobiles and the need for critical mass to be trained for that purpose is now being felt. CABI, a more than 100 years old, not for profit science based organization (www.cabi.org), has launched Plant wise, a global initiative aimed at improving food security and the lives of the poor by reducing crop losses in South Asia countries (www.cabi.org). The program is broadly composed of a network of plant clinics to be established globally, and a knowledge bank comprising of worldwide data on crops and crop pests. CABI has been at the leading edge of information science and technology from its first abstract journal published in 1913. Similarly, it has helped to pioneer new approaches to help farmers and their environments, such as integrated pest management in the 1980s and recently plant health clinics and knowledge management through 'Plantwise' which provide support through national and community structures to farmers globally, who otherwise would lack reliable plant health services. Currently, there are clinics operating in India, Bangladesh Sri lanka and Nepal. The clinics, operated by trained local personnel, advise farmers on pests in a manner similar to the way a health center does for humans. Another initiative is the mobile agro-advisory services wherein there are presently around 5 million farmers in 8 States of India as their subscribers. Farmers can use the mobile phones and mobile phone based applications, for real-time expert advisory, receiving pro-active warnings or uses it as a tool for establishing effective market linkages. This is very critical for farmers who live in places which are dispersed, difficult to reach or isolated.

Besides, countries have yet to develop a strong programme of awareness generation on biosecurity for various stakeholders such as academia, policy makers, custom officials, students and general public. In nutshell, there is a need for enhancing the national and regional biosecurity engagements.

IMPORTANCE OF KNOWLEDGE MANAGEMENT FOR PLANT BIOSECURITY IN SOUTH ASIA

The problem of plant disease, particularly in developing countries, is aggravated by the dearth of resources devoted to their management. This may be as a result of poor governance but it also arises from the difficulty of quantifying plant disease and relating this to the failure to reach achievable yields. The pace of research and development had been reasonable in plant protection, but this stream of science in relation to its importance, continues to be grossly overlooked by the policy makers probably because of the lack of appropriate data on economic losses. Besides, technically the major constraint

faced by plant protection specialists is the correct identification of the causal agents to the species level. The need for correct identification of plant pests and pathogens can hardly be exaggerated since it is fundamental to control. Fortunately, a number of tools are available which enable to narrow down the findings and assist in coming to appropriate conclusions. At the field level, there are a certain compendia developed by CABI like CABI's Crop Protection Compendium and CABI's invasive species compendium beside the globally known CAB abstract, which equips the researcher to have rich and validated information.

Presently, a sectorial approach is being adopted to address the issues of biosecurity (Swaminathan, 2008) and this is true more so in South Asian countries. There is a lack of integrated approach to deal with its various components. The countries do not have their information on various aspects of plant protection that impinges upon plant biosecurity at one platform. To begin with there is a need for a strong legislative mechanism, reorganization of infrastructure facilities and synergy of expertise from various organizations under the different concerned ministries (Agriculture, Environment, Commerce, Tourism etc), databases of pests and diseases, research findings and the extension programmes all at one place.

South Asia countries have varying strength in terms of regulation, institutions, infrastructure, expertise and funding for plant health management in its national programmes. There are number of Acts related to biosecurity and biodiversity impinging upon plant health management. The Ministry of Agriculture in South Asia countries do have national programmes on quarantine, integrated pest management, registration of new molecules, locust and rodent control and capacity building. Besides, in some countries there are large numbers of research institutions and universities for a technical back-up to the ministries programme on extension and development. As an example, India has one of the largest agricultural scientific manpower in the world spanned across 99 Institutes of Indian Council of Agriculture Research and 70 State Agriculture Universities many of which deal with plant health management. There are also large numbers of R&D on plant health management by various private corporate of national and multinational origin. Also it has been observed that South Asia, is recently becoming increasing aware of integration of its policies, research, general plant protection measures and quarantine to develop an effective plant health management system. India has already ventured into a National Agricultural Biosecurity System, the recent outcome of which is the Integrated Agricultural Biosecurity Bill which is now tabled in the Parliament.

It is thus amply evident that in order to manage the growing technical, regulatory and infrastructure needs for an efficient biosecurity system a national hub on knowledge bank needs to be envisaged.

KNOWLEDGE MANAGEMENT STRATEGY

A constant threat from pests and diseases hangs over South Asian countries thus challenging the biosecurity system that are still not in place in the real sense as mentioned above. It is thus important to move in the following direction:

1) The countries need to strategize to develop an integrated communications and knowledge management approach for plant health to cover policies, regulations, diagnostics, certification, surveillance, early warning systems, forecasting, teaching, training, etc. Ensuring that the relevant audiences know where to find and how to access and use these significant knowledge resources is a challenge, but essential to build evidence based policy and interventions. A national knowledge resource would help deliver appropriate and customized information for policy makers, researchers and those implementing the public and private sector programmes. Any knowledge resource should aim to collect and collate the rich but scattered and often inaccessible information on a single platform; information should be customized to stakeholders in trade, field management, academia, etc., making it accessible to key national and international actors engaged in enhancing food production.

2) It would be of immense use in exploring the development of a 'knowledge repository' in biosecurity in a partnership mode. A knowledge repository should cover the broadest possible range of plant health issues from pre-crop to on-farm and postharvest issues and cover transboundary diseases. A pilot study would at first have a restricted scope; a focus on a limited range of crops, which also have export potential and where the impact of a reduction in crop losses can also be measured in terms of increased export earnings. The 'knowledge repository' should document the latest research information published and of relevance to South Asian countries and present analyses of research knowledge thereby helping to direct future research agendas. Survey information will help in the surveillance of pests and diseases in the region and assist in risk analysis and pest and disease management; potentially in association with new plant clinic networks. The site and associated activities will support capacity building in plant health management, and support those capacity building activities being carried out by relevant organizations.

3) It is imperative in the interest of the food security and economy of the region that the countries come together To brainstorm on the very need for developing a knowledge management platform for biosecurity where the more countries who are more advanced in the field such as India may take a lead through platform of SAARC or other regional bodies.

CONCLUSION

The knowledge management hub in plan biosecurity will

not only permit to collate and harness the information on pests but will also lead to capture the market for export as well be able to customize information as per requirement. This will also lead to a timely and accurate diagnosis of pests and will thus it will be an indispensable tool for early warning system and for developing a rapid response strategy in case epidemics break out. The South Asia countries need to come on one platform to strategize collectively in this aspect to harness the natural and knowledge resources in the region to ensure an effective plant biosecurity in the region. For this, the countries/organizations need to align their strategies with the knowledge base of the country by keeping pace with technology and using SAARC, Asia Pacific Plant Protection Commission and other regional bodies as a platform to achieve the objectives. What we need is just a political and a professional will to begin with.

Conflict of Interest

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

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Scientific Research and Essays

Full Length Research Paper

Influence of nanosilica and a polycarboxylate superplasticizer on the rheological and electrokinetical properties of cement pastes

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The effect of individual and combined addition of both nanosilica (NS) and polycarboxylate ether plasticizer (PCE) admixtures on cements pastes was studied. The sole incorporation of NS increased the water demand, as proved by the mini-spread flow test. An interaction between NS and hydrated cement particles was observed in fresh mixtures by means of particle size distribution studies, zeta potential measurements and optical microscopy, giving rise to agglomerates. On the other hand, the addition of PCE to a cement paste increased the flowability and accelerated the setting process. PCE was shown to act in cement media as a deflocculating agent, reducing the particle size of the agglomerates through a steric hindrance mechanism. Mechanical strengths were improved in the presence of either NS or PCE, the optimum being attained in the combined presence of both admixtures that involved relevant microstructural modifications, as proved by pore size distributions and SEM observations. The results indicate also the effectiveness of NS and polycarboxylate superplasticizer in producing high packing density and in accelerating the pozzolanic activity to produce more C-S-H gel by consuming calcium hydroxide $Ca(OH)_2$ in order to improve the mechanical properties of cement pastes.

Key words: Cement pastes, polycarboxylate plasticizer, nanosilica, optical microscopy, rheological and electrokinetical properties.

INTRODUCTION

During the mixing, the cement particles are dispersed and suspended in water. However in the presence of a superplasticizer, the cementitious paste becomes more stable and the shortage of water which affects the maniability of cementitious pastes in time will be resolved in the presence of a superplasticizer. In addition, many research studies have shown that several factors affecting the rheological stability of the cement pastes namely; the concentration of solids, the cement characteristics and the nature and dosage of the superplasticizer used. The essential purpose of the use of a superplasticizer is to disperse the cement grains.

Due to the dispersing effect of a superplasticizer, the fluidity of the paste is increased, and shear stress and

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Table 1. Chemical and mineralogical composition of different cements.

Composition	Cement		
	C_1	C_2	C_3
SiO_2	21.65	18.81	22.68
Al_2O_2	03.38	04.91	06.05
Fe_2O_3	04.94	02.95	03.81
CaO	64.39	64.16	60.49
MgO	01.29	01.06	01.26
K_2O	00.54	00.43	00.63
Na_2O	00.14	00.31	00.49
SO_3	01.66	01.34	02.79
CaO_{Free}	00.85	00.69	01.50
Cl^-	0.0014	0.0024	0.0075
C_3S	57	57	56
C_2S	19	18	19
C_3A	02	12	10
C_4AF	15	07	09
Specific Surface (SSB) (cm^2/g)	3300	3726	3624
Setting times; (min)	189-260	149-210	189-260
% limestone addition in clinker	-	11	-
% pozzolan addition in clinker	-	-	08

plastic viscosity are reduced (Tattersall and Banfill, 1983; Justnes and Vikan, 2005; Vom Berg, 2009; Vikan, 2005; Neubauer et al., 1998; Collepardi, 2005; Uchikawa et al., 1997). The superplasticizer is adsorbed on the cement particles and changes the degree of flocculation in one of three ways: the increase in zeta potential and the repulsive forces between the cement particles (electrostatic repulsion of the double layer); improvement of the solid-liquid affinity and presence of a steric hindrance.

Several researchers have studied the effect of the nature and superplasticizer dosage on the rheological behavior of cementitious pastes. The superplasticizer action consists of two physical and chemical phenomena (Vom Berg, 2009; Vikan, 2005; Neubauer et al., 1998). The physical phenomenon includes non-specific effects of adsorption, electrostatic repulsion and steric repulsion (Neubauer et al., 1998; Uchikawa et al., 1997). Chemical phenomenon was assigned to the reactive nature of the cement particles. The preferential adsorption (selective) (Neubauer et al., 1998; Collepardi, 2005; Uchikawa et al., 1997; Kauppi et al., 2005; Flatt and Houst, 2001; Zhang et al, 2001) to chemisorption and chemical reactions to form new hydrated phases (Zhang et al, 2001; Griesser et al., 2005; Plank and Hirsch, 2007). The importance of steric forces dispersed cement suspension was highlighted in recent years, for various types of superplasticizers. This depends on the zeta potential of the suspension. Also, the sulfate concentration (sulfate content in cement, as a setting regulator) in the solution has a significant influence on the adsorption of superplasticizer during the first minutes of the process of hydration. Griesser has proved that the shear stress (yield stress) of the cement paste reaches a minimum with a certain amount of sodium sulphate (Na_2SO_4), which depends on the type of cement and superplasticizer (Griesser et al., 2005; Kheribet et al., 2012). This was explained by the competitive adsorption on the hydro-calcium aluminate C_3A between sulphate ions and molecules of the superplasticizer. This phenomenon is observed much more with the superplasticizers of the polynaphthalenes type (PNS) and polycarboxylates PCE (Houst et al., 2002; Yamada et al., 2001; Alonso et al., 2007; Palacioset al., 2009; Chandra and Björnström, 2002; Mäder et al., 2004; ACI, 2003; Johnson et al., 2000).

For this, the present study comes be given more information about the effect of superplasticizer type on the essentials properties of cement mortar (concrete) such as rheological and mechanical properties and the sustainability. The use of superplasticizer Aeternum1 (containing nanosilice + polycarboxylate) with cements (CEM II/A) will be the object of our study. We propose to integrate the adjuvant Aeternum1 of new generation to cements compounds CEMII / A, in order to assess the influence of superplasticizer on rheological and physico-mechanical properties of these cements in aggressive environments. The results obtained allow us to estimate substitution possibilities of CRS cement by these cements with superplasticizer of Aeternum1.

METHODOLOGY

Materials used

Cement: In order to assess the effectiveness of the superplasticizer Aturnum1, three cement types were chosen. For this, the cement C1 (Artificial Cement Portland (CPA)), cement C2 (Cement Resistant to the Sulfates (CRS)) and cement C3 (Cement with pozzolan addition of type CEMII/A 42.5) were used in this work. The chemical and mineralogical characteristics of cements used are given in Table 1. All used cements are according standard norm NF EN 197-1 (EN 197-1 and 197-2, 2000). The XRD pattern of the used cement confirms that these cements are according to standard norm (Figure 1).

Sand: The sand is standard sand for making mortar. It is also according to standard norm EN-v.

Superplasticizer (SP): The superplasticizer Aeternum-1 is used as a superplasticizer in cement pastes and mortar mixtures at different percentages by cement weight. Figure 2 gives the chemical and mineralogical composition presented in a XRD-pattern of the chosen superplasticizer. Also, the IR spectroscopy analysis is carried on this superplasticizer. The obtained results are given in the Figure 3.

The XRD showed that Aeternum-1 is a new generation superplasticizer, which is an hydration accelerator based on carbon

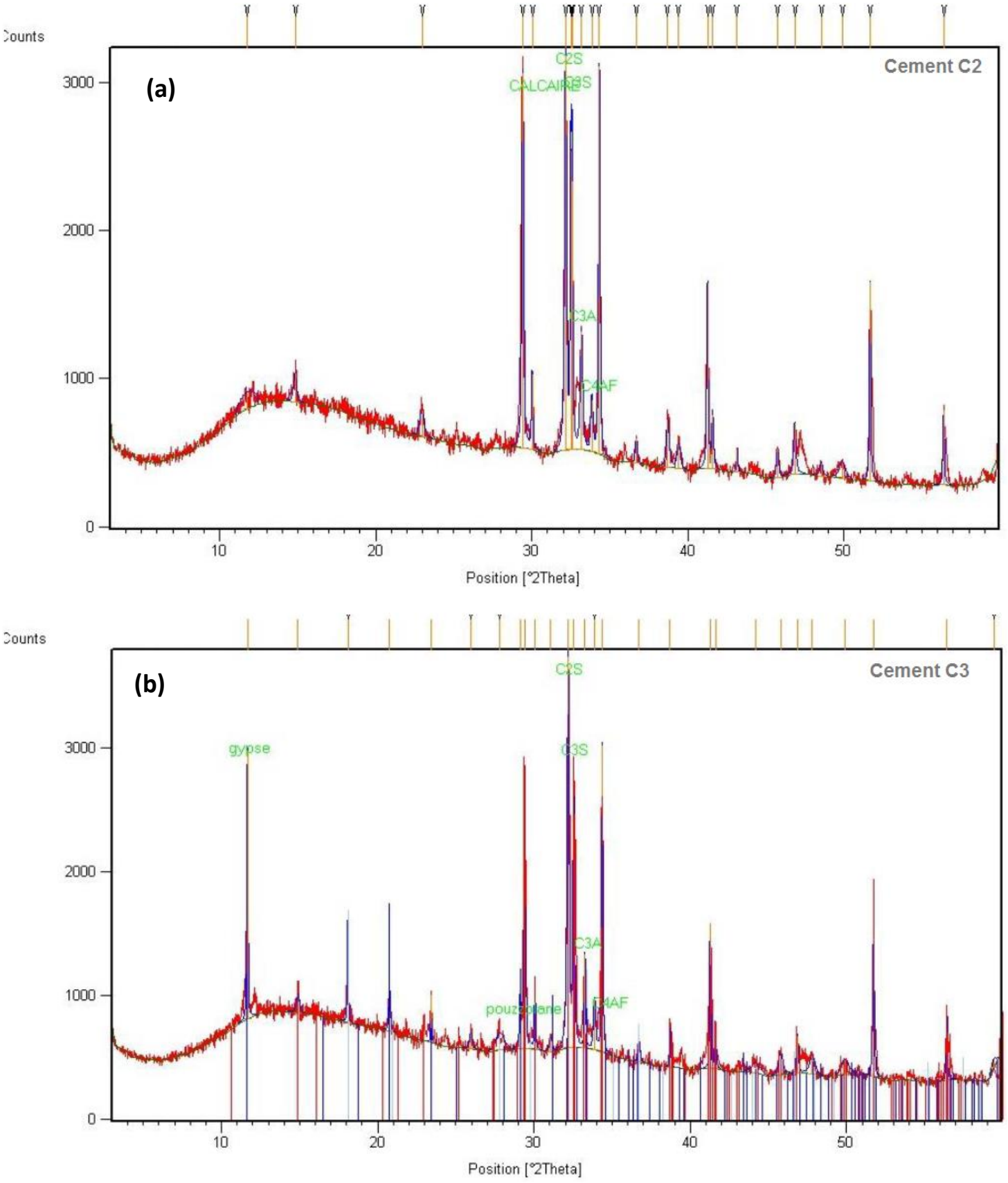

Figure 1. XRD pattern of all cements used in this work (a) C2 (b) C3.

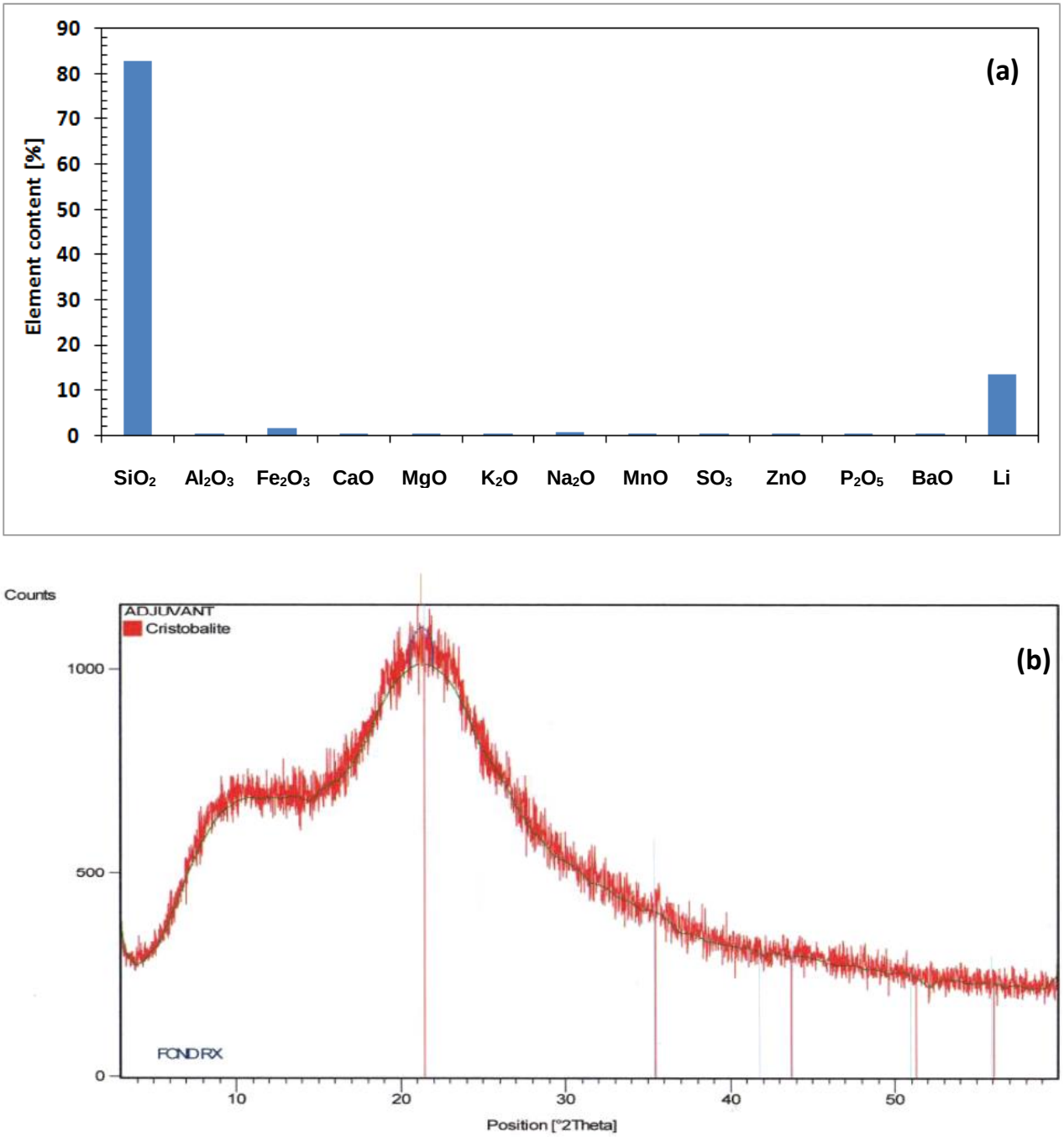

Figure 2. Chemical composition (a) and XRD analysis (b) of the adjuvant Aeternum-1.

and silicate in powder, infused in active nano-micro-silicates, it combines with the high pozzolanic activity of the latters, ensuring good rheological properties, fluidity (without segregation), waterproofness and compressive strength as well as good stability to the chemical aggressions.

The viewpoint of the physical characteristics consists of spherical particles having as size a few tenths of microns, and specific surface area of about 220000 cm^2/g. This feature allows a high dispersion and reaction on the cement particles, and a large capacity to capture and fix the calcium hydroxide ($Ca(OH)_2$) and transform it in a first time to an hydrated silicate and then to a stable and irreversible calcium hydrosilicate (C-S-H) tobermorite type.

The results provided by X-ray fluorescence allowed us to know the content of the various chemical constituents. Silica has an elevated content (82.67%), a high value of loss on ignition (13.7%), and a value of 6% carbon. The other constituents have very low values. The result of the XRD showed that the adjuvant is an amorphous body in high proportion and present a single peak of crystalline silica, as can be be seen on the crystallographic radiogram. This XRD graph is very similar to that of the silica fume.

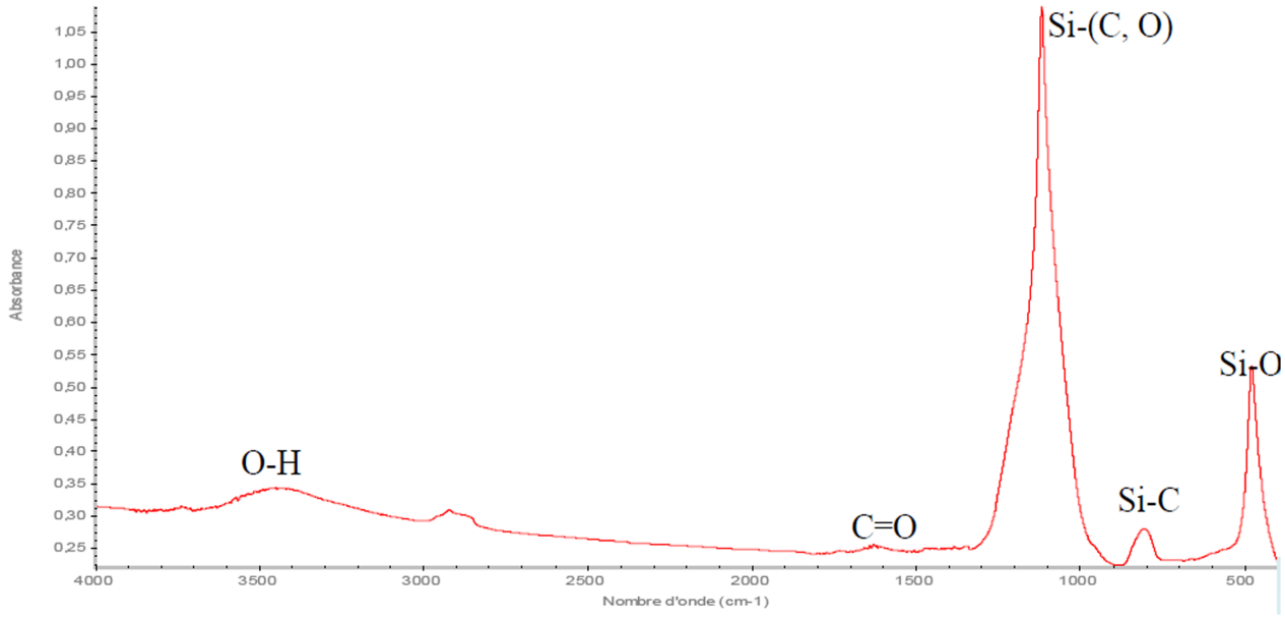

Figure 3. IR spectroscopy analysis of the superplasticizer used.

The Infrared spectrometry consists in irradiating the sample in the range 4000-400 cm^{-1} and detecting the frequencies absorbed by the latter (Figure 3). Only the vibrational normal modes inducing a displacement of the barycenter charges of the atomic group are active in infrared spectrometry. Figure 4 shows the IR spectrum of the adjuvant used, and illustrates some types of links, such as-Si-(O-C), Si-O and Si-C which are the most visible, indicating that this adjuvant is based on silicate and carbon; it is also organic. The C=O link is not significant as revealed in this adjuvant being without a carboxyl function.

Test methods

To perform this work, an experimental study was conducted according to the following work plan.

Rheological tests and fresh properties

Rheological tests: All rheological tests were carried using a Viscosimeter (VT550) equipped by coaxial cylinder geometry. Rheological measurements were conducted according to following protocol used by Kheribet et al. (Safi et al., 2011; Kheribet et al., 2012). It was proved by these authors that this protocol can be used for the cementitious pastes containing the superplasticizer (Safi et al., 2011). To compare the effect of superplasticizer used in this study, the cementitious pastes based on (C2 and C3), were prepared with a ratio W/C = 0.38 which is kept constant. The result obtained were compared to cement pastes with a ratio W/C = 0.5.

Slump test: All studied cement pastes were also tested for flowability immediately after mixing. The slump was measured at 20°C, using a mini-cone it was carried on fresh cement paste for each mixture.

Electrokinetic properties: The effect of different dosages of superplasticizer on the zeta potential of cementitious pastes was carried using a Zetameter (ZETASIZER 2000) of Malvern Instrument which has frequently used for determining the zeta potential of cement particles. For this, 1 cm^3 of the cementitious suspension is diluted in 30 cm^3 of distilled water, after which 5 ml of this suspension is injected into the analyzer (Kheribet et al., 2012).

Physical and mechanical properties

Setting time: The setting time was measured according to standard norm ASTM C191–13 (ASTM C191, 2013) same results as the time of setting of hydraulic cement paste measured by other methods, or the time of setting of mortar or concrete.

Casting and curing of mortars specimens: All the mixtures were mixed and prepared using a mortar mixer. All mortar compositions are prepared with the normal sand, according to the European standard EN 196-1 (EN 196-1, 2000). Before casting, slump-flow test is attempted as workability tests on fresh mortar for each mixture. Thereafter, three (3) specimens were cast in prismatic molds of (40×40×160 mm^3), for each concrete mixture. One day after casting, samples were stored in water under 21±1°C.

Mechanical tests: The mechanical tests (flexural strength and uniaxial compression) were conducted at 28 days of curing age according to European Standard EN 196-1 (EN 196-1, 2000). To study the effect of the superplasticizer on mortars, durability was based on the three cement types; the flexural and compressive strength was also measured on all mortar specimens that have been cured in different environments (trap water, sea water and chemical solution) under 21±1°C. The obtained results were compared to result obtained on the mortar samples stored in water.

RESULTS AND DISCUSSION

Rheological study of cement pastes with and without adjuvant

Rheological tests are performed for cement pastes based

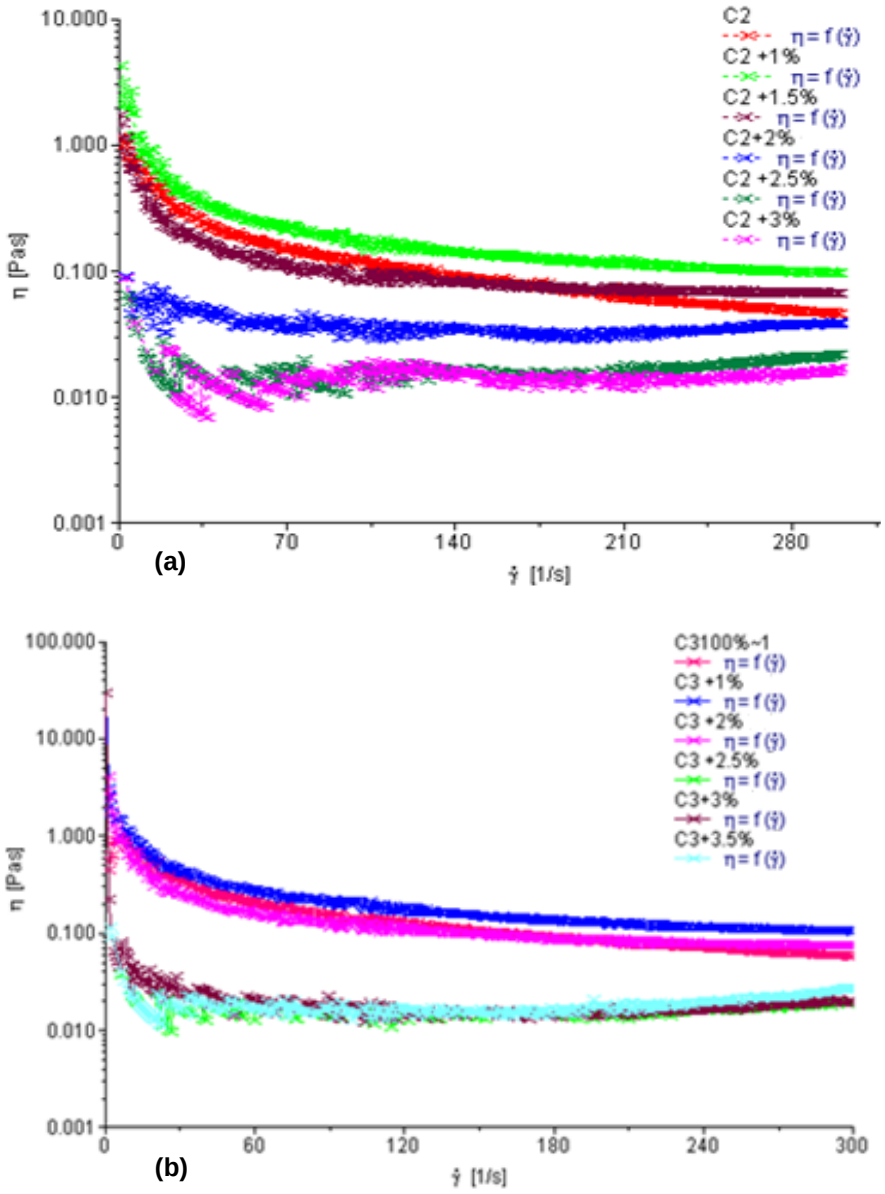

Figure 4. Apparent viscosity according to the shear rate of the cement pastes (C2 and C3) in dosages with adjuvant (0, 1, 1.5, 2, 2.5 and 3%, respectively) (a) C2 (b) C3.

on two types of cement C2 and C3 at different dosages of adjuvant to study the behavior of cement pastes and determining the saturation point for each type of cement. In this part we will also study the workability of cement grout, before ending with a study of the zeta potential measurements.

Determining saturation point of the different cements

To determine the saturation point we proceeded according to the protocol formulation of cement pastes, of fixing the amount of water and varying the amount of adjuvant in the mixture and the rheological measurements using the viscometer VT 550 with imposed speed. A composition of the cement paste was prepared for the witness cement grout with W/C ratio = 0.50 and the other pastas were prepared with a W/C ratio = 0.38. The results obtained are shown in Figure 4.

The saturation point is obtained from the curve of apparent viscosity according to shear rate. After testing the two types of cement C2 and C3 with different amounts of adjuvant (0, 1, 1.5, 2, 2.5, 3 and 3.5%) it was observed that:

The cement slurry with W/C=0.5 at the beginning have a higher viscosity value, but decreases with increasing shear rate, that is the same for both studied cements. For adjuvanted cement grout (cement + water +% adjuvant, with W/C= 0.38), we find that:

The adjuvant Aeternum-1 significantly affects the viscosity of the cement paste, and its content increases more, the viscosity of the paste decreases more. The apparent viscosity decreases with the shear rate of the cements grout with added limestone + 1% adjuvant, but with higher viscosity values relative to that of the grout without adjuvant. For 1.5% of adjuvant, the viscosity decreases initially and increases with increasing shear rate, such that it exceeds the values obtained by the grout without adjuvant.

This behavior was observed for grout with added pozzolan percentages for 1% and 2% successively. For a percentage equal to 2% superplasticizer, the cement slurry with added limestone has a constant viscosity with shear rate, which is a Newtonian flow. For the cement with added pozzolan, the Newtonian flow is obtained with 2.5% adjuvant, where the viscosity is constant as a function of shear rate. Beyond these percentages, it no longer affects the flow of grout. It can be seen that as the percentage of Aeternum-1 increases, the flow approaches the Newtonian flow, until it reaches the saturation point beyond which the adjuvant has no influence on the flow, which can be explained by the supersaturation of the cement grains. The saturation point varies from one cement to another – that of superplasticizer of C2 is 2% and the one of C3 is 2, 5%. The variation of the shear rate is a function of shear rate gradient of cements at different percentages of adjuvant:

According to the results, we observe that all studied pastas have a Binghamian behavior (1) following the model of Hershel-Bulckley described by the equation:

$$\tau = \tau_0 + k.\dot{\gamma}^n \quad (1)$$

τ_0 is the yield stress of the material; *k* is a parameter of consistency and n is a flow index. If *n* <1, the material is said to be shear-thinning; If *n*> 1, the material is said to be shear thickening.

The Figure 5 shows clearly that there is a correlation between the Hershel Bulckley model, and the results obtained (Banfill, 2003; Collepardi, 2005). The flow of the material is in steady state if it remains homogeneous (no segregation of the particles).

Spreading- test with mini cone

Spreading with mini cone is typically used to study evolution of the handling of the grout as a function of time. This involves measuring the diameter of the spreading of the slurry on the plate at various time intervals after the preparation of the grout (10, 20, 30, 40, 60, 90 and 120 min). All cements were tested for a W/C ratio = 0.38 in the presence of different dosages of Aeternum-1 adjuvant. The obtained results are given in Figure 6.

When the superplasticizer contacts with water, the dissolution of the constituents of cement begins, which increases the concentration of calcium (Ca^{2+}), alkali (K^+, Na^+), hydroxides (OH^-) and sulphates (SO_4^{2-}). These alkali are initially present in the clinker phases (Na_2O and K_2O) or sulfated form (Na_2SO_4, K_2SO_4, K_2Ca (SO_4) 2H2O. The presence of these salts in the pore water, especially alkalis hydroxides (Na, K) induces a decrease in the relative fluidity (Griesser et al., 2005; Kheribet et al., 2012) as have been noticed for C3, which loses its workability over time; and measuring the reduction in dosage of adjuvant due to the high content of alkali (Na_2O, K_2O) and SO_3, unlike the C2 cement that has good workability over time regardless of the percentage of adjuvant. The nature of cement also affects the workability; the cement C3 contains natural pozzolan which has the porous character and C2 cement contains the limestone filler which is known for its physical properties (better handling and workability).

Spread measurement after different times of rest revealed increase in viscosity after application of a stress or shear rate, the system returns to its initial state (reversible phenomenon), sees its viscosity decreases and spread of cement grouts.

Electrokinetic study of cements at different percentages of superplasticizer

The electrostatic surface potential is another important feature of cement suspensions for adsorption of superplasticizers. Zetametry measurements allow determining the potential (zeta) of ζ particle, which is defined as the measured electrostatic potential at the shear plane of the particle. The potential near the isoelectric point (pH for which the zeta potential is zero) are difficult to measure. Slightly positive or negative values can be found in the literature and depend on the cement composition.

From Figure 7 we see that the zeta potential has values that do not exceed 4.9 mV in absolute value. Potential of cement slurries C2 have negative charges with and without adjuvant, for the cement slurries C3, the zeta potential changes its sign from positive to negative, in which the isoelectric point is obtained for a dosage of 1% adjuvant. Based on the results we can see that Aeternum-1 is better adsorbed by active sites.

Effect of superplasticizer content on the physico-mechanical properties

Evolution of physical properties depending on superplasticizer dosage

Setting time: In this graphical presentation (Figure 8), it

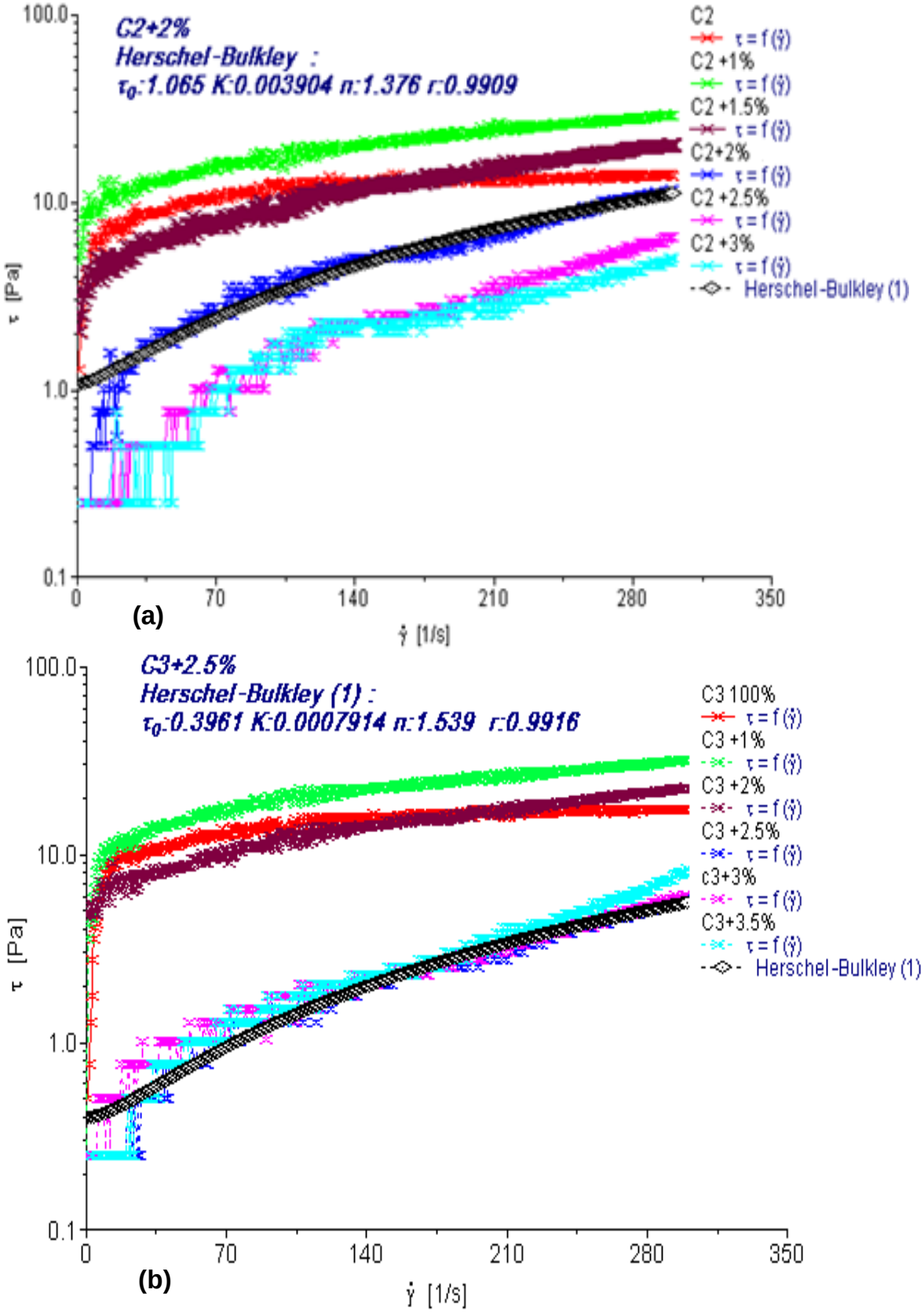

Figure 5. Shear stress as function as shear rate of the cement pastes (C2 and C3) with dosages in adjuvant (0, 1, 1.5, 2, 2.5 and 3%, respectively) (a) C2 (b) C3.

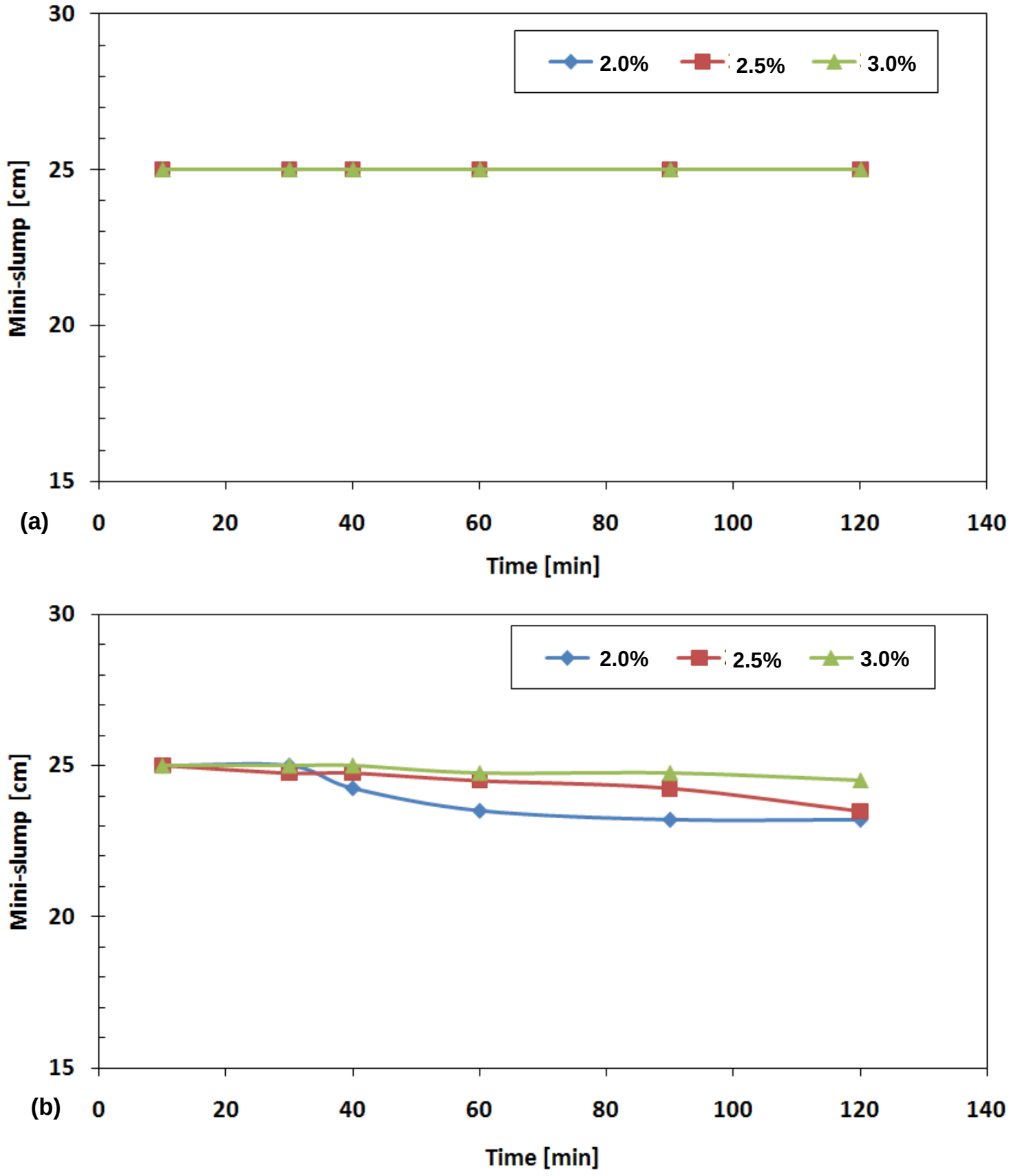

Figure 6. Graphical representations of the results of the spreading with mini-cone (a) C2 (b) C3.

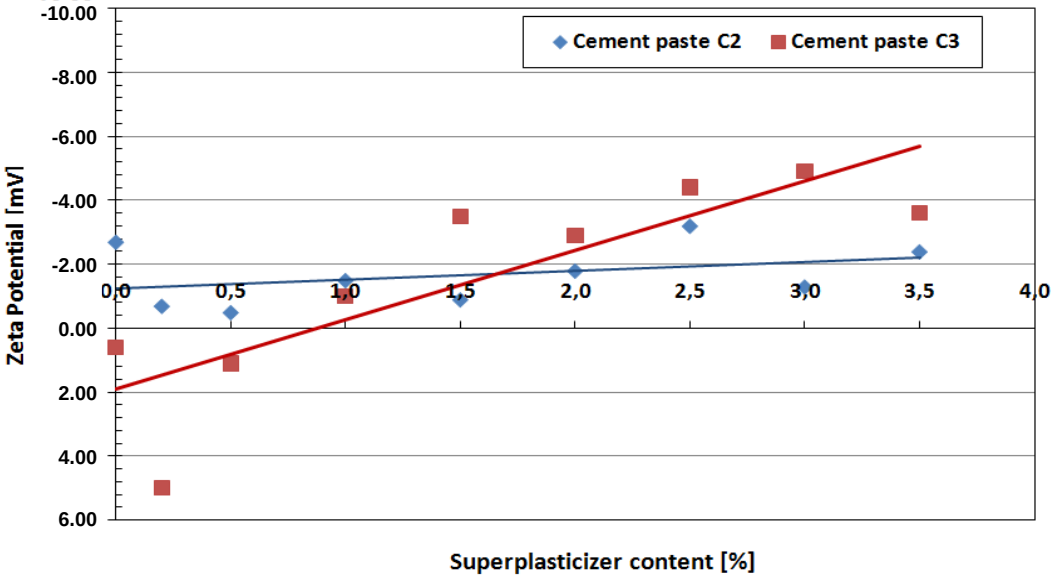

Figure 7. Graphical representation of the results of zeta potential.

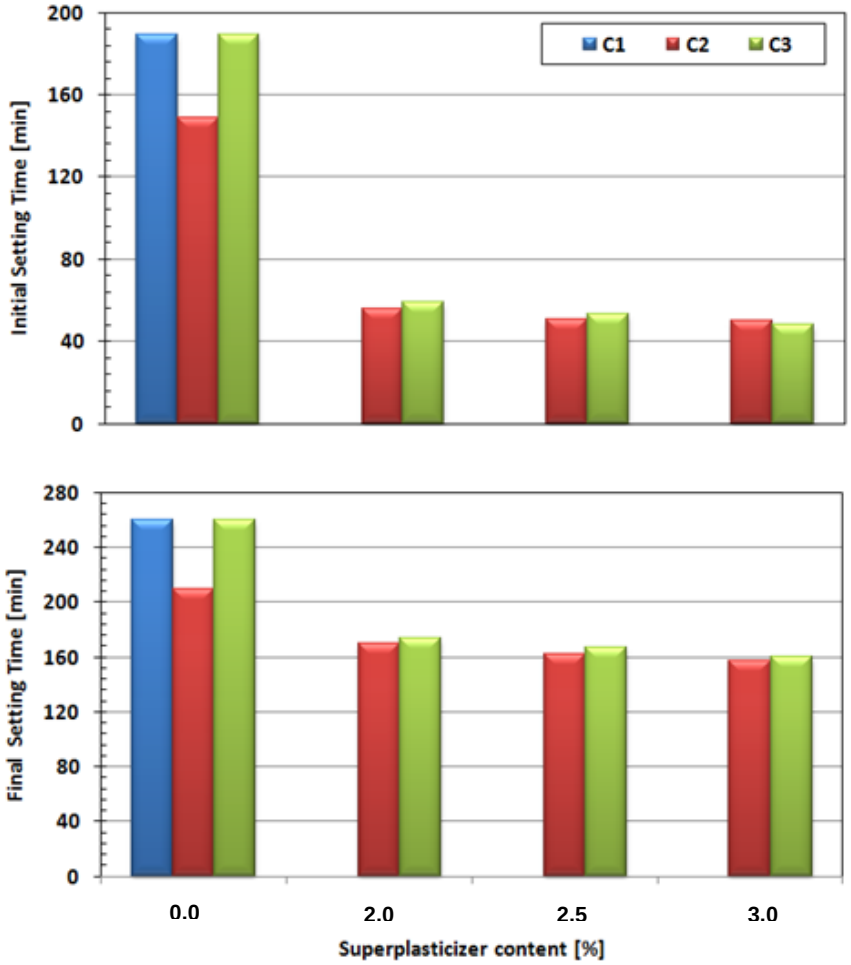

Figure 8. Setting times of cementitious pastes with superplasticizer.

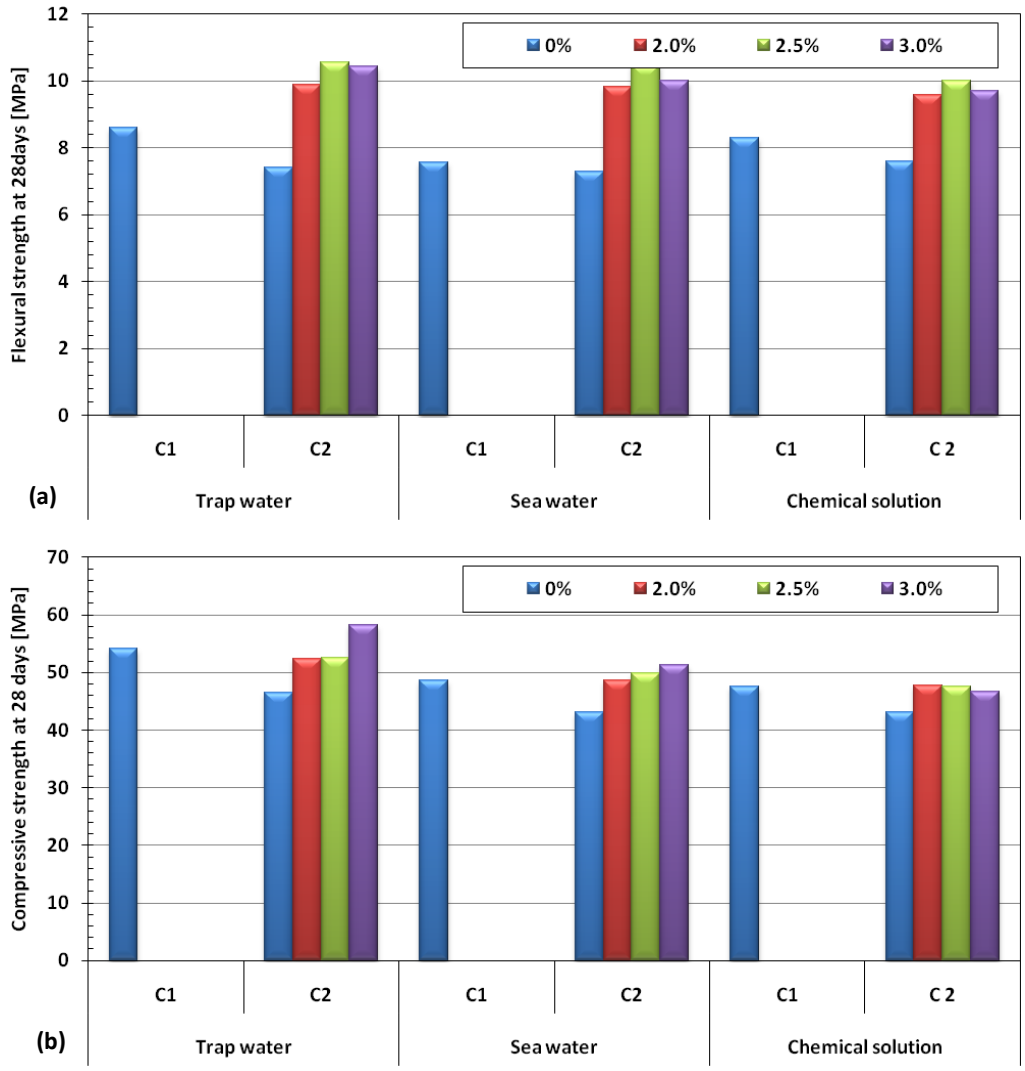

Figure 9. Mechanical strengths at 28 days based mortars C2 (a) Flexural strength (b) Compressive strength.

was noted that: The cement setting time depends on the ratio W/C of temperature and of nature of cement. In the presence of superplsticizer, it appears to take acceleration depending on the dosage of the superplasticizer in cements C2 and C3. The mineralogical composition has a high content of C3S, so there is a large heat of hydration, which involves its rapid dissolution and a precipitation of CSH.

This can be explained by the presence of the superplasticizer which acts as a setting accelerator by nucleation or germination on the surface of the cement grains. This germination causes a rapid setting time, and also by the deflocculating effect of the superplasticizer on cement, thus accelerating the setting and hydration.

Evolution of mechanical strength depending on the age of the test specimens of adjuvanted mortars stored in different environments: To properly study the evolution of mechanical strength in the presence of adjuvant Aeternum-1 various mixtures were carried out whose compositions are as follows: With three storage agressive environments: potable water; seawater; chemical solution, (we consider here a solution dosed at 50 mmol.l^{-1} of magnesium sulphate as aggressive environment. According to NF 18011, such a solution has a degree of aggressiveness A3 by both sulfate and magnesium ions).

Determination of mechanical strengths for the C2 - Comparison between C1 and C2 (with addition of limestone): Figures 9 and 10 show a comparison of flexural and compression strengths of test specimens of a cement mortar with added limestone in the presence of varying percentages of superplasticizer (0, 2, 2.5 and 3%, respectively), with a witness cement (sulphate cement resistant, CRS), preserved in the different environments

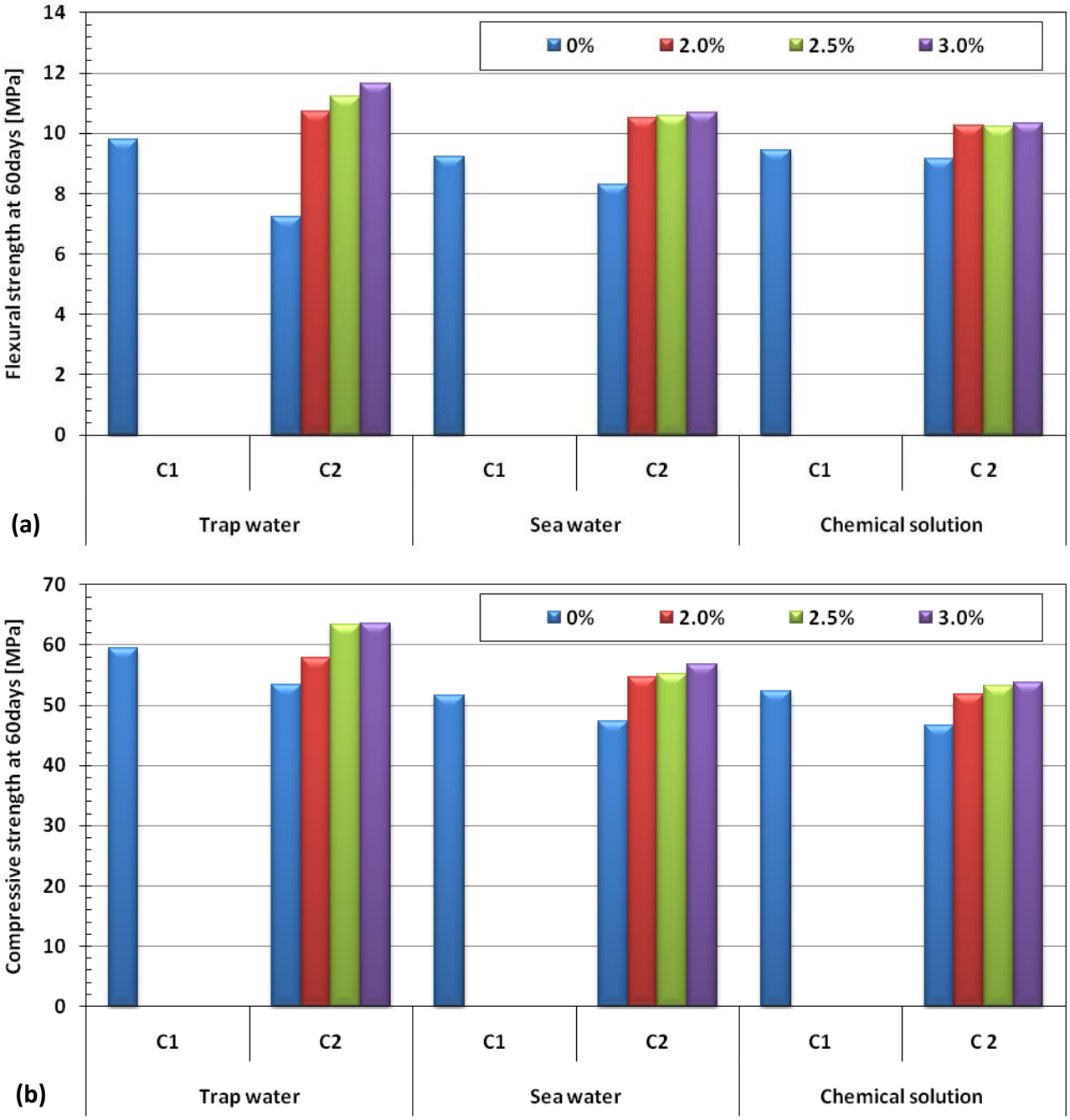

Figure 10. Mechanical strengths at 60 days based mortars C2 (a) Flexural strength (b) Compressive strength.

to different ages (2, 7, 28 and 60 days, respectively).

In the short term, it is noted that flexural and compressive strength of cement C2 is always smaller than the cement witness, but after incorporation of the adjuvant, an increase in resistance is observed. Thus, the test pieces preserved in the drinking water give better strength values than in the sea water and the chemical solution which is due to the aggressiveness of the environment. The increase in resistance in the presence of superplasticizer is caused by making the accelerating effect and rapid hydration of C_3S.

After 28 days of storage, it was noted that the cement witness (C1) shows better resistance in relation cement C2 without superplasticizer, and a decrease in resistance following the aggressiveness of the environment. The adjuvanted mortars give a slight improvement of resistance in aggressive environments; however, we saw very good resistances similar to those obtained by the

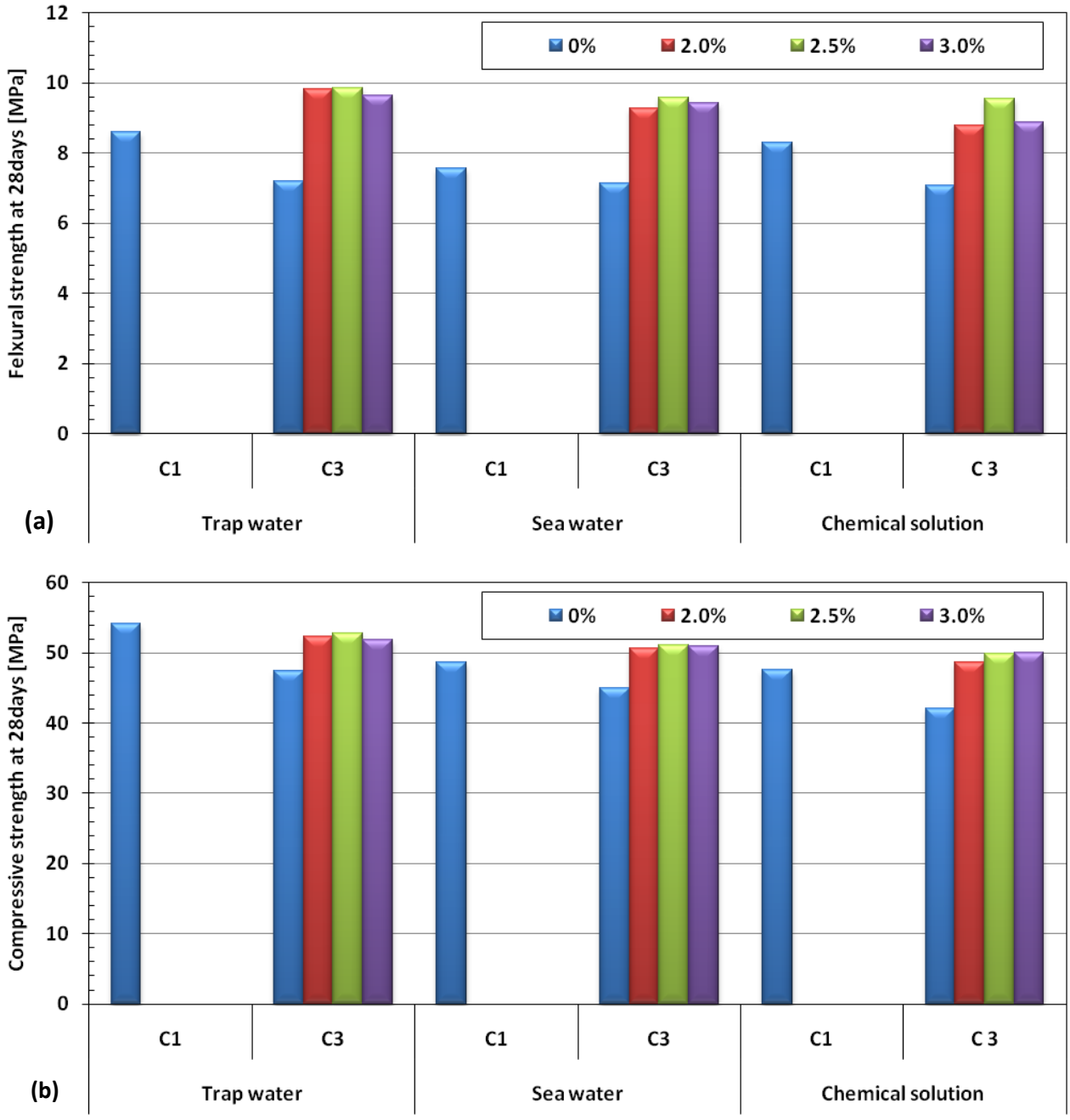

Figure 11. Mechanical strengths at 28 days based mortars C3 (a) Flexural strength (b) Compressive strength.

CRS in drinking water.

At 60 days of storage, we noted that the adjuvanted mortars C2 have better resistances depending on the dosage of superplasticizer and comparable to those of C1 in all environments, which shows the reliability of the plasticizer used.

The two types of cement addition increase resistances to all ages (Kamel et al. 1991; Read et al., 1991; Ghrici et al., 2005). Resistances at early age are due to the acceleration of the hydration of cement, while those at long term develop through the pozzolanic reaction (Kamel et al. 1991; Read et al., 1991) causing refinement of the pores and by replacing portlandite by the CSH.

Determination of mechanical strengths for the C3 (Comparison between C1 and C3 (with addition of pozzolan): Figures 11 and 12 show a comparison of flexural and compression strength of test specimens of cement mortar with addition of pozzolan with various percentages of superplasticizer (0, 2, 3 and 2.5%,

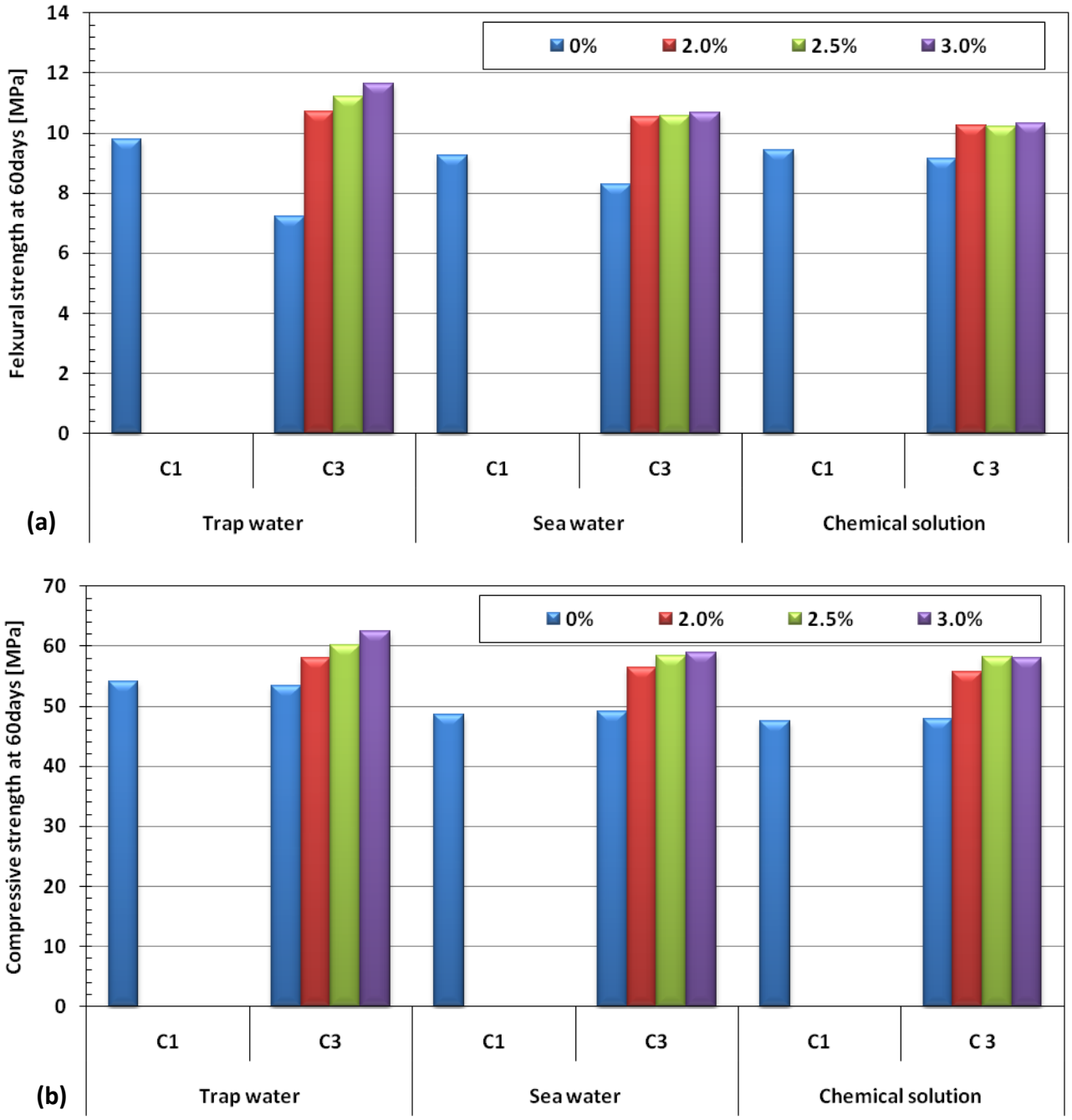

Figure 12. Mechanical strengths at 60 days based mortars C3 (a) Flexural strength (b) Compressive strength.

respectively), with a witness cement (sulphate resistant cement, CRS), preserved in different environments for ages (2, 7, 28 and 60 days, respectively).

From these figures, we note that the non-adjuvanted cement mortars C3 develop flexural and compressive strengths always lower than the witness mortar, and that at all age, and a reduction in the resistance according the environment aggressivity. For samples of mortars containing adjuvant dosages (2, 2.5 and 3%, respectively), mechanical resistances develop as follows:

After 28 days of storage we noted that the adjuvanted mortars have resistances comparable to those of the witness in the different environments.

At 60 days of storage, we note that the adjuvanted mortars C3, have better resistances depending on the dosage of adjuvant and are comparable to those of C1 in all environments, which shows the reliability of the

adjuvant used.

The two types of cement addition increase mechanical strengths to all ages. Mechanical strengths at an early age are due to the acceleration of the hydration of cement, while those at long term develop through the pozzolanic reaction causing refinement of the pores and by replacing portlandite by the CSH (Read et al., 1991; Ghrici et al., 2005).

Conclusion

The use of the adjuvant with a percentage (2, 2.5 and 3%), substantially reduces the value of W/C deflocculated the cement particles; this reduction results in an increase in resistance to a maximum value (63.55 MPa). Age is a predominant parameter, given the observed results, particularly on the mechanical resistance. The cement with addition of pozzolan, gives the best mechanical resistances; this is due to the pozzolanic reactivity, (natural pozzolan contained in the cement and amorphous silica present in the superplasticizer).

Beyond 28 days of storage, the mortars containing pozzolan have high mechanical strength compared to the C1 (sulphate resistant cement) through the double pozzolanic activity of the pozzolan and silica fume contained in the adjuvant; also, the structure of the cement paste is modified by the formation of the CSH and decrease in portlandite. That is to say, the additional hydrate formation CSH, which precipitate in the pores, decreases the porosity and increases the compactness of the cement paste.

Cement with limestone addition and adjuvanted, present significant mechanical resistances. Indeed, the presence of fine limestone consolidates and densifies the cementitious matrix by filling the pores of the structure and CSH formation, and graced the pozzolanic reactivity of the amorphous silica of the adjuvant.

The behavior of cement with limestone addition to the inverse of the pozzolan develops its mechanical resistance to short-term (7 days), but they are less important compared to those obtained by the cement with pozzolan addition to long term (28j and 60j).

Finally, this study opens new perspectives on the possibility of using composite cements CEM II-A with adjuvant/superplasticizer (Aeternum-1) in aggressive environments.

Conflict of Interest

The authors have not declared any conflict of interests.

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Scientific Research and Essays

Full Length Research Paper

Nutritional composition, functional properties and sensory evaluation of breads based on blends of 'orarudi' (*Vigna* sp) and wheat flour

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The proximate composition, functional and physical properties, as well as sensory evaluation of breads based on blends of wheat and 'orarudi' (*Vigna* sp) flour were investigated. Batches of 'orarudi' (*Vigna* sp) were separately put in a container and subjected to natural lactic acid fermentation in deionized water in a ratio of 1:3 (w/v) at 28 ± 2°C for 24 h. The fermented samples were manually dehulled and the cotyledons dried at 55 ± 2°C in a drought air oven, hammer milled into flour (500 µm mesh screen) and stored in a refrigerator (5 ± 2°C). The blends were formulated thus, the 'orarudi' flour (ORF) substituted 5, 10, 15, and 20% wheat flour (WF). The 100% WF served as the control. The parameters investigated were evaluated using standard methods. The data obtained were statistically analyzed. The results showed that fermentation and food supplementation enhanced both the proximate, minerals and vitamin contents of the experimental breads. The physical parameters indicated that fermentation and type of supplements had negative effect on the test breads relative to the control. The results revealed that the experimental breads had higher nutrient quality than the 100% wheat bread, probably due .to food-to-food fortification/supplementation.

Key words: Nutritional composition, functional properties, breads, composites, fermentation, nutrient quality.

INTRODUCTION

Consumption of baked products is greatly increasing due to the ever increasing urbanization, the products' cost competitiveness, their ready-to-eat convenience, availability of various products (bread, biscuit, cake, cookies) with varying taste and textural characteristics as well as their high nutritional profile and longer shelf life (Onoja et al., 2011; Mastromatteo et al., 2013). In particular, bread is an important food product that is cherished across the entire continents because of its sensorial and textural properties. Bread has been used as human food since ancient times and has been contributing over 50% of dietary energy due to its high carbohydrate content (Reebe et al., 2000; Dhingra and Jood, 2000; Onoja, 2007, 2011; Akubor, 2008; Mastromatteo et al., 2013). It is rich in both macro and micro nutrients, especially, proteins, carbohydrates, fibre

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as well as iron, magnesium, sodium, phosphorus, and some vitamins (B-vitamins). It has been shown that the rate of bread carbohydrate digestion greatly affects the absorption of glucose and consequently regulates the metabolic reactions that alter the glycemic and lipidemic postprandial responses in humans (Usha et al., 1989; Boby and Leelamma, 2003). For example, it has been reported that the slower digestion and absorption of bread carbohydrates helps maintain regular blood glucose which helps prevents non-communicable diseases associated with hyperglycemia and hyperlipidemia (Englyst et al., 2003). Moreover, many researchers have reported that the amylose/amylopectin ratios, the starch granule structure as well as protein matrix characteristics, all play an important role in determining the pattern of their hydrolysis and digestibility, and consequently affect the glycemic index of bread (Englyst et al., 2003; Mastromatteo et al., 2013). In addition, protein as well as starch/carbohydrate contents have been shown to influence both the loaf volume and the appearance of the bread (Honda and Jood, 2005). Nevertheless, these baked foods do not have sufficient essential nutrients required for good health (FAO/WHO, 2004).

Supplementation of cereal-based foods with legume for the production of bakery products to improve their nutrient quality has been reported (Impar, 1977; Nout, 1977; Macwatters, 1982; Natalie, 1988; Dhingra and Jood, 2000; Onoja, 2007; Akubor, 2008). These works showed that composite flour produced bakery products that were higher in nutrient quality compared with the 100% wheat products. This is because legume protein is high in lysine, an essential limiting amino acid in most cereals. Cereals on the other hand, are high in methionine and cystine which are deficient in legumes (FAO, 2004). Therefore, blending legume with cereal will provide desirable protein pattern that would help to enhance nutritional status of the population. Moreover, the high mineral and vitamin contents of these food crops are responsible for the increased nutritive quality of the supplemented products (Hotz and Gibson, 2007; Uwaegbute and Anyika, 2008). In particular, the functional properties of the composite flour have been found to be suitable for the production of bakery products (Hamad and Fields, 1979; Raidi and Klevin, 1983; Honda and Jood, 2005; Akubor, 2008). Due to their high fibre content, legumes have also been included within the group of functional foods due to their hypocholesterolemic and hypoglycemic effects (Usha et al., 1989; Boby and Leelamma, 2003).

The production of any food product depends on its raw material availability. The major problem facing the bakery industry in sub-Saharan Africa is the total dependence on importation of wheat to sustain its production. It is, therefore, imperative that alternatives to wheat which is traditionally used for bakery products be developed either as an extension or a replacement. Nigeria is a rich agricultural country but a higher percentage of food produced is wasted through post-harvest losses (Oyenuga, 1968). The application of fermentation to produce legume flour for bakery products will help to enhance nutrient quality and decrease anti-nutrients. There is no available report in the literature on the use of composite flour produced from 'orarudi' *(Vigna* sp) and wheat flour (WF) for the production of bakery products, notably, bread. The study was conducted to investigate the use of flour blends from these food crops for the production of bread. In particular, the functional, physical and sensory properties were evaluated; in addition, proximate, mineral and vitamin composition was determined. The characteristics of the test breads were compared with the 100% WF.

MATERIALS AND METHODS

Wheat (*Triticum aestivum*) flour was purchased from Nsukka main market. The 'orarudi' (*Vigna* sp), a special type of beans grown in Nsukka area (that lies latitude 6° N and longitude 7° E) was equally bought from Nsukka main market, Enugu State, Nigeria. Milk, water, compressed yeast, margarine, sugar and salt were bought from the local market.

Preparation of 'orarudi' flour (ORF)

Two kilograms (2 kg) of 'orarudi' (*Vigna* sp) grains were cleaned by sorting to remove extraneous materials, washed under running water and shade dried. The different batches were separately put in a container and subjected to natural lactic acid fermentation in deionized water in a ratio of 1:3 (w/v) at 28 ± 2°C for 24 h. The fermented samples were dried at 55 ± 2°C in a hot drought air oven (Gallenkamp, BS Mode l 250 size 2 UK), decorticated/dehulled and milled in hammermill into fine flour (500 µm mesh screen) and stored in a refrigerator (5 ± 2°C, 50% RH) until used for the production of breads.

Preparation of wheat (*Triticum aestivum*) flour

Two kilograms (2 kg) of white wheat (*T. aestivum*) flour (about 72% extraction rate) was purchased already milled as sold in Nsukka main market. The flour was made by Nigerian Flour Mills (Golden Penny). The bread produced from the 100% WF served as the control.

Evaluation of functional properties of flour

Water and oil absorption capacities were determined following the methods of Sosulski et al. (1976). Foaming capacity (FC) and foam stability (FS) were determined by the method of Sathe et al. (1982). The volume of foam at 30 s of whipping was expressed as FC. The volume of foam was recorded 1 h after whipping to determine FS as percent of the initial foam volume. Bulk density was determined by the method of Onimawo and Egbekwun (1998). Emulsion activity (EA) and emulsion stability (ES), least gelation concentration (LGC), swelling power and solubility was determined by the method of Okaka and Potter (1977).

Formulation of flour blends

The four (4) blends were formulated by replacement as follows:

ORF was used to substitute 5, 10, 15, and 20% WF. The 100% white WF served as the control flour.

Bread baking process

The bread samples were prepared using straight dough method as described by Ceserani et al. (1995). The recipe of the doughs included: 200 g of the composite flour, 125 ml (milk and water mixed), 5 g of compressed yeast, 10 g of margarine, 5 g of sugar and 2 g of salt. Each appropriately weighed composite flour (screened with 500 µm mesh) and Ingredients were thoroughly mixed in a mixer using a modified straight dough mixing method to produce the dough. The mixer was operated at a low speed for 5 min followed by high speed mixing for 20 min. The dough obtained after the mixing process was weighed, cut into uniform sizes, manually kneaded, molded, brushed with egg and covered with a cheese cloth and left to proof (ferment) at 35°C and 85% relative humidity for 36 min in a thermostatically controlled oven. The leavened dough was carefully transferred to the thermostatically controlled baking oven at 180°C for 35 min. The breads produced were cooled to ambient temperature and packaged in polyethylene bags for analyses. The baking process was performed in triplicate.

Chemical analysis

The breads were analyzed for proximate, mineral, vitamin and physical properties using standard methods (AOAC, 2005). The Kjeldahl method was used to estimate the protein nitrogen (N) which was then multiplied by the factor 6.25 to get the percent protein content (N × 6.25%). Ash was estimated by incinerating 1 g of the sample at between 550 to 600°C for 6 h in a muffle furnace until ash was obtained. Fat was estimated by extraction with petroleum ether using Tecator apparatus.

Subsequent extractions and weighing were continued until a constant weight was obtained. The carbohydrate content was obtained by difference, thus: 100 – (% protein + % fat + % ash + % fibre + % moisture). Energy values were calculated using Atwater's factor (% protein × 4 + % CHO × 4 + % fat × 9 kcal). Mineral estimation was done using wet digestion with nitric and perchloric acids. The values were then read out in Atomic Absorption Spectrophotometer (Latta and Eskin, 1980). The vitamins B_1, B_2 and niacin content were estimated according to the method of Pearson (1976). All analyses were performed in triplicate.

Physical properties

The physical characteristics of the breads investigated included height, breadth, weight, length, oven spring, proofing ability and specific volume were determined according to the method described by Ceserani et al. (1995). The height, breadth and length were measured by a metal rule. The weight was determined using a weighing balance. The proofing ability was measured by subtracting the initial height of the dough before proofing from the final height after proofing and multiplying the value by 100. Specific volume was determined using the formula by Ceserani et al. (1995). Thus,

$$\text{Specific volume (cm}^3\text{/g)} = \frac{L \times B \times H}{W}$$

Where L = Bread length; B = bread breadth; H = bread height; W = bread weight.

Oven spring was estimated as the difference in dough height before and after baking.

Sensory evaluation

Using a 9 point Hedonic scale (Retapol et al., 2006), where 9 represented the highest score and 1 the lowest was employed to evaluate the product for flavor, texture (crumb, crust), color (crumb, crust) and the general acceptability. A- 40 person taste panel randomly selected from students and lecturers of the Home Science Department, University of Nigeria (Nsukka), participated in the tasting sessions. Loaf samples were sliced evenly without removing the crust. Each sample was placed on white plates and identified with random three-digit numbers. Each judge (panel member) was seated in an individual compartment free from noise and distraction. The properly coded breads were served to the panelists for evaluating taste, flavor, color (crumb, crust), texture (crumb, crust), mouth feel and the general acceptability. Each judge was presented with a glass of water after each tasting session to rinse the mouth in order to prevent a carry-over effect.

Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 17) was used to analyze the data. The Duncan's New Multiple Range Tests (DNMRT) was used to test the significance of the difference among means. The significance was accepted at a $p < 0.05$. (Steel and Torrie, 1980).

RESULTS

Functional properties

The functional properties of the flour are presented in Table 1. The ORF had significantly ($p < 0.05$) higher water absorption capacity (WAC) (132.23%) than the WF (126.67%). There was a similar trend in the oil absorption capacity (OAC) between the flour (Table 1). On the other hand, the EA and ES slightly differed ($p < 0.05$). The FC of the WF (11.2%) was significantly ($p < 0.05$) lower than the ORF (17.76%), while FS showed similar trend for the two flours (WF: 39.57%; ORF: 51.52%) ($p < 0.05$). The bulky density did not differ significantly ($p > 0.05$) between the two flours (0.58%; 0.47%). The swelling power of ORF (3.55%) was significantly ($p < 0.05$) lower than the WF (13.23%). The solubility of the two flour differed (7.88%; 10.34%) for WF and ORF, respectively. The LGC characteristics between the two flour differed ($p < 0.05$).

Proximate composition of the breads

The proximate composition of the breads produced from the flour blends and the control is presented in Table 2. The moisture content of the samples varied. The control sample had the highest value that differed from the test groups ($p < 0.05$) (Table 2). The protein content of breads produced from the flour blends and the control ranged from 8.20 to 14.56%. The wheat and orarudi (WOR_4) blend had the highest protein content (14.56%) that was significantly different ($p < 0.05$) from the rest including the control (8.20%). The control had much higher ($p < 0.05$)

Table 1. Functional properties of wheat and 'orarudi' flour.

Flour/property	Wheat Flour(WF)	'Orarudi' Flour (ORF)
Water absorption capacity(%)	$126.67^{b} \pm 0.01$	$132.23^{a} \pm 0.02$
Oil absorption capacity (%)	$130.26^{a} \pm 0.03$	$135.44^{b} \pm 0.01$
Emulsion activity (%)	$14.54^{b} \pm 0.01$	$16.58^{a} \pm 0.03$
Emulsion stability (%)	$6.84^{a} \pm 0.01$	$7.07^{a} \pm 0.01$
Foaming capacity (%)	$11.20^{b} \pm 0.03$	$17.76^{a} \pm 0.02$
Foam stability (%)	$39.57^{b} \pm 0.01$	$51.52^{a} \pm 0.03$
Bulk density (g/cm^3)	$0.58^{a} \pm 0.01$	$0.47^{a} \pm 0.02$
Swelling power (%)	$13.23^{a} \pm 0.01$	$3.55^{b} \pm 0.01$
Solubility (%) least gelation	$7.88^{b} \pm 0.03$	$10.34^{a} \pm 0.02$
concentration (%.W/N)	$9.20^{b} \pm 0.01$	$5.98^{a} \pm 0.02$

Data are means of 3 determinations. Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 2. Proximate composition and energy content of breads prepared from different blends and the control (per 100 g sample).

Parameters/composites/ratios	WOR_1 95:5	WOR_2 90:10	WOR_3 85:15	WOR_4 80:20	Wheat bread 100
Moisture (%)	$12.32^{d} \pm 0.02$	$13.88^{d} \pm 0.03$	$31.12^{b} \pm 0.01$	$30.35^{d} \pm 0.01$	$33.72^{a} \pm 0.03$
Protein (%)	$9.72^{d} \pm 0.01$	$11.23^{c} \pm 0.02$	$13.30^{b} \pm 0.01$	$14.56^{a} \pm 0.01$	$8.20^{e} \pm 0.02$
Total CHO (%)	$69.41^{b} \pm 0.01$	$75.70^{c} \pm 0.01$	$74.40^{d} \pm 0.01$	$73.30^{d} \pm 0.03$	$82.40^{a} \pm 0.02$
ASH (%)	$3.44^{d} \pm 0.02$	$4.80^{c} \pm 0.01$	$5.60^{b} \pm 0.02$	$5.84^{a} \pm 0.03$	$3.42^{d} \pm 0.02$
FAT (%)	$3.25^{b} \pm 0.01$	$2.60^{c} \pm 0.01$	$2.42^{d} \pm 0.02$	$2.30^{d} \pm 0.01$	$3.46^{a} \pm 0.02$
Total fibre (%)	$1.76^{d} \pm 0.01$	$2.56^{c} \pm 0.01$	$4.78^{b} \pm 0.01$	$6.10^{a} \pm 0.01$	$1.14^{e} \pm 0.01$
Energy (kcal)	$381.73^{b} \pm 0.03$	$371.12^{c} \pm 0.03$	$368.02^{d} \pm 0.02$	$367.10^{d} \pm 0.03$	$391.74^{a} \pm 0.02$

*Data are means of 3 determinations. Values in the same row with different superscripts are significantly different ($p \leq 0.05$). Energy calculation was based on Atwater factor (protein x 4, CHO x 4, Fat x 9 kcal). CHO by difference that is, 100 - {protein + fat + ash + fibre}. W = wheat, OR = 'orarudi' (*Vigna* sp), CHO = carbohydrate. Blend1 (WOR_1), Blend2 (WOR_2), Blend 3 (WOR_3), Blend4 (WOR_4) and 100% wheat bread (control).

carbohydrates (82.40%) than the rest. The experimental breads variation ranged between 73.30 and 78.40%. The ash content of the WOR_1 and the control were similar ($p > 0.05$) but differed significantly ($p < 0.05$) from the other test breads. The values varied from 3.44 to 5.84%. The control had the highest fat content (3.46%) compared with the test samples ($p \leq 0.05$). The WOR_1 bread had higher fat content (3.25%) relative to the other test samples ($p \leq 0.05$). The control had the least total fibre (1.14%) that differed significantly ($p < 0.05$) from the test samples. The experimental breads ranged from (1.76% to 6.10%). The energy content of the control bread (391.74 kcal) was the highest compared with the test samples ($p < 0.05$). The values of the test samples ranged from 367.10 to 381.73 kcal.

Physical properties

The physical properties of the breads are shown in Table 3. The control bread had the lowest weight (136.20 g) than the test breads and was significantly ($p < 0.05$) different. The values for the test breads ranged from 139.0 to 145.20 g. The WOR_4 bread had the highest weight (145.20 g). The control had the highest width value (5.30 cm) relative to the test breads. The WOR_1 bread had the highest height compared with the other test samples ($p < 0.05$). Similarly, the control (WF bread) had the overall highest height (6.90 cm) that differed significantly ($p < 0.05$) from the test breads. The loaf lengths (dimensions) of the test samples differed significantly ($p < 0.05$) from the control (15.20 cm). The oven spring of both the test breads and the control were similar ($p > 0.05$). The spread ratios of both the control sample and the WOR_1 bread were similar ($p > 0.05$) and were higher than other samples. The value of the specific volume for the control bread was the highest (3.86%) which was significantly ($p < 0.05$) different from the test samples. The proofing ability of the control bread (96%) was higher than other test breads.

Minerals and vitamins content

Table 4 presents the mineral and vitamin composition of the breads. The mineral content of the test breads were

Table 3. Physical properties of the experimental breads and the control.

Blends/ratios/parameters	WOR_1 95:5	WOR_2 90:10	WOR_3 85:15	WOR_4 80:20	Wheat bread 100
Weight (g)	$139.0^b \pm 0.03$	$140.60^c \pm 0.01$	$142.0^a \pm 0.02$	$145.20^b \pm 0.01$	$136.20^d \pm 0.03$
Width (cm)	$4.90^a \pm 0.01$	$4.62^b \pm 0.01$	$4.60^b \pm 0.01$	$4.65^b \pm 0.02$	$5.30^a \pm 0.01$
Height (cm)	$5.82^b \pm 0.01$	$4.60^d \pm 0.03$	$4.70^d \pm 0.01$	$5.30^c \pm 0.02$	$6.90^a \pm 0.03$
Length (cm)	$14.30^b \pm 0.03$	$11.90^c \pm 0.02$	$10.40^d \pm 0.01$	$10.60^d \pm 0.01$	$15.20^a \pm 0.02$
Oven spring (cm)	$0.83^a \pm 0.01$	$0.76^b \pm 0.01$	$0.72^c \pm 0.01$	$0.71^c \pm 0.01$	$0.90^a \pm 0.01$
Spread ratio	$7.10^b \pm 0.02$	$6.40^b \pm 0.02$	$5.32^c \pm 0.01$	$5.30^c \pm 0.01$	$6.69^b \pm 0.03$
Specific volume (%)	$2.56^b \pm 0.01$	$1.20^c \pm 0.01$	$1.30^c \pm 0.02$	$1.42^c \pm 0.01$	$3.86^a \pm 0.01$
Proofing ability (%)	$91^b \pm 0.03$	$89^c \pm 0.14$	$85^d \pm 0.12$	$78^e \pm 0.16$	$96^a \pm 0.03$

*Data are means of 3 determinations. Values in the same row with different letter superscripts are significantly different ($p \leq 0.05$). W = wheat, OR = 'orarudi '(*Vigna* sp). Blend1 (WOR_1), Blend2 (WOR_2), Blend3 (WOR_3), Blend4 (WOR_4) and 100% wheat bread (control).

Table 4. Mineral and vitamin composition* of breads produced from the blends and the control (per 100 g sample).

Blends/ratios/parameters (mg)	WOR_1 95:5	WOR_2 90:10	WOR_3 85:15	WOR_4 80:20	Wheat bread 100
Fe	$0.16^e \pm 0.01$	$0.46^d \pm 0.02$	$1.62^b \pm 0.01$	$1.92^a \pm 0.01$	$1.06^c \pm 0.01$
Cu	$0.38^d \pm 0.01$	$0.42^c \pm 0.01$	$0.48^b \pm 0.01$	$0.64^a \pm 0.01$	$0.38^d \pm 0.01$
Ca	$43.20^d \pm 0.02$	$48.26^c \pm 0.01$	$66.00^b \pm 0.03$	$87.30^a \pm 0.01$	$42.50^d \pm 0.04$
P	$67.26^d \pm 0.12$	$75.40^b \pm 0.14$	$74.30^c \pm 0.12$	$84.42^a \pm 0.03$	$66.40^d \pm 0.02$
I_2	$0.01^a \pm 0.001$	$0.01^a \pm 0.001$	$0.01^a \pm 0.001$	$0.01^a \pm 0.001$	$0.01^a \pm 0.00$
K	$178.30^c \pm 0.13$	$182.20^b \pm 0.12$	$181.20^b \pm 0.02$	$188.40^a \pm 0.03$	$177.56^c \pm 0.02$
Mn	$0.29^b \pm 0.01$	$0.30^b \pm 0.01$	$0.32^b \pm 0.01$	$0.33^b \pm 0.02$	$0.31^b \pm 0.01$
Na	$728.20^c \pm 0.13$	$736.30^b \pm 0.16$	$738.26^b \pm 0.12$	$744.12^a \pm 0.11$	$722.20^d \pm 0.22$
Zn	$0.62^d \pm 0.01$	$0.64^d \pm 0.02$	$0.58^d \pm 0.02$	$0.66^d \pm 0.01$	$0.63^d \pm 0.01$
Mg	$23.20^c \pm 0.12$	$24.20^b \pm 0.23$	$23.23^c \pm 0.24$	$28.40^b \pm 0.13$	$20.20^d \pm 0.0112$
Cd	$0.001^c \pm 0.0001$	$0.002^c \pm 0.0001$	$0.001^c \pm 0.0001$	$0.002^c \pm 0.0001$	$0.001^c \pm 0.0001$
Cr	$0.034^a \pm 0.01$	$0.034^a \pm 0.01$	$0.033^a \pm 0.02$	$0.036^a \pm 0.02$	$0.033^a \pm 0.01$
B_1	$0.28^c \pm 0.01$	$0.29^c \pm 0.02$	$0.34^b \pm 0.02$	$0.42^a \pm 0.01$	$0.078^d \pm 0.01$
B_2	$0.27^b \pm 0.02$	$0.26^b \pm 0.01$	$0.26^b \pm 0.01$	$0.32^a \pm 0.01$	$0.075^c \pm 0.02$
Niacin	$1.11^c \pm 0.01$	$1.14^c \pm 0.01$	$1.44^b \pm 0.01$	$1.56^a \pm 0.01$	$0.52^d \pm 0.01$

*Data are means of 3 determinations. Values in the same row with different letter superscripts are significantly different ($p \leq 0.05$). Data expressed as mg/100g product. W = wheat, OR = 'orarudi' (*Vigna* sp). Blend1 (WOR_1), Blend2 (WOR_2), Blend 3 (WOR_3), Blend4 (WOR_4) and 100% wheat bread (control).

comparable with the control. The vitamins (B_1, B_2 and niacin) content of the samples were moderate relative to the bakery products.

Sensory evaluation

Table 5 presents the mean sensory evaluation scores of the breads. The control bread had higher general acceptability, followed by WOR_1 sample. There was a significant ($p < 0.05$) difference in crumb and crust color among the test products. The judges preferred the crust color of the WOR_1 bread to the other test samples ($p < 0.05$) including the control. Although some of the sensory attributes of the breads from the other test blends were lower than those of the control and the WOR_1 bread, they were, however, acceptable. All the test samples recorded over 60% of overall acceptance. However, there was a slight difference in the degree of acceptance amongst the breads.

DISCUSSION

The higher WAC of ORF compared to WF might be ascribed to the higher amounts of hydrophilic constituents particularly, proteins, carbohydrates and fiber it contains. Kinsella (1987) and Akubor (2008) showed that WAC mainly depends on the amount and nature of the hydrophilic constituents present in the samples. In particular, it has been reported that WAC of dough is influenced by the protein content and quality as well as the extent to which the starch is damaged mechanically (the greater the damage the more the absorption (Bushuk and Hlynka, 1964). Also, it has been shown that fiber is characterized by high water holding capacity as reported

Table 5. Mean sensory attributes of breads made from different blends and the control.

Blends/ratios/parameters	WOR_1 95:5	WOR_2 90:10	WOR_3 85:15	WOR_4 80:25	Whole wheat 100
Loaf shape	$8.40^{b} \pm 0.01$	$7.60^{c} \pm 0.02$	$7.60^{c} \pm 0.03$	$6.40^{d} \pm 0.01$	$8.80^{a} \pm 0.02$
Mouth feel	$8.82^{a} \pm 0.02$	$7.60^{b} \pm 0.03$	$7.40^{c} \pm 0.01$	$7.10^{d} \pm 0.01$	$8.80^{a} \pm 0.12$
Taste	$8.60^{a} \pm 0.01$	$7.40^{c} \pm 0.02$	$7.60^{b} \pm 0.01$	$6.20^{d} \pm 0.03$	$8.70^{a} \pm 0.02$
Flavor	$7.80^{b} \pm 0.02$	$7.20^{c} \pm 0.01$	$7.30^{c} \pm 0.02$	$6.40^{d} \pm 0.01$	$8.20^{a} \pm 0.13$
Crust					
Color	$8.60^{a} \pm 0.02$	$7.66^{c} \pm 0.01$	$7.56^{c} \pm 0.03$	$5.10^{d} \pm 0.01$	$8.20^{b} \pm 0.02$
Texture	$7.20^{c} \pm 0.01$	$7.10^{d} \pm 0.02$	$7.60^{b} \pm 0.01$	$5.60^{e} \pm 0.01$	$7.80^{a} \pm 0.03$
Crumb					
Color	$8.46^{b} \pm 0.02$	$7.10^{d} \pm 0.11$	$7.40^{c} \pm 0.02$	$6.20^{e} \pm 0.01$	$8.60^{a} \pm 0.03$
Texture	$7.60^{b} \pm 0.03$	$7.26^{c} \pm 0.01$	$6.60^{d} \pm 0.01$	$6.20^{e} \pm 0.03$	$7.98^{a} \pm 0.02$
General acceptability	$8.50^{b} \pm 0.01$	$7.66^{c} \pm 0.03$	$6.66^{d} \pm 0.04$	$6.40^{d} \pm 0.01$	$8.60^{a} \pm 0.02$

*Data are means of 3 determinations. Values in the same row with different superscripts are significantly different ($p<0.05$). Values were based on a 9 – point Hedonic scale (where 9 represented the highest and 1 the lowest). W = wheat, OR = 'orarudi' (*Vigna* sp). Blend1(WOR_1), Blend2(WOR_2), Blend 3(WOR_3), Blend4(WOR_4) and 100% wheat bread (control).

by Houoway and Grieg (1984). The low fat contents of the blends may have enhanced their WAC. Fat has been shown to decrease the hydration capacity of flour used in the formulation of the blends and the control. It has been reported that WAC is critical in bulking and consistency of products as well as in baking processes (Akubor et al., 2013). Many researchers have reported that water also plays a significant role in the major changes that take place during the baking process which include starch gelatinization, protein denaturation, yeast and enzyme inactivation, as well as flavor and color formation (Bushuk and Hlynka, 1964; Pomeranz, 1985; Czuchajowska et al., 1988). Moreover, water content and its distribution has been shown to affect the shelf life of bread which is directly caused by microbial spoilage, softness of crumb, crispness of the crust, crumb hardening, crumbliness, etc associated with staling and overall lowered acceptability by the consumers (Pomeranz, 1985).

The higher OAC of ORF suggested that it contained higher amounts of a polar amino acids (normally present in legume proteins) than WF. Akubor et al. (2013) attributed OAC mainly to the physical entrapment of oils depicting the role at which proteins complex with fat in food formulations. It is suggested that OAC is important in bakery products (Onimawo and Egbekwun, 1998) and this study showed that WF and ORF would have potential for bakery products. Fat acts as flavor enhancer and increases the mouths feel of foods. It has also been shown to increase the leavening power of the baking powder in the dough and improves the texture of the baked products particularly, bread (Dhingra and Jood, 2000). The EA and ES were low, with the ORF exhibiting the highest activity. The low protein content of WF and high fiber levels in ORF may have discouraged the formation and stabilization of emulsions (Kinsella, 1987). The high protein content of legumes including ORF may explain its good ability to form and stabilize foams. Akubor et al. (2013) noted that FC and FS depends on protein concentration, protein solubility, swelling power, among other factors. Foams are used to improve texture, consistency and appearance of foods. Therefore, blending WF with ORF would improve their applications in baking processes.

The WF and the ORF had comparable bulk densities probably due to their similar particle sizes. Bulk density has been reported to be a function of particle size because particle size is inversely proportional to bulk density (Onimawo and Egbekwun, 1998). The low bulk density of the flour is beneficial for cost effectiveness in packaging design. The LGCs of the flour was significantly ($p < 0.05$) lower in the ORF than WF. Sathe et al. (1982) associated the variations in the gelling properties of flour to the different ratios of protein, carbohydrate and fat that makes up the flour. Interaction among these components was also noted to play a significant role in functional properties as it affects gelation. The rate of gelling and gel firmness was reported to be influenced by temperature, time of heating and protein content (Houoway and Grieg, 1984; Kinsella, 1987). Flour like WF and ORF with low values of LGC could be good thickening agents and might be useful in products which require thickening and gelling such as complementary foods.

The high protein values of the test breads could be ascribed to the synergistic effect of mutual food supplementation. It could as well be attributable to synthesis of new proteins from hydrolyzed free amino acids during fermentation by microflora enzymes. It is

known that when legumes supplement cereals, they provide a protein quality comparable to or higher than that of animal (Impar et al., 1977; Hotz and Gibson, 2007). The higher protein for the WOR_4 blend over the other blends is probably due to its lower level of carbohydrates. The protein drop in other test samples could be attributed to a dilution of protein by the increased level of carbohydrates in them. In particular, the WOR_1 blend having the lowest protein value (9.72%) showed the highest carbohydrates value (78.4%). This observation agrees with the findings of other researchers (Sathe, 1982; Kibite and Evans, 1984). The high carbohydrate levels of the test breads though lower than the control (WF) might be ascribed to either individual food materials and/or microflora enzyme hydrolysis that led to the synthesis of complex carbohydrates from other nutrients carbon skeletons due to fermentation and synergistic effect of food supplementation. This condition was also applicable to other nutrients (Odunfa, 1985; Hotz and Gibson, 2007). The high ash content of the test breads and the control was an indication that the products are good sources of mineral (Reebe et al., 2000). The low lipid level was expected since legumes and cereals store energy in form of starch rather than lipids. The low lipid values are beneficial as it guarantees longer shelf life for the breads because it has been reported that the higher the lipid content of a given food, the higher are the chances for rancidity (Beuchat and Worthington, 1974).

The mineral and vitamin contents of the test breads relative to the control suggest their superiority over the control. For instance, iron complexes with tannin and phytate present in beans but during the fermentation of these foods, these complexes are broken down by the hydrolyzing enzymes to improve the availability of iron (Hamad and Fields, 1979; Reebe et al., 2000; Dhingra and Jood, 2000; Honda and Jood, 2005; Akubor, 2008). The improved phosphorus level might be due to the release of phosphorus from its organic complex by the microflora enzymes and this could address the problem of osteoporosis in the elderly (Reebe et al., 2000). However, fermentation negatively influenced the physical characteristics of the test breads produced relative to the control as observed in the higher weight and dull color of the experimental breads compared with the control. The fibre levels are good and the products could be potential candidate in the management of diabetes (Usha et al., 1983; Boby and Leelamma, 2003; Englyst et al., 2003). The width and length of the test samples had comparable values however, they were lower than the control. This is because the spread of bread was affected by the competition of the ingredients for the available water. Flour which absorbs water during mixing will tend to reduce it (Raidi and Klevin, 1983; Singh et al., 1991). The WF used in producing control bread might have absorbed more water than the test blends.

The low level of gluten in the composite flour no doubt affected the weight, height, proofing ability, specific volume and the oven spring of the test products as less carbon IV oxide (CO_2) would be retained (less bubbles) by the dough. It has been shown that crumb firmness is influenced by crumb structure which is closely related to the gluten content, the degree of starch gelatinization and moisture redistribution (Mastromatteo et al., 2013). Moreover, during fermentation, fat, protein and carbohydrate are hydrolyzed (diluted) and would influence the products and resulting in the above observations. The present findings were in agreement with those for fermented wheat - cowpea blend (Akubor, 2008), fermented composite flour blend (Onoja, 2007), African yam-bean/wheat blend (Onyechi and Nwachi, 2008). Fermentation and supplementation affected the physical properties (Table 3) of the products when compared with the control.

The preference of crumb and crust color, texture, flavor, mouth feel and taste of bread from WOR_1 blend and the control could be due to their soft crumb with large bubbles, and good crust firmness that resulted in pleasant appearance. It has been shown that flour constituents, particularly protein is critical for the loaf volume and appearance (Kihlberg et al., 2004). WOR_3 and WOR_4 recorded lower scores of general acceptability compared with other test samples, but were still above 60% of the sensory score. This observation could be due to higher incorporation of the legume (orarudi) resulting in firmer crumb with few large bubbles (characteristic of composite bread) as well as beany flavor associated with beans (Patel et al., 2005). The bread from the control and WOR_4 that had better flavor, taste and color than others were much more acceptable. This phenomenon is expected because it has been reported that appearance of food evokes the initial response however, the flavor determines the ultimate final acceptance or rejection by the consumer (Retapol and Hooker, 2006). Although the other test samples had lower overall score, they were higher in nutrient quality/density. This high acceptability of the test breads are in agreement with the reports of other researchers like Hamad and Fields (1979) and Onoja (2007) who equally reported higher acceptability of corn chips, rice chips and bakery products from fermented composite flour.

CONCLUSION AND RECOMMENDATIONS

Fermentation and supplementation improved nutrient quality of the products. Supplementation of cereals with legume to produce enriched reads increased acceptability in the experimental bread. The selected blends could be incorporated into the traditional dishes of those who prefer natural enhancement of nutrients than artificial fortification. The blends and their products have greater promise for increased use of other under-utilized food crops in Nigeria ecosystem. Future study should focus on the shelf life of the products.

Conflict of Interest

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

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