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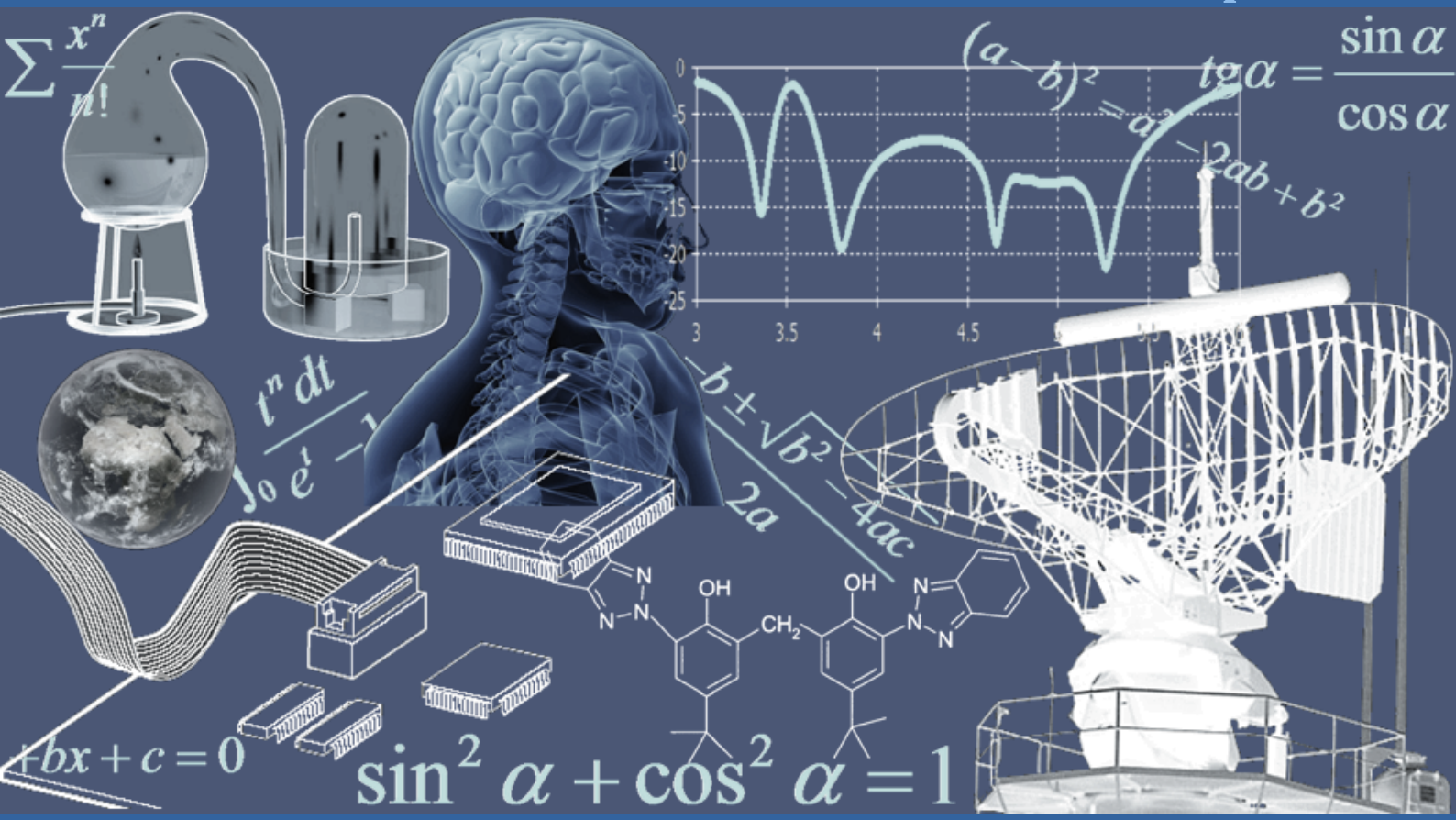

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Risk factors of road crash: An empirical analysis among an Italian drivers sample

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ABSTRACT: With the aim of increasing information about risk factors for crashes in the area of Parma, North Italy, a total of 1489 road accidents occurred in the year 2008 was analyzed. Logistic regression was used to evaluate the association between drivers, accidents characteristics and accident outcomes (killed, severely, and mildly injured). Age classes much involved in road crash were 26-25 and 36-35 years. Men were much frequently responsible for accident than women. The hourly distribution of crash for working days, Saturday and Sunday showed that the prevalence was higher during the late night hours (0-3 on Sunday and 20-23 on Saturday, respectively). The youngest age class was involved in a greater number of accidents especially on 0-3 time of day class. About half of road crashes was directly attributed to violations. High-speed, alcohol and drug abuse affected only a small portion of cases. The highest combined risk of dying or being severely injured was found in males, driving a motorcycle. These results will influence transport and local safeties measures and policies, which will change inappropriate behaviors of drivers and protect the least experienced road users.

KEYWORDS: vehicle accidents, risk variables, driver behavior, accident outcomes.

1 BACKGROUND

Road accidents are complex events resulting primary from human, technical, and environmental contributing factors. The notion of factor can be used to pinpoint possible actions on the condition of transportation system components [1], [2], [3] [4], [5], [6] [7], [8], [9]. For example, a level of blood alcohol greater than the legal norm gives rise to actions in the fields of information, control and enforcement [10], [11], [12], [13]. Investigations carried out on accident phenomena have shown the complexity, dynamic character and the significance of many dimensions in accident production.

The present study is focused on the causal factors that have determined road accidents basing on the task-capability interface (TCI) model [14]. In this model, task difficulty arises out of the dynamic interface between the demands of the driving task and the capability of the driver. Where the capability exceeds demand, the task is easy; where capability equals demand the driver is operating at the limits of his/her capability and the task is very difficult. Where demand exceeds capability, then the task is by definition just too difficult and the driver fails at the task, loss of control occurs, and this perhaps leads to a collision. Thus, in essence, task difficulty is inversely proportional to the difference between task demand and driver capability. With a static level of capability, any event that pushes up task demand will therefore reduce this critical difference, increase task difficulty and potentially challenge safety. For instance, the use of a mobile phone can be an

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additional task, which pushes demand beyond driver capability. Driver capability is initially constrained by biological characteristics of the driver, such as information processing capacity and speed, reaction time, physical reach, motor coordination. Built on top of these characteristics are knowledge and skills arising out training and experience. Such knowledge includes formal elements such as rules of the road, procedural knowledge defining what to do under what circumstances and a representation of the dynamic of road and traffic scenario which enables prediction of how this scenario will develop. Skills include control capabilities associated with basic vehicle control as well as handling skills in challenging conditions. Together, these biological characteristics and acquired characteristics through training and experience determine the upper limit of competence of the driver. However, this competence is not necessarily what is delivered at any moment of time because capability is vulnerable to a host of human factor variables including attitude, motivation, fatigue, drowsiness, time-of-day-drugs, distraction, emotion and stress [15]. Any of these can detract from driver competence to yield a somewhat lower level of capability [14], [16].

Added to environmental factors, such as visibility, road alignment, road marking, road signs and signals or other road users who can occupy critical areas in the projected path of the driver, are elements of task demand over which the driver has immediate and direct control, such as speed selection [17], [8], [18] [9], [19], [20]. Task demand and capability are not independent elements. Capability is determined by many variables and one of these is the driver's level of arousal or activation, partly caused by endogenous factors such as the circadian rhythms but under the influence of external stimulation. Also, driver behavior is determined by task difficult perception; in other words, a driver will determine a range of task difficulty that she/he is prepared to accept, a kind of target margin or envelope of task difficulty. What determines the preferred level will be motivation for speed, perceived capability and effort motivation [21]. Drivers' ability to recognize the relationship between the demands of the driving task and their own capability was defined "calibration" and the importance in driver training of putting less emphasis on specific skills training but more on developing a reliable evaluation of the relationship between task demand and capability or feelings of risk should be stressed [1], [22], [9]. The TCI model and the associated hypothesis of task difficulty homeostasis are both descriptive of the interaction of key factors, which influence driver behavior and provide a dynamic control-motivational framework for understanding driver action, very useful in order to design safety network improvements or local safety policies.

Analyzing the underlying psychological mechanisms leading to the errors, it was estimated that approximately 40% of the accidents were due to attentional problems (e.g. lack of care, distraction, failed to look, and lack of attention), approximately 25% were due to perceptual problems (e.g. looked but failed to see, misjudgment of speed and distance) and approximately 15% were due to judgment problems (e.g. lack of judgment and wrong decision). It was also concluded that in 28% of the accidents, road and environmental factors were identified as contributory factors; in 8.5% of the accidents, vehicles features were identified as contributory factors and in 65% of the accidents, the road user was identified as the sole contributor. Despite this, compared to other domains in which human error was considered as a major trouble (e.g. air transport), there has been only limited investigation focusing on causal factors that contribute to accidents [9]. A number of factors consistently emerge in the international literature as contributors to driver crashes. Driver characteristics include age, gender, license status [23], [12], [24], [25], driving experience, consumption of alcohol or drugs [10], [11], [13], [8], fatigue [26], [3], [18], [19], inattention or not wearing seat belts [20].

The main purpose of the present study was to identify the most probable factors for crashes in the area of Parma, North of Italy, and to suggest possible preventive measures. In addition to cars and motorcycles, in this area also bicycles and mopeds are very common means of transportation; they are frequently used by the elderly and by the youth (mopeds can be ridden by anyone aged $\geq$ 14 years without license). Within the European Commission strategy, reducing serious injuries and deaths from alcohol related road accidents is a main priority. Target of European Union is 50% reduction of road death and 40% of serious injures by 2020. Thus, our primary purpose was to determine which factors or combinations of factors play a role in influencing crash occurrence and suggesting possible safety campaigns [27].

2 MATERIAL AND METHOD

2.1 DATA SAMPLING

In Italy, the ISTAT (Istituto Nazionale di Statistica) collects data on all accidents occurred on roads open to public traffic, in which at least one person was killed or injured and in which at least one moving vehicle was involved. The Police officers (Traffic Policemen, Carabineers or Municipal Policemen) who arrive at the site of the accident are responsible for filling the appropriate structured form (Rapporto Statistico di Incidente Stradale or ISTAT/CTT/INC) and for sending it to ISTAT. Information reported includes time and place of the accident, characteristics of the involved vehicles, gender and age of drivers, injured passengers and pedestrians. The ISTAT definition of 'trucks' includes vehicles used for carrying objects only,

trailer trucks with tow, articulated vehicles, semitrailers, vehicles equipped with special instruments, tractors and vehicles used for towing only. On the contrary, vans and pickups are considered as cars. Information about seat belt and helmet use at the time of accident is collected but neither on airbags nor on uses of child restraint systems. Complete information is acquired for up to three drivers. If more than three vehicles are involved in the same incident, the exceeding drivers can only contribute to the total number of injured persons, with no distinction from other vehicles occupants. Consequences of accidents are defined as non-fatal injury (regardless of its severity) or fatal injury (if death occurs within 7 days from the date of the accident). Police reports grossly underestimate the current prevalence of driving under the influence of alcohol (DUI) and a few individuals are tested for levels of blood alcohol concentration (BAC) since such an exam was not performed by forensic pathologists on the accident victims.

Demographic, accident and injury characteristics on all road crash occurred in the province of Parma during the year 2008 were obtained from police records. Since the goal of our analysis was to identify factors influencing road accidents, we chose to use the driver, rather than any subject involved in the accident, as the unit of analysis. In fact, the driver, but generally not the passenger, may hold some responsibility of the accident and should be the main target of prevention. Initially we considered the following characteristics: a) gender, b) age, c) nationality, d) type of vehicles, e) road surface, f) road type, g) road lighting, h) day time, i) day of the week, l) road characteristics, m) weather condition, n) accident causes, o) traffic density, p) injury, q) death. The severity of injuries was graded according to the "*New Injury Severity Score*" (NISS) [28]. We asked to four master degree students to independently classify each crash. These students had previously had road safety training, including practical work on analyzing accident cases. They had also trained on a different sampling of accidents and coding systems until the total agreement on occurrence or non-occurrence of specific variables in police reports was gained. JIAS hopes that Researchers, Graduate students, Developers, Professionals and others would make use of this journal publication for the development of the scientific research. Accepted papers are available freely with online full-text content upon receiving the final versions, and will be indexed at major academic databases. There is no submission or publication fee [5], [6], [7].

2.2 STATISTICAL ANALYSIS

For all accidents combined, we estimated the likelihood of being involved in a severe versus non-severe or fatal crash. For car, motorcycles and truck drivers, we estimated both the likelihood of being severely injured versus not severely injured. The odds ratio (OR) was used to estimated the likelihood of the more severe outcome as compared with the lesser serious, under the condition that a road crash with at least one lesion had occurred. Therefore, the ORs we report are estimates of the relative risk of people being injured given that they have been involved in an accident. Multivariate logistic models included terms for gender and age of involved persons, vehicle type, road type, time of day and weekday. Two tailed 95% CI were also computed. The models were tested against the global null hypothesis using the log likelihood ratio test. Their goodness of fit was tested using the Hosmer and Lemeshow test [29]. According to traffic police reports, a series of personal characteristics and road variables were investigated. As certain circumstances were rare, the factor levels for the analysis of variance were grouped as in previous investigation [3]:

- Gender (*male, female*);
- Seven age classes (*< 18, 18-25, 26-35, 36-45, 46-55, 56-65, over 65 years*);
- Nationality (*Italian, non-Italian*);
- Three classes of type of motor vehicles: *motorcycle, car and truck* (that includes: *ambulance, bus, and lorry with trailer*) while *agricultural machine* and *velocipede* were eliminated;
- Two classes for road surface (*dry* and *wet*, that includes: *wet, damp and frozen*);
- Road type (*urban, extra-urban, tangential, highway*);
- Two classes of road lighting: *good, insufficient* (that includes *insufficient* and *poor*);
- Six time intervals within a day (*0-3, 4-7, 8-11, 12-15, 16-19, 20-23*);
- Three classes for the day of the week (*working day, Saturday* and *Sunday*);
- The variable "road characteristics" was eliminated;
- Weather condition (*rain, fog, snow*);
- Accident causes (*violations, speed*, *alcohol or drug, loss of control, dangerous driving, block on roadway, lack of attention, stroke, doze off, failure, not reported*);
- Traffic density (*normal, poor* and *intense*);
- Injured prevalence (*slight* and *severe injured*);
- Death prevalence.

Possible differences in proportions of categorical variables were subjected to a chi-square (χ^2) test. To calculate the association between crashes and any probable risk factor, adjusted odds ratios (OR) with 95% confidence interval (95% CI) were obtained through a multivariate unconditional logistic regression [29]. All potential confounding factors were entered into the logistic model. In this model the odds ratio can be considered as a relative risk. Unadjusted ORs are not calculated since the interest of the present study was not aimed to establish a causal relationship for each single factor. Data were subjected to the statistical analyses by means of SPSS 14.0 software.

Table 1. Characteristics of crash cases and distributions of demographic variables, vehicle, road and factors derived from police records

***Gender of driver* n (%)**	
Female	357 (24.0%)
Masculine	1132 (76.0%)
Age of driver classes	
< 18	14 (0.9%)
18-25	248 (16.7%)
26-35	384 (25.8%)
36-45	346 (23.2%)
46-55	226 (15.2%)
56-65	128 (8.6%)
>65	141 (9.5%)
Unknown	2 (0.1%)
Nationality	
Italian	1102 (74.0%)
Non Italian	244 (16.4%)
Unknown	143 (9.6%)
Type of Vehicle	
Motorcycle	94 (6.3%)
Car	1199 (80.5%)
Truck	150 (10.1%)
Unknown	46 (3.1%)
Road surface	1173 (78.8%)
Asphalted	21 (1.4%)
Slipping	295 (19.8%)
Unknown	
Road condition	986 (66.2%)
Dry	201 (13.5%)
Wet	302 (20.3%)
Unknown	
Traffic	655 (44.0%)
Normal	340 (22.8%)
Poor	188 (12.6%)
Intense	306 (20.6%)
Unknown	
Day of the week	1074 (72.1%)
Working days	216 (14.5%)
Saturday	199 (13.4%)
Sunday	
Road Type	848 (57%)
Urban	499 (33.5%)
Extra-urban	100 (2.6%)
Tangential	100 (6.7%)
Highway	3 (0.2%)
Unknown	

2. RESULTS

3.1. FREQUENCIES DISTRIBUTION OF ROAD CRASHES

In Table 1. are reported the characteristics of cases, the distributions of demographic variables, accidents, the vehicle, the road and the other factors obtained from police records. Drivers mean age was 40.86 years (SD=15.819). As far as the gender is concerned, 1132 (76%) drivers were males, 357 (24%) were females (see Table 1). Accidents appeared not to be severe at first sight and those involving a single vehicle were more likely. A significant difference was shown for incident occurrence in the different age classes (χ^2=472.31, p< .01): The most frequency was found both in 26-35 years (25.8%) and 36-45 years (23.2%). Figure 1. shows the frequencies distribution of road crashes in relation to gender and age. Male drivers were more frequently involved in accident than female drivers but statistical analysis did not reveal any age difference between genders: the majority of road crashes occurred in the same age classes (26-35 and 36-45). As far as the variable "*Nationality*", the great majority of accident (74%) occurred to Italian drivers contrasting with the 16.4 percent occurred to non-Italian drivers Concerning the "*Vehicle type*" we found the maximum number of accident for "*Car"* (80.5%), followed by "*Truck"* drivers (10.1%). The general conditions and driving situations more frequently represented included: "*Asphalted*" (78.8%) and "*Dry*" (66.2%) road, in *"Normal*" (44.0%) traffic condition, and in "*Urban*" road (57%). Concerning the variable "*Lighting*", the great majority occurred in "*Good*" lighting conditions (68.2%).

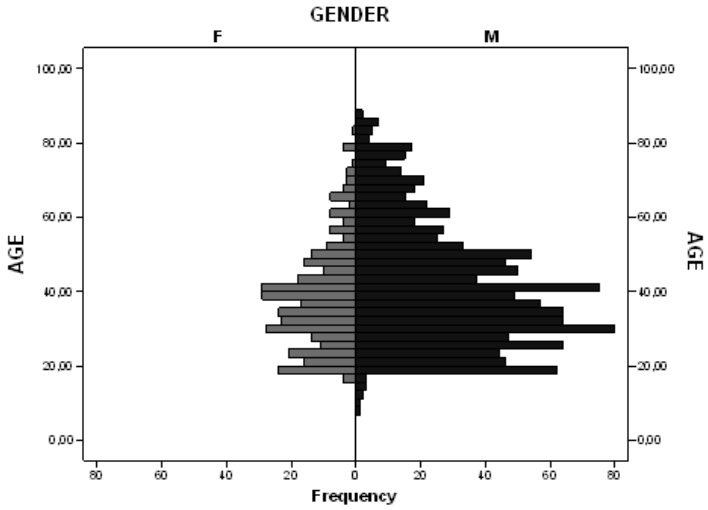

Fig. 1. Distribution of road crashes for gender and age

3.2. DISTRIBUTION OF ACCIDENT FOR DAYS AND TIME RANGES

Relative to the day of the week, the maximum number of accidents occurred on working days (n=1074), while 216 and 199 accidents occurred during Saturday and Sunday, respectively. The day of the week was marked as slightly significant in the statistical analysis but interacted significantly with other factors, producing different time and age patterns. A significant difference shows that crash occurrence is different along the different time of the days of the week (χ^2=58.99, p<.001). The hourly distributions of accidents are compared in Table 2 where standardized cell residuals scores are reported. During working days, 0-3 hour range leads to the fewest road accidents. On the contrary, on Saturday between 20-23 hour range and particularly between 0-3 on Sunday, the impact of road crashes was significantly higher.

Table 2. Distribution of accident for days and time ranges

	Accidents: timetable					
	0-3	**4-7**	**8-11**	**12-15**	**16-19**	**20-23**
Working days	**5.7** ***-2.3***	11.3 *.0*	21.1 *1,2*	23.3 *.6*	28.9 *.6*	9.7 *-1.5*
Saturday	**11.2** *1.8*	12.6 *.6*	15.8 *-1.2*	18.6 *-1.2*	25.1 *-.8*	**16.7** ***2.4***
Sunday	**14.6** ***3.5***	9.6 *-.7*	14.6 *-1.5*	21.7 *-.2*	25.8 *-.6*	13.6 *1.0*

In other words, the highest risk was found from Saturday night to the first hours of Sunday. The counts of accidents occurred on Saturday and Sunday nights exceeded the values of Friday and working days. A significant association is also showed in the accidents occurrence between time and gender (χ^2=19.708, p<.001) where a different distribution is shown in the 20-23 class with the most accidents frequency for male drivers (84.1%). A remarkable association was found among the age distribution and the time of day (χ^2=81.672, p<.001): while all age classes show accidents majority in 8-11, 12-15 and 16-19 day span, the youngest drivers were involved in a greater number of accident which greatly increased on 0-3 time of day class (standardized cell residuals: 3.6). As far as the causes of road crashes, 739 (49%) were attributed to violations. High-speed was the second more frequent road crashes cause (12.8%) surpassing alcohol and drug abuse (11.6%), and loss of vehicle control (9.7%).

3.3. ACCIDENT OUTCOMES AND RISK FACTORS

Concerning accident outcome, four consequences have been considered (dead, severe injuries, mild injures, and no injuries). A significant association between type of vehicle and risk involvement in injures outcome was found. If vehicle was a motorcycle, the risk involvement in severe injuries is very high (OR=2.85; 95% IC:1.72-4.76), and the probability to not report any injury notably low (OR=.432; 95% IC:.155-1.205). About twenty five percent of all motorcycle drivers resulted severely injured in comparison with truck (13.5%) and car drivers (10.9%). The majority (69.1%) of the people who drove a motorcycle reported also mild injuries. Truck drivers had a greater chance to not report any injury (OR=2.12; 95% IC:1.10-4.0). Driving a car showed a high risk for dying (even if this finding did not reach statistic significance). Moreover, women are more likely not seriously injured and less likely seriously injured, in other words they are less at risk of severe injury (OR= .465, 95%IC: .288-.752) and death (OR= .753; 95%IC: .415-.837). No significant relationship was found between drug abuse and accident outcomes.

3. DISCUSSION

The present study had both an applied and a theoretical focus, investigating the role of different factors within the context of road accident. Road crash reports can be a very useful and efficient means for studying driving behavior and incident factors and causes. But at their best Police reports can't provide reliable in-depth information about behaviors, as well as about the motives leading to risky driving and errors. The statistical analysis of our data revealed that predictors of road accident were violations, gender, age, two-wheeled vehicles, days and time of the days. Violations and fast driving were reported in many studies more frequently by males than by females. Women were involved in fewer accidents than men. In addition, female drivers were less likely to die or be severely injured. The most likely explanation is the difference in risk-taking behavior between males and females [30]. The age ranges comprised between 26-35 years (25.8%) and 36-45 years (23.2%) resulted more involved in accidents, particularly when men riding motorcycles than when driving cars or trucks.

Our study showed that Saturdays and Sundays are particularly dangerous for drivers, particularly during late night hours; the consumption of alcohol, that is a well-known risk factor for severe motor vehicles crashes, may be particularly high during night hours. Moreover, alcohol intake may be increased by the cold because of its warming effects on drinkers. Alcohol dependence may lead to higher risk of injury and accidents, but acute effects among inexperienced drinkers, such as adolescents, may put the person at a greater, short term, risk. As to the influence of time of the day, our data put into evidence clear circadian and semi-circadian effects with evidence of high incidence of sleep-related accident around 0 and 3 a.m. and in the early afternoon. Such a pattern is highly correlated with the well-known circadian and semi-circadian rhythm of alertness-sleepiness, reported by several laboratories [3]. Sleepiness is a typical manifestation of the biological need of sleep and increased sleepiness is associated with a decrement in reaction time, psychomotor coordination, information processing and decision making which influence the probability of having accidents. Although the lack of appropriate norms

for road use, day of the week and time of day do not allow discriminating between the exposure and the risk of sleep related-accidents at the level of detail used in this study, our data account for a specific risk for younger drivers. We can infer that old drivers are less exposed to risk of accidents during the night hours and are more likely to drive during the afternoon, so that the high incidence of accidents for younger adults during the night could be interpreted as a mixture of higher risk and higher exposure. This view is corroborated by data on the difference between working days and weekends: the occurrence of late night socializing and poor sleep habits on Saturdays and Sundays are likely to prevent younger drivers from getting sufficient sleep thus increasing their risk and their exposure at the same time. These data could be the expression of a strong rise in accidents due to the increased number of young people adopting a lifestyle that involves behaviors which put them at risk, i.e., late night socializing, early morning driving after sleep deprivation, fatigue due to dancing and noisy environment and possible interference due to alcohol and psychotropic substances.

4. CONCLUSION

The findings of this study may be used as starting point for speculations about possible attitude- and behavior-change strategies. In terms of interventions designed to reduce aggressive behavior on the road, the finding suggests that interventions helping drivers to increase self-awareness about human factors variables, managing stress and relaxing while driving may help to reduce these behaviors, particularly with reference to younger, male, and high-mileage drivers. Some methodological limitations of the present investigation that may limit the generalizability of our findings should be acknowledged. Although we are unable to address personal risk factors for fatal accidents such as risk-taking behavior or DUI because of lack of information, we identified groups of subjects and conditions that were associated with higher road crash risk. In conclusion, our data presents important implications, both for road-safety researchers and for social psychologists. Because the contributory role of many risk factors on Italian roads is still underestimated, a more detailed assessment of road incident and related factors is necessary. Our findings promote the inclusion of crash circumstances, individuals and environmental data. By achieving a greater understanding of the specific types of aberrant behavior on the roads and by investigating the groups that perform these various behaviors to a greater or lesser extent, safety campaigns can be designated to target particular individuals in relations to specific target groups together with their beliefs, norms, and attitudes.

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REFERENCES

[1] A. Bergomi, G. Violi, S. Rovesti, P. Bussetti, A., Ferrari and R. Vivoli, “Ruolo di alcuni fattori psico-fisiologici sulla sicurezza alla guida”, *Annali di Igiene, Medicina Preventiva e di Comunità*, no. 22, pp. 387-400, 2010.

[2] C. A., Field and G. O’Keefe, “Behavioural and psychological risk factors for traumatic injury”, *Journal of Emergency Medicine*, no. 26, pp. 27-35, 2004.

[3] S. Garbarino, L. Nobili, M. Beelke, F. De Carli, V. Balestra and F. Ferrillo “Sleep related vehicle accidents on Italian highways”, *Giornale Italiano di Medicina del Lavoro ed Ergonomia*, no. 23, pp. 430-434, 2001.

[4] E. Javouhey, A.C. Guérin and M. Chiron, “Incidence and risk factors of severe traumatic brain injury resulting from road accidents: A population-based study”, *Accident Analysis and Prevention,* no 38, 225–233, 2006.

[5] T. Kontogiannis, Z. Kossiavelou and N. Marmaras “Self-reports of aberrant behaviour on the roads: Errors and violations in a sample of Greek drivers” *Accident Analysis and Prevention*, no. 34, pp. 381–399.

[6] R. Lawton, D. Parker, A: S. R. Manstead and S. Stradling, “The role of affect in predicting social behaviours: The case of road traffic violations”, *Journal of Applied Social Psychology*, no. 27, pp. 1258–1276.

[7] D. Parker, R. West, S. Stradling and A. S. R. Manstead, “Behavioural characteristics and involvement in different types of traffic accident”, *Accident Analysis and Prevention*, no. 27, pp. 571-581, 1995.

[8] E. Petridou and M. Moustaki, “Human factors in the causation of road traffic crashes”, *European Journal of Epidemiology*, no. 16, pp. 819–826, 2000.

[9] N. A. Stanton and P. M. Salmon, “Human error taxonomies applied to driving: A generic driver error taxonomy and its implications for intelligent transport systems”, *Safety Science*, no. 47, pp. 227-237, 2009.

[10] G. Borges, C. Cherpitel and M. Mittleman, "Risk of injury after alcohol consumption: A case-crossover study in the emergency department", *Social Science & Medicine*, no. 58, 1191-1200, 2004.
[11] D. Giovanardi, C.N. Castellana, S. Pisa, B. Poppi, D. Pinetti, A. Bertolini and A. Ferrari "Prevalence of abuse of alcohol and other drugs among injured drivers presenting to the Emergency Department of the University Hospital of Modena, Italy", *Drug and Alcohol Dependence*, no. 80, pp. 135-138, 2005.
[12] Z. A. T. Harrel and N. Karim, "Is gender relevant only for problem alcohol behaviours? An examination of correlates of alcohol use among college students", *Addictive Behaviors*, no. 33, pp. 359-365, 2008.
[13] K. L. Movig, M. P. Mathijssen, P. H. Nagel, T. van Egmond, J. J. de Gier, H. G. Leufkens and A. C. Egberts, "Psychoactive substance use and the risk of motor vehicle accidents", *Accident Analysis and Prevention*, no. 36, pp. 631-636, 2004.
[14] R. Fuller, "Towards a general theory of driver behavior", *Accident Analysis and Prevention*, no. 37, pp. 461-472.
[15] P. Ulleberg "Personality subtypes of young drivers. Relationships to risk-taking preferences, accident involvement, and response to a traffic safety campaign", *Transportation Research Part F: Traffic Psychology and Behaviour*, no. 4, pp. 427-443, 2001.
[16] J. A. Horne and L. A. Reyner, "Sleep related vehicle accidents", *British Medical Journal*, no. 310, pp. 565-567, 1995.
[17] J. F. O'Hanlon and E. R. Volkerts, "Hypnotics and actual driving performance", *Acta Psychiatrica Scandinavica*, no 332, pp. 95–104, 1986.
[18] P. Philip, J. Taillard, P. Sagaspe, C. Valtan, M. Sanchez-Ortuno, N. Moore, A. Charles and B. Bioulac, "Age, performance and sleep deprivation", *Journal of Sleeping Research*, no. 13, pp. 105-110, 2004.
[19] H. Summala, and T. Mikkola, "Fatal accidents among car and truck drivers: Effects of fatigue, age and alcohol consumption", *Human Factors*, no. 36, pp. 315-326, 1994.
[20] F. Valent, F. Schiava, C. Savonitto, T. Gallo, S. Brusaferro and F. Barbone "Risk factors for fatal road traffic accidents in Udine, Italy", *Accident Analysis and Prevention*, no. 34, pp. 71-84.
[21] P.N. Blockey and L.R. Hartley, "Aberrant driving behaviour: Errors and violations", *Ergonomics,* no. 38, pp. 1759–1771, 1995.
[22] Fisher, D.L., & Pollatsek, A, Novice driver crashes: Failure to divide attention or failure to recognize risks, In A.F. Kramer, D.A. Wiegmann and A. Kirlik (Eds.), *Attention: From theory to practice*, pp. 134–153, New York, NY, Oxford University Press, 2007.
[23] P.R. Chapman and G. Underwood, "Visual search of driving situations: Danger and experience", *Perception,* no. 27, pp. 951-964, 1998.
[24] F. Lucidi, A. M. Giannini, R. Sgalla, L. Mallia, A. Devoto and S. Reichmann, "Young novice driver subtypes: Relationship to driving violations, errors and lapses", *Accident Analysis and Prevention,* no. 42, pp. 1689–1696, 2010.
[25] A. Vorko-Jovic, J. Kern and Z. Biloglav, "Risk factors in urban road traffic accidents", *Journal of Safety Research,* no. 37, pp. 93 – 98.
[26] J. Connor, G. Whitlock, R. Norton and R. Jackson, "The role of driver sleepiness in car crashes: A systematic review of epidemiological studies", *Accident Analysis and Prevention,* no. 33, 31-41, 2001.
[27] P. Delhomme, W. De Dobbeleer, S. Forward and A. Simoes, "*Manual for designing, implementing and evaluating road safety communication campaigns"*, Belgian Road Safety Institute, Brussels, 2009.
[28] T. Osler, S. P. Baker and W. Long, "A modification of the Injury Severity Score that both improves accuracy and simplifies scoring", *Journal of Trauma-Injury Infection & Critical Care*, no. 6, pp. 922-926, 1997.
[29] D. W. Hosmer and S. Lemeshow, S., *Applied Logistic Regression*, New York, Wiley, 1989.
[30] C. Turner, R. McClure and S. Pirozzo, "Injury and risk-taking behavior – a systematic review", *Accident Analysis and Prevention*, no. 19, pp. 133-140, 2004.

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Validity and Reliability of the Big Five Personality Traits Scale in Malaysia

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ABSTRACT: This study examined the validity and reliability of the Big Five personality traits scale in Malaysia. The sample of the study comprised 343 nurses in the health tourism hospitals. Administered on-site method was used for data collection. The respondents were required to rate their degree of agreement in the questionnaires with regard to their personality traits. Initial pilot test results showed an excellent internal reliability for each of the subscales. However, validity test extracted only four factors of the Big Five Inventory with factor loadings ranging from 0.573 to 0.803. The four factors were extraversion, conscientiousness, neuroticism and openness to experience. The reliability coefficients for all the extracted factors were above 0.7. Thus, the validated measures of the Big Five Inventory were deemed consistent and reliable throughout the study. Plausible reasons were stated in this study to explain the outcome of the factor analysis. Although many researchers recognized the Big Five Inventory as necessary and adequate to describe the structure of personality globally, this paper suggests that conducting validity and reliability test for Big Five Inventory is necessary when the study is conducted in countries with different cultural perspectives. In addition, it also offers suggestion to healthcare managers to identify their nurses' personality traits by using the validated measures. By knowing the nurses' personality traits, one can predict their work behaviors.

KEYWORDS: Openness to experience, conscientiousness, extraversion, agreeableness, neuroticism, nurses.

1 BACKGROUND OF PERSONALITY TRAITS

Starting in the 1930's, Allport has conducted a lexical study of the personality-relevant terms in unabridged English dictionary. Four major categories of personality traits were identified [1]. The first category defined personality traits as personalized and generalized determining stable modes and tendencies-consistent of an individual's adjustment to his environment. The second category encompassed temporary activities, moods and states. The third category comprised highly evaluative judgments of personal conduct and reputation and the last category embraced physical characteristics, talents, capacities and terms that could not be assigned to any of the other three categories [1]. Since then, Cartell's structured-based systems theory describes personality as a system with regard to the environment and attempts to explain the complicated transactions between them as they generate change and growth in the individuals [2]. Cartell viewed theory of personality as a theory which must explain and analyze the goal-directed motivation of individuals because motivational learning always guides one's actions. In 1949, Cartell successfully reduced 99 percent of Allport's work and introduced a sixteen Personality Factor Scale (16 PF Scale) as a way to measure personality through a self-assessment instrument [3]. Cartell's pioneering study and the accessible of short list of variables has stimulated other researchers to further investigate the dimensions of personality and lead to the discovery of Big Five dimensions [4]. Some psychological staffs have summarized findings by Cartell by classifying those highly similar and correspond to what would later become known as the Big Five. This five factor structure has been initially named extraversion, agreeableness, conscientiousness, emotional stability and culture [4].

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Further work by [5] showed that words identified by Allport earlier also fit into the Five Factor Model (FFM). This model encapsulates the main tenets of human personality into five main descriptors that are used to categorize the variety of human behaviors into an organized taxonomy [6]. The five factors are commonly labeled as openness to experience, conscientiousness, extraversion, agreeableness and neuroticism. They are recognized as the Big Five [7]. Several theories have conceptualized the Big Five as relational constructs. Interpersonal theory emphasizes the interpersonal motives of agency and communion in relationships. It interprets the Big Five dimensions in terms of their interpersonal implications owing to agreeableness and extraversion is the most obvious interpersonal dimensions [8]. Socio-analytic theory stresses on the social functions of self and other perceptions [9]. According to [9], traits are socially constructed to assist interpersonal functions and used as a tool to encode and communicate reputations whereas personality is generally defined as the ability to impress others and to get along well with people [2]. However, defining personality in terms of personable and sociable behaviors alone is not sufficient as there are many other behaviors associated with personality in the wider sense. Hence, in personality psychology, trait concept was utilized to explain the consistent patterns of a person's behaviors [10].

The Big Five has provided support for the existence of the Five Factor Model (FFM) and its universal application [11]. The Big Five factors were found positively associated with distinct aspects of contextual performance as accordance to meta-analytic research studies [12]. The validity of the Big Five is strongly supported by empirical evidence specifically when it represents taxonomy to describe human personality [13]. Owing to the wide acceptance of Big Five to describe the most salient aspects of personality [1], it has been comprehensively used in recent organizational and other applied research. However, despite the strong validity of Big Five, it is necessary to re-examine the measures validity by performing factor analysis especially when the research is conducted in countries with different cultural perspectives. This is to reasonably ensure that the instrument is measuring the concept the researcher intends to measure within the research context.

2 THE BIG FIVE INVENTORY

Measures of the personality traits in this study were based on the Big Five Inventory (BFI) developed by [1]. There were a total of 44 items of measurement. The researcher adapted these measures because they were short instruments and easy to understand by the respondents. In addition, BFI also shows high convergent validity with other self-report scales and with peer ratings of the Big Five [14]. Previous tested reliability of the BFI was typically ranged from 0.79 to 0.88 [15]. The measurement items are listed in Table 1.

Table 1. Big Five Inventory by John and Srivastava (1999)

Dimensions	Items
Openness to Experience	1. I am curious about many things. 2. I always come up with new ideas. 3. I am creative and a deep thinker. 4. I have an active imagination. 5. I am inventive. 6. I value artistic experiences. 7. I prefer work that is routine.(R) 8. I like to reflect and play with ideas. 9. I have few artistic interests.(R) 10. I am advanced in art, music or literature.
Conscientiousness	1. I can be somewhat careless.(R) 2. I carry out my job thoroughly. 3. I am a reliable worker. 4. I tend to be disorganized.(R) 5. I tend to be lazy.(R) 6. I persevere until the task is finished. 7. I do things efficiently. 8. I make plans and follow through with them. 9. I am easily distracted. (R)
Extraversion	1. I see myself as someone who is reserved.(R) 2. I am talkative. 3. I am full of energy. 4. I am passionate and spirited. 5. I tend to be quiet.(R) 6. I have an assertive personality. 7. I am sometimes shy and inhibited.(R) 8. I am outgoing and sociable.
Agreeableness	1. I am helpful and unselfish with others. 2. I tend to find fault with others.(R) 3. I tend to start quarrel with others.(R) 4. I have a forgiving nature. 5. I am generally trusting. 6. I can be cold and isolated.(R) 7. I am considerate and kind to almost everyone. 8. I am sometimes rude to others.(R) 9. I like to cooperate with others.
Neuroticism	1. I am relaxed and can handle stress well.(R) 2. I am depressed and blue. 3. I can be tense. 4. I worry a lot. 5. I am emotionally stable and not easily upset.(R) 6. I can be moody. 7. I remain calm in tense situation.(R) 8. I get nervous easily.

3 METHOD

Participants were consisted of 343 nurses in the health tourism hospitals in Malaysia (295 females, 48 males, age range from 18 to 55 years old). In order to have a better response rate, administered on-site method by [16] was used for data collection. This method is very efficient in generating large amount of data especially when the survey is conducted during meeting with the respondents [17]. Respondents were required to rate their degree of agreement by assigning point on a

Likert scale ranging from 1 (*Strongly disagree*) to 5 (*Strongly agree*). They were also reminded that there is no right or wrong answer to the questions and confidentiality of their answers is guaranteed.

4 RESULTS AND DISCUSSIONS

4.1 PILOT TEST

According to [18], data collected from a small sample of 15 to 30 subjects of the study served as a guide for the larger study. The survey instruments were pilot tested on a small scale sample of respondents in one of the selected health tourism hospitals. A total of 35 questionnaires were distributed to the respondents to collect data. The pilot test was completed with a total of 30 responses. The instruments were then tested for internal reliability based on the data collected from the pilot survey. Table 2 shows the overall internal reliability for each of the instruments during the pilot test.

Table 2. Internal Reliability for Personality Traits

Instrument	Item	Cronbach's Alpha
Personality Traits		
• Openness to Experience	10	0.859
• Conscientiousness	9	0.888
• Extraversion	8	0.774
• Agreeableness	9	0.904
• Neuroticism	8	0.736

The results of the internal reliability showed an excellent reliability coefficient for each of the instruments. The Cronbach's Alphas obtained were above the minimum acceptable level of 0.7 as suggested by [19], [20] and [21]. Therefore, all the items require no modification and ready to be administered in the survey to the remaining of the sample.

4.2 VALIDITY TEST

Upon receiving all the responses in the survey, factor analysis was conducted for the Big Five personality traits scale. Prior to conduct factor analysis, inspection of the correlation matrix was done and it indicated that most of the item coefficients were 0.3 and above. The analysis of Measure of Sampling Adequacy and Partial Correlations showed that the value of Kaiser-Meyer-Olkin for measuring of sampling adequacy (KMO/MSA) was 0.806. It exceeds the minimum value of 0.6 for a great factor analysis (0.8 – 0.9) [22]. Bartlett's test of sphericity was significant at $p<0.001$ where it supported the factorability of the correlation matrix. Table 3 shows that the Principal Component Analysis (PCA) extracted four factors compared to the original of five factors after cross loadings were deleted and loadings below 0.35 were discarded. These 4 factors cumulatively captured 48.572% of the variance. Factor 1, 2, 3 and 4 contributed 20.178%, 11.589%, 9.638% and 7.167% of the common variance with Eigenvalues of 4.237, 2.434, 2.024 and 1.505 respectively. The factor loading values for the scales were in the range of 0.573 to 0.803. Drawing upon the factor analysis results, items that loaded on Factor 1 (7 items) were labeled as openness to experience, Factor 2 (5 items) were categorized as extraversion, Factor 3 (5 items) were named as neuroticism and Factor 4 (4 items) were designated as conscientiousness.

Table 3. Factor Analysis for Personality Traits

Items	Description	Factor Loading			
		1	2	3	4
E2	I am talkative.		0.701		
E3	I am full of energy.		0.698		
E4	I am passionate and spirited.		0.718		
E5	I tend to be quiet (R).		0.616		
E6	I have an assertive personality.		0.682		
C2	I carry out my job thoroughly.				0.803
C3	I am a reliable worker.				0.685
C7	I do things efficiently.				0.645
C8	I make plans and follow through with them.				0.690
N3	I can be tense.			0.681	
N4	I worry a lot.			0.618	
N5R	I am emotionally stable and not easily upset.			0.717	
N6	I can be moody.			0.671	
N7R	I remain calm in tense situation.			0.717	
O2	I always come up with new ideas.	0.620			
O3	I am creative and a deep thinker.	0.615			
O4	I have an active imagination.	0.631			
O6	I value artistic experiences.	0.646			
O8	I like to reflect and play with ideas.	0.721			
O9R	I have few artistic interests.	0.686			
O10	I am advanced in art, music or literature.	0.573			
Eigenvalue		4.237	2.434	2.024	1.505
Percentage of Common Variance		20.178	11.589	9.638	7.167
Cumulative Percentage (%)		20.178	31.767	41.405	48.572
KMO/MSA		0.806			
Bartlett's Test of Sphericity (sig.)		0.000			

The result of the factor analysis has omitted the dimension of agreeableness. This indicates that the measuring items of agreeableness were not reflecting the concept it is intended to measure. Agreeableness, which is described as likeability or compliance, does not exist as one of the traits possess by the respondents although it is regarded as an obvious interpersonal dimension in the Big Five [8]. The exclusion of agreeableness may due to the respondent's behavior, nature of work and culture in the organization. In other words, agreeable personnel were not found in the sample of the study. Since the measures of agreeableness has been discarded during factor analysis, the remaining four factors of the Big Five (i.e. openness to experience, conscientiousness, extraversion and neuroticism) shall represent the respondents' personality traits. One plausible reason to explain openness to experience as descriptor of the respondents' personality trait is that they may be good at generating new ideas to help patients to solve their problems. Comparatively, conscientiousness is found to be one of the personality traits because the nursing profession requires responsible, meticulous, hardworking and dependable work behaviors. Previous investigations by past researchers like [23], [24], [25], [26] and [27] have shown conscientiousness is positively linked to service performance. Among other possible reasons to explain extraversion as the respondents' personality trait is that extroverted people are friendly, outspoken, confident and overbearing [25], [28]. Nurses always perform tasks involving extensive interaction with the patients where they have to communicate with them very often. Neuroticism exists among the respondents because nurses usually encounter stressful events in the hospital. According to [29], neurotic personnel have the propensity to experience distress that might affect their behavioral actions. Therefore, there is a necessity to identify nurses' personality traits because by knowing their personality traits, one can predict their work behaviors.

4.3 RELIABILITY TEST

The reliability test results show that the reliability coefficient (Cronbach's Alpha) for each factor of the personality traits was 0.779 (openness to experience), 0.727 (conscientiousness), 0.725 (extraversion) and 0.716 (neuroticism) (see Table 4). Since all the reliability coefficients have surpassed the minimum value of 0.7, the measures were deemed consistent and reliable throughout the study.

Table 4. Reliability Coefficients for Personality Traits

Extracted Factors	Number of Items	Cronbach's Alpha
Openness to Experience	7	0.779
Conscientiousness	4	0.727
Extraversion	5	0.725
Neuroticism	5	0.716

5 THEORETICAL AND PRACTICAL IMPLICATIONS

This study attempts to perform validity and reliability test for the Big Five personality traits scale in Malaysia. In terms of theoretical implication, the results of factor analysis and reliability test have added to the literature of personality traits in relation to its scale validity and reliability. Although many researchers recognized BFI as necessary and adequate to describe the structure of personality globally, it is suggested that conducting another validity and reliability test is necessary when the study is conducted in countries with different cultural perspectives. Therefore, the outcome of this study shall guide future personality research especially in the healthcare context in Malaysia.

In terms of practical implication, the present study offers suggestion to healthcare managers to identify their nurses' personality traits. Managers can use the validated measures in this study to identify their personality traits and assign them into different groups according to their traits. Those nurses with positive traits can be assigned to guide and motivate those with negative traits. By doing so, it can improve their work behaviors and enhance the organization's service performance.

6 CONCLUSION

In conclusion, the validated measures in the present study have accurately measured the concept of Big Five personality traits in the Malaysian context. The reliability of the measures has been found consistent and indicates the homogeneity of the items in the measures. The validated measures can be used by future researchers in similar empirical research.

REFERENCES

[1] O.P. John and S. Srivastava, "The Big-Five trait taxonomy: History, measurement and theoretical perspective." In L.A. Pervin & O.P. John (Eds.), *Handbook of personality: Theory and research* (102-138). New York: Guilford, 1999.

[2] R.M. Ryckman, *Theories of Personality (8th Ed.)*. United States of America: Thomson Learning, Inc, 2004.

[3] J. Feist and G.J. Feist, *Theories of Personality* (5th ed.). New York: McGraw Hill, 2002.

[4] O.P. John, R.W. Robins and L.A. Pervin, *Handbook of Personality: Theory and Research (3rd Ed.)*. New York: Guilford Press, 2008.

[5] L.R. Goldberg, "An alternative description of personality: The Big-Five factor structure," *Journal of Personality and Social Psychology*, vol. 59, pp. 1216-1229, 1990.

[6] J.J.A. Denissen and L. Penke, "Motivational individual reaction norms underlying the Five-Factor model of personality: First steps towards a theory based conceptual framework [Electronic version]," *Journal of Research in Personality,* vol. 42, pp. 1285-1302, 2008.

[7] R.R. McCrae and P.T.Jr. Costa, "Validation of the five factor model of personality across instruments and observers," *Journal of Personality and Social Psychology,* vol. 60, *no.* 2, pp. 329-361, 1987.

[8] J.S. Wiggins and P.D. Trapnell, "A dyadic-interactional perspective on the five factor model," In Wiggins, J.S. (Ed.), *The five factor model of personality: Theoretical perspectives*, pp. 180-207. New York: Guilford Press. 1996.
[9] R. Hogan, "A socio-analytic perspective on the five factor model," In J.S.Wiggins (Ed.), *The five factor model of personality: Theoretical perspectives*, pp. 180-207. New York: Guilford Press, 1996.
[10] D.G. Winter, O.P. John, A.J. Stewart, E.C. Klohnen and L.E. Duncan, "Traits and motives: Toward an integration of two traditions in personality research," *Psychological Review,* vol. 105, pp. 230-250, 1998.
[11] R.R. McCrae and P.T. Costa, "Personality trait structure as a human universal," *American Psychologist,* vol. 52, pp. 509-516, 1997.
[12] J. Hogan and B. Holland, "Using theory to evaluate personality and job-performance relations: A socioanalytic perspective," *Journal of Applied Psychology,* vol. 88, pp. 100-112, 2003.
[13] B.P. O'Connor, "A quantitative review of the comprehensiveness of the five-factor model in relation to popular personality inventories," *Assessment*, vol. 9, pp. 188-203, 2002.
[14] S.D. Gosling, P.J. Rentfrow and Jr.W.B. Swann, "A very brief measure of the Big-Five personality domains," *Journal of Research in Personality,* vol. 37, pp. 504-528, 2003.
[15] V. Benet-Martinez and O.P. John, "Los Cinco Grandes across cultures and ethnic groups: Multi-trait multi-method analysis of the Big Five in Spanish and English," *Journal of Personality and Social Psychology*, vol. 75*, no.* 3, pp. 729-750, 1998.
[16] D. Miller, M.F.R. Kets de Vries and J. Toulouse, "Top executive locus of control and its relationship to strategy-making, structure and environment," *Academy of Management Journal,* vol. 25*,* no. 2, pp. 237-253, 1982.
[17] C.C. Snow and J.B. Thomas, "Field research methods in strategic management: Contributions to theory building and testing," *Journal of Management Studies*, vol. 41, no. 4, pp. 457-480, 1994.
[18] N. Malhotra and M. Peterson, *Basic Marketing Research: A Decision Making Approach*, NJ: Pearson Education, 2006.
[19] J.C. Nunnally, *Psychometric theory*. New York: McGraw Hill, 1978.
[20] J.P. Robinson, P.R. Shaver and L.S. Wrightsman, *Measures of Personality and Social Psychological Attitudes*. San Diego: Academic Press, 1991.
[21] R.F. DeVellis, *Scale development: Theory and applications* (2nd ed.), California: Sage, 2003.
[22] G. Hutcheson and N. Sofroniou, *The multivariate social scientist*. London: Sage, 1999.
[23] H. Liao and A. Chuang, "A multilevel investigation of factors influencing employee service performance and customer outcomes," *Academy of Management Journal*, vol. 47, no. 1, pp. 41-58, 2004.
[24] T.F. Brown, J.C. Mowen, D.T. Donavan and J.W. Licata, "The customer orientation of service workers: Personality trait effects on self-and supervisor performance ratings," *Journal of Marketing Research*, vol. 39, no. 1, pp. 110-119, 2002.
[25] N. Lin, H. Chiu and Y. Hsieh, "Investigating the relationship between service providers' personality and customers' perception of service quality across gender," *Total Quality Management*, vol. 12, no. 1, pp. 57-67, 2001.
[26] R.F. Hurley, "Customer service behavior in retail settings: a study of the effect of service provider personality," *Journal of the Academy of Marketing Science*, vol. 26, no. 2, pp. 115-27, 1998.
[27] A.A. McBride, J.L. Mendoza and S.M. Carraher, "The development of biodata instrument to measure service orientation," *Psychological Reports,* vol. 81, pp. 1395-407, 1993.
[28] K.H. Ehrhart, "Job characteristic beliefs and personality as antecedents of personality of subjective person-job fit," *Journal of Business and Personality,* vol. 21, no. 2, pp. 193-227, 2006.
[29] R.R. McCrae and O.P. John, "An introduction to the five-factor model and its applications," *Journal of Personality,* vol. 60, pp. 175-215, 1992.

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Optimizing the Bread Formulation of Sudanese Wheat Cultivars

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ABSTRACT: The objective of this study was to develop an effective bread formulation to achieve high loaf volume with good quality breads for Sudanese wheat cultivars. The response of Sudanese commercial wheat flour to different additives was studied. Alpha amylase, ascorbic acid (AA) and diacetyl tartaric esters of monoglyceride (DATEM) were tested in combination to produce bread with high loaf volume and good quality. Combination of AA (50 ppm) and DATEM (0.25%) with alpha amylase (0.05%) had a marked effect on the dough rheology. Dough development time, water absorption, and stability were reduced considerably. However the degree of softening, resistance to extension and energy were significantly increased. Incorporation of the combined improvers significantly increase the bread specific volume from 2.95 to 3.92 cm^3/g for Argeen, 2.85 to 4.28 cm^3/g for WadiElneel, 2.60 to 4.51 cm^3/g for Nepta, and 3.40 to 5.07 cm^3/g for Australian wheat (control). The high response of the Sudanese wheat flours to the improvers investigated indicated the possibility of producing high loaf volume with good quality breads from Sudanese wheat. However, the overall quality scores showed considerable improvement when these improvers were used in the formula in combination. Further research should be done to encourage using locally available ingredients as bread improvers.

KEYWORDS: Argeen, Dough rheology, Nepta, Sudanese wheat, Wadi Elneel.

1 INTRODUCTION

Wheat cultivars produced in different parts of the world differ greatly in their intrinsic protein qualities and quantities, the quantity is influenced mainly by environmental factors, but the quality of protein is mainly a heritable characteristic [1]. Baking quality is determined by the physical properties of dough, its oxidative properties, the flour water absorption, bread volume, and the color of the bread crumb and crust. The baking properties of a dough sample depend on the flour's ability to form dough that, after mixing and during fermentation, has appropriate physical properties. The strength thus contributed to the dough is an important part of the bread making quality of the flour [2]. For several thousand years, bread has been one of the major constituents of the human diet, making the baking of yeast-leavened and sourdough breads one of the oldest biotechnological processes. In wheat bread making, flour, water, salt, yeast and/or other microorganisms are mixed into a visco-elastic dough, which is fermented and baked [3]. During all steps of bread making, complex chemical, biochemical and physical transformations occur, which affect and are affected by the various flour constituents. In addition, many substances are nowadays used to influence the structural and physicochemical characteristics of the flour constituents in order to

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optimize their functionality in bread making [3]. The optional ingredients such as acids, enzymes, surfactants, sugar, milk or milk solids and improvers are included in bread formulation to improve nutritional, sensory and keeping quality of bread [4]. These ingredients also have a significant effect on rheological and bread making properties. The salt imparts flavor, taste and strength to the dough [4]. Fat is used for providing softness to the bread. It acts as a plasticizer, improves volume and imparts antistaling properties to bread. Dairy ingredients are included in bread formulation for nutritional benefits through increasing calcium content and improving protein efficiency ratio. Ascorbic acid is an acceptable oxidizing agent to replace potassium bromate in natural, organic and health breads. Addition of ascorbic acid increased dough strength, reduced dough stickiness [5], improves crust characteristics, and crumb structure and colour [6]. Bread volume is associated with the use of alpha amylase in bread making. Alpha amylase also associated with an improvement of crumb grain, and antistaling effect [7], [8]. Diacetyl tartaric esters of monoglycerides (DATEM) are added to the dough to improve the mechanability of the dough and the quality of the baked product by increasing bread volume [5], [9] and produce finer crumb structure [10]. In Sudan, wheat is a strategic field crop, since it constitutes the main staple food for most of the urban and rural population. Wheat cultivation in Sudan expanded recently and occupying the largest area in Sudanese irrigated schemes, and it is the second most important cereal crop after sorghum in the country [11]. The consumption of wheat bread in Sudan is increasing in both rural and urban areas as a consequence of changing taste, convenience and consumer subsidies. However, bread can only be made from imported high gluten wheat which is not suitable for cultivation in the tropical areas for climatic reasons [12]. Generally Sudanese wheat cultivars gave dough with relatively low elasticity and low alpha – amylase activity, which results in a low bread volume. Since Sudanese wheat are generally of poor bread making quality, which is attributed to the low protein and gluten quantity and quality, in addition to low alpha amylase activity. Thus improvement of flour quality is very essential for production of good quality bread. Therefore, the aim of the present study was to investigate the effect of different improvers on the rheological and bread making properties of three local Sudanese wheat cultivars.

2 MATERIALS AND METHODS

2.1 MATERIALS

Three commercial Sudanese wheat cultivars namely Nepta, Wadi Elneel, and Argeen (season 2008/2009) were obtained from Dongla Research Station, Elmultaga Research Station, and Khartoum (Sondos Scheme), respectively. Imported Australian wheat was used as control. The wheat grains were milled in Quadrumat junior mill. The patent flour was adjusted to extraction rate of (72%). The produced flour was used for chemical analysis and bread making. Ascorbic acid (AA), diacetyl tartaric esters of monoglycerides (DATEM) and alpha –amylase were obtained from local companies, Khartoum, Sudan.

2.2 CHEMICAL COMPOSITION

The determination of moisture, protein and ash were carried out on the samples according to AOAC standard methods [13].

2.3 GLUTEN QUANTITY AND QUALITY

Gluten quantity and quality of wheat flours with and without improvers were carried out according to the revised standard ICC method No. 155 and 158 [14] by using Glutomatic 2200 system (Perten Instruments AB, Huddinge, Sweden). Ten grams of the sample was mixed into dough with 5 ml distilled water in a test chamber with bottom sieve. The dough was then washed with 2% solution of sodium chloride. The gluten ball obtained was centrifuged at maximum speed by centrifuge (Type 2015) and quickly weighed. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the gluten index. The total wet gluten was dried in heater (Glutork, 2020) to give the dry gluten. The weight of gluten was multiplied by ten to give the percentage of wet or dry gluten.

2.4 FALLING NUMBER

Alpha – amylase activity of wheat flours with and without improvers was determined according to Perten [15]. Appropriate flour sample weight, was weighed and transferred into falling number tube and 25 ml distilled water was added, the stopper was fitted into the top of the viscometer, and shaked well until a homogenous suspension was formed. The viscometer tube was placed in the boiling water bath, and locked into position. The test automatically starts. The sample was stirred for 60 seconds, and then the viscometer stirrer was stopped in up position, released and sinked under its own weight through the uniform gelatinized suspension. The time in seconds for the stirrer to fall through the suspension was recorded as the falling number (seconds), the required flour sample weight (RFW) was obtained from the correction tables of sample

weight to 14% moisture basis [15], corresponding to 7 g at 14% moisture, no change is made in the quantity of the water used (25 ml).
Calculations:

$$\text{Required Flour Weight (g)} = 7 \times \frac{100 - 14}{100 - \text{Actual moisture content}}$$

2.5 SEDIMENTATION VALUE

Sedimentation value of wheat flours with and without improvers was carried out according to the official standard methods [16]. About 3.2 g of fine flour samples were placed in 100 ml glass stoppered graduated cylinder, simultaneously timing started when 50 ml distilled water containing bromophenol blue was added. Then the flour and water were thoroughly mixed by moving stoppered cylinder horizontally length wise, alternately right and left, through space of 7 in 12 times in each direction in 5 seconds, then flour was completely swept into suspension during mixing. At the end of first 2 min period, the contents were mixed for 30 seconds, in this manner the cylinder was completely inverted then righted up, as if it were pivoted at center, this action was performed smoothly 18 times in the 30 seconds then was let to stand 1.5 min. After that 25 ml of isopropyl alcohol lactic acid were added, mixed immediately by inverting cylinder four times as the latest step then was let to stand 1.75 min., mixed again for 15 sec, then the cylinder was immediately placed in upright position and let to stand for 5min.The factor to obtain sedimentation value was brought from table on 14% moisture basis, [16].

2.6 FARINOGRAPH AND EXTENSOGRAPH

Brabender farinograph method was carried on wheat flours with and without improvers according to AACC method [16]. Extensograph method was carried out according to the standard method [17].

2.7 BAKING QUALITY TESTS

The dough formulation used in this study were comprised of; flour 250 g, dry yeast 2.5 g, salt 1.5 g, sugar 3 g, oil 1% and water based on farinograph optimum absorption according to Badi *et al*. [18]. The ingredients were mixed in mono-universal laboratory dough mixer at medium speed. The dough was allowed to rest for 5 minutes at room temperature (25 ºC) and then scaled to three portions (120 g each). The three portions were made into round balls and allowed to rest for another 5 minutes and then molded, put into pan and placed in the fermentation cabinet for final proof which varies according to the fermentation power of the different dough's. Baking was done in Simon Rotary Test Oven at 250 ºC .Baking time was 13 minutes. After one hour, the loaves were weighed in grams and the volumes were measured in ml using the millet seed displacement method (Volumeter).

2.8 SENSORY EVALUATION

The loaves were sliced with an electric knife and prepared for sensory evaluation at the same day. The sensory evaluation of bread samples (aroma, taste, crumb texture, crumb color, crumb cell uniformity, general acceptability) was carried out by 10 semi trained panelists according to the method of Lwe [19]. The surrounding conditions were kept the same all through the panel test.

2.9 STATISTICAL ANALYSIS

The analysis of variance (ANOVA) was performed to examine the significant effect in all parameters measured [20]. Duncan Multiple Range Test was used to separate the means.

3 RESULTS AND DISCUSSION

3.1 FLOUR CHARACTERISTICS

The chemical characteristics of Sudanese and Australian wheats are presented in Table 1. With exception to Wadi Elneel cultivar, moisture content of Sudanese wheat cultivars (Nepta and Argeen) and Australian wheat cultivars showed insignificant differences. Argeen showed higher (13.66%) moisture content compared to other wheat cultivars including the Australian one. These results are similar to those of Mutwali [21] who reported a range of 10.21 to 13.13 for several Sudanese wheat cultivars grown in three different locations. Whereas, Ahmed [22] reported that the moisture content of

Sudanese wheat cultivars ranged from 6.33 to 8.6%. However, Mohamed [23] found that moisture content of four Sudanese wheat cultivars Debaira, Elneelain, Condor and Sasaraib range between 7.5 and 7.95%. The slight variation of the moisture content between these cultivars could be attributed to the differences in the environmental and soil conditions in wheat production sites as well as variation in genotypes. Moisture content is greatly affected by relative humidity at harvest and during storage. It is well known that moisture content is one of the most important factors affecting the quality of wheat. Since it has direct economic impact, higher moisture content of Sudanese wheat cultivars (Nepta and Argeen) compared to that of Australian wheat might be preferable in milling industry as well as bread making. The ash content of the flour of Australian and Sudanese wheats is shown in Table 1. Analysis of variance indicated that there are insignificant differences between all flours of the cultivars under the study. Ash content of Sudanese wheat flours and Australian wheat is ranged between 0.60% and 0.67%. These results were well agreed with the data reported by Mutwali [21] who found that the ash content of 20 Sudanese wheat cultivars was ranged between 0.47 to 0.85%. Furthermore, the ash contents in white flour of Pakistani spring wheats cultivars were ranged from 0.41 to 0.55% [24].The variation of these results could be attributed to differences in soil conditions, temperature, water and fertilizers. Ash content has been considered an important indicator of flour quality. It gives some indication of the miller's skill and the degree of refinement in processing and it is directly related to the amount of bran in the wheat, and hence has a rough inverse relationship to flour yield [25].

Table 1. Chemical characteristics of the Sudanese and Australian wheat flours.

Parameter	Australian	Nepta	Wadi Elneel	Argeen
Moisture (%)	13.30 [a]	13.49 [a]	12.96 [b]	13.66 [a]
Ash (%)	0.66 [a]	0.67 [a]	0.60 [a]	0.67 [a]
Protein (%)	13.70 [a]	13.29 [a]	11.610 [c]	12.81 [b]
Wet gluten (%)	36.00 [a]	32.40 [b]	30.70 [b]	34.80 [b]
Dry gluten (%)	12.21 [b]	10.80 [b]	10.30 [b]	11.60 [b]
Falling No (sec)	868.00 [a]	597.00 [b]	471.00 [c]	833.00 [a]
Sedimentation values (ml)	32.00 [a]	21.00 [b]	22.00 [b]	20.00 [b]

**Means values within the row having different superscripts letters are significantly different (P < 0.05)*

Grain protein is of primary importance in determining the bread making quality of wheat. Variations in both protein content and composition significantly modify the flour quality for bread making. The protein content of Sudanese wheat flour is ranged between 11.61% and 13.29% (Table 1). Among Sudanese wheat cultivars, Nepta showed the highest protein content (13.29%) whereas Wadi Elneel showed the lowest value (11.61%). The protein content of Nepta cultivar is comparable to that of Australian wheat cultivar (13.70%). The results of the present study lies within the range obtained by Mutwali [21] who reported that the protein content of white flours of 20 different Sudanese cultivars grown in three different locations ranged between 9.59% to 14.06%. Moreover, the current results are in consistent with the results reported by Anjum *et al*. [26] and Khan *et al*. [24] who reported variation in protein content among Pakistani wheat varieties from 9.68 to 13.45 % and from 10.23 to 11.60 %, respectively. The results were also within the optimum range reported by Mailhot and Patton [27] who stated that flours with protein content between 11-14% were considered acceptable for bread making. Thus, with regards to protein content Sudanese wheat cultivars could possibly be used for bread making. Protein content and quality are of vital importance in flour milling. They are the characteristics that make wheat unique and are the main factors on which wheat is traded, where higher protein wheats commanding a higher price. Regarding the quality of the protein of local wheat cultivar, the wet gluten values were found to be ranged between 30.7and 34.8% (Table 1). The minimum value (30.7%) was found for Wadi Elneel whereas the maximum value (34.8%) was observed for Argeen wheat flour. Australian wheat flour on the other hand showed the maximum (36%) wet gluten compared to Sudanese wheat cultivars. It has recently been reported that the wet gluten content of Pakistani spring wheat cultivars are ranged between 28.47 and 38.83% [24]. Moreover, Mutwali [21] reported that the wet gluten value of 20 Sudanese cultivars is ranged between 28.63% and 46.94%. However, Sudanese Standard Specifications (SDS) recommended minimum wet gluten value of 27% for bread making [28]. These results demonstrated that the local cultivars could efficiently be used for bread making as Australian wheat is the major wheat flour used in baking industry in Sudan. Dry gluten values of wheat flours are ranged between 10.30% and 12.21% (Table 1). These results are in a good agreement with range 10.49 to 13.60% of Pakistani spring wheat [24]. Similar results were also obtained by Mutwali [21] who reported that the dry gluten content of Sudanese wheat cultivars grown in three different regions are ranged between 8.96 and 16.76 %. The sedimentation values of local Sudanese and Australian wheat flours ranged from 20 to 32 ml (Table 1). Australian flour had significantly higher sedimentation values (32 ml) fallowed by Wadi Elneel (22 ml), Nepta (21 ml) and lower value by Argeen (20 ml). Recently, Mutwali [21] reported a range of 19.0 to 40.3 ml for the sedimentation value of 20 Sudanese wheat cultivars grown at three different locations. While, Mohamed [23] showed that, the sedimentation value of Sudanese wheat cultivars Debaira, Elneelian, Sasaraib, and

Condor ranged between 21 and 24 ml. The variation in these results might be due to the variation in the growing seasons and/or conditions. Sedimentation value, however, should be more than 20% for optimum bread making quality [28]. The sedimentation test was based on the fact that gluten imbibes water and swells greatly when treated with dilute lactic acid under standard conditions. The amount of water imbibes and volume occupied by a weight of flour depends on the quality of gluten. Strong gluten swells the most and occupies the bigger volume [29]. The falling number of the three Sudanese cultivars and Australian wheat flours was shown in Table 1. Alpha – amylase activity of the cultivars is found to be in the range of 868 to 471 seconds. Similarly, higher falling numbers in the range of 508.0 to 974.7 sec were reported by Mutwali [21] for 20 Sudanese wheat cultivars. This higher falling number may be attributed to dry harvest season which consequently affect the activity of alpha-amylase. By contrast Ahmed [22] showed that the falling number values of some Sudanese wheat cultivars ranged between 396 and 486 seconds. However, Mohamed [23] found that the falling number values of four Sudanese wheat cultivars Debaira, Elneelain, Condor and Sasaraib ranged between 425 and 675 seconds. The difference in the falling number of Sudanese wheat in these studies could be attributed to the variation in the genotypes and environmental conditions. The falling numbers above 400 second indicated that the flour is deficient in alpha- amylase and that the flour should be supplemented with a form of amylase to achieve the desirable level of enzyme activity [30]. High values of falling number for all cultivars indicated their very low alpha amylase activity. These results bring out the necessity for the use of alpha amylase as one of additives in bread recipe to improve the bread making quality of Sudanese wheat flours.

3.2 OPTIMIZING BREAD IMPROVER RECIPE

Our preliminary experiments to optimize the bread formulation for Debaira cultivar flour season 2006-2007 (protein 11.2% , falling number 529 sec, sedimentation value 22 ml, and loaf specific volume 3.33 cm^3/g), had shown that the combination of AA(50ppm) and DATEM (0.25%) with alpha- amylase (0.05%) produced bread with significantly higher specific volume of 4.12 cm^3/g (Table 2), hence this form of combination had been used as the optimum improver level in Sudanese wheat cultivars (Nepta, Wadi Elneel, and Argeen) in addition to Australian wheat (control) to evaluate their responses to these improvers.

Table 2. Effects of combinations of dough improvers on average loaf volume (cm^3/g) of Debaira bread

Alpha-amylase (%)	DATEM (%)	AA(ppm)	
		50	100
0.025	0.25	3.89 [b]	3.94 [ab]
0.050	0.25	4.12 [a]	3.87 [bc]
0.025	0.50	3.84 [c]	3.59 [e]
0.050	0.50	3.68 [d]	3.92 [ab]

**Means values within the row having different superscripts letters are significantly different (P < 0.05)*

3.3 FARINOGRAM CHARACTERISTICS

The farinogram characteristics of the flours tested with and without improvers are presented in Table 3. Water absorption values of the cultivars with and without improvers ranged from 68.4 to 55.9%. The highest value (68.4%) was observed in Nepta without improvers, while the lowest value (55.9%) was found in Wadi Elneel wheat flour with improver. These results were within the range 57 to 62% obtained by Mutwali [21] for Sudanese wheat cultivars grown in three different locations. Similar observation of water absorption was recently reported for Iranian wheat that used for the preparation of leavened flat bread locally known as Barbari [31].

Table 3. Farinogram characteristics of the flours of the three local wheat cultivars and Australian wheat flour with and without improvers

Samples	Water absorption (%)	Development time(min)	Stability(min)	Degree of softening(fu)
Australian without improver	67.4	7.3	13.3	36
Australian with improver	61.9	2.2	9.5	56
Nepta without improver	68.4	5.2	6.1	84
Nepta with improver	59.2	1.5	3.2	126
WadiElneel without improver	60.5	5.5	8.5	48
WadiElneel with improver	55.9	1.2	1.6	110
Argeen without improver	63.3	2.5	1.6	99
Argeen with improver	59.3	2.0	1.4	162

Furthermore, these findings were in agreement with that reported by Kaur and Bains [32], and Ravi *et al*. [9]. From the results it is clear that addition of improver to the cultivars exhibited decrease in water absorption compared with the same cultivars without improvers. Generally high farinograph water absorption of flour is considered an indication of good baking performance. The reason could be that high protein content causes good baking performance and high water absorption [33]. The development time of all flour with and without improver were ranged from 7.3 min to 1.2 min (Table 3). These results agreed with the findings of Mutwali [21] who reported that dough development time of Sudanese wheat cultivar in the range of 1.68 – 5.16 min. The Australian wheat without improvers gave the highest value, while Wadi Elneel with improver gained the lowest value. From the present results it is clear that the dough development time was decreased in the flour with improvers. Faubion and Hoseney [34] reported that, the full bread making potential of the dough is attained only at the optimum point of dough development. The dough stability values of all cultivars are ranged from 13.3 to 1.4 min (Table 3). In Sudanese cultivars with and without improver the dough stability was ranged between 9.5 and 1.4 min, whereas that of Australian wheat flour was 13.3 min. Recently, it is reported that the dough stability of Sudanese wheat cultivars are ranged between 6.2 and 2.0 minutes [21]. Compared to Australian wheat the Sudanese wheats showed considerably lower dough stability. It is well known that weak flour gives dough of low elasticity and stability, while the strong flour gives elastic dough with high stability. Mailhot and Patton [27] recommended a minimum dough stability of 7.5 min for bread making. Thus, the Sudanese cultivar such as Wadi Elneel (8.5 min) might efficiently be used for bread making. The degree of softening of all cultivars was ranged from 36 FU to 162 FU (Table 3). Consistent with these results, the degree of softening of Sudanese wheat cultivars is recently reported to be in the range of 301 FU to 62.5 FU [21]. The highest degree of softening was observed for Argeen with improvers, whereas the lowest value was obtained for Australian wheat without improver. Sudanese wheat flours showed significantly higher degree of softening compared to that of Australian wheat flour. This indicated that the Sudanese wheat cultivars are of hard type wheat. Obviously, addition of improvers increased the degree of softening and reduced water absorption, dough development time and stability. This may be due to the incorporation of alpha –amylase in the formula.

Table 4. Extensogram characteristics of the flours of the three local wheat cultivars and Australian wheat flour with and without improvers

Sample	Extensibility (mm)	Resistance to extension	Ratio	Energy(cm2)
Australian without improvers	199	284	2.3	125
Australian with improvers	183	436	4.4	184
Nepta without improvers	141	270	2.4	67
Nepta with improvers	142	302	3.1	81
WadiElneel without improver	148	336	2.9	92
WadiElneel with improvers	158	348	3.7	118
Argeen without improvers	193	134	0.8	46
Argeen with improvers	233	133	0.8	64

3.4 EXTENSOGRAM CHARACTERISTICS

The extensograph measures the stretching properties of wheat flour dough for determining the flour quality and for checking flour treatment with additives like ascorbic acid, proteinase or emulsifiers. Table 4.shows the extensogram characteristics of the dough of wheat with and without improvers. With exception of Australian wheat, the extensibility of the dough of all Sudanese cultivars was significantly improved with the addition of the formulated improvers. These results disagree with those of Rao *et al*. [35] who reported a decrease in extensibility with addition of surfactant gels to wheat flour. The variation in these results could be attributed to the differences in genotypes and its nutritional constituents. Uthayakumaran *et al*. [36] reported that the increase in protein content is associated with an increase in mixing time, mixograph peak resistance, and resistance to extension, extensibility and loaf volume.

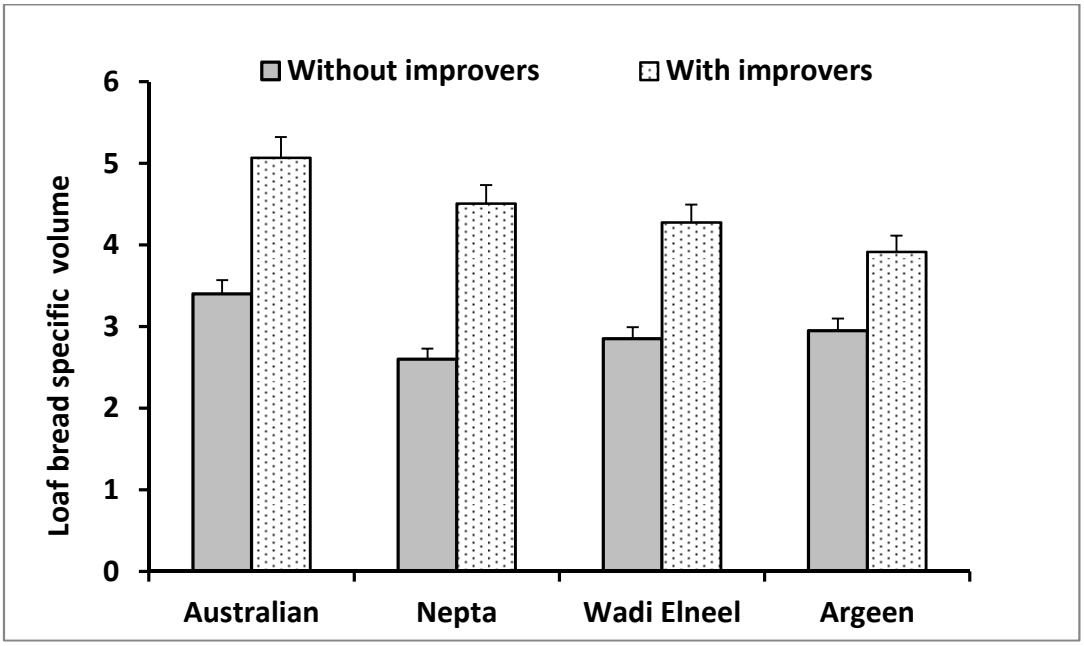

Fig. 1. Loaf bread specific volume (cm^3/g) of Sudanese wheat cultivars and Australian wheat flours with and without improvers

In the flours of all wheat cultivars the resistance to extension of the doughs significantly increased with the addition of bread improvers. This effect is in agreement with study of Rao *et al*. [35] who reported an increase in resistance to extension by addition of guar gum to wheat flour. While the addition of improvers to the same cultivars flours revealed an increase in

resistance/ extensibility ratio compared to the cultivars without improvers. Perhaps these improvers contain oxidizing agents causing more s – s groups in the dough resulting in high resistance to extension. Kieffer [37] has published results from comparative investigations of dough rheology and dough yield and he concluded that only resistance is positively related to baked volume. However, the strengthen effect of the improvers indicated by increased energy in all the flours. This may be due to the incorporation of ascorbic acid and DATEM in the formula. The results were in close agreement with the previous findings of Ravi *et al*. [9] and Aamodt *et al*. [5].

Fig. 2. loaf bread of Sudanese wheat cultivars and Australian wheat flours with and without improvers

3.5 BREAD MAKING QUALITY

The main factor, which places wheat in the front position among the world crops, is its bread-making quality. Wheat is used for several purposes, but the traditional staple food is bread, which is produced in many forms by different processes. The results of loaf bread specific volume made from flours with and without improvers are presented in figure 1. Incorporation of the combined improvers significantly increased the bread specific volume from 2.95 to 3.92, 2.85 to 4.28, 2.60 to 4.51 and 3.40 to 5.07 for Argeen, Wadi Elneel, Nepta and Australian flours, respectively. This results are well agreed with those of Mutwali [21] who reported a range of 2.4 to 3.54 cm3/g that the bread specific volume of eight Sudanese wheat cultivars grown at three different location. These results are also supported by the photos in figure 2, in which it is clearly demonstrated that addition of the formulated improvers significantly enhanced the bread volume. Sudanese wheat cultivars flour produced bread with low specific volume, in spite of their high values of protein (ranged between 11.61 and 13.29%).This confirm the fact that the high protein quantity of Sudanese wheat does not compensate for the poor bread quality , and thus underlines the importance of gluten quality in baking. From the present results, it is clear that the specific volume of the loaf bread was affected by the addition of improvers and by wheat quality as indicated by the amount of protein content, gluten quantity and quality and sedimentation value. Cauvain and Chamberlain [38] stated that, loaf volume increase is attributed to improved gas retention and to extending the period of dough expansion during the baking stage. Perten [39] stated that, quality factors such as loaf volume and water absorption are related to gluten quality and quantity.

Higher gluten quantity values generally give a greater bread volume. Basically, strong flours must be used for making good bread.

3.6 SENSORY EVALUATION

The data on sensory evaluation of the bread made from flours with and without improvers are presented in Table 5. Addition of improvers significantly enhanced the taste preference of all wheat cultivars with the highest being for Australian cultivars and the lowest for Argeen cultivar. In one hand, flavor, crumb texture, crust colour and general acceptability were also significantly improved by the incorporation of the formulated improvers in the bread making flours from all wheat cultivars. Interestingly, the addition of improvers to the flours enhanced all the sensory characteristics of the bread compared to the wheat flour without improvers. Similar findings have been reported by Junge *et al*. [10], Yamada and Preston [6], Ravi and Rao [40], and Ravi *et al*. [9] who observed that addition of improvers to various wheat flours significantly enhanced the sensory attributes of the bread.

Table 5. Sensory evaluation of the bread made from the three local wheat cultivars and Australian wheat flour with and without improvers

Sample	Taste	Flavour	Crumb texture	Crust colour	General acceptability
Australian without improvers	5.14 ^{d}	5.14 ^{d}	5.42 ^{d}	5.28 ^{d}	5.42 ^{d}
Australian with improvers	8.28 ^{a}	8.42 ^{a}	8.14 ^{a}	8.28 ^{a}	8.28 ^{a}
Nepta without improvers	4.42 ^{e}	4.14 ^{e}	3.71 ^{e}	4.28 ^{e}	4.28 ^{d}
Nepta with improvers	7.57 ^{ab}	6.71 ^{b}	7.14 ^{b}	7.00 ^{c}	6.85 ^{b}
WadiElneel without improver	4.42 ^{e}	4.28 ^{e}	3.71 ^{e}	3.85 ^{f}	4.00 ^{d}
WadiElneel with improvers	7.28 ^{b}	6.57 ^{bc}	6.71 ^{c}	5.28 ^{d}	6.14 ^{b}
Argeen without improvers	4.14 ^{e}	4.00 ^{e}	4.00 ^{de}	3.85 ^{f}	4.14 ^{d}
Argeen with improvers	6.42 ^{c}	6.00 ^{c}	5.71 ^{d}	7.14 ^{b}	5.91 ^{c}

**Mean values having different superscript letter in each column differ significantly at ($p \leq 0.05$).*

4 CONCLUSIONS

The results clearly revealed that the Sudanese wheat cultivars investigated are of poor bread making quality in spite of their relatively high protein values due to their low gluten strength as indicated by their lower sedimentation values and loaf specific volume. The high response of the Sudanese wheat flours to the improvers investigated indicated the possibility of producing high loaf volume with good quality breads from Sudanese wheat. Further research should be done to encourage using locally available ingredients as bread improvers.

REFERENCES

[1] J. Bordes, G. Branlard, F. X. Oury, G. Charmet and F. Balfourier, "Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection," *Journal of Cereal Science,* vol. 48, no. 3, pp. 569–579, 2008.

[2] M. Menkovska, D. Knezevic and M. Ivanoski, "Protein Allelic Composition, Dough Rheology, and Baking Characteristics of Flour Mill Streams from Wheat Cultivars with Known and Varied Baking Qualities," *Cereal Chemistry,* vol. 79, no. 5, pp. 720–725, 2002.

[3] H. Goesaert, K. Brijs, W. S. Veraverbeke, C. M. Courtin, K. Gebruers and J. A. Delcour, "Wheat flour constituents: how they impact bread quality, and how to impact their functionality," *Trends in Food Science and Technology,* vol. 16, no. 1–3, pp. 12–30, 2005.

[4] Hoseney, R. C., *Principals of cereal science and technology*, 1st Ed. American Association of Cereal Chemists Inc., St. Paul, Minnesota, USA, 1986.

[5] A. Aamodt, E. M. Magnus and E. M. Faergestad, "Effect of flour quality, ascorbic acid and DATEM on dough rheological parameter and hearth loaves characteristics," *Journal of Food Science,* vol. 68, no. 7, pp. 2201–2210, 2003.

[6] Y. Yamada and K. R. Preston, "Sponge and dough bread –effects of oxidants on bread and oven rise properties of a Canadian red spring wheat patent flour," *Cereal Chemistry,* vol.71, no. 3, pp. 297–300, 1994.
[7] E. Armero and C. Collar, "Crumb firming kinetics of wheat breads with anti-staling additives," *Journal of Cereal Science,* vol. 28, no. 2, pp. 165–174, 1998.
[8] H. S.Gujral, M. Haros and C. M. Rosell, "Starch hydrolyzing enzymes for retarding the staling of rice bread," *Cereal Chemistry,* vol. 80, no. 6, pp. 750–754, 2003.
[9] R.Ravi, R.S. Manohar and P. H. Rao, "Influence of additives on the rheological characteristics and baking quality of wheat flours," *European Food Research and Technology,* vol. 210, no. 3, pp. 202–208, 2000.
[10] R.C. Junge, R.C. Hoseney and E. Marston, "Effect of surfactants on air incorporation in dough and the crumb grain of bread," *Cereal Chemistry,* vol. 58, no. 4, pp. 338–342, 1981.
[11] Ishag, H. M., *Genotype differences in heat stress in wheat in Gezira Scheme*. In: D. A. Saunders, and G. P. Hettel (Eds.), Wheat in Heat- Stressed Environment, Irrigated, Dry Areas and Rice-Wheat Farming Systems, Mexico, D.F.: CIMMYT, pp. 170–174, 1994.
[12] M. O. Edema, L. O. Sanni and A. I. Sanni, "Evaluation of maize-soybean flour blends for sour maize bread production in Nigeria," *African Journal of Biotechnology,* vol. 4 no. 9, pp. 911-918, 2005.
[13] AOAC, *Official methods of analysis of AOAC international,* 17 Ed. Association of the Official Analytical Chemists (AOAC) International, Gaithersburg, MD, USA, 2003.
[14] ICC, ICC Standard No. 155. Determination of wet gluten quantity and quality (Gluten Index ac. To Perten) of whole wheat meal and wheat flour (*Triticum aestivum*). Verlas Moritz Schafer: Detmold, West Germany, 1994.
[15] Perten, H., *Manual of falling number 1500 as a measurement of alpha-amylase activity*, (ISO – standard No. 3093, 1974), ICC standard No. 10711 (1995), and AACC method 56-81B (1992), Hudding, Sweden, 1996.
[16] AACC, *Approved Methods of the American Association of Cereal Chemists* (Method 38-12), 10th Ed. American Association of Cereal Chemists, St. Paul, MN, USA, 2000.
[17] ICC, *International Association for Cereal Chemistry*, (No. 114/1) Vienna, Austria, 2001.
[18] S. M. Badi, H. A. Elfaki and H. Perten, "Evaluation of Sudanese wheat varieties (harvested 1975)," *Sudan Journal of Food Science and Technology,* vol. 10, no. 1 pp. 5–11, 1978.
[19] Lwe, M.O., *Handbook of sensory methods and analysis*. 1st Ed. Projoint communications service Ltd. Enugu, 2002.
[20] Mead, R., and Gurnow, N.R., *Statistic methods in agricultural experimental biology*, 1st Ed. Great Britain, 1990.
[21] Mutwali, N. I. A. K., *Quality of wheat cultivars grown in different locations of Sudan for bread making* (PhD thesis), University of Khartoum press, 2011.
[22] Ahmed, S. E., *Proximate composition and flour quality of wheat cultivars grown in the Sudan* (M.Sc. Thesis), University of Khartoum press, 1995.
[23] Mohamed, E.A., *Evaluation of four local wheat cultivars with special emphasis on protein fractions* (M.Sc Thesis), University of Khartoum press, 2000.
[24] M. R. Khan, F. M. Anjum, T. Zahoor and H. Nawaz, "Biochemical and Technological characterization of Pakistani spring wheats" *Pakistan Journal of Agricultural Science,* vol. 46, no. 4, pp. 2076–2086, 2009.
[25] Zeleny, L., *Criteria of wheat quality*, In: Y. Pomeranz (Eds.), Wheat Chemistry and Technology, St. Paul, MN: American Association of Cereal Chemists, pp. 26, 1971.
[26] F. M. Anjum, I. Ahmad, M. S. Butt, M. A. Sheikh and I. Pasha, "Amino acid composition of spring wheats and losses of lysine during chapatti baking," *Journal of Food Composition and Analysis,* vol. 18, no. 6, pp. 523–532, 2005.
[27] Mailhot, W. C., and Patton, J. C., *Criteria of flour quality*. In: Y. Pomeranz (Eds.), Wheat: Chemistry and Technology, St. Paul: American association of Cereal Chemists, pp. 69–90, 1988.
[28] SDS, Wheat flour Standard (037/2007), Sudanese Standard and Specification (SDS), 2007.
[29] J. Williams, "Brief notes on wheat and flour evaluation," *Journal of Food Science and Technology,* vol. 2, no.1, pp. 38–42, 1970.
[30] Cauvain, S., and Young, L., *Flour,* In: S. Cauvain , and L. Young (Eds.), Baking problems solved (pp 19- 37). Cambridge: Wood Head Publishing Limited, 2001.
[31] M. Ghanbari and J. Farmani, "Influence of Hydrocolloids on Dough Properties and Quality of Barbari: An Iranian Leavened Flat Bread," *Journal of Agricultural Science and Technology,* vol.15, no.3, pp. 545–555, 2013.
[32] B. Kaur and G. S. Bains, "Effect of amylase supplements on the rheoiogical and baking quality of Indian wheat's," *Journal of Food Science and Technology,* vol.13, no. 6, pp. 328–332, 1976.
[33] A. H. Bloksma, "Flour composition, dough rheology, and baking quality," C*ereal Science Today,* vol. 17, no. 3, pp. 380–386, 1972.
[34] Faubion, J. M., and Hoseney, R. C., *The viscoelastic properties of wheat flour doughs*, In: H. Faridi, and J. M. Faubion (Eds), Dough rheology and baked product texture, AVI, New York: Springer Ltd., 1990.

[35] G.V. Rao, D. Indrani and S. R. Sharpalakar, "Guar gum as an additives for improving the bread making quality of wheat flours," *Journal of Food Science and Technology,* vol. 22, no. 1, pp. 101-107, 1985.
[36] S. Uthayakumaran, P. W. Gras, F. L. Stoddard and F. Bekes, "Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough," *Cereal Chemistry*, vol. 76, no. 3, pp. 389‑395, 1999.
[37] R. Kieffer, "Die elastizitat von weizenteig cin hufig uberschatztes qualitatsmerkmal," *Getrcide Mehl Bront,* vol. 57, no. 6, pp. 335–339, 2003.
[38] S. P. Cauvain and N. Chamberlain, "The bread improving effect of fungal alpha amylase," *Journal of Cereal Science,* vol. 8, no. 3, pp. 239–248, 1988.
[39] Perten, H., *Manual glutomatic system. The gluten index method* (ICC standard methods No. 158, AACC method 38-12), Huddinge, Sweden, 1995.
[40] R. Ravi and P. H. Rao, Factors influencing the response of improvers to commercial Indian varieties, *Journal of Food Science and Technology,* vol. 32, no. 1, pp. 36–41, 1995.

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Validation of a Method for Determining Heavy Metals in Some Ethiopian Spices By Dry Ashing Using Atomic Absorption Spectroscopy

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ABSTRACT: The optimal conditions for determination of Pb, Cd and Cr in spices sample by FAAS after dry mineralization 1g spice samples for 5hr in a temperature of 500 °C followed dissolution in 4 mL HNO_3 in Fenugreek, Black cumin, garlic and ginger. Working linear ranges are given: for Cd, 0 -6mg/l, for Cr, 0.2-1mg/l and for Pb, 2-20µg/l. Found detection limits are 0.2mg/l, 0.13mg/l and 0.02µg/l for Cd, Cr and Pb, respectively. The result obtained Lead in Fenugreek, Black cumin, garlic and ginger ranged from 0.0126 to 0.0155, 0.0205 to 0.0254, 0.0046 to 0.0066 and 0.0161 to 0.0178 mg/kg respectively. Cadmium is detected only in Fenugreek in the ranged of ND to 0.0175mg/kg where as Chromium in Fenugreek, Black cumin, Garlic and Ginger ranges from 0.0187 to 0.0219, 0.0134 to 0.0152, 0.0014 to 0.0016 and 0.0258 to 0.0346 mg/kg respectively which is under the values recommended for concentration levels present in the analyzed samples.

KEYWORDS: dry ashing, fenugreek, black cumin, ginger, garlic, atomic absorption spectrometry.

1 INTRODUCTION

Spices are dried parts of plants, which have been used as dietary components of food often to improve its color, aroma, palatability and acceptability. They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plant. There have been many definitions for 'spices' which are often used interchangeably with 'herbs'. Spices are defined as the aromatic parts of any plants that are used to add flavor to food. But herbs are the aromatic leaves of any plant that can add flavor to food. The origins of herbs are leaves of plants that have soft stems or are shrubs but not trees. In general, spices are defined as un-leafy dried substances whereas herbs are leafy un-dried substances [1].

In Ethiopia, there are between 6,000 to 7,000 higher plant species, out of about 500 (about 8%) are edible [1]. According to [2]. on 50 species of aromatic and medicinal plants collected from eastern Ethiopia, it was indicated that a sizable number of local merchants were involved in the trading of spices along with other aromatic and medicinal plants. Of these plant species studied as such, a significant number of the plants (over 25) were spices.

Heavy metals are those metals which are toxic for our body even if at their lower concentration include cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), and chromium (Cr). Most of these metal ions (Cd, Cu, Zn, Hg, As, Ag, Cr, and Fe) can be release from the industries are in simple cationic forms [3], [4].

Many analytical methods including Atomic Absorption Spectrometry for trace element determination in plant materials require the digestion of the sample [5].Because of its sensitivity, specificity, simplicity and precision, Atomic Absorption Spectrometry (AAS) is the most widely recommended instrument utilized in analytical procedures for trace heavy metal analysis. In order to separate the analyte from the matrix and to avoid organic matter which may react with the metal ions or chemical reagents and interfere with the analyte in acid digestion methods are very important step. The most commonly used methods for the sample treatment of spices are dry ashing, wet ashing and microwave assisted treatment.

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Dry ashing procedures use a high temperature muffle furnace. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. The advantage of Dry ashing is safe, few reagents are required, many samples can be analyzed simultaneously and not labor intensive. The disadvantage of this method is, it requires relatively expensive apparatus (platinum crucibles and muffle furnace),losses due to volatilization, resistance to ash by some materials, difficult dissolution of ashed material and high risk of contamination[6].

In this study, the authors compare the efficacy of differing digestion procedures dry ashing on commercial spice samples. The contents of Lead, Chromium and Cadmium in spice samples produced in Ethiopia were determined by flame atomic absorption spectrometry after dry ashing.

2 MATERIALS AND METHODS

2.1 APPARATUS

Buck scientific 210 VGP flame atomic absorption spectrometer was used in the experiments. A deuterium background corrector was used for background corrections. The operating parameters for the elements were set as recommended by the manufacturer (Table 1). For flame measurements, a 10-cm long slot-burner head, a lamp and an air-acetylene flame were used.

Furnace (type 1500) for dry ashing was used to ash the sample for the analysis of Heavy metals concentration. Platinum and porcelain Crucibles was used to dry the sample at Furnace. Sample volume, digestion times for the drying, ashing, atomization and cleaning temperatures were optimized prior to analysis in order to obtain the maximum absorbance with minimum background.

Table 1. Working Conditions of Atomic Absorption Spectroscopy

Element	Wavelength nm	Slit Width nm	Lamp Current mA	IDL mg/L	MDL mg/L	Flame type
Cd	228.9	0.7	2	0.005	0.0062	Rich/yellow
Cr	357.9	0.7	2	0.05	0.051	Lean/blue
Pb	217.0	1.0	5	0.01	0.016	Lean/blue

2.2 REAGENTS

All reagents were of analytical reagent grade. Double distilled deionized water (Milli-Q Millipore 18.2 MΩ-cm resistivity) was used for all dilutions. HNO_3 was of suprapure quality (E. Merck, Darmstadt). All plastic and glassware were cleaned by soaking in diluted HNO_3 (10% v/v) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting stock solutions of 1000 mg/L of each element.

2.3 SAMPLING

Total of four spice sample types were collected randomly from different traders and Samples were washed thoroughly with tap water followed by de-ionized water and dried in the oven at a temperature of 105 ^{o}C for 24 hr for Fenugreek, Ginger, and Garlic. But black cumin was dried at a temperature of 105 ^{o}C for 48 hr. The dried samples were ground in a stainless steel mill till obtaining fine particles that pass through a 0.5 mm mesh and kept dry in a polyethylene bag in desiccators until analysis.

2.4 DIGESTION PROCEDURE

Dry ashing was applied for digesting the spice samples produced in Ethiopia. Optimum digestion conditions are given below.

2.5 DRY ASHING

One gram of each spice sample was placed into porcelain crucible. The furnace temperature was slowly increased from room temperature to 500˚C. The sample was ashed for about 5 hr until a white or grey ash residue was obtained. The residue was dissolved in 5 mL of HNO_3 (25%, v/v). The solution was transferred to a 10 mL volumetric flask and made up to the mark [7].

2.6 DIGESTION CONDITIONS

Different procedures for spices sample digestion were assessed based on varying reagent volume, digestion time and digestion temperature. For optimizing the procedure Fenugreek *(Trigonella foenumgraecum* L.) was selected and it was digested with the three digestion methods. The selection for the respective optimum digestion methods was made based on the particular procedure which resulted to the following conditions: clear digestion solution, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested spice samples [8]. The results are given in Table 2. The comparison of three digestion methods showed statistically significant differences in results. The recovery values were nearly (≥ 95%) for all digestion methods. **ANOVA** was used in this study ($p < 0.05$). The relative standard deviations were less than 10% for all elements. The approximate time required for dry ashing was 10 hr.

2.7 PREPARATION OF STANDARD SOLUTIONS

Determination of the metal concentration in the experimental solution was based on the calibration curve. In plotting the calibration curves lead, cadmium and chromium stock solutions of 1000 ppm were prepared by dissolving 1.6 g of $Pb(NO_3)_2$, 2.74 g $Cd(NO_3)_2.4H_2O$ and 2.83 g $K_2Cr_2O_7$ in de- ionized water respectively. Blank solutions were prepared for the methods and, for the standard working solutions, to prepare 100 ppm, 10 mL of the standard $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$ and $K_2Cr_2O_7$ stock solution were pipetted and added into 100 mL calibrated flasks finally diluted with de-ionized water and the solution was mixed thoroughly. Next, to prepare 50 ppm standard solution of each metal, 50 mL of each of 100 ppm stock solution was pipetted into 100 mL volumetric flasks and diluted with de-ionized water. Finally to prepare 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 ppm aliquots of this standard working solution 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 mL was pipetted from 50 ppm standard solution into 50 mL calibrated flasks and made up to volume with De-ionized water [9].

2.8 VALIDATION OF EXPERIMENTAL RESULTS

2.8.1 DETERMINATION OF DETECTION LIMITS

Detection limit is the lowest concentration level that can be determined at 95% confidence level [10] or the minimum concentration that can be detected by the analytical method with a given certainty [11] a general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal [12]. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σ blank, n = 5).

2.8.2 RECOVERY TESTS

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0.02, 0.02 ppm of Pb, Cr and 0.2 ppm Cd, respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 92 to 103 %. The results are given in Table 3. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except Fenugreek were not calculated due to results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

$$\%\,\mathbf{Recovery} = \frac{\mathbf{Amount\ after\ Spike - Amount\ before\ Spike}}{\mathbf{Amount\ Added}} \mathbf{x\,100} \quad [2]$$

2.8.3 OPTIMIZATION OF WORKING PROCEDURE

The optimal procedure chosen on the basis of these criteria for dry ashing 5 hr digestion time at a temperature 500^0C in 4 mL HNO_3 was employed 4 mL HNO_3 was added after white ash is obtained. The optimized operating conditions were compared with literature report on similar study made by Mustafa *et al*., (2004). The work had improved especially dry ashing and wet digestion methods employed there significantly as far as digestion time is concerned. as it listed in Table 2.

Table 2. Optimum working conditions for the digestion methods used

Digestion Methods	Previous work optimum parameters (Mustafa *etal*., 2004)			Current work optimum Parameters			Difference		
	T (^{o}C)	V(mL)	Time(hr)	T (^{o}C)	V(mL)	Time(hr)	T(^{o}C)	V(mL)	Time (hr)
Dry Ashing	450	5	8	500	4	5	50	1	3

Note; T = temperature; V = volume DA= dry ashing

3 RESULTS AND DISCUSSION

The method detection limit (MDL) is defined as the concentration corresponding to three times the standard deviation of blanks. Method detection limit values of the investigated elements for AAS were found to be 0.016 mg/L for Pb, 0.0062 mg/L for Cd and 0.051 mg/L for Cr.

Trace metal levels in the analyzed samples are given in Table 4. The metal contents in the samples studied depended on the specific species. Levels of the essential metals in the spice samples were found to be higher than those of the non-essential metals. The lowest and highest contents of copper were found in 4.1 µg/g for *Diantus sp.* and 28.7 µg/g for *Rhus coriaria*, respectively. The lowest and highest levels of zinc were found as 7.84 µg/g for *Capsicum annuum* and 47.6 µg/g for *Papaver somniferum*, respectively. Copper and zinc values in spices of Nigeria have been reported in the range of 0.40-13.3 µg/g and 0.20-53.7 µg/g, respectively(7). The highest cadmium level was found as 0.93 µg/g for *Piper nigrum*, whereas the lowest cadmium level was 0.10 µg/g in *Nigella sp.* The lowest and highest contents of nickel were found as 0.65 µg/g for *Cassia sp.* and 8.69 µg/g for *Menta sp*, respectively. The concentrations of cadmium and nickel in spices of Nigeria have been reported in the range of 0.12-0.36 µg/g and 1.03-3.47 µg/g, respectively(8). The concentration of lead varied from 0.47 µg/g in *Rhus coriaria* to 1.89 µg/g in *Nigella sp [13]*

3.1 OPTIMIZATION OF WORKING PROCEDURE

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3.2 RECOVERY TESTS

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0.02, 0.02 ppm of Pb, Cr and 0.2 ppm Cd respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 92 to 103 %. The results are given in Table 3. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except Fenugreek were not calculated since results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

Table 3. Recovery test of the methods at P≤0.0

Metal		Dry Ashing			
		Fenugreek	Black Cumin	Garlic	Ginger
Pb	Amount Recovered	0.032	0.040	0.025	0.035
	percentage recovery	95	96	97	96
	concentration in spice	0.0132	0.0209	0.0055	0.0154
Cr	Amount Recovered	0.037	0.032	0.021	0.054
	concentration in spice	0.0187	0.0134	0.0016	0.0346
	percentage recovery	94	93	95	96
Cd	Amount Recovered	0.196	0.168	0.178	0.180
	concentration in spice	0.0125	-	-	-
	percentage recovery	92	-	-	-

Since Cd is not detected in all except fenugreek the %R is also not calculated

Table 4. Metal Concentration (mg/kg) in Spices from Dire Dawa Market (Mean± S.D)

Digestion Methods	Spices	Pb	Cr	Cd
DA	Fenugreek	0.0132±0.0006	0.0187±0.0008	0.0125±0.0016
	Black cumin	0.0209±0.0003	0.0134±0.0005	ND
	Garlic	0.0055±0.0006	0.0016±0.0002	ND
	Ginger	0.0154±0.0002	0.0346±0.0018	ND

Note; DA= dry ashing and ND = not detected at P≤0.

4 SUMMARY AND CONCLUSION

4.1 SUMMARY

The general objective of the study was to compare the efficiency of digestion methods for determination of Cr (VI) Pb (II) and Cd (II) in some selected Ethiopian spices using AAS. All experiments were done at Haramaya University soil science and central laboratory. The Pb concentrations were found to be in the range of 0.0132 to 0.0140, 0.0209 to 0.0224, 0.0055 to 0.0058, and 0.0154 to 0.0172 mg/kg for Fenugreek, Black Cumin, Garlic and Ginger respectively. Whereas Cr concentrations ranged from 0.0187 to 0.0269, 0.0134 to 0.0152, 0.0014 to 0.0016, 0.0258 to 0.0346 mg/kg in Fenugreek, Black Cumin, Garlic and Ginger respectively, Cd was not detected in any of the samples except in Fenugreek which is ranging from 0.0125 to 0.0175 mg/kg. The results obtained showed that the spices in this study are not likely to pose any health risk to the public through consumption of the spices for both the toxic elements determined. The recoveries of the trace metals were in the range of 92 to 103% and the standard deviations were less than 10%.

4.2 CONCLUSIONS

This study is focused on check the extraction efficiency of dry ashing methods in different spices including Garlic, Ginger, Black Cumin and Fenugreek for the determination of heavy metals like Cd, Pb and Cr by using Atomic Absorption spectrometry (AAS).

Spice samples were ashed by using furnace and digested by using HNO_3 as digestion reagents. The concentrations of these reagents were optimized to minimize possible matrix interferences. Different temperature programs, reagent volume and digestion time were investigated to obtain maximum digestion efficiency with minimum digestion reagent consumption for all digestion methods. All methods gave almost similar results for the elements studied.

But dry ashing methods are more time consuming and complicated than the other digestion methods in terms of digestion efficiency. Example the use of microwave digestion system in sample digestion provides very fast, safer, simple and

cleaner method of sample preparation, increases analyte recoveries and useful volatile elements. Effects of HNO_3 and H_2O_2 concentrations on Pb (II), Cd (II) and Cr (VI) signals were investigated to see the effect of matrix matching on AAS and it was found that increasing concentrations of HNO_3 has suppression effect on Pb and Cr concentration whereas the effect on Cd concentration were not detected by using neither direct calibration method or standard addition method by AAS.

Based on this knowledge direct calibration method was used for Pb and Cr determination whereas for Cd determination standard addition method was applied but similar result was obtained. In the last part of the study the results of previous analyses for Cd, Cr and Pb determination in spice samples were evaluated together with the results of this study for statistical analysis. For each spice sample the results of all elements obtained by different digestion methods were compared by using ANOVA at 95% confidence interval.

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REFERENCES

[1] Wahid and S.K Durrani, "Concentration of selected heavy metals in spices, dry fruits and plant nuts," *Plant Foods for Human Nutrition*. 39(3) 279-286 ,1989**.**

[2] Asfaw Z. and M.Tadesse, "Prospects for sustainable use and development of wild food plants in Ethiopia," *Economic Botany*. 55 (1):47-62, 2001.

[3] Letchamo, W. and H. Storck, "Spices and Medicinal Plants Trade in Eastern Ethiopia,"

[4] *ISHS. Acta Horticulturae*. (270): 247-53, 2004,1991.

[5] AOAC (Association of Official Analytical Chemists), Official Methods of Analysis, 16th ed., Arlington, Virginia, USA, 1995.

[6] Mustafa S., T.Mustafa, N.Ibrahim and S.Hayati, "Comparison of digestion procedures for the determination of trace metal contents in spice samples produced in Turkey," *Journal of Food and Drug Analysis, Vol. 12(3), P.254-258.*

[7] Association of Official Analytical chemists/AOAC, "Determination of metals in plant using ICP-OES." *Journal of Association of Official Analytical Chemists*.68:499

[8] Kingston H.M., L.B Jassie ,1986. microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. *Journal of Analytical Chemistry* , 58 (12) , 2534-2541, 1985.

[9] Tuzen, M., "Determination of some metals in honey samples for monitoring environmental pollution,"*Bulletin of Fresenius Environment;* 11: 366-370, 2002.

[10] Polkowska-M. H., B.Danko, R.Dybczynski, A. K.-Ammerlaan and P. Bode, "Effect of acid digestion method on cobalt determination in plant materials," *Analytical Chemical Acta* 408: 89-95,2000.

[11] Vandecasteele, c., and, C.B., Block,. "Modern method for trace element determination ,"*John Wiley and Sons* Ltd.pp33, 1993.

[12] Tuzen, M., H. Sari and M. Soylak, "Microwave and wet digestion procedures for atomic absorption spectrometric determination of trace metals contents of sediment samples," *Analytical Letters* 37: 1949-1960,2004.

[13] Volesky, B and H. May-Phillips, "Biosorption of heavy metals by *Saccharomyces cerevisiae*," *Journal of Applied Microbiology Biotechnology*. 42: 797-806, 1995.

[14] Wang, JL, "Biosorption of copper (II) by chemically modified biomass of Saccharomyces cerevisiae," *Process Biochemistry.* 37:847–50, 2006.

[15] White, T.R and G.E.Douthit, "Use of microwave oven and nitric acid-hydrogen peroxide digestion to prepare botanical materials for elemental analysis by inductively coupled argon plasma emission spectroscopy," *Journal Association of Analytic Chem*istry,68:766-69,1985.

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Potential use of flow cytometry in microalgae-based biodiesel project development

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ABSTRACT: The decrease of petroleum reserves and the global increase in energy demand has resulted in the focus of research toward exploration of alternate fuels using biological renewable sources. Biodiesel is one of these renewable energy forms. Microalgae are considered one of the most promising sources for biodiesel production. However, the potential use of microalgae in this field still needs to be explored since only about twenty species has been studied from the approximately 30.000 known species. Thereby, screening work on new potentially lipid overproducer strains is actually an active field. An overview of potential applications of multi-parameter flow cytometry in development of a biodiesel production strategy using microalgae is detailed in this review. Rapid and simultaneous measurements of different physiological parameters indicating diversity in marine and freshwater microalgal communities, biomass quality such as biochemical composition and viability of individual cells, isolation of targeted cells and obtention of axenic cultures are applications of flow cytometry that enable monitoring and optimisation of production of lipids from microalgae.

KEYWORDS: microalgae, biodiesel, flow cytometry, fluorescence, screening.

1 INTRODUCTION

Development of biofuels using microalgae as a feedstock is increasingly studied worldwide. This is a consequence of an increase in global energy demand, a decline in global petroleum reserves as well as the increased global awareness towards the development of renewable energies respectful of the environment. Microalgae are a desirable source for biofuels production compared to other biofuel feedstocks in view of the following strengths: **(i)** microalgae have a fast growth rate, **(ii)** microalgae culture can use non fresh water (marine or waste water) and non-arable lands **(iii)** microalgal based biofuels do not interfere with food security concerns, in comparison with first generation biofuels, **(iv)** lipid metabolism can be easily modulated by modifying growth conditions allowing a significant increase in lipid content, and finally **(v)** intracellular lipid content can rise 80% of cell dry weight making microalgae an attractive source for biodiesel production [1], [2], [3]. Thereby, microalgae seem to be a promising renewable biofuel source capable of meeting the global demand for transportation fuels [1].

Studies on microalgae candidates for biodiesel projects have focused only on around twenty species [1, Tab. 1], which indicates a limited exploration of the wide algal biodiversity. This could be due to: **(i)** the ease of working with known strains whose taxonomy, culture requirements are well described and related documentation available, **(ii)** culture conditions (media, light intensity, CO_2 supply, response to stress, etc.) have been widely studied which means time and effort saving, **(iii)** metabolic profiles are well known in most of the cases, (**iv)** some species are considered models for genetic manipulations and engineering, **(v)** Some of these species have even been tested in pilot scale and used by industrials, which encourages researchers to work on, for improving productivity to the detriment of new species. However, it is essential to expand the

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spectrum of studied strains, in order to develop economically viable biofuel projects. This can lead to find new lipid-rich species, in addition to isolating indigenous homologues of known species. In consequence, various projects around the world have undertaken programs of high-throughput screening in order to isolate homologues of known species and to explore new ones [4], [5].

Table 1. Oil content of some microalgae [1]

Microalga	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecica	15–23

Despite the significant progress in the field of microalgae-based biodiesel, several technical barriers need to be overcome for a biofuel project to be profitable and scalable to an industrial level [6]. This includes lipid content and quality of microalgae strains, control of biomass production at a large scale in highly variable outdoor conditions, productivity improvement, etc. To improve the use of microalgae in biofuel production, it is important to introduce techniques allowing fast, easy, and effective monitoring of intracellular and extracellular features related to their productivity. Flow CytoMetry (FCM) has, in this sense, many advantages that can contribute to successful development of microalgae-based biofuel projects given the analytical capabilities it offers.

The essence of FCM is the simultaneous measurement of different optical cell properties (light scatter and multicolor fluorescence emission). This allows an exhaustive characterization and classification of individual cells in a natural or treated sample. FCM was developed in the middle of last century and was exclusively used for medical applications. The first applications of FCM in aquatic sciences seem to have been started in the late 1970s [7], [8], [9], [10], [11]. FCM was applied to phytoplankton analysis initially for monitoring the populations in natural samples based on their auto-fluorescent properties. Use of this technique has contributed to knowledge improvement of spatial-temporary distribution of microalgal populations as well as population dynamics linked to the environment [12]. It is only recently that new application of FCM applied to microalgal biotechnology started to be developed [4], [5], [13], [14].

2 APPLICATIONS OF FCM IN BIOFUEL PROJECTS DEVELOPMENT

2.1 BIODIVERSITY MONITORING

The enormous biodiversity of microalgae makes them suitable candidates for biofuel production [15]. This natural biodiversity is estimated at 30.000 species [16] distributed in different habitats such as freshwater, seawater, brackish, wastewater, hyper saline, etc [15]. These ecosystems harbour a number of high lipid producer's microalgae strains, most of which not yet exploited.

Know microalgae population's distribution gives us a clear idea about how many and whose species can be found in a determined sampling point (period/region). According to Mutanda *et al*. [15], search, collect and identify interesting strains are first and crucial steps for a successful microalgae-based biofuels project. In this way, multi-parameter FCM analysis allows the fast and real time monitoring of microalgae populations contained in an environmental sample.

Figure 1 represents an example of intrinsic features monitoring of microalgal population within a mixed sample using FCM. In this case, the mainly used parameters are auto-fluorescence of photosynthetic pigments and cell size. Chlorophyll and phycobiliproteins (phycoerythrin and phycocyanin) that are typical of cyanobacteria (cryptophytes and rhodophytes) are the most common photosynthetic pigments of microalgae [17]. Content and composition of these pigments depend on taxon but also on environmental conditions (illumination, mineral composition of water, pH, etc.) and can be monitored by FCM [18], [19]. This allows FCM to facilitate a first and fast taxonomical identification of microalgae populations in a natural sample (Tab. 2).

Table 2. Fluorescence excitation/emission of different fluorescent pigments using FCM. LP: long pass filter [17], [20]

Pigment	Excitation	Channel (emission)	Taxonomic group
Chlorophyll	Argon 488nm	FL3 (670LP) Red	Majority of phytoplankton and microalgae
Phycoerythrin (PE)	Argon 488nm	FL2 (585/30nm) Orange	Cyanophyceae, Cryptophyceae, Rhodophyceae
Phycocyanin (PC)	Red diode 633 nm	FL4 (661/16nm) Dark red	Cyanophyceae, Cryptophyceae

Chlorophyll: Chlorophyll is present in all photoautotrophic microalgae species. After excitation with 488 nm using the argon laser, chlorophyll emits in both orange (optical filter FL2) and red (optical filter FL3) and fluorescence emission can be observed at 570 nm or 690 nm respectively [17]. The signal can be collected in FL2 and FL3 channels although FL3 channel seems to be the most used. PhycoErythrin *(PE)*: is also excited using argon laser 488 nm and emits in orange. Fluorescence is then measured at 585 nm (FL2) [17], [20]. PhycoCyanin *(PC):* generally excited at 620 nm using diode laser and emits at 640 nm (optical filter FL4) [17], [20].

Two-dimensional profiles of FL2 against FSC (Forward Scatter, indicating cell size) and FL3 against FSC are plotted and the parameter setting adjusted to monitor all chlorophyll-having populations present in the sample (Fig. 1).

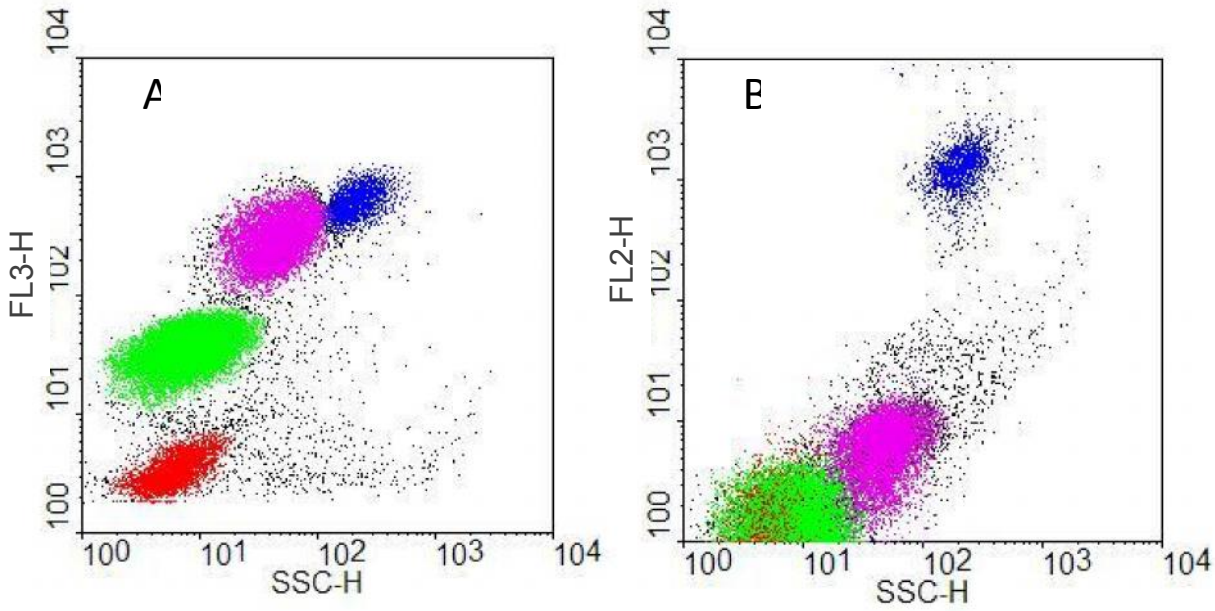

Fig. 1. Cytograms of some microalgal species analyzed using FACSCalibur cytometer, Synechococcus sp. (red), Nannochloropsis gaditana (green), Isochrysis galbana (pink) and Rhodomonas salina (blue). Dot plots presents fluorescence of chlorophyll: FL3 (A) and phycoerythrin: FL2 (B), vs celular structure. Strains belong to Culture Collection of Marine Microalgae (CCM-ICMAN-CSIC, Spain). Data not published

2.2 CELL SORTING

Once microalgae populations in an environmental sample are characterized, comes the isolation and purification of interesting populations or strains. Those steps are tedious and time-consuming. A development of fast and efficient method, for microalgae strains isolation from natural samples, becomes a mandatory given the development rate of biofuels programs worldwide.

Cell sorting coupled to FCM is based on the multi-dimensional distribution of algal populations for red fluorescence that represent essentially chlorophyll against forward-light scatter representing cell size. The first step is to identify the parameter settings that allow the best visualization of all populations contained in the fresh unfixed sample [21]. These parameters settings include voltages of FSC, SSC (Side SCatter), PE channel (FL2), chlorophyll channel (FL3), PC channel (FL4) and threshold applied to FL3 (to separate chlorophyll fluorescence from auto-fluorescence signals of other molecules). Once all sorting conditions are mastered, the population of interest is gated and sorting launched using the adequate mode. The sorting operation can take from some minutes to few hours (when the cell number of interesting populations in the sample is very low), followed by culturing individual sorted cells in culture wells using an appropriate medium. Cell sorting module present in new generation of flow cytometers offers the possibility of rapid and efficient isolation of microalgal cells from the original natural sample (Fig. 2). With this method pure populations can be isolated in few hours versus months with classical methods such as single cell isolation, dilution or micromanipulation [21], [22]. In addition, the sorter module coupled to FCM offers also the possibility to carry out an axenic sorting. To achieve an axenic sorting, the fluidic system should be thoroughly disinfected using, for instance, 70% ethanol for 20 min followed by a flushing with 0.2 µm filtered or autoclaved sheath fluid. The cell sorting is carried out using sterile sheath fluid and sterile vials to collect sorted cells.

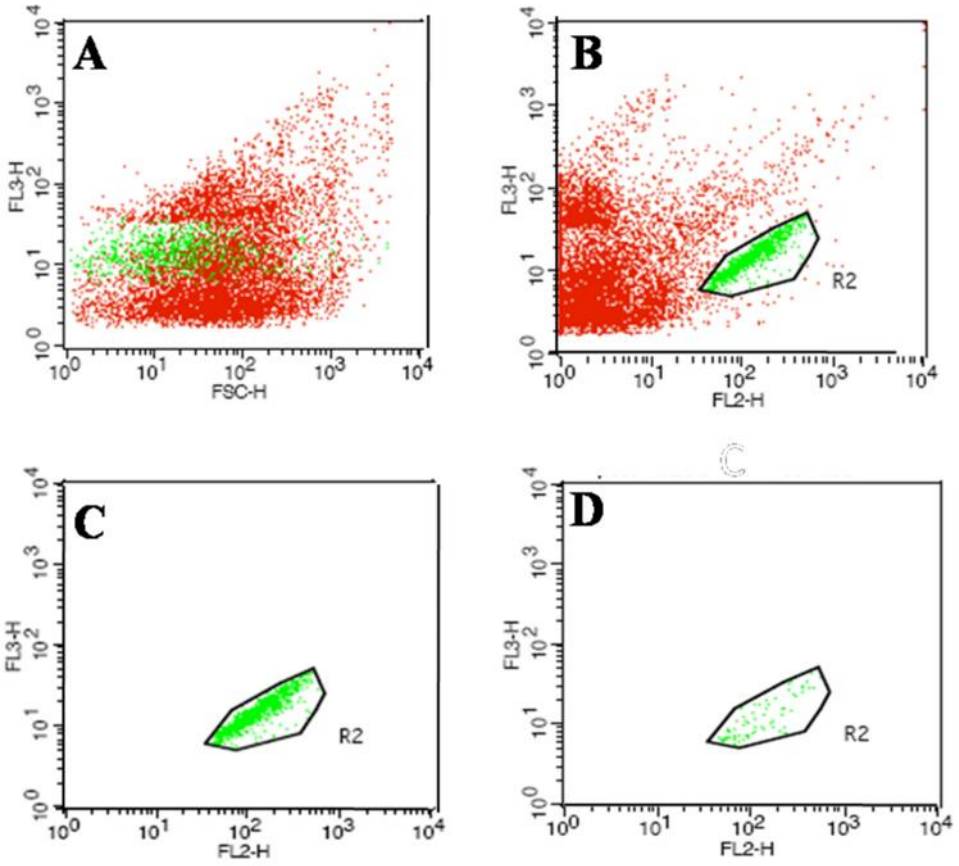

Fig. 2. Flow cytometric dot plots of sorted microalgae isolated from natural sea water from North of Morocco, using single-cells sorting mode of sorter coupled to a FACSCalibur cytometer. Experiment was carried out in our laboratory. (A) Dot plot windows based on cell size (FSC) and chlorophyll (FL3) auto-fluorescence of microalgae. (B) Chlorophyll (FL3) vs. phycoerythrin (FL2) auto-fluorescence of gated population previous to sorting. (C) Sorted cells monitoring during sorting and (D) analysis of sorted sample after centrifugation and resuspension in filtered natural sea water. Data not published

Several researchers have used FCM features for microalgae isolation in microalgae-based biodiesel project. High-lipid producing strains of *Tetraselmis* have been isolated using this method [5]. Furthermore, Doan *et al*. [4] isolated about 96 strains from costal water of Singapore for a biodiesel project.

However, a probably adverse effect of using cell sorting coupled with FCM has been described. It was reported that sorting can affect the cell viability by electrical, mechanical or optical stress (laser contact with the cells). Nevertheless, this effect is not dramatic at least in the analyzed species belonging to diatoms and green microalgae [4] when 50-75% of sorted cells remained viable. In the same manner, other researchers showed high viability (65%) of sorted *Tetraselmis* cells [5]. The viability of microalgae after sorting was significantly improved in comparison with earlier data that reported that only 20-30% of sorted cells grew successfully [23]. It's probably due to the improvement of sorting technology and also to the cell resistance against laser treatment; some species with rigid or additional cell wall like diatoms can be protected against this side effect [20].

It is important to know that for increasing the viability of sorted cells, some precautions have to be taken in consideration; firstly the sheath fluid must be appropriate for the growth of sorted microalgae, this prevents additional stress to microalgae. The laser power is another important factor affecting cell viability; it should be turned down as far as possible to minimize photo-damage of the sorted cells [20].

Another challenge of using cell sorting lies in use of natural samples containing pluricellular or filamentous microalgae communities, which cannot pass through the needle of the cytometer.

The cell-sorting coupled to FCM can also be used for re-isolation or re-purification of selected strains used for biodiesel production (i.e., when a culture becomes contaminated with bacteria, fungi or with autochthones non-lipid producing microalgae strains). Contamination occurs frequently since open culture systems are the most used for microalgae culture because of their profitability [1].

2.3 CELL COUNTING

Defining number of microalgae cells in a culture is very important during all steps of microalgae culture, mainly when the exact number of cells needs to be known. Microalgae, like most microorganisms can be counted using optical microscopy. However, the need for the use of faster and more precise systems has been noted by researchers. FCM represents an automated system, allowing precise and fast determination of microalgae number in a culture. In addition to this, it offers the possibility to distinguish smallest microalgae (picoplankton: phytoplankton <2 to 3 µm) from bacteria which is an advantage over optical microscopy. Counting microalgae, as well as other applications, is based on their auto fluorescent properties, due to photosynthetic pigments. This allows discriminating them from other microorganismds and non-living particles. For microalgae cell counting, fresh unfixed samples are preferably used, so that cells conserve their fluorescent properties. Fluorescent beads with known concentration and size can be used to measure the flow rate. This data is then used to calculate, based on flow rate, the number of fluorescent particles (microalgae) analysed by time and volume units [17]. This FCM application is a useful tool for microalgae culture destined to biofuels production, since it allows the control of microalgae growth and evolution during the culturing period.

2.4 CELL VIABILITY MONITORING

It is usually necessary to evaluate cell viability during microalgae culture for biofuel production as well as after treatments for lipids enhancement. Since microalgae are very sensitive to culture conditions, it becomes essential to develop an easy and fast method to evaluate the effect of culture conditions variations on cell viability. In open culture systems (open ponds) microalgae are exposed to medium evaporation (salinity and nutrient stress), shaking stress (agitation is generally mechanical), temperature variation between day and night (thermal stress) and other risks like biological contaminations. In closed systems like photobioreactors these risk factors are more controlled. However other problems like photodamage of cells due to illumination are more frequent. For this reason, easy and continuous control of physiological state of microalgae is very important. Specific dyes like Propidium Iodide (PI) or SYTOX Green cannot cross walls of living cells, and only once membrane integrity affected the dyes can associate with DNA and exhibit fluorescence. When a microalgae sample is stained with PI solution, it penetrates the nonviable cells, binds to DNA and after excitation with 488 nm, the fluorescence is measured at 585 nm (FL2 channel), contrary to living cells which emit no fluorescence in FL2 (Fig. 3). This method has been successfully used in various microalgae strains implied in biodiesel projects to monitor cell viability [24].

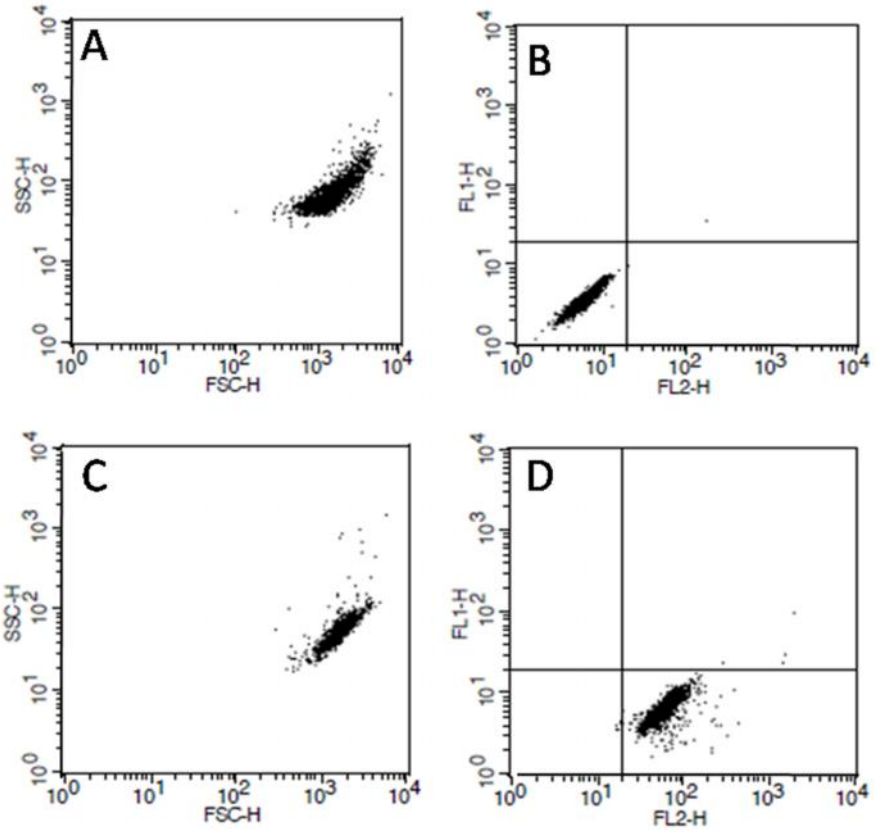

FIG 3: An example of PI staining comparison between living and died cells of Dunaliella salina isolated from saline water located in north of Morocco. Experiment was carried out in our laboratory. (A) Dot plot FSC/SSC of one population corresponding to living cells, (B) no staining of cells after incubation with PI. After heat treatment (10 min in water bath at 100°C for), no major changes in size /structure has been observed (C), but PI stained killed cells and signal is collected on FL2 (D). Data not published

2.5 INTRACELLULAR LIPIDS CONTENT

Microalgae stores lipids bodies as a form of energy reserve represented by neutral lipids, and as essential components of membranes like glycol and phospholipids. It is well established that, in most microalgae strains (mainly lipid accumulative strains), neutral lipids concentration increase significantly with culturing time and in response to culture conditions changes (nutrients starvation, light stress, CO_2 supply, etc.). This property is mainly exploited in biodiesel projects since successful and economically viable algae-based biofuels industry depends on selection of appropriate strains and their culture conditions. One of the most critical properties of strains candidates of a biofuel project are lipids content and quality. In this way, rapid and efficient methods allowing determination of lipid content are needed. In comparison with chromatographic and gravimetric techniques, that needs previous lipid extraction, fluorometric measurements are fast, cost-effective [25] and only few microliters of culture are needed *vs* milliliters to litters for lipid extraction. Thereby, use of specific dye and FCM technique offers an attractive tool. Solvatochromic dye Nile Red (NR) (9-diethylamina-5H-benzo[a]phenoxazine-5-one) stains intracellular lipids selectively allowing the quantification of neutral and polar lipids [26]. Lipid measurement of intracellular lipids using NR fluorescence is produced in highly hydrophobic environments and quenched in hydrophilic ones [27], [28]. The efficiency of NR fluorescence and the total lipid content has been shown linear [25], [27], [29].

In a flow cytometer, lipids' straining with NR is traduced to different fluorescence emissions after excitation with argon laser at 488 nm. When NR is dissolved in neutral lipids it emits an intense yellow fluorescence collected in channel FL2 (560-640 nm in a FACSCalibur™ system, Becton Dickinson Instruments, equipped with a 488 nm argon laser and 635 nm red diode laser) (Fig. 4), and when dissolved in polar lipids it exhibits red fluorescence (>650 nm) collected in channel FL3 [27], [30].

NR fluorescence showed to be an efficient high-throughput screening tool to select a favourable microalgae strain based of their intracellular lipid content, for biofuel projects [4], [13]. Different aspects can be studied: firstly kinetic of neutral lipids accumulation during the growth cycle to identify the peak of lipid production, comparison of lipid content in stationary phase between different species, and/or various growth conditions and the effect of some treatments and metabolic orientation on the lipid production. Several works have used this technique efficiently to develop biofuel projects [4], [13]. Doan *et al*. [4] screened 96 strains isolated from natural samples and found that about more than half of studied strains contained more than 25% lipids.

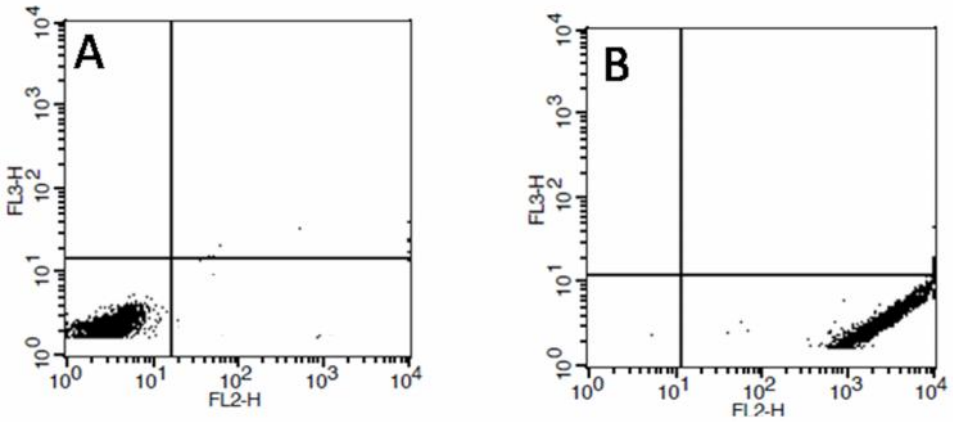

Fig. 4. NR staining dot plots of Nannochloropsis sp. isolated from North of Morocco. Experiment was carried out in our laboratory. (A) Microalgae sample before NR staining, parameter settings are adjusted to place population in negative area. (B) After NR addition, sample is incubated 2 min at 37°C and an intense signal is collected at FL2 channel indicating that this strain has an interesting neutral lipids accumulation. Data not published

The possibility to use the multi-parameter flow cytometry for fast and real time monitoring of intracellular lipids content has been studied for some microalgae species belonging to different taxonomic groups (Tab. 3).

TABLE 3: Species candidates for biodiesel project analysed by FCM after NR staining.

Microalgae	Lipid content (% of DW)	References
Skeletonema costatum	9.5	[4]
Thalassiosira sp.	17.8	[4]
Nannochloropsis sp.	40.3-44.8	[4]
Chaetoceros sp.	16.3	[4]
Achnanthes sp.	44.5	[4]
Heterosigma sp.	39.9	[4]
Tetraselmis suecica	17.7-29.9	[5]
Chlorella prothecoides	27.5	[13]
Scenedesmus obliquus	12.8	[24]
Neochloris oleoabundans	16.5-52	[24]

This technique offers multiple applications in this field such as fast, in situ and real time estimation of intracellular lipids (mainly neutral ones) between several microalgae species without the laborious stage of lipid extraction and chromatographical analysis. In addition, it gives an idea of microalgae lipid content in order to decide if the strain is interesting or not for a biodiesel project. In case of lipid rich strains, a confirmation is needed by chromatographical analysis [13]. However some species like some chlorophyceae, diatoms, etc. presents rigid cell walls that decrease the NR penetration into the cell. In these cases, when gravimetric measurements show lipid presence, improved methods have to be used for lipid analysis using FCM (Fig. 5). Permeation of cell walls using some chemical reagents is the most used methods applying

different concentrations of DMSO (5-30% v/v), ethanol or glycerol [4, 13]. Also, FCM allows easy monitoring of lipid content during the microalgal growth and the identification of maximal lipid accumulation phase, monitoring of the effect of growth conditions (culture media, light, CO_2 supply, shaking, etc.) on lipid accumulation, giving the possibility to rapidly change culturing conditions to maintain maximal productivity. When the cell lipid content information is available during the culturing process, these parameters can be controlled.

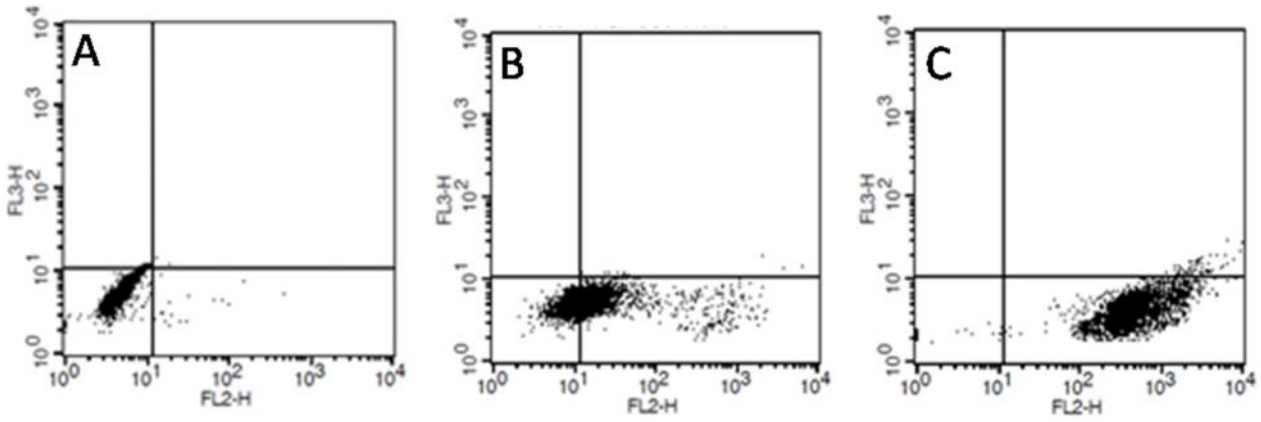

Fig. 5. Improvement of Dunaliella sp. staining efficiency using DMSO. This strain has been isolated from saline in North Morocco, and experiment was carried out in our laboratory. A: microalgae sample without staining, parameter settings are adjusted to place population in upper left area. B: Nile red addition and incubation at 37°C during 10 min. C: NR staining improvement after addition of DMSO 20% simultaneously to NR addition. Data not published

As described, FCM can be used in the most decisive steps during biodiesel project development (Fig. 6); isolation of strains from their natural habitats, screening and selection of microalgae candidates for the biodiesel production.

Both at laboratory or large scale levels, use of FCM leads to fast and efficient control of critical parameters changes such as biomass and lipids evolution in term of quality and quantity. This offers the possibility to act immediately to adjust culturing conditions, determine optimal conditions for growth and increase productivity.

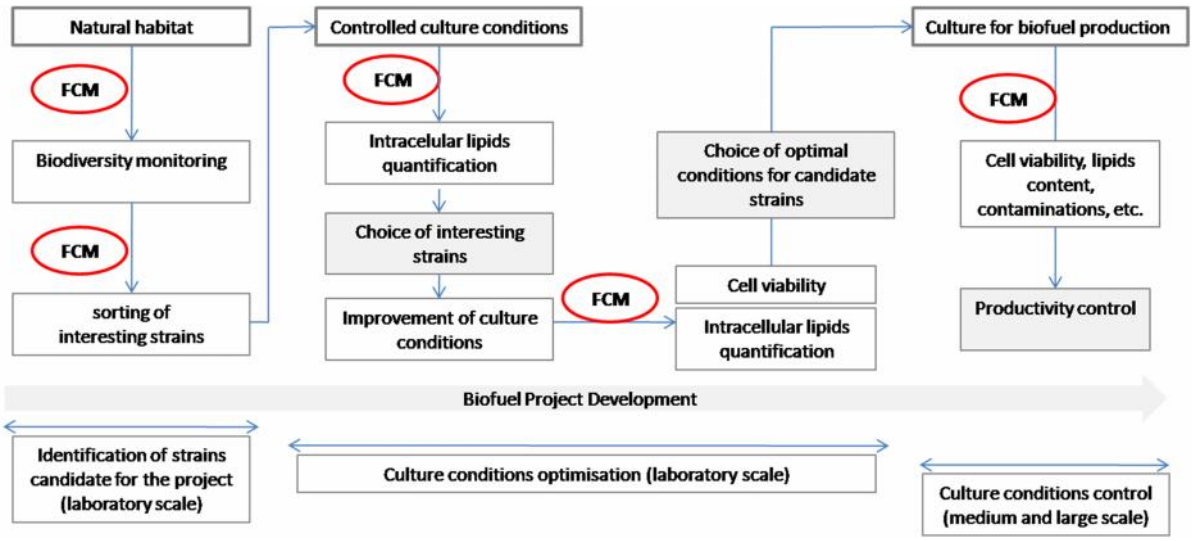

Fig. 6. Applications of FCM during steps of biodiesel project development

3 CONCLUSION

Despite the considerable increase towards the use of microalgal lipids for biodiesel production, the development of a project that is profitable at large scale still needs further investigation. In this context, many researchers are opting for development of microalagal biotechnology as a powerful way to improve productivity of microalgae. In this review, FCM is described as a multiparameter technique that can contribute efficiently in microalgal biotechnology development and represent a helpful tool for a better exploitation of microalgae as feedstock for biodiesel production.

REFERENCES

[1] Y. Chisti, "Biodiesel from microalgae," Biotechnology Advances, vol. 25, no. 3, pp. 294-306, 2007.

[2] Y. Chisti, "Biodiesel from microalgae beats bioethanol," Trends in Biotechnology, vol. 26, no. 3, pp. 126-131, 2008.

[3] A. Banerjee, R. Sharma, Y. Chisti and U.C. Banerjee, "*Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals," Critical Reviews in Biotechnology, vol. 22, no. 3, pp.245-279, 2002.

[4] T. T. Y. Doan, B. Sivaloganathan, and J. P. Obbard," Screening of marine microalgae for biodiesel feedstock," Biomass and Bioenergy, vol. 35, no. 7, pp. 2534-2544, 2011.

[5] M. F. Montero, M. Aristizàbal, and G. G. Reina, "Isolation of high-lipid content strains of the marine microalga *Tetraselmis suecica* for biodiesel production by flow cytometry and single-cell sorting," Journal of Applied Phycology, vol. 23, no. 6, pp. 1053-1057, 2011.

[6] M. C. Radakovits, R. E. Jinkerson, A. l. Darzins, and M. C. Posewitz, "Genetic engineering of algae for enhanced biofuels production," Eukaryotic cell, vol. 9, no. 4, pp. 486-501, 2012.

[7] A. S. Paau, J. Uro, and J. R. Cowles, "Applications of flow cytometry to the study of algal cells and isolated chloroplasts," Journal of Experimental Botany, vol. 29, pp.1011-1020, 1978.

[8] B. J. Trask, G. J. van den Engh and J. H. B. W. Elgerhuizen, "Analysis of phytoplankton by flow cytometry," Cytometry , vol. 2, no. 4, pp. 258-264, 1982.

[9] C. M. Yentsch, P. K. Horan, K. Muirhead, Q. Dortch, E. M. Haugen, L. Legendre, L. S. Murphy, D. Phinney, S. A. Pomponi, R. W. Spinrad, A. M. Wood, C. S. Yentsch, and B. J. Zahurenec, "Flow cytometry and sorting: a powerfull technique with potential applications in aquatic sciences," Limnology and Oceanography, vol. 28, pp. 1275-1280, 1983.

[10] R. J. Olson, S. L. Frankel, S. W. Chisholm and H. M. Shapiro, "An inexpensive flow cytometer for the analysis of fluorescence signals in phytoplankton: chlorophyll and DNA distributions," Journal of Experimental Marine Biology and Ecology, vol. 68, no. 2, pp. 129-144, 1983.

[11] R. J. Olson, D. Vaulot, and S. W. Chisholm, "Marine phytoplankton distributions measured using shipboard flow cytometry," Deep Sea Research Part A. Oceanographic Research Papers, vol. 32, no. 10, pp. 1273-1280, 1985.

[12] F. Partensky, W. R. Hess, and D. Vaulot, "*Prochlorococcus*, a marine photosynthetic prokaryote of global significance," Microbiology and Molecular Biololy Reviews, vol. 63, no. 1, pp. 106-127, 1999.

[13] T. Lopes da Silva, A. Reis, R. Medeiros, A. C. Oliveira and L.Gouveira, "Oil production towards biofuel from autotrophic microalgae semicontinuous cultivations monitorized by flow cytometry," Applied Biochemistry and Biotechnology, vol. 159, no. 2, pp. 568-578, 2009 a.

[14] H. Mendoza Guzmàn, A. de la Jara Valido, L. C. Duarte and K. F Presmanes, " Estimate by means of flow cytometry of variation in composition of fatty acids from *Tetraselmis suecica* in response to culture conditions," Aquaculture International, vol. 18, no.2 pp. 189-199, 2010.

[15] T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj and F. Bux, "Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuels production," Bioresource Technology, vol. 102, no. 1, pp. 57-70, 2011.

[16] S. A. Scott, M. P Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith and A. Smith, "Biodiesel from algae: challenges and prospects," Current Opinion in Biotechnology, vol. 21, no. 3, pp. 1-10 2010.

[17] Marie, D., Simon, N. and Vaulot, D., Phytoplankton cell counting by flow cytometry, In: R. A Andersen (Ed.), Algal Culturing techniques, Chap. 17, pp. 53-267, 2005.

[18] S. Demers, S. Roy, R. Gagnon and C. Vignault, "Rapid light-induced changes in cell fluorescence and in xanthophyll-cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae): A photo-protection mechanism," Marine Ecoligy Progress series, vol. 76 , no. 2, pp. 185-193, 1991.

[19] J. Toepel, C. Wilhelm, A. Meister, A. Beckert and M. Martinez-Ballesta, "Cytometry of freshwater phytoplankton," Cytometry: New Developments, vol. 75, pp. 375-407, 2004.

[20] M. Reckermann, "Flow sorting in aquatic ecology," Scientia Marina., vol. 64, no. 2, pp. 235-246, 2000.

[21] A. Kacka, and G. Donmez, "Isolation of *Dunaliella* spp. From hypersaline lake and their ability to accumulate glycerol," Bioresource Technology, vol. 99, no.17, pp. 8348-8352, 2008.

[22] Andersen, R., Algal culturing techniques. Elsevier Academic Press, Burlington, 2005.
[23] C. Sensen, K. Heimann and M. Melkonian, "The production of clonal and axenic cultures of microalgae using fluorescence-activated cell sorting," European Journal of Phycology, vol. 28, no. 2, pp. 93-97, 1993.
[24] T. Lopes da Silva, C. Amarelo Santos and A. Reis, "Multi-parameter flow cytometry as a tool to monitor heterotrophic microalgal batch fermentations for oil production towards biodiesel," Biotechnology and Bioprocess Engineering, vol. 14, no. 3, pp. 330-337, 2009.
[25] S. J. Lee, B. Yoon and H. Oh, "Rapid method for the determination of lipid from the green alga *Botryococcus braunii*," Biotechnology Techniques, vol. 12, no. 7, pp. 553-556, 1998.
[26] F. Alonzo and P. Mayzaud, "Spectrofluorometric quantification of neutral and polar lipids in zooplankton using Nile red," Marine Chemistry, vol. 67, no. 3-4, pp. 289-301, 1999.
[27] A. De la Jara, H. Mendoza, A. Martel, G. Molina, L. Nordstron, V. De la Rosa and R. Diaz, "Flow cytometric determination of lipid content in a marine dinoflagellate, *Crypthecodinium chonii*," Journal of Applied Phycology, vol. 15, no. 5, pp. 433-438, 2003.
[28] Pulz, O., "Photobioreactors: production systems for phototrophic microorganisms". Applied Microbiology and Biotechnology, vol. 57, no. 3, pp. 287-293, 2001.
[29] K. E. Cooksey, J. B. Guckert, S. A. Williams and P. R. Callis, "Fluorometric-determination of the neutral lipid content of microalgal cells using Nile Red". Journal of Microbiological Methods, vol. 6, no. 6, pp. 333-345, 1987.
[30] Shapiro, H.M., Practical flow cytometry. 3 rd ed. New York: Wiley-Liss, 1995.
[31] W. Chen, C. Zhang, L. Song, M. Sommerfeld and Q. Hu, "A high throughput Nile Red method for quantitative measurement of neutral lipids in microalgae," Journal of Microbiological Methods, vol. 77, no. 1, pp. 41-47, 2009.

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Diversity and Dominance of Ectomycorrhizal Fungi on After Burned and Unburned Forests in Kutai National Park (Indonesia)

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ABSTRACT: Ectomycorrhizal fungi has long been known as benefit microorganism for plants, because the fungi supply nutrients and water from soil to their hosts. But ectomycorrhizal fungi are very sensitive to environmental change of their habitat, thus can be used as bioindicator. One of the change that influence on their presence is forest fire that kill the trees as their hosts. Forest fire is almost occur every year in the dry season in Indonesia, not exception Kutai National Park (KNP), where the fire comes from the society gardens surrounding it. This study aimed to determine species diversity and dominance of ectomycorrhizal fungi on the after burned (Prevab) and unburned forests (Sangkimah). The results showed that the number of species of ectomycorrhizal fungi at Prevab were 12 species and 37 individuals, while at Sangkimah there were found 34 species of 87 individuals, this meant that the numbers of species and individuals of fungi at Sangkimah were more than at Prevab. However, based on biodiversity index, at both locations were low (H'<1), respectively was 0.51528 at Prevab and 0.50233 at Sangkimah. At Prevab, species of fungus with high levels of dominance was *Clavulina* sp., moderate level of dominance were *Mycena pura*, *Coprinus atramentaria*, *C. comatus* and *Russula decolorans*, whereas other species were in the level of low dominance. At Sangkimah, fungi with a high level of dominance was *Clitocybe* sp4, moderate level of dominance were *Leucocoprinus flos-sulfuris* and *Cantharellus* sp3, whereas other species were in the level of low dominance.

KEYWORDS: dipterocarps, ectomycorrhiza, bioindicator, Prevab, Sangkimah.

1 INTRODUCTION

Various studies indicate that many species of fungi are sensitive to changes in habitat environments, such as macro fungi that grow in the ground (ectomycorrhizal), therefore ectomycorrhizal fungi can be used as bio-indicators of forest quality. The assumption is that when many species of ectomycorrhizal fungi found, then the condition of the forest or habitat is good and when bit species of ectomycorrhizal fungi found, the forest conditions are not good (damaged). The growth of ectomycorrhizal fungi are also influenced by environmental factors such as light intensity, temperature, humidity, soil fertility and aeration and plant root exudates.

Reference [1] noted that there were 60 species of ectomycorrhizal fungi in East Kalimantan (Bukit Soeharto, Wanariset Samboja and ITCI area), where mostly of the forest were secondary forest that were dominated by the trees of large dipterocarps. The presence of the fungi were through the adaptation process influenced by environmental conditions. Reference [2] reported that in the unburned forest in Sungai Wain forest had a number of species of ectomycorrhizal fungi much more than the burned forest. Relation to the rate of deforestation was known that in habitats with low deforestation rates (due to logging concessions and illegal logging) had a number of ectomycorrhizal fungi species much more than the highly degraded habitats.

Kutai National Park (KNP) had suffered a huge fire in 1982/1983 and 1997/1998. The data from KNP noted that until 2004 fire caused by human negligence had damaged about 146,080 ha (80%) of the total area. The damage was compounded by illegal logging and encroachment, proven during 2001-2004 the number of illegal timber seized reach 246,082 m^3 [3]. The fire will also affect the macro soil animals, fungi and various species of plants and animals that play an important role in the decomposition and recycling of nutrients [4].

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This research was conducted to determine the diversity and dominance of ectomycorrhizal fungi in burned (Prevab) and unburned forests (Sangkimah) in KNP.

The expected outcome of this research were to provide important information about the species of ectomycorrhizal fungi in the region of Prevab and Sangkimah of KNP in the development and enhancement of the area, and also as material input and consideration for the government and relevant agencies especially the KNP authority in the preservation of species of ectomycorrhizal fungi .

2 METHODE OF RESEARCH

This research was conducted in the area of KNP, precisely at Prevab (forest burned in 1981/1982 and 1997/1998) and Sangkimah (unburned forest) East Kutai regency of East Kalimantan Province and in the Laboratory of Forest Protection, Faculty of Forestry, Mulawarman University, Samarinda. The time required for research reach 4 months (September to December 2012) which included primary data collection in the field as well as the identification of species of fungi in the laboratory.

Sample plots were developed purposively. The size of each plot was 50 m x 50 m and replicated 4 times, so the number of all plots were 2 forest condition (burned and unburned) x 4 replicates = 8 plots (2 ha).

Only big size fruit bodies of ectomycorrhizal fungi were taken (macro fungi/mushroom) with a diameter of hood of at least 0.5 cm. Fungal fruiting body that is too small is not recorded because of the difficulty in the identification process. Collected data were consisted of:

a. Species of fungi found through direct identification in the field and in the laboratory with the help of literatures.
b. The number of species of fungi and the number of individuals, i.e counting the number of species of fungi and the number of individuals of each species.
c. Characteristics of fungi, which describe the morphological characteristics of fruit bodies such as the hood, stem (stipe) and other important characteristics.

2.1. Diversity of Ectomycorrhizal Fungi

To determine species diversity, a diversity index (H') of Shannon-Wiener was used with the formula according to reference [5] as follows:

$$H' = -\Sigma\{(ni/N) \log (ni/N)\} \quad (1)$$

H' = diversity index
ni = sum of individu of each species
N = sum of individu of all species

When H'<1, then species diversity is low, when H' = 1–3 show that species diversity is moderate and when H'>3 show that species diversity is high [6].

2.2. Dominance of Ectomycorrhizal Fungi

To determine which fungi is dominance at each location, it is used dominance index of Simpson as follows [7]:

$$D = ni/N \quad (2)$$

D = dominance index of species
ni = sum of individual of each species
N = sum of individual of all species

Dominance index ranges between 0 to 1, the smaller the value (near 0), it shows that a species is not dominant, but the higher the value (near 1) then the species is dominant.

Based on the determination, the criteria are as follows:

0.00-0.33 = domination level is low
>0.33-0.66 = domination level is moderate
>0.66-1.00 = domination level is high

3 RESULTS AND DISCUSSION

3.1. Diversity of Fungi on Different Location

Number of species of ectomycorrhizal fungi found at Prevab and Sangkimah showed in Table 1. The number of ectomycorrhizal fungi at each location and research plots were different, i.e ranged between 2 to 17 species. At Prevab found only 12 species and 37 individual and the most abundant was at plot 2 as many as 5 species 15 individual. At Sangkimah found 34 species 87 individual and the most abundant was at plot 1 as many as 17 species 49 individual.

Different number of species and individual at each location was caused by different in vegetation. At Sangkimah generally was no dense lower vegetation such as shrubs, wild *Alpinia galanga* (galangal) and other species of non host of ectomycorrhizal fungi, but there were many big trees dominated by dipterocarp species that served as symbiont of ectomycorrhizal fungi. Dipterocarps are the most important species for living of ectomycorrhizal fungi. Low intensity of sun light was also still reach soil surface. Generally for formation fruit body, the fungi need light although with low intensity.

At Prevab, where the site establihed research plots, the dipterocarp species were not found. According to information of KNP authority, formerly illegal logging and forest fire had occured, so the present trees are the result of succession that do not reach climax yet, hence belong to young secondary forest. Young regeneration of dipterocarps species at research plots was not present because there was no mother tree.

Based on environment condition, i.e altitudes at Prevab and Sangkimah were 37–57 m and 32–78 from sea level respectively, while air temperatures at Prevab and Sangkimah at the noon ranged between 29.2–30.0°C and 29.0–31.0°C respectively. Relative air humidities were 80.4–94.5% and 83.0–86.1% respectively. Temperatures, sum of species and individual were far different between both locations. This meant that species of host trees are the most important for development of ectomycorrhizal fungi. In the research plot at Sangkimah found many big trees such as *Shorea johorensis*, *S. laevis*, *S. leprosula* and *Dryobalanops* sp., where the species act as main host of ectomycorrhizal fungi, while at Prevab there was no such species, but dominated by non dipterocarps such as *Cananga odorata* (kenanga), *Croton* sp., *Polyaltia* sp., *Vitex pinnata* (laban) and *Dracontomelon dao* (sengkuang).

Table 1. Number of species and individual of ectomycorrhizal fungi found at Prevab (burned forest) and Sangkimah (unburned forest)

Location	Plot number	Plot position	Altitude (m from sea level)	Air temperature (°C)	Air humidity (%)	Sum of species	Sum of individual
Prevab	1	0.0°32'02"N-117°27'25.8"E	42	29.2	94.5	3	14
	2	0.0°31'57.5"N-117°27'50"E	57	29.6	81.0	5	15
	3	0.0°31'35"N-117°27'30"E	46	29.7	80.4	3	6
	4	0.0°32'3.7"N-117°27'49.8"E	37	30.0	82.3	2	2
	Sum					12*	37
	Average		45.5	29.6	84.6		
Sangkimah	1	0.0°21'51.7"N-117°28'13.4"E	32	29.0	83.0	17	49
	2	0.0°22'3.1"N-117°28'26.5"E	78	30.4	86.1	9	16
	3	0.0°21'59.8"N-117°28'21.2"E	48	30.8	85.1	4	10
	4	0.0°22'1.6"N-117°28'12.7"E	44	31.0	84.3	5	12
	Sum					34*	87
	Average		50.5	30.3	84.7		

*same species was present at different plots

The differences of vegetation species growing in both locations make ectomycorrhizal fungi are also different in species diversity, hence ectomycorrhizal fungi can be used as bioindicator of forest condition, where when abundant species of ectomycorrhizal fungi found, it means that the forest is still good indicated by the presence of big trees as their host, but when few ectomycorrhizal fungi found, it means that the forest is damaged, usually indicated by rare or no big trees as the host of fungi. Although there are only presence small trees, but when the trees are suitable for the host of ectomycorrhizal fungi, then the fungi will grow well. Reference [8] reported that ectomycorrhizal fungi in Czechoslovakia are very sensitive against air pollution, the influence are as follow: i) inhibition of fruitbody formation, ii) reduction of fungal species diversity and iii) lost of part or all of symbiotic fungi. It can also be used to assess the level of forest destruction in connection with the fungal sensitivity to air pollution, the higher the air pollution, the damaged forest and the symbiotic fungi more and more disappear. Reference [9] noted that at three locations i.e forests of Mului, Mount Lumut and Rantau Layung in Paser District (East Borneo), almost all species of fungi growing in soil were ectomycorrhizal fungi and the most commonly found were at Mului as many as 44 species, followed by forest in Mount Lumut 20 species, whereas in Rantau Layung foret only 14 species. These results indicated that the environmental conditions in the forest habitat of Mului was better than at the other two locations. In Mului forest were still many dipterocarp trees with a diameter greater than 50 cm, whereas forest trees at Rantau Layung such trees were rare, but only weathered residual illegal logging, so it can be said, that forest of Rantau Layung in the condition of severely damaged.

If the host is not suitable for the growth of ectomycorrhizal fungi, the fungal fruiting body formation does not occur. This is according to reference [10], that in Muaralawa (West Kutai District), that in land rehabilitation of the former coal mine PT Trubaindo Coal Mining did not find ectomycorrhizal fungi, whereas in secondary forests with lots of dipterocarp tree species found 49 species of ectomycorrhizal fungi.

Reference [11] stated, that ectomycorrhizal fungi can be life threatened by climate change and habitat destruction; reduction ectomycorrhizal fungal species detected in the last few decades in various studies in Europe, where it is an alarming development. The loss of species diversity in ectomycorrhizal fungi are harmless, given the important role of these fungi in forest ecosystems. Ectomycorrhizal fungi in the forest should be preserved because of high species diversity is an important prerequisite for healthy forests and healthy forests are very important for maintaining high species diversity and productivity of the fungi.

When seen from the number of all species found, then the fungi found at Prevab as many as 12 and at Sangkimah 34 species is almost identical to the findings in Mului by reference [9] on 12 November to 3 December 2005 as many as 45 species, which at that time was rainy season. Although the number of species is almost the same, but there were many differences in the species name. This could be due to differences in location, altitude above sea level, weather conditions, especially temperature and humidity as well as the host tree species found in the study site, which was the last seen more real cause.

Research in the KNP was conducted during the dry season month of September 2012. When seen the average rainfall for 11 years from 2000 to 2010, then in September was the least month of rainfall, i.e 108.0 mm, it meant that species on ectomycorrhizal fungi found in KNP were species of resistant to lower precipitation. It is possible that the other species will grow in the months of higher rainfall. Therefore, the species of fungi can be recommended as a seedling-inoculation of the seedling planted in reforestation programs such as Intensive Silviculture (Silin) program which prioritizes the species of dipterocarps to be planted.

In Table 2 is shown ectomycorrhizal fungal species diversity index at Prevab and Sangkimah. The table shows, that the diversity index (H') of the ectomycorrhizal fungi at Prevab (burned forest) of plots 1 to 4 ranged from 0.2211 to 0.5339 so that the value of H'<1 showed that species diversity in each study plot at Prevab was low. Likewise diversity index at Sangkimah (unburned forest) from 1 to 4 replicates ranged from 0.3096 to 1.0912, so that the value of H'<1 to between 1 and 3, it showed that the diversity of ectomycorrhizal fungi in plots 1 was moderately, while in plots 2, 3 and 4 were low level of diversity.

Table 2. Diversity index (H') of ectomycorrhizal fungi found in each plot research

Location I: burned forest (Prevab)

Nr.	Species	Family	Sum of individual	ni/N	Log ni/N	ni/N log ni/N	H’
Plot (replication) 1							
1	*Lepiota* sp	Agaricaceae	1	0.0714	-1.1461	-0.0819	
2	*Clavulina* sp	Clavulinaceae	12	0.8571	-0.0670	-0.0574	
3	*Agaricus* sp1	Agaricaceae	1	0.0714	-1.1461	-0.0819	
	Sum		14			-0.2211	0.2211 (I)
Plot (replication) 2							
1	*Amanita rubescens*	Amanitaceae	1	0.0667	-1.1761	-0.0784	
2	*Mycena pura*	Mycenaceae	8	0.5333	-0.2730	-0.1456	
3	*Amanita* sp	Amanitaceae	1	0.0667	-1.1761	-0.0784	
4	*Agaricus* sp2	Agaricaceae	1	0.0667	-1.1761	-0.0784	
5	*Coprinus atramentaria*	Agaricaceae	4	0.2667	-0.5740	-0.1531	
	Jumlah		15			-0.5339	0.5339 (I)
Plot (replication) 3							
1	*Coprinus atramentaria*	Agaricaceae	4	0.6667	-0.1761	-0.1174	
2	*Rhizopogon* sp	Rhizopogonaceae	1	0.1667	-0.7781	-0.1297	
3	*Coprinus comatus*	Agaricaceae	1	0.1667	-0.7781	-0.1297	
	Jumlah		6			-0.3768	0.3768 (I)
Plot (replication) 4							
1	*Coprinus comatus*	Agaricaceae	1	0.5000	-0.3010	-0.1505	
2	*Russula decolorans*	Russulaceae	1	0.5000	-0.3010	-0.1505	
	Sum		2			-0.3010	0.3010 (I)
	Grand total						1.4328
	Average						0.3582 (I)

Table 2 (continuation)

Location II: unburned forest (Sangkimah)

Nr.	Species	Family	Sum of individual	ni/N	Log ni/N	ni/N log ni/N	H'
Plot (replication) 1							
1	*Hygrophorus* sp	Hygrophoraceae	4	0.0816	-1.0882	-0.0888	
2	*Lyophyllum* sp1	Tricholomataceae	1	0.0204	-1.6902	-0.0345	
3	*Clitocybe* sp1	Tricholomataceae	1	0.0204	-1.6902	-0.0345	
4	*Myrlostoma coliforme*	Geastraceae	1	0.0204	-1.6902	-0.0345	
5	*Laccaria laccata*	Hydnangiaceae	1	0.0204	-1.6902	-0.0345	
6	*Suillus* sp1	Boletaceae	1	0.0204	-1.6902	-0.0345	
7	*Agaricus placomyces*	Agaricaceae	1	0.0204	-1.6902	-0.0345	
8	*Calostoma fuscum*	Sclerodermataceae	8	0.1633	-0.7871	-0.1285	
9	*Cantharellus* sp1	Cantharellaceae	1	0.0204	-1.6902	-0.0345	
10	*Omphalina* sp	Tricholomataceae	3	0.0612	-1.2131	-0.0743	
11	*Clitocybe* sp2	Tricholomataceae	8	0.1633	-0.7871	-0.1285	
12	*Gliophorus* sp	Hygrophoraceae	1	0.0204	-1.6902	-0.0345	
13	*Russula* sp1	Russulaceae	1	0.0204	-1.6902	-0.0345	
14	*Collybia acervata*	Tricholomataceae	4	0.0816	-1.0882	-0.0888	
15	*Boletus* sp	Boletaceae	1	0.0204	-1.6902	-0.0345	
16	*Inocybe* sp	Cortinariaceae	3	0.0612	-1.2131	-0.0743	
17	*Naematoloma* sp	Strophariaceae	1	0.0204	-1.6902	-0.0345	
18	*Tricholoma* sp	Tricholomataceae	8	0.1633	-0.7871	-0.1285	
	Sum		49			-1.0912	1.0912 (m)
Plot (replication) 2							
1	*Lycoperdon perlatum*	Agaricaceae	1	0.0625	-1.2041	-0.0753	
2	*Agaricus placomyces*	Agaricaceae	1	0.0625	-1.2041	-0.0753	
3	*Lyophyllum* sp2	Tricholomataceae	1	0.0625	-1.2041	-0.0753	
4	*Cantharellus* sp2	Cantharellaceae	1	0.0625	-1.2041	-0.0753	
5	*Lepiota sp1*	Agaricaceae	2	0.1250	-0.9031	-0.1129	
6	*Clitocybe sp3*	Tricholomataceae	1	0.0625	-1.2041	-0.0753	
7	*Collybia sp1*	Tricholomataceae	1	0.0625	-1.2041	-0.0753	
8	*Collybia butyracea*	Tricholomataceae	1	0.0625	-1.2041	-0.0753	
9	*Leucocoprinus flos-sulfuris*	Agaricaceae	6	0.3750	-0.4260	-0.1597	
10	*Lepiota* sp2	Agaricaceae	1	0.0625	-1.2041	-0.0753	
	Sum		16			-0.8747	0.8747 (l)
Plot (replication) 3							
1	*Clitocybe* sp4	Tricholomataceae	7	0.7000	-0.1549	-0.1084	
2	*Agaricus placomyces*	Agaricaceae	2	0.2000	-0.6990	-0.1398	
3	*Lepiota* sp3	Agaricaceae	1	0.1000	-0.1000	-0.1000	
	Sum		10				0.3482 (l)
Plot (replication) 4							
1	*Cantharellus* sp3	Cantharellaceae	6	0.5000	-0.3010	-0.1505	
2	*Suillus* sp2	Boletaceae	2	0.1667	-0.7781	-0.1297	
3	*Cantharellus* sp4	Cantharellaceae	1	0.0833	-1.0790	-0.0899	
4	*Collybia* sp2	Tricholomataceae	1	0.0833	-1.0790	-0.0899	
5	*Russula* sp2	Russulaceae	2	0.1667	-0.7781	-0.1297	
	Sum		12			-0.3096	0.3096 (l)
	Grand total						2.6237
	Average						0.6559 (l)

l = low level of diversity. m = moderately level of diversity

When viewed from the average diversity index that describes the diversity of species in two different locations, the species diversity at Prevab was lower than at Sangkimah, respectively 0.3582 and 0.6559, where the diversity of species at both locations were in lower criteria. This is in accordance with the provisions of reference [6], if H'<1, then the species diversity is low, if H' = 1-3 the diversity of species classified as moderate and when H'>3 relatively high species diversity.

In Table 2, the difference of low index of species diversity in two locations due to differences in the number of individuals among species is quite large. According to reference [12], high and low species diversity is influenced by the evenness of the number of individuals of each species within a community, the smaller the difference in the number of individuals among species mean numbers more evenly, thus the higher species diversity.

3.2. Dominance Species of Fungus on Different Locations

In Table 3 is shown the dominance index of each species of ectomycorrhizal fungi in the location Prevab (burned forest) and Sangkimah (unburned forest). Judging from the dominance index range from 0 to 1, where the smaller the dominance index value (close to 0), it indicates that a species is not dominant, whereas if the greater dominance index (close to 1), it indicates the species of dominant [13]. This provision further divided into low-level dominance when the index from 0.00 to 0.33; moderate when > 0.33 to 0.66 and high if > 0.66 to 1.00. Of these details can be seen in Table 3, that at Prevab (burned forest), the species of fungi that contained high levels of dominance in plot 1 was *Clavulina* sp, level of moderately dominance was found on plot 2 i.e *Mycena pura*, *Coprinus atramentaria* on plots 3, *Coprinus comatus* and *Russula decolorans* plot 4, while other species were included in a low level of dominance.

Table 3. Dominance index (Di) species of ectomycorrhizal fungi found in Kutai National Park

Location I: burned forest (Prevab)

Nr.	Species	Sum of individual	H’	Di = ni/N	Level of dominance
Plot (replication) 1					
1	*Lepiota* sp	1		0.0714	l
2	*Clavulina* sp	12		0.8572	h
3	*Agaricus* sp1	1		0.0714	l
	Sum	14	0.2211 (l)	1.0000	
Plot (replication) 2					
1	*Amanita rubescens*	1		0.0667	l
2	*Mycena pura*	8		0.5333	m
3	*Amanita* sp	1		0.0667	l
4	*Agaricus* sp2	1		0.0667	l
5	*Coprinus atramentaria*	4		0.2667	l
	Sum	15	0.5339 (l)	1.0000	
Plot (replication) 3					
1	*Coprinus atramentaria*	4		0.6667	m
2	*Rhizopogon* sp	1		0.1667	l
3	*Coprinus comatus*	1		0.1667	l
	Sum	6	0.3768 (l)	1.0000	
Plot (replication) 4					
1	*Coprinus comatus*	1		0.5000	m
2	*Russula decolorans*	1		0.5000	m
	Sum	2	0.3010 (l)	1.0000	

Location II: unburned forest (Sangkimah)

Plot (replication) 1

Nr.	Species	Sum of individual	H'	Di = ni/N	Level of dominance
1	*Hygrophorus* sp	4		0.0816	l
2	*Lyophyllum* sp1	1		0.0204	l
3	*Clitocybe* sp1	1		0.0204	l
4	*Myrlostoma coliforme*	1		0.0204	l
5	*Laccaria laccata*	1		0.0204	l
6	*Suillus* sp1	1		0.0204	l
7	*Agaricus placomyces*	1		0.0204	l
8	*Calostoma fuscum*	8		0.1633	l
9	*Cantharellus* sp1	1		0.0204	l
10	*Omphalina sp*	3		0.0612	l

Table 3 (continuation)

Nr.	Species	Sum of individual	H'	Di = ni/N	Level of dominance
11	*Clitocybe* sp2	8		0.1633	l
12	*Gliophorus* sp	1		0.0204	l
13	*Russula* sp1	1		0.0204	l
14	*Collybia acervata*	4		0.0816	l
15	*Boletus* sp	1		0.0204	l
16	*Inocybe* sp	3		0.0612	l
17	*Naematoloma* sp	1		0.0204	l
18	*Tricholoma* sp	8		0.1633	l
	Sum	49	1.0912 (l)	1.0000	
Plot (replication) 2					
1	*Lycoperdon perlatum*	1		0.0625	l
2	*Agaricus placomyces*	1		0.0625	l
3	*Lyophyllum* sp2	1		0.0625	l
4	*Cantharellus sp2*	1		0.0625	l
5	*Lepiota sp1*	2		0.1250	l
6	*Clitocybe* sp3	1		0.0625	l
7	*Collybia* sp1	1		0.0625	l
8	*Collybia butyracea*	1		0.0625	l
9	*Leucocoprinus flos-sulfuris*	6		0.3750	m
10	*Lepiota* sp2	1		0.0625	l
	Sum	16	0.8747 (l)	1.0000	
Plot (replication) 3					
1	*Clitocybe* sp4	7		0.7000	h
2	*Agaricus placomyces*	2		0.2000	l
3	*Lepiota* sp3	1		0.1000	l
	Sum	10	0.3482 (l)	1.0000	
Plot (replication) 4					
1	*Cantharellus* sp3	6		0.5000	m
2	*Suillus* sp2	2		0.1667	l
3	*Cantharellus* sp4	1		0.0833	l
4	*Collybia* sp2	1		0.0833	l
5	*Russula* sp2	2		0.1667	l
	Sum	12	0.3096 (l)	1.0000	

l = level of species diversity or level of species dominance is low. m = moderate. h = high

At Sangkimah (unburned forest), the species of fungi with a high level of dominance contained in plot 3 was *Clitocybe* sp4, level of moderately dominance were found on plots 2 and 4, respectively *Leucocoprinus flos-sulfuris* and *Cantharellus* sp3, while other species were included in the rate low dominance. The dominance species of fungi in each of the study plots was dependent on the number of individuals of each species of fungi itself, the more the number of the individual, the greater the dominance index if there were other species found in the plot itself. But on plot 4 (Prevab), *Coprinus comatus* and *Russula decolorans* equally dominant, although each only 1 individual, because only 2 species that were found in the plot. A large number of individuals of each species of ectomycorrhizal fungi depending on the number of suitable host within a particular area, when the same host species present in significant amounts, the number of individuals of the same species of fungi will also be a lot, but when its host tree species varied, the species of fungi and the number of individuals also vary widely, so the dominance of each species of fungi is reduced approaching the lower level of criteria.

Based on the dominance index of each species of fungi in Table 3, it can be concluded the level of dominance by location of research, that at Prevab, the species of fungus with high dominance level was *Clavulina* sp, moderately level of dominance were *Mycena pura*, *Coprinus atramentaria*, *Coprinus comatus* and *Russula decolorans*, while other species were included in a low level of dominance. At Sangkimah, species of fungi with a high level of dominance was *Clitocybe* sp4, moderately level of dominance was *Leucocoprinus flos-sulfuris* and *Cantharellus* sp3, while other species were included in a low level of dominance.

The number of individuals in a species of ectomycorrhizal fungis itself depends on the species of fungi and the suitability of the environment. There is a species of fungi that grows singly (solitary) and some are clustered in one clump. Biotic environment such as the host species and the abiotic environment such as temperature and humidity, and altitude above sea level are also instrumental in the formation of fruiting bodies of an ectomycorrhizal fungus species. According to reference [14], for growth, mycelium of fungi need water, otherwise the fruit body begins to form when the conditions for the growth of the mycelium become unprofitable, such as the availability of water as a solvent nutrients needed for growth to be reduced. As if the mycelium was almost dead, so that all the energy reserves should be kept for a good season to produce fruit body which in turn will produce spores that are necessary for the continuation of the species concerned.

Reference [14] also stated, that the various species of *Morchella*, *Volvaria*, *Coprinus* and *Peziza* growing in undisturbed soil. In contrast to the treated soil, especially if fertilized with chemical fertilizers, then very few mushrooms will be found. The area of contact with the salty spray of sea water is not a good habitat for many species of fungi, but *Psalliota bernardi*, *Bovista plumbea* and *Geaster nanus* growing on sand dunes along the coast, while *Coprinus atramentaria* and some species of the genus *Psalliota* grows in meadows and gardens vegetables fertilized with manure.

4 CONCLUSION

The number of ectomycorrhizal fungi at Prevab (burned forest) were 12 species of 37 individuals, whereas at Sangkimah (unburned forest) found 34 species of 87 individuals, hence the number of species and individuals at Sangkimah was more than at Prevab. However, when viewed from the species diversity index in both locations, including low (H'<1), respectively at Prevab was 0.51528 and at Sangkimah 0.50233.

At Prevab, species of fungi with high level of dominance was *Clavulina* sp., moderately level of dominance were *Mycena pura*, *Coprinus atramentaria*, *Coprinus comatus* and *Russula decolorans*, while other species were included in low level of dominance. At Sangkimah, species of fungi with a high level of dominance was *Clitocybe* sp4, moderately level of dominance were *Leucocoprinus flos-sulfuris* and *Cantharellus* sp3, while other species were included in low level of dominance.

5 RECOMMENDATION

The species of fungi that exist in KNP can be utilized for inoculation on seedlings to be planted on land that has been disturbed such as fire scars, post-mining land, *Imperata cylindrica* land and land with overgrown shrubs.

Preservation of the existence of ectomycorrhizal fungi in the KNP needs to be done for example by preventing forest fires, planting tree species that are host of ectomycorrhizal fungi on burned land, preventing the felling of host trees and dissemination of leaflets and information boards about forest potential of KNP in the entrance counters and so forth.

To determine the species diversity of ectomycorrhizal fungi more, further research needs to be conducted at the same location at different times, because it is possible still to be found other than the species found in this research.

REFERENCES

[1] W. Jülich, "Dipterocarpaceae and Mycorrhizae", *Special Issue, GFG Report of Mulawarman University* no. 9, 103 p, 1988.

[2] M. Noor. *Diversity of Ectomycorrhizal Fungi in Burned and Unburned Forest Area in Sungai Wain Protection Forest, Balikpapan City* (in Indonesian). Master Thesis, Study Program of Forestry Science, University of Mulawarman, Samarinda, 2002.

[3] I. Zulkarnaen, *The Fate of the Present Kutai National Park* (in Indonesian), 2008. [Online] Available: http://www.antaranews.com/view/?i=1211365046&c=ART&s= (February 7, 2013).

[4] J. Arjuna, *Forest Fire and Land* (in Indonesian), 2008. [Online] Available: http://www.terranet.or.id/tulisandetil.php?id=1393.

[5] C.E. Shannon, C.E. "A Mathematical Theory of Communication". *Bell System Technical Journal* no. 27, pp 379–423, 1948.

[6] C.J. Krebs, *Ecological Methodology.* Harper & Row Publ., Inc., New York, 1989.

[7] E.H. Simpson, "Measurement of Diversity". *Nature* no. 163, p 688, 1949.

[8] R. Fellner, "Mycorrhiza-forming Fungi as Bioindicators of Air Pollution", *Journal of Agriculture, Ecosystems & Environment,* vol. 28, no. 1–4, pp 115–120, 1990.

[9] D. Mardji and M. Noor, *Mushrooms Diversity in Mount Lumut Protection Forest*. Research Report for Tropenbos International Indonesia Programme (MoF – Tropenbos Kalimantan Programme), 2006.

[10] D. Mardji, Sugiarto and D. Triadiawarman, *Identify the Species and Potential Ectomycorrhizal Fungi to Support Ex-Mining Land Rehabilitation Program* (in Indonesian). Research Report, Samarinda, 2009.

[11] S. Egli, "Mycorrhizal Mushroom Diversity and Productivity - An Indicator of Forest Health?", *Annals of Forest Science,* no. 68, pp 8–88, 2011.

[12] Anonymous, Ecological Sampling Methods, 2000. [Online] Available: http://www.countrysideinfo.co.uk/ biol_sampl_cont.htm.

[13] E.P. Odum and G. Barrett. *Fundamentals of Ecology*. W.B. Saunders Company, Philadelphia, 1971.

[14] G. Pace, *Mushrooms of the World*. Firefly Books Ltd., Spain, 1988.

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Modeling of the lock-in thermography process through finite element method for measuring of the thermal diffusivity

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ABSTRACT: In industry, especially in the high technology sector such as aerospace, we produce and we use increasingly new materials for the construction of new structures that have good thermal and mechanical properties. The characterization of these materials requires knowledge of their thermo-physical properties. Thermal diffusivity is an important parameter in the materials characterization. Lock-in thermography is widely used in the materials thermal characterization. It involves applying on the front face sample a heater in the form of a sine wave and analyzing the phase difference or the amplitude difference between the incident thermal wave and the transmitted thermal wave. Indeed, the passage of the thermal wave through a material is influenced by its thermal diffusivity. We used the finite element method, in three dimensions, to calculate the instantaneous temperatures of the front and rear faces of the inspected sample, and deduct their phase shifts and therefore the sample thermal diffusivity. Our contribution in the lock-in thermography technique is the development of a new model for the thermal diffusivity evaluation with good precision. The results for polystyrene are very satisfactory. Indeed, the thermal diffusivity calculated by our new model is very close to the value reported in the literature. The proposed new model can be used in the characterization of new materials.

KEYWORDS: Lock-in thermography, Finite element method, Thermal diffusivity, Modeling, Simulation.

1 INTRODUCTION

The thermal diffusivity is one of the most important parameters which affects the efficiency of heat transfer. Several techniques have been developed for measuring the thermal diffusivity. The use of infrared thermography as a nondestructive evaluation technique is becoming increasingly attractive for measuring the thermal diffusivity [1], [2], [3], [4], [5]. Thermography offers several advantages over other non-destructive techniques in that it is non-contact, able to inspect wide areas and produce easily interpreted results.

Lock-in thermography is based on analyzing the response of the inspected sample which has been subjected to a variable excitation. The principle is to apply a heat flow or a temperature which varies sinusoidally in time. The passage of the transmitted thermal wave by conduction is influenced by the thermal properties of the characterized sample. This technique is used in determining the material thermal diffusivity.

When the considered material is uniform of thickness d and heated over wide plane surface (z=0) by applying at z=0 a temperature $T_m \exp(i\omega t)$ varying at angular frequency ω and assuming that heat flux is zero at z=d and providing d > μ, the phase difference Δϕ between the temperature variation at z = d and at z = 0 is simply as [6]:

$$\Delta\varphi \approx -\frac{d}{\mu} \qquad (1)$$

The thermal length μ is related to the material thermal diffusivity by the following equation:

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$$\mu = \sqrt{\frac{2\alpha}{\omega}} \qquad (2)$$

Using a 3D finite element simulation, we are going to highlight in the first part of this work the difference between the actual value of the thermal diffusivity and its estimated value by the relation (1).

In the second part, we propose a model for estimating the thermal diffusivity with better accuracy.

2 FINITE ELEMENT MODELING

The general equation of heat diffusion is given by:

$$\nabla.[k\nabla T(r,t)] - \rho c_v \frac{\partial T(r,t)}{\partial t} = -Q(r,t) \qquad (3)$$

Where T(r,t) is the temperature (K), at the position r(m) and at the moment t(s), k is the thermal conductivity (W/m.K), ρ is the density (kg/m^3), cv is the specific heat capacity (J/kg.K), and Q(r,t) is the density of input energy.

Solving equation (3) is generally inaccessible, an approximate solution is obtained by a finite element calculation. Our considered model is a thin plate of dimensions (40mm x 32 mm) and of a variable thickness d (Figure 1).

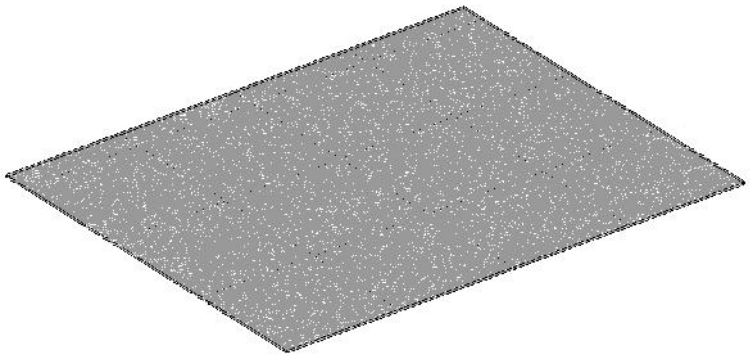

Fig. 1: 3D model of a thin plate

We applied on the front face of the considered sample a sinusoidal temperature assuming that the flux through the rear surface is zero and that the sides are thermally insulated.

The thermal properties of the used materials are as follows:

Table 1: Thermal properties of materials at 25 ° C

	Density kg/m^3	Thermal conductivity W/mK	Specific heat J/kg K	Thermal diffusivity m2/s
Polymethyl Methacrylate PMMA[7]	1190	0.193	1420	$1.1421\ 10^{-7}$
Polypropylene PP[8]	900	0.1	2000	$5.5556\ 10^{-8}$

The transient thermal analysis was performed with a step of 0.01s for each sample and each value of excitation frequency.

3 RESULTS AND DISCUSSION

For each considered material and for each value of excitation frequency, we calculated, by the finite element method, the phase difference Δϕ. The thickness of PMMA sample is d=1.93mm and the thickness of polypropylene (PP) sample is d=1.95mm. Figures 2-a and 2-b show the evolution of the phase difference opposite -Δϕ as a function of d/μ.

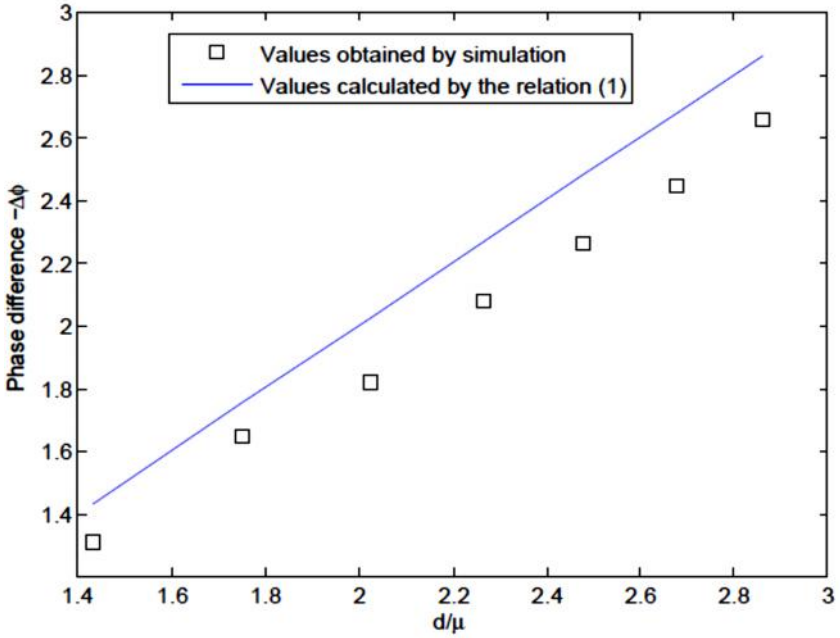

Fig. 2-a: Variation of phase difference opposite -Δφ versus d/μ, Case of PMMA, Maximal gap =0.2354 rad

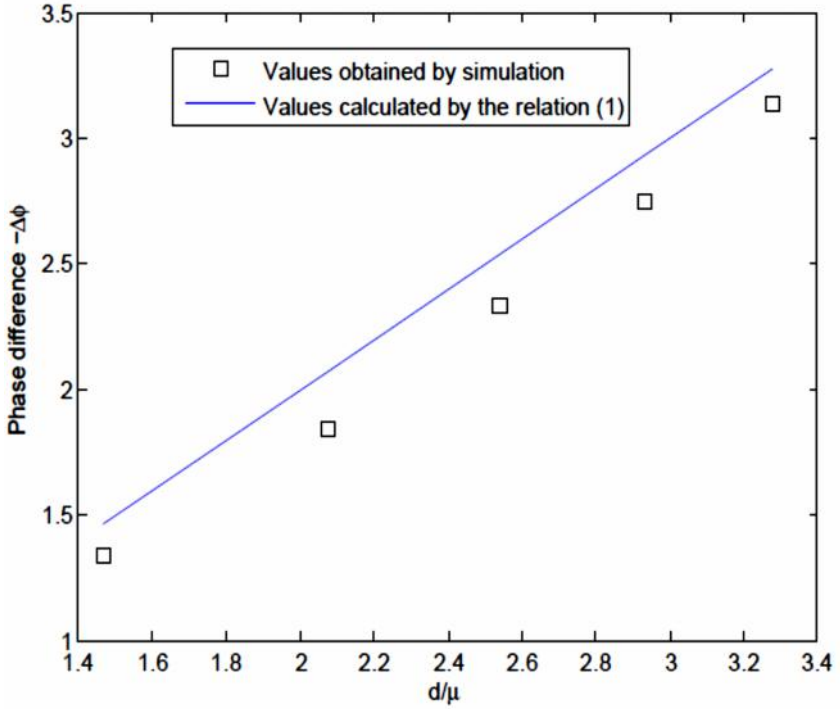

Fig. 2-b: Variation of phase difference opposite -Δφ versus d/μ, Case of polypropylene (PP), Maximal gap =0.2298 rad

We observe the difference between the values calculated by finite elements method and their estimated by relation (1), this difference is reflected in the estimation of the thermal diffusivity. A correction of the relation (1) is necessary to

approximate the actual values. In order to have a better accuracy in estimating the thermal diffusivity, we propose a new model that allows estimating of phase difference from sample thickness and thermal length.

The model is as follows:

$$\Delta\varphi = -0.9593\frac{d}{\mu} + 0.09 \qquad (4)$$

In Figures 3-a and 3-b, we superimposed the results obtained by simulation and the results obtained by the proposed model in the relationship (4). We plotted the variations of the phase difference as a function of ratio of sample thickness and thermal length.

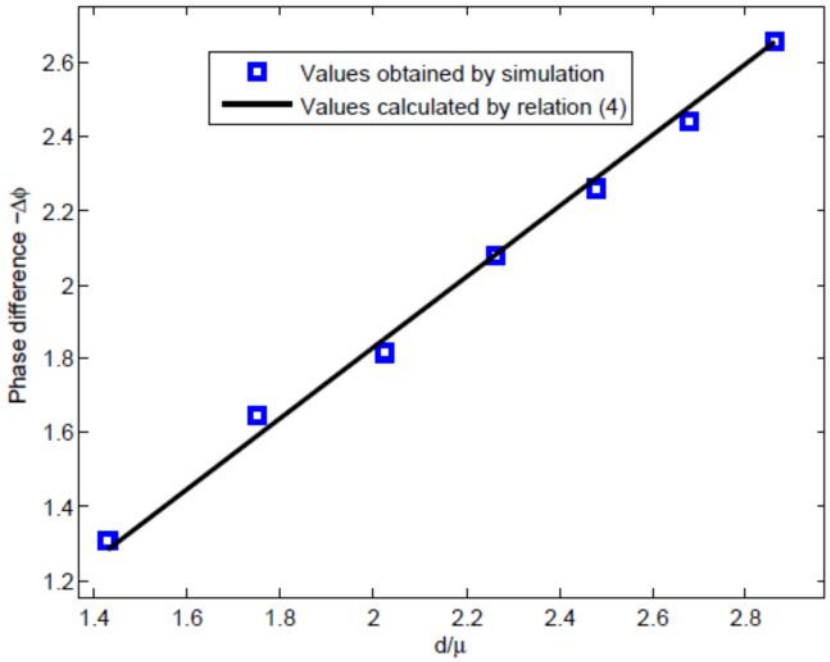

Fig. 3-a: Results of the proposed model for PMMA sample, Maximal gap = 0.0541 rad.

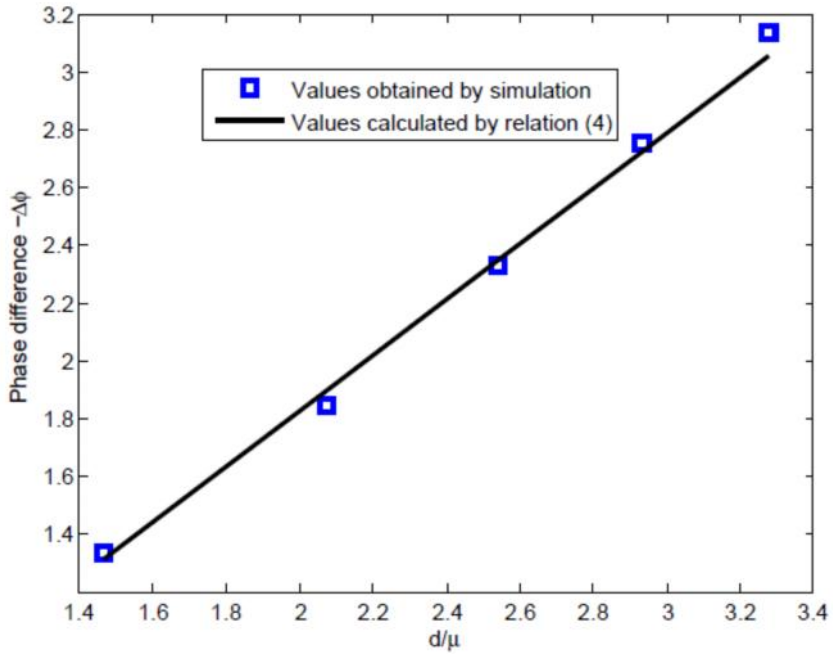

Fig. 3-b: Results of the proposed model for polypropylene sample, Maximal gap = 0.0844 rad.

The relation (4) gives a good approximation of the phase difference Δϕ for both materials, PMMA and polypropylene. Indeed, the obtained error values remain low for both tested samples.

From relations (2) and (4), the thermal diffusivity may be evaluated by equation (5) as follow:

$$\alpha = \frac{\omega}{2}\left(\frac{0.9593d}{\Delta\varphi - 0.09}\right)^2 \qquad (5)$$

To evaluate the proposed improvement in this work, we apply the formula (5) to the phase difference value obtained for a Polystyrene sample (Table 2):

Table 2: Improvement of accuracy estimation of polystyrene thermal diffusivity

	Frequency f (Hz)	**Thickness d (mm)**	**phase difference -Δϕ (rad)**	**Diffusivity without correction (10^{-7} m^2/s)**	**Thermal diffusivity by relation (5) (10^{-7} m^2/s)**	**Thermal diffusivity in literature (10^{-7} m^2/s) [7]**
Polystyrene	0.05	1.94	2.4049	1.0222	0.8740	0.87

We note that the thermal diffusivity estimated by equation (5) is closer to the value cited in literature.

4 CONCLUSION

In this work, the objective was to improve the estimation of thermal diffusivity of a sample by the use of lock-in thermography. In the first part, we have highlighted the need for a correction of the approximation formula of the diffusivity from the phase difference value. For this, a finite element simulations were performed and results show the necessity of correcting the estimation formula published in the literature. In this context, we have proposed a model for estimating the thermal diffusivity with better accuracy. In the end, we have applied the proposed model to determine the thermal diffusivity of a polystyrene sample. The obtained value is close to the actual value with a good accuracy.

REFERENCES

[1] Martin Rides, Junko Morikawa, Lars Halldahl, Bruno Hay, Hubert Lobo, Angela Dawson, and Crispin Allen, "Intercomparison of thermal conductivity and thermal diffusivity methods for plastics," *Polymer Testing*, vol. 28, pp. 480-489, 2009.

[2] Jian-Jun Zheng, Xin-Zhu Zhou, Yu-Fei Wub, and Xian-Yu Jin, "A numerical method for the chloride diffusivity in concrete with aggregate shape effect," *Construction and Building Materials*, vol. 31, pp. 151-156, 2012.

[3] F. Cernuschi, P. Bison, S. Marinetti, and E. Campagnoli, "Thermal diffusivity measurement by thermographic technique for the non-destructive integrity assessment of TBCs coupons," *Surface & Coatings Technology*, vol. 205, pp. 498-505, 2010.

[4] I.O. Mohamed, "Development of a simple and robust inverse method for determination of thermal diffusivity of solid foods," *Journal of Food Engineering*, vol. 101, pp. 1-7, 2010.

[5] Masanori Monde, Masataka Kosaka, and Yuichi Mitsutake, "Simple measurement of thermal diffusivity and thermal conductivity using inverse solution for one-dimensional heat conduction," *International Journal of Heat and Mass Transfer*, vol. 53, pp. 5343-5349, 2010.

[6] C. Boué, and S. Holé, "Infrared thermography protocol for simple measurements of thermal diffusivity and conductivity," *Infrared Physics & Technology*, vol. 55, no. 4, pp. 376-379, 2012.

[7] M. F. Couturier, K. George, and M. H. Schneider, "Thermophysical properties of wood-polymer composites," *Wood Science and Technology*, vol. 30, pp. 179-196, 1996.

[8] Xiangtao Yin, Scott A. Morris, and William D. O'Brien Jr, "Ultrasonic Pulse-Echo Subwavelength Defect Detection Mechanism: Experiment and Simulation," *Journal of Nondestructive Evaluation*, Vol. 22, No. 3, 2003.

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Metacognitive Awareness of Reading Strategies among Iranian EFL Learners in an "Input-poor" Environment

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ABSTRACT: The importance of language learner strategies, metacognitive awareness of these strategies, and reading comprehension has been well documented in the first as well as second language research. By contrast, little investigations have been conducted to highlight the role of these components, especially language learners' metacognitive knowledge or awareness of strategies, in the foreign language learning contexts; most notably in *"input-poor"* environments. The study aimed at investigating the frequency of the use and types of reading strategies employed by Iranian EFL university students in an input-poor environment. In doing so, a questionnaire, namely the Survey of Reading Strategies (SORS) was administrated to 144 EFL students at Tonekabon Islamic Azad University, Iran. The analyses of the SORS through using descriptive statistics showed that these students were almost moderately aware of reading strategies. They most frequently used Problem-solving strategies (Mean= 2.67), followed by Global strategies (Mean= 2.54) and Support strategies (Mean= 2.40). Based on the results, several implications and suggestions for further research are offered for improving EFL learners' metacognitive awareness of reading strategies in an input-poor environment where conscious attention to learner strategies certainly merits closer consideration than unconscious acquisition caused by exposure to ample foreign language input outside the classroom.

KEYWORDS: Metacognition, Metacognitive Awareness, Metacognitive Strategy Instruction, Language Learner Strategies, Learner Autonomy, Self-regulation, Strategic-based instruction, Input-poor Environment.

1 INTRODUCTION

Reading comprehension as a highly convoluted and multifaceted skill requires readers to possess the knowledge of skills and strategies and ability to apply them effectively in arriving at writer's intended meaning. It is well documented that self-regulated and accomplished readers enjoy a variety of cognitive and metacognitive skills and demonstrate a remarkable capacity to exploit them before, during, and after reading for meaning construction and learning purposes ([1], [2]). "Skilled readers often engage in deliberate activities that require planned thinking, flexible strategies, periodic self-monitoring… [whereas] novice readers often seem oblivious to these strategies and [even] the need to use them" ([3]).

Metacognition appears to be the cornerstone of thoughtful, active, and successful reading (e.g., [4], [5]). Such metacognition "entails knowledge of strategies for processing texts, the ability to monitor comprehension, and the ability to adjust strategies as needed" ([4]). Therefore, if metacognitive strategies are a key distinctive characteristic of skilled readers from unskilled or less skilled ones as well as an integral part of reading comprehension , and this is truly so, so as to develop independent self-regulated and proficient readers, metacognitive strategies instruction deems essential and is an imperative that should be integrated into reading comprehension instruction.

This study is an attempt to find out what learner strategies students use in their reading comprehension in an input-poor environment as well as to what extent they have developed those strategies. Mahdavi (2013) [6] postulated that the English language learning environment in Iran can be characterized as an "*input-poor*" environment defined by Kouraogo (1993) as

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those learning environments in which little opportunities were provided for the learners to practice and learn the language outside or even inside the classroom" ([7]).

1.1 LANGUAGE LEARNER STRATEGIES

Learning strategies are "special thoughts or behaviors that individuals use to comprehend, learn, or retain new information" ([8]). There is a general consensus among scholars that teaching learner strategies to less successful language learners would help them become more active and independent language learners ([9], [10], [11], [12]). "The use of strategies embodies taking active, timely, coordinated responsibility for learning. This is both learnable and teachable" ([13]).

It is most probable that a language is "the most complex set of skills one could ever seek to acquire" ([14]); hence, what Brown calls 'strategic investment' deems essential for learners to gain mastery over language.

Oxford (2008) noted that "learning strategies are generally signs of learner autonomy" ([13]). Hsiao and Oxford (2002) acknowledged that "[l]earning strategies for L2s help build learner autonomy, which requires the learner to take conscious control of his or her own learning process" ([12]). What seems to be quite clear is that proficient L2 learners show strong tendency to possess and employ a wide array of strategies than less proficient learners ([10], [13]).

As to the importance of learning strategies in FLLIPE (foreign language learning in input-poor environments), Kouraogo (1993) maintains that in such environments the use of conscious strategies would afford better chances for improving language learning than unconscious language acquisition through input ([7]) .

1.2 METACOGNITION

To put it simply, metacognition refers to "thinking about thinking" and regulation of this thinking or our ability to know what we know and what we don't know ([15], [16]) and it is comprised of metacognitive knowledge and metacognitive regulation. Metacognition was also characterized by Flavell as a "promising new area of investigation" ([15]).

O'Malley and Chamot (1990) emphasized the crucial role that metacognition plays in learning by noting that "students without metacognitive approaches are essentially learners without direction or opportunity to plan their learning, monitor their progress, or review their accomplishments and future learning directions ([8]).

Metacognition nurtures independent thinkers and lifelong learners who are able to grapple with new situations and learn how to learn and continue to learn throughout their lifespan in this hectic pace of life ([17], [18]). However, learning how to be mindful and manager of one's own learning is not inherited, nor does it happen naturally and overnight, it necessitates specific instruction of basic metacognitive skills and strategies.

Metacognition "has the potential to empower students to take charge of their own learning and to increase the meaningfulness of students' learning" ([19]), it also encourages learners to 'learn what to do when they don't know what to do' ([20], [21]). Similarly, Chamot et al. (1999) stated that "metacognition or reflecting on one's own thinking and learning is the hallmark of the successful learner" ([22]).

1.3 READING COMPREHENSION AND METACOGNITION

Undoubtedly, the penultimate goal of reading is comprehension or the reconstruction of meaning from the text, and understanding text necessitates knowledge and execution of a variety of strategies deliberately and independently. If not the most important, students' metacognitive awareness (knowledge) and control (regulation) of reading, beyond any dispute, are one of the most important ability pertinent to reading comprehension. To ensure that comprehension is occurring, the reader draws on the power of metacognitive knowledge and regulation, a volitional process that empowers him or her to exploit a variety of strategies which deems essential for monitoring and taking charge of the reconstruction of meaning while reading.

Thus, metacognition is the student's capacity to plan, monitor, and, if necessary, re-plan various reading strategies in the service of comprehension. Baker and Brown (1984) contended that metacognition is an integral part of reading comprehension largely due to the fact that in understanding a text, readers must be aware of and control the cognitive activities necessary to communicate with the text and author's intended meaning ([23]).

As Duffy (2005) puts it, metacognition in reading instruction is linked to reading strategies [24]. To be more precise, metacognition is about flexible knowledge of reading strategies, selecting well-matched strategies consistent with specific situations, and implementing and orchestrating a variety of strategies. Students who consistently demonstrate good

comprehension tend to possess a repertoire of well-developed metacognitive skills and flexibly use them; moreover, they are aware and assume a degree of control over their intellectual activities as they read. In a nutshell, effective readers are metacognitive, and metacognitive readers know what strategies to apply, how, when, and why to apply them; furthermore, they plan, monitor, evaluate, and regulate their own reading ([25]).

Metacognition is comprised of two basic elements when applied to reading context, namely metacognitive knowledge or awareness and metacognitive regulation or control. Metacognitive knowledge is further subdivided into three other categories: declarative knowledge or knowing how (knowledge about self-characteristics, task characteristics, and task-relevant strategies), procedural knowledge or knowing how to use the different strategies involved in the construction of meaning from the text successfully, and conditional knowledge which refers to knowing when and why to apply strategies. Metacognitive regulation also encompasses planning, monitoring, and revising strategies. In teaching readers to be metacognitive, what seems to be vital is the incorporation of both aspects of metacognition into a training program.

O'Malley and Chamot (1990) classified metacognitive strategies for a successful reading into these five categories:

1. Advance organization which involves previewing the main ideas or concepts of the material, often by skimming, to create a linkage between the previous knowledge and the new topic is going to be learned.
2. Organizational planning refers to planning the reading tasks, identifying the parts, the sequences or main ideas that would help comprehending of a text.
3. Selective attention is deciding in advance to focus on specific aspects of input, often by scanning for key words, concepts, and discourse markers.
4. Self-monitoring relates to one's monitoring and checking one's comprehension during reading.
5. Self-evaluation refers to one's assessment on how well the reader has accomplished a reading or learning activity after its completion. ([8])

The study endeavors to answer the following questions:

Research question 1: Are Iranian EFL students high, medium, or low reading learner strategy users?

Research question 2: What are the most and the least frequently used categories of reading learner strategies by Iranian EFL learners in an "input-poor" environment?

Research question 3: What are the most and the least frequently used individual reading learner strategies by Iranian EFL learners in an "input-poor" environment?

2 MATERIALS AND METHODS

2.1 INTRODUCTION

This section provides a brief account of the design of the study, the participants, instrument, procedures, and data analysis methods. This study examined whether metacognitive strategy training enhanced the EFL students' reading proficiency in an input-poor environment.

2.2 DESIGN OF THE STUDY

To gain new insights into vocabulary learner strategies EFL students in an input-poor environment apply, a qualitative research design has been used to serve the objectives of this study.

2.3 PARTICIPANTS

The students who took part in the study were consisted of 144 university students (80 males and 64 females), 18-24 year-old majoring in biology at Islamic Tonekabon Azad University, Iran. Selection of the participants for the study was based on a simple random sampling from the five hundreds freshmen university students enrolled in biology faculty.

2.4 INSTRUMENTATION

The researcher in the present study used a self-report measure, Survey of Reading Strategies (SORS) developed by Mokhtari and Sheorey (2002) with the intent to "measure adolescent and adult ESL students' metacognitive awareness and perceived use of reading strategies while reading academic materials such as textbooks" ([26]). The Survey of Reading Strategies (SORS) was field-tested by its originators on a population of ESL students studying at two universities in the United States and revealed consistent results relative to the instrument's reliability (internal reliability= .89 or better) which is indicative of a reasonable degree of consistency in measuring awareness and perceived use of reading strategies among non-native students of English (see [27], for more details).

The students read each statement of the original 30-item SORS and rated how often they used the strategy depicted in the questionnaire items using a 5-point Likert-type scale ranging from 1 (I *never* or *almost never* do this) to 5 (I *always* or *almost always* do this). The SORS measures three broad categories of reading strategies: global reading strategies, cognitive strategies, and support strategies. Global Reading Strategies (GLOB) or metacognitive strategies refer to those conscious and planned methods for the purpose of monitoring and managing reading comprehension such as having a purpose in mind or using typographical aids (e.g., tables, figures) and contains 13 items ([26]).

Problem Solving Strategies (PROB) or cognitive strategies "are the actions and procedures that readers use while working directly with the text. These are some techniques that learners make use of them when there are some problems in understanding passages such as guessing the meaning of unknown words or rereading the text for improving the comprehension and includes 8 items ([26]).

And finally Support Strategies (SUP) are those supportive techniques which help the reader to comprehend the passage such as dictionary use or highlighting textual information ([26]).

2.5 PROCEDURES

About 160 copies of SORS were distributed among the participants at the individual class periods and were administered with the help of the classroom instructors. The students were informed of the purpose of the questionnaires and of the fact that there were no right or wrong answers, and asked to express their honest opinion by choosing the appropriate number printed on the right side of each SORS statement. The completion of the SORS took about 15 minutes. Each completed survey was manually examined, and, after discarding the incomplete ones, 144 participants managed to complete the questionnaires appropriately and then those questionnaires were coded for statistical analysis.

2.6 DATA ANALYSIS

Descriptive statistical procedures were used to analyze the data obtained from the SORS to provide insight into EFL learners' metacognitive awareness and use of reading strategies. It was sought to examine reading strategy use among the students on the SORS scales, which ranged from 1 to 5, through three types of usage levels proposed by Oxford and Burry-Stock (1995, p. 12) for general language learning strategy usage: **high** (mean of 3.5 or higher), **medium** (mean of 2.5–3.4), and **low** (2.4 or lower) [29].

3 RESULTS

The basic idea behind the survey study was to throw light on Iranian EFL learners' metacognitive awareness of reading strategies and whether they are high, medium, or poor vocabulary strategy users. The data regarding to research questions were analyzed and tabulated in the following tables. To interpret the results of the SORS, Oxford and Burry-Stock's usage levels have been used: **high** (mean of 3.50 or higher), **medium** (mean of 2.50–3.49), and **low** (2.49 or lower).

Research question 1: Are Iranian EFL students high, medium, or low reading learner strategy users?

The mean value for the learners' overall reading strategy use was 2.54. Therefore, the analysis of data obtained from SORS revealed that Iranian EFL learners are **medium** reading strategy users based on three usage levels proposed by Oxford and Burry-Stock (1995) ([28]): **high** (mean of 3.50 or higher), **medium** (mean of 2.50–3.49), and **low** (2.49 or lower).

Table 1. Score analysis for overall vocabulary strategy use

Mean	S. D.	Min	Max
2.54	0.323	2.06	3.13

Research question 2: What are the most and the least frequently used category of reading learner strategies by Iranian EFL learners in an "input-poor" environment?

Table 2 shows the most and the least frequently used category of reading learner strategies on the SORS by Iranian EFL learners. The most frequently used category of strategies was problem-solving strategies with the mean value of 2.67 followed by global strategies (M= 2.54) and support strategies (M= 2.40).

Table 2. Rank order of categories of strategies from the most to least frequently used category

General categories on SORS	Mean	S. D.
Problem-solving Strategies	2.67	0.485
Global Reading Strategies	2.54	0.440
Support Reading Strategies	2.40	0.366

Research question 3: What are the most and the least frequently used individual reading learner strategies by Iranian EFL learners in an "input-poor" environment?

Table 4a shows the least frequently used reading strategies by the learners. Translation from L2 to L1 (M= 3.13), Re-reading for better understanding (M= 3.00), Setting a purpose for reading (M= 3.00), Staying focused on reading (M= 2.96), Using typographical aids (M= 2.93), and Using prior knowledge (M= 2.93) were among the most frequently used individual reading strategies by the Iranian EFL learners.

Table 3a. Rank order of the most frequently used reading strategies

Strategies	Categories	Mean	S. D.	Rank	Levels
Translation from L2 to L1	SUP	3.13	0.994	1	**Mid**
Re-reading for better understanding	PROB	3.00	0.946	2	**Mid**
Setting a purpose for reading	GLOB	3.00	1.144	3	**Mid**
Staying focused on reading	PROB	2.96	0.614	4	**Mid**
Using typographical aids (e.g. italics)	GLOB	2.93	1.048	5	**Mid**
Using prior knowledge	GLOB	2.93	0.944	6	**Mid**

In terms of individual reading learner strategies, the learners reported to use Using context clues (M= 1.96), Asking oneself questions (M= 2.06), Reading aloud when text becomes hard (M= 2.10), Going back and forth in text (M= 2.20), Comprehension monitoring (M= 2.20), and Pausing and thinking about reading (M= 2.20) less frequently than any other reading strategies.

Table 3a. Rank order of the least frequently used vocabulary strategies

Strategies	Categories	Mean	S. D.	Rank	Levels
Using context clues	GLOB	1.96	0.964	1	**LOW**
Asking oneself questions	SUP	2.06	1.014	2	**Low**
Reading aloud when text becomes hard	SUP	2.10	0.999	3	**Low**
Going back and forth in text	SUP	2.20	0.935	4	**Low**
Comprehension monitoring	GLOB	2.20	0.996	5	**Low**
Pausing and thinking about reading	PROB	2.20	1.096	6	**Low**

4 DISCUSSIONS AND CONCLUSIONS

The findings from this study on the reading learning strategies used by EFL learners in an "input-poor" environment suggested that the students showed awareness of almost all of the reading strategies included in the SORS, but their awareness of those strategies was not highly developed, and most of them were used at a low or medium frequency. No individual strategy was used with high frequency. The most frequently used strategy was translation from English into Persian with the mean of 3.13 and the least often used individual strategy reported to be using context clues with the mean of 1.96.

To conclude, Strategic-based instruction (SBI) in reading strategies deems essential for improving students' reading comprehension in this input-poor environment. The students learn to apply these strategies successfully through practice with different texts, and over a long period of time. Knowing the strategies (what the strategy is) may not be translated into actual use of those strategies ([27]). Strategy instruction can provide a lot of opportunities for the readers practicing these strategies to deepen their understanding of them and to make them aware of howness, whyness, whenness, and whereness of their use. In the long run, placing emphasis on as well as devoting energy and attention to teaching strategies which enhances self-regulation empowers learners to become active learners.

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REFERENCES

[1] Garner, R. Strategies for reading and studying expository text. Educational Psychologist, 22(3-4), 299-312, 1987b.
[2] Pressley, M., Johnson, C. J., Symons, S., and McGoldrick, J. A. Strategies that improve children's memory and comprehension of text. Elementary School Journal, 90 (1), 3-32, 1989.
[3] Paris, S. G and Jacobs, J. E. The benefits of informed instruction for children are reading awareness and comprehension skills. Child Development, 55, 2083-2093, 1984.
[4] Auerbach, E. R., and Paxton, D. "It's Not the English Thing": Bringing Reading Research into the ESL Classroom. TESOL QUARTERLY, Vol. 31, No. 2, 237-261, 1997.
[5] Carrell, P., Pharis, B., & Liberto, J. Metacognitive strategy training for ESL reading. TESOL Quarterly, 23, 647-678, 1989.
[6] Mahdavi, M. Language Learner Strategies Used by Iranian EFL Learners in an Input-poor Environment. Anglisticum Journal, Vol. 2, issue 4, pp. 173-179, 2013.
[7] Kouraogo, P. Language Learning Strategies in Input-poor Environments. System, Vol. 21, No. 2, pp. 165-173, 1993.
[8] O'Malley, J. M. and Chamot, A. U. Learning Strategies in Second Language Acquisition. USA: Cambridge University Press, 1990.
[9] Chamot, A. U. Language Learning Strategy Instruction: Current Issues and Trends. Annual Review of Applied Linguistics, 25, 112-130, 2005).
[10] Oxford, R. L. Language Learning Strategies: What Every Teacher Should Know. New York: Newbery House Harper Collins, 1990.
[11] Wenden, A. How to be a successful language learner: Insight and prescriptions from L2 learners. In A. Wenden, & J. Rubin (Eds.), Learner strategies in language learning (pp. 103-117). Englewood Cliffs, NJ: Prentice Hall, 1987.
[12] Hsiao, T.-Y. and Oxford, R. L. Comparing Theories of Language Learning Strategies: A Confirmatory Factor Analysis. The Modern Language Journal, 368-383, 2002.
[13] Oxford, R. L. Hero with a Thousand Faces: Learner Autonomy, Learning Strategies and Learning Tactics in Independent Language Learning. In S. H. Lewis, Language Learning Strategies in Independent Setting Bristol: Multilingual Matters pp. 41-63, 2008.
[14] Brown, H. D. Teaching by principles: an Interactive Approach to Language Pedagogy. New York: Longman, 2001.
[15] Flavell, J. H. Metacognition and Cognitive Monitoring: A new area of cognitive developmental inquiry. American Psychologist, 34, 907-11, 1979a.
[16] Livingston, J. A. Metacognition: An Overview.
[Online] Available: http://www.gse.buffalo.edu/fas/shuell/cep564/Metacog.htm, 1-5 (1997).
[17] Eggen, P., & Kaucbak, D. Strategies for Teachers: Teaching Content and Thinking Skills. Boston: Allyn and Bacon, 1995.
[18] Papaleontiou-Louca, E. The Concept and Instruction of Metacognition. Teacher Development, Vol 7, No. 1, 9-30, 2003.

[19] Amado Gama, C. Integrating Metacognition in Interactive Learning Environments. Unpublished Ph.D. Thesis. Sussex, the US: University of Sussex, (March 2005).
[20] Wade, S. E. Using think-aloud to assess comprehension. The Reading Teacher, 43, 7, 442-451, 1990.
[21] Claxton, G. Building learning power. London: TLO Ltd, 2002.
[22] Chamot, A. U., Barnhardt, S., El-Dinary, P. B., and Robbins, J. The learning strategies handbook. White Plains, N.Y.: Longman, 1999.
[23] Baker, L. and Brown, A. L. Metacognitive skills and reading. In D. P. Pearson,M. Kamil, R. Barr, and P. Monsenthal (Eds.), Handbook of Reading Research. New York: Longman, 353–394, 1984.
[24] Duffy, G. G. Developing metacognitive teachers: Visioning and the expert's changing role in teacher education and professional development. In S. E. Israel, Metacognition in literacy learning: Theory, assessment, instruction, and professional development. Mahwah, NJ: Lawrence Erlbaum Associates, 299–314, 2005.
[25] Salataci, R. and Akyel, A. Possible effects of strategy instruction on L1 and L2 reading. Reading in a Foreign Language, 14 (1), 2002.
[26] Mokhtari, K. and Sheorey Assessing students' metacognitive awareness of reading strategies. Journal of Educational Psychology, 94(2), 249-259, 2002.
[27] Sheorey, R. and Mokhtari, K. Differences in the metacognitive awareness of reading strategies among native and non-native readers. System 29, 431-449, 2001.
[28] Oxford, R.L. & Burry-Stock, J.A. Assessing the use of language learning strategies worldwide with the ESL/EFL version of the Strategy Inventory for Language Learning. System, 23(2), 153–175, 1995.