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CONTENT

THE UPTAKE OF HEAVY METALS IN *Phaseolus vulgaris* AND *Zea mays* SEEDS HARVESTED FROM POLLUTED AND UNPOLLUTED AREAS

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ABSTRACT

The uptake of heavy metals is a process dependent on the resistance/tolerance of plants against chemical stress. The nature of the metal and the particularities of each species influence the uptake capacity. In order to test the uptake capacity of some species, seeds of *Phaseolus vulgaris* and *Zea mays* from polluted and respectively unpolluted sites were selected for the experiment following. The authors' previous experiments on germination in the presence of metals (Fe, Cu, Zn and Pb) revealed that seeds from metalliferous fields behave differently against chemical stress compared with seeds from non-metalliferous fields. The differential behavior of the two types of seeds was observed even starting from imbibition. The aim of experiments is to investigate the accumulation abilities of non-metallophytes species such as *Phaseolus vulgaris* and *Zea mays*, analyzing the first growth stage. The analysis of the experimental data thus obtained demonstrates that harvested from polluted soil has lower uptake capacity less than the plants grown from seeds harvested from unpolluted soils.

Keywords*: heavy metals, Phaseolus vulgaris, uptake capacity, Zea mays*

1. Introduction

A growing pool of research shows that metabolic reactions to stress (heat, wounds, oxidative or chemical) are alike. Therefore, there are warning signs of a biochemical or biophysical nature, specific to each stress agent, that make the cell to trigger tolerance mechanisms. Establishing the ways of living organisms to adapt and understanding their mechanisms to gain resistance against adverse factors is of tremendous importance.

As open systems, in steady substance and energy exchange with their environment, plants respond appropriately to inauspicious contexts. Pollutants trigger a so-called stress status in vegetal organisms, translating into the alteration of growth and development, of the photo-synthesis, respiration, hormonal activity and of other processes calibrated at molecular level. At cellular level, the metabolic disorder grows stronger as the degree of pollution is higher. The resilience and the capacity to adapt determine the ability to survive in adverse environment. By means of specific biochemical reactions, plants incorporate, metabolise and partially detoxify pollutants, thus mitigating the risk of environmental pollution.

Plants behave in different ways depending on their sensitivity to deficient or toxic supply with micro-nutrients. The concentration in excess of essential or incidental metals is a proxy for the phyto-toxicity of different metals. The relative toxicity of heavy metals depends on the genotype of the plant and on the condition of soil. The intensity of absorption of heavy metals grows gradually from reproduction organs toward leaves and roots.

The metabolic feedback of the plant to the exposure to heavy metals in various concentrations determines its survival capacity. The genetic structure impacts the future behaviour of the individual, namely his adaptive response, his survival and reproduction capacity. The natural selection of genotypes capable to trigger tolerance-enabling mechanisms gives birth to tolerant ecotypes, and even to metal-tolerant populations of species [1, 2].

As plants are unable to avoid the absorption of heavy metals, various species or ecotypes have developed multiple mechanisms to tolerate them. Herbaceous

species are the most common metallophyte plants, as also are a number of rapidly-growing trees (*Betula* sp., *Salix* sp. and *Acer* sp.), apt to colonize metalliferous soil. The tolerance thus reached relies on the detoxification mechanisms inside or outside the cytoplasm. The capacity to eliminate metals is not a general characteristic of metal-tolerant organisms. Instead of eliminating metals, many plants accumulate them in roots and leaves, in high concentrations. Superior plants have systems that prevent the access, respectively the accumulation of metals in cells.

The focus of the authors' previous research was the germination stage of *Phaseolus vulgaris* and *Zea mays* seeds originating from metalliferous and non-metalliferous areas in Maramures, a county in the North-West of Romania. The experiments during germination revealed that even during imbibition (the incipient stage of germination), seeds from polluted areas (referred to following as PB - polluted beans and PM – polluted maize) display the capacity to adapt against chemical stress. In such seeds there is low accumulation of Zn, Cu, Fe or Pb as compared to seeds from areas that are not polluted, referred to following as UPB – unpolluted beans and UPM – unpolluted maize [3-6].

The focus of this research is to investigate the behaviour of plants during the growth stage. At low concentrations of heavy metals, the plants grown from unpolluted seeds absorb a higher quantity of metal than the plants grown from polluted seeds. At high concentrations, plants grown from polluted seeds absorb nearly the same quantity as plants grown from unpolluted seeds.

2. Materials and methods

The seeds used in this experiment, focused on the growth stage, were harvested in 2012. The crops of beans and maize prevail in the agricultural output of farmers in Maramures. Therefore, seeds of beans and maize were harvested from two carefully selected sites. One of the sites is heavily polluted because of intensive mining and ore processing activities. In the other site there is no any mining or industrial source of pollution. Besides seeds, the authors have collected soil samples from the two sites as above.

In order to measure the concentration of metals in seeds, soil and vegetative organs the atomic absorption spectrometry method was applied.

3. Experimental

The experiment includes four types of seeds: UPB, PB, UPM and PM (abbreviations as defined above). Thirteen experimental versions were conceived for beans and thirteen versions for maize respectively. Each of the thirteen experimental versions includes one witness experimental version. The witness experimental version consists in ten seeds harvested from polluted soil and ten seeds from unpolluted soil, germinated in the same container and grown up in nutrient solution exclusively. In the other twelve experimental versions, ten polluted and ten unpolluted seeds have germinated and grown up in nutrient solution containing different metallic ions (Zn, Cu, Fe and Pb) at various concentrations (three concentrations for each metallic ion).

The seeds were disinfected in alcohol, washed in distilled water and introduced in germination containers with a mix of nutrient solution and metallic ion solution. Solutions with metal ions were prepared using $FeSO_4$, $(CH_3COO)_2Pb$, $CuSO_4$ and $ZnSO_4$ salts. The concentrations of the metallic ion in the nutrient – metal ion mix were: 10, 50 and 250 mg/L for Fe^{2+}; 10, 20 and 25 mg/L for Cu^{2+}; 5, 10 and 15 mg/L for Zn^{2+}; 100, 1000 and 10000 mg/L for Pb^{2+}. These specific experimental concentrations of the metallic ions were

selected in order to match the concentration of each respective metallic ion in the polluted soil from which we harvested the seeds of beans and maize.

The parameters for germination were: 22^0C, 16 hours/day of light, 8 hours/day of darkness. After 7 days, the seedlings were transferred in test tubes with the mix of nutrient solution and metallic ions solution. The concentration of metallic ions in the nutritive solution was identical with that during germination. A Knop solution was selected as nutrient solution with the composition as follows: 1 g/L $Ca(NO_3)_2 \cdot 4H_2O$, 0.25 g/L KH_2PO_4, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L KCl and 0.0125 g/L $FeCl_3 \cdot 6H_2O$ [7]. After 14 days of growth in the mix of nutrient solution and metallic ions solution, the plants were washed in distilled water. The vegetative organs were separated and dried at 105^0C for 72 hours in a US-made Binder drying oven.

A Retsch RM-100 grinding machine was used to prepare the samples for mineralization. The mineralization took place in a Berghof MWS-2 microwave system. The parameters for mineralization were: in stage 1 - 145^0C, 5 minutes, power 75%; in stage 2 - 190^0C, 10 minutes, power 90%; in stage 3 - 100^0C, 10 minutes, power 40%. For the mineralization of soil the authors have established the following parameters: stage 1 - 180^0C, 25 minutes, power 99%; stage 2 - 100^0C, 10 minutes, power 99%. A mix of 10 mL HNO_3 65% (d = 1.4 kg/L, Lach-Ner) with 0.3 g plant powder, respectively with 4 g of dried soil was introduced in a microwave system. For the mineralization of all samples the authors have complied with the methodology provisions in the users' guide of the microwave oven. After mineralization, the samples were brought to 100 ml volumetric flask with distilled water. A Perkin Elmer AAS-800 Spectrometer has allowed for the application of the spectrometric method in order to measure the concentration of metals in seeds, soil and vegetative organs.

4. Results and discussion

The seeds of beans and maize, both part of the humans' food intake, uptake metallic ions from polluted soils, in concentrations as revealed in the following table:

Table 1. Content (mean value ± standard deviation) of heavy metals in seeds and soil

Metal (mg/g DW[*])	PB polluted beans seeds	PM polluted maize seeds	PS polluted soil	UPB unpolluted beans seeds	UPM unpolluted maize seeds	UPS unpolluted soil
Zn	0.009±0.002	0	0.836±0.086	0	0	0.039±0.009
Cu	0.007±0.001	0.004±0.001	0.173±0.054	0.006±0.002	0.002±0.001	0.014±0.004
Fe	0.075±0.010	0.015±0.003	13.343±0.110	0.054±0.013	0.009±0.003	0
Pb	0.064±0.008	0.049±0.007	0.583±0.015	0.044±0.012	0.040±0.008	0.058±0.010

[*] DW = dry weight.

Table 1 shows, on one hand, how polluted is the soil, in mg/g DW terms (column marked as PS), and on the other hand the concentration of the polluting agents in the seeds of beans and maize harvested from polluted and respectively unpolluted soils, seeds on which the authors have conducted their experiments during growth.

Fig. 1 shows the distribution pattern of Zn in mg/g DW terms, in the vegetative organs of beans grown up from the witness samples (W in both graphs in Fig.1) and from polluted and unpolluted seeds (PB and UPB) exposed to Zn-generated

stress, in concentrations growing from   5 mg/L to 15 mg/L.
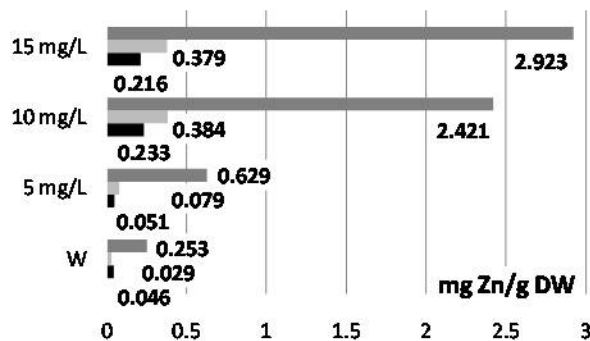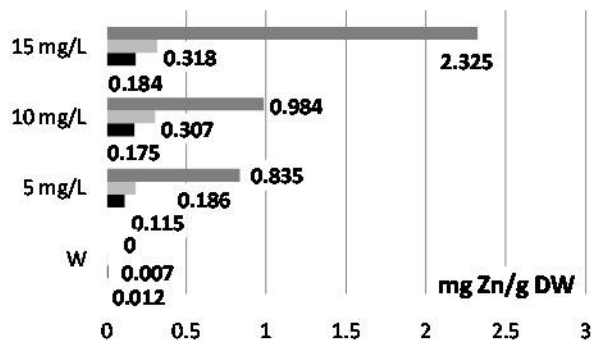
Figure 1. Pattern of accumulation of Zn in the vegetative organs of beans grown up from the witness samples, polluted (1a) and respectively unpolluted seeds (1b)

The quantity of Zn the beans uptake in the roots, in the stem or in leaves, is the difference between the quantity measured in the roots, in the stem or in leaves of the plant subjected to stress and the equivalent quantity in the roots, in the stem or in leaves of the witness sample. At small concentrations of Zn (5 mg/L) plants grown up from beans harvested from polluted soils uptake less metallic ions in roots and in the stem than plants grown up from seeds harvested in unpolluted soil. At 5 mg Zn/L, the plants grown up from unpolluted beans (UPB) accumulate 2.2 times more metallic ions in roots, 3.6 times more metallic ions in the stem, and 21 times more metallic ions in leaves, as compared to the plants grown from polluted beans (PB).

At higher Zn concentrations, the plants grown up from seeds harvested in polluted soil uptake most of the metallic ions in the roots. At 10 and respectively 15 mg Zn/L the beans from polluted seeds accumulate Zn prevalently in the roots, while beans from unpolluted seeds accumulate metallic ions in the roots only at 15 mg Zn/L in nutrient solution. Under such chemical stress, the concentration of Zn in the stem is comparable to the concentration in leaves. These remarks let the authors conclude that beans from polluted seeds have the capacity to adapt against the chemical stress that Zn generates.

The qualitative conclusions of the experiments with Zn stand also true for all the other metallic ions considered, namely Cu, Fe and Pb.

Figures 2(a-d) and 3(a-d) show the content of heavy metals (Zn and Cu, respectively Fe and Pb) accumulated in various organs of plants subjected to various concentrations of metallic ions.

Besides the concentrations indicated in the graphs, it is to note that the authors have experimented with concentrations of 25 mg Cu/L, 250 mg Fe/L and 10000 mg Pb/L. Both the polluted and unpolluted seedlings of beans and maize have not survived 14 days of exposure at the above mentioned concentrations. This is why those respective concentrations do not appear in the graphs in Fig.2 and Fig.3. Even more, at 1000 mg Pb/L only the seedlings from polluted seeds have survived for 14 days. This happened because of the high toxicity of this metal and because of the plant missing the capacity to accommodate to such harsh environment.
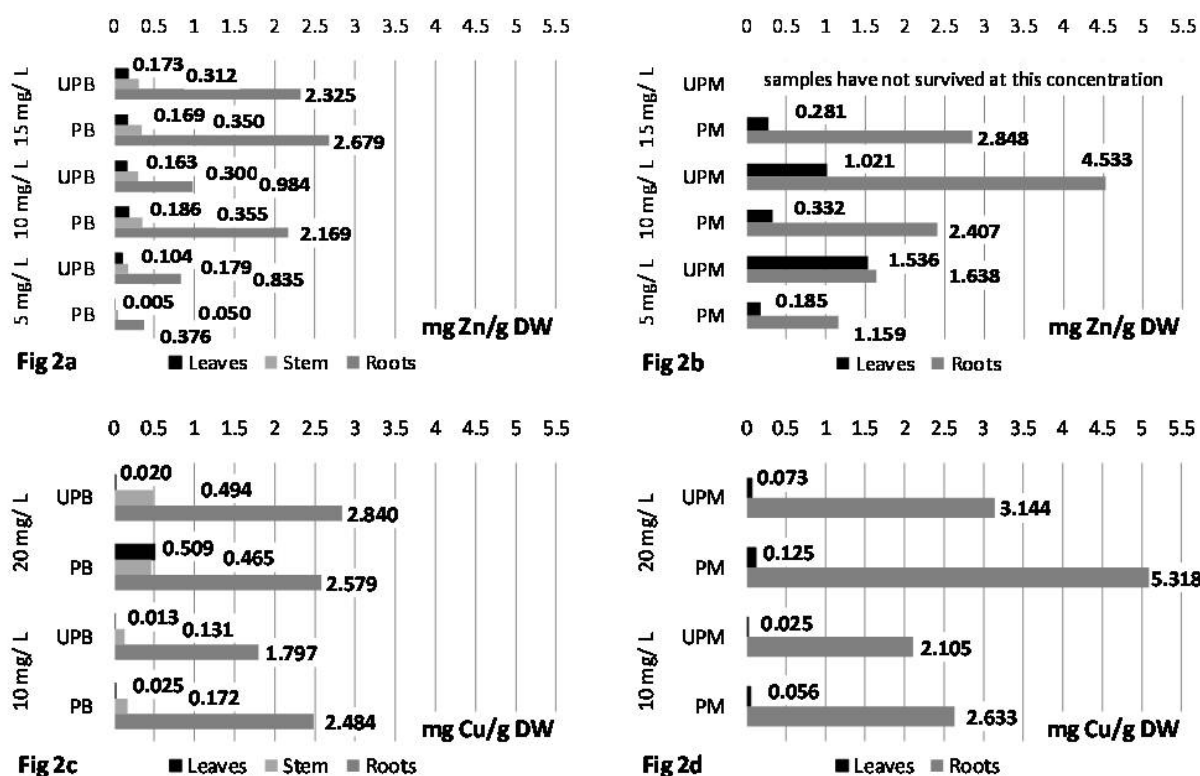
Figure 2. Content of copper and zinc in various organs of the plants grown up from polluted and unpolluted beans and maize seeds, subjected to different levels of chemical stress

Figure 3. Content of iron and lead in various organs of the plants grown up from polluted and unpolluted beans and maize seeds, subjected to different levels of chemical stress

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As shown in Figs. 2(a) and (b), the accumulation pattern in beans and maize reveals that the maize is able to accumulate more Zn than beans, both in roots and in the stem. At 5 mg Zn/L the polluted maize (PM) accumulates 3.1 times more Zn in the roots and 3.4 times more in the over-ground part of the plant than the polluted beans. Subjected to the same chemical stress, the unpolluted maize (UPM) accumulates only 2 times more Zn in the roots and respectively 5 times more in the over-ground parts of the plant than the unpolluted beans. Thus maize, and particularly maize from unpolluted seeds, accumulates Zn, but is not Zn-tolerant. With Zn in high concentrations, only a few plants have survived.
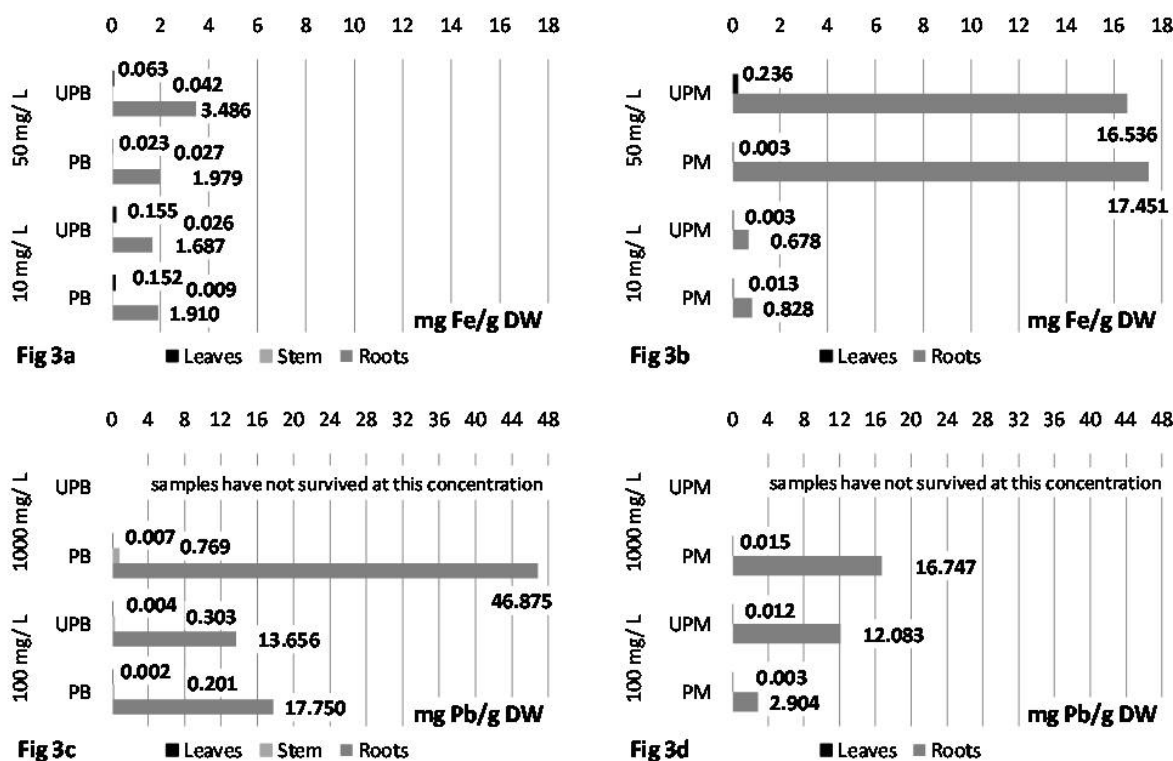
At 10 mg/L of chemical stress, the accumulation of Zn is two times higher in the stem and 7 times higher in the roots of PB as compared to Cu. In UPB, there is 2.3 times more Zn than Cu in the roots and 12.5 times more Zn than Cu in leaves. In terms of the accumulation of Zn in the over-ground parts of the beans, our conclusions match those by Gupta et al. [8]. The tendency to accumulate Zn in the over-ground parts of the plant stands also for maize.

One of the conclusion of the research undertaken by Davies`s et al. [9] about the tolerance of plants to Zn reveals that *Deschampsia caespitosa* is Zn-tolerant. In this species Zn is particularly concentrated in the vacuole, as opposite to other species which do not tolerate this metal.

Silene and *Rumex acetosa* are known as Zn-tolerant species, displaying high concentrations of malate in the upper parts of the plant, as opposite to other ecotypes which are not Zn-tolerant. The malate removes the Zn in excess in the cytoplasm, relocating it toward the vacuole, where ligands (citrates or oxalates) bind Zn [10]. The cellular wall of *Agrostis tenuis*, another Zn-tolerant species, has the capacity to bind Zn. A higher capacity of the cellular wall to bind Zn translates into a higher resilience of the plant against this metal [11-13].

The behaviour of beans was compared with the behaviour of maize against Cu-induced chemical stress. Up to a certain threshold of the concentration of Cu, maize reacts as Cu-tolerant and accumulates it. The maize locks the metallic ions of Cu in roots. The accumulation of Cu is 40-45 times higher in roots than in the over-ground parts of maize and respectively only 3-10 times higher in the roots than in the over-ground parts of beans.

Herbaceous plants, vegetables, vine, trees and shrubs have the capacity to tolerate Cu in excess. Plants differ in terms of their capacity to accumulate heavy metals depending on the plants' genotype, on the soil's conditions and on the micro-organisms associated to the soil. The microorganisms play the role of pollutants absorbers. Plant characteristics (species, the development of the organ with the highest absorption capacity of the metallic compound, age), the presence in soil of other ions, the pH and the redox potential of soil are also factors influencing the degree of concentration of micro-elements in vegetal tissues.

Exposed to Fe-induced chemical stress, the polluted beans have developed adaptive capacity as revealed in Fig. 3(a). As the concentration of the metallic ion went 5 times up, there is irrelevant change (1.04 times up) in terms of accumulation in the roots. Neither the PB nor the UPB accumulates Fe, but both are Fe-tolerant. The accumulations of Fe in the roots double as the concentration goes 5 times up; this demonstrates the existence of adaptive mechanisms in UPB, but those mechanisms are not as well developed as the equivalent mechanisms in PB. Maize accumulates Fe and locks the Fe ions in the roots.

Beans accumulate Pb, a metal with high degree of toxicity, in higher quantity than maize. Despite this, there is about 1000 times more Pb in the roots as compared to

the over-ground parts of maize, while the same ratio is about 65 for beans. Therefore the maize is Pb-tolerant, while beans accumulate Pb.

Pb accumulates in the cellular walls of *Oryza sativa* plants cultivated in environment with toxic concentration of Cu and Zn [14, 15]. This is also true for *Lupinus lutelus* cultivated in environment with toxic concentration of Cu and Zn [16-18].

5. Conclusions

The following key conclusions come out as a summary of the above:

− subjected to severe stress, induced by Cu (25 mg/L), Fe (250 mg/L) or Pb (10000 mg/L), neither beans nor maize survive, regardless of the origin of seeds; at 1000 mg Pb/L in concentration in nutrient environment, neither UPB nor UPM survived, because of the high toxicity that the concentration and the nature of this heavy metal trigger, and because UPB or UPM have no adaptive mechanisms against such harsh conditions;

− beans are more resilient against Zn than the maize; PB have adaptive mechanisms against Zn-induced chemical stress; Zn accumulates particularly in the over-ground parts of beans and maize; maize, especially from unpolluted seeds, accumulates Zn but is not Zn-tolerant;

− within a certain range of concentrations of the metallic ion, the maize is Cu-tolerant, accumulates Cu and locks the metal in roots;

− both the polluted and unpolluted beans are Fe-tolerant; UPB have an inferior adaptive capacity against Fe as compared to PB; the maize accumulates Fe and locks the metal in the roots;

− the seeds of beans accumulates Pb, while those of maize are Pb-tolerant.

6. References

1. Baker A.J.M., (1987), Metal tolerance, *New Phytologist*, 106, 93-111.
2. Meharg A.A., (1993), The role of plasmalemma in metal tolerance in angiospermes, *Physiologia Plantarum.*, 88, 191-198.
3. Varga C., Marian M., Peter A., Boltea D., Mihaly-Cozmuta L., Nour E., (2009), Strategies of heavy metal uptake by *Phaseolus vulgaris* seeds growing in metalliferous and non-metalliferous areas, *Studia Universitatis Babes-Bolyai Chemia,* LIV, 223-234.
4. Nicula C., Marian M., Mihaly-Cozmuta L., Peter A., Mihaly-Cozmuta A., (2010a), *Adaptive mechanisms of plants grown in agrocenoses prone to pollution with heavy metals,* 19th International Workshop of European Vegetation Survey, „Flora, vegetation, environment and land-use at large scale", Pecs.
5. Nicula C., Marian M., Mihaly-Cozmuta L., Peter A., Mihaly-Cozmuta A., (2010b), Adaptive mechanisms of *Phaseolus vulgaris* and *Zea mays* grown in agrocenoses prone to pollution with heavy metals, The Annals of Oradea University, Biology Fascicle, Tom XVII, 152-157.
6. Pop A., (2010), Influence of heavy metal ions concentration on germination and plant growth, *Carpathian Journal of Food, Science and Technology,* 2, 1-6.
7. Strepp R., Scholz S., Kruse S., Speth V., Reski R., (1998), Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin, *Proceedings of the National Academy of Sciences,* 95(8), 4368-4373.
8. Gupta A.K., Dwivedi S., Sinha S., Tripathi R.D., Rai U.N., Singh S.N., (2007), Metal accumulation and growth performance of *Phaseolus*

vulgaris grown in fly ash amended soil, *Bioresource Technology*, 98, 3404-3407.

9. Davies K.L., Davies M.S., Francis D., (1992), Zinc-induced vacuolation in root meristematic cells of cereals, *Annals of Botany*, 69, 21-24.

10. Harmens H., Koevoets P.L.M., Verkleij J.A.C., Ernst W.H.O., (1994), The role of low molecular weight organic acids in the mechanism of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke, *New Phytologist*, 126, 615-621.

11. Wainwright S.J., Woolhouse H.W., (1977), Some physiological aspects of copper and zinc tolerance in *Agrostis tenuis* Sibth.: cell elongation and membrane damage, *Journal of Experimental Botany*, 28, 1029-1036.

12. Marques A.P.G.C., Rangel A.O.S.S., Castro P.M.L., (2007), Zinc accumulation in plant species indigenous to a portuguese polluted site, *Journal of Environmental Quality*, 36, 646-653.

13. Mbila M.O., Thompson M.L., (2004), Plant-available zinc and lead in mine spoils and soils at the mines of Spain, Iowa, *Journal of Environmental Quality*, 33, 553–558.

14. Muramoto S., (1989), Heavy metal tolerance of rice plants (*Oryza sativa* L.) to some metal oxides at the critical levels, *Journal of Environmental Science and Health*, 24, 559-568.

15. Shi J., Li L., Pan G., (2009), Variation of grain Cd and Zn concentration of 110 hybrid rice cultivars grown in a low-Cd paddy soil, *Journal of Environmental Sciences*, 21, 168-172.

16. Kopyra M., Gwozdz E.A., (2003), Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*, *Plant Physiology and Biochemistry,* 41, 1011-1017.

17. Jomova K., Morovic M., (2009), Efect of heavy metal treatment on molecular changes in root tips of *Lupinus luteus* L., *Czech Journal of Food Sciences*, 27, S386-S389.

18. Ximenez-Embun P., Madrid-Albarran Y., Camara C., Cuadrado C., Burbano C., Muzquiz M., (2001), Evaluation of lupines species to accumulate heavy metals from waste waters, *International Journal of Phytoremediation,* 3, 369-379.

NANOSENSOR BASED ON TiO₂ FOR DETECTION OF OXYGEN IN DAMAGED VACUUM PACKAGES

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ABSTRACT

This study aims to prepare and test the capacity of a sensor based on TiO₂ to detect the presence of oxygen in the damaged vacuum packages. The oxygen indicator is described, comprising nanoparticles of titania added to a sacrificial electron donor, glycerol and to a redox indicator, methylene blue (MB). The indicator is blue colored in the absence of UV light, however upon exposure to UV light its blue color becomes light blue and until it is exposed to oxygen, whereupon its original color is restored.

Keywords: *nanosensor, titania, vacuum packages*

1. Introduction

Often, the reduced damages of the vacuum packages are not easily observable. Even so, the presence of small amount of oxygen in the damaged vacuum packages promotes degradations of lipids, proteins and glucides, affecting the food safety. Thus, it is imperiously need to develop a procedure to detect the integrity of the vacuum packages.

There are many established methods for the detection of oxygen, which include the Clark electrode [2] and gas chromatography [3], however, such methods are too expensive and time consuming to allow 100% quality assurance.

Some commercial colorimetric oxygen indicators are already on the market, for example Ageless EyeTM [4].

Recently, have been prepared and tested different types of oxygen sensors, based on titania dispersed in different polymer matrix [1-4]. Mills et al. [1] have tested an indicator that appears susceptible to appreciable photodegradation when UV-activated under ambient (21% O₂) conditions.

The aim of this research is to test the capacity of the nanosensor based on TiO₂ to detect the presence of oxygen in the damaged vacuum packages.

2. Materials and methods
2.1. Nanosensor preparation

An amount of 0,1 g commercial nanocrystallized TiO₂ (CarloErba, Spain) was added to 0,25 g glycerol (Chimactiv, Romania) and subsequenlty to 0,0025 g methylene blue (Merck, Germany). The mixture was homogenized and was kept for rest for 3 days. Then, the obtained ink was coated in the shape of a dot on a paper sheet and dried 1h at 60°C. Figure 1 illustrates the obtained nanosensor before UV light activation.

Figure 1. Nanosensor before UV light activation.

2.2. Nanosensor activation

The obtained nanosensor was activated by irradiation generated by a mediu pressure Hg lamp (150W). The irradiation time was one hour (Figure 2).
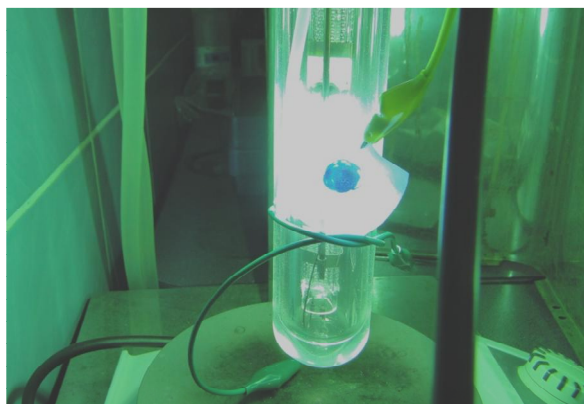
Figure 2. Installation used for activation

2.3. Preparation of vacuum packages

Vacumm packages containing summer salami (30 g) purchased from Ferma Zootehnica, Romania were prepared using a vacuum machine (Figure 3).

Figure 3. Vacuum machine

Four different packages containing the nanosensor were prepared as follows:
• package with non-activated nanosensor– reference;
• vacuum package with non-activated nanosensor;
• vacuum package with activated nanosensor;
• damaged vacuum package with activated nanosensor. The damage was realized by cutting a corner of the package.

The reason for the including of a food type (summer salami) into the vacuum packages was to observe if the humidity of the food sample will affect the functionallity of the nanosensor.

The packages were kept at 22^0C, humidity 35% (measured with a Thermo sensor for humidity) and after 5 and 8 days of storage were analyzed.

3. Results and discussions

3.1. Nanosensor efficiency

Before including of the piece of paper with ink dot (nanosensor) into the vacuum packages, the functionally of the nanosensor was established. The piece of paper was irradiated for 1 hour (Figure 4).
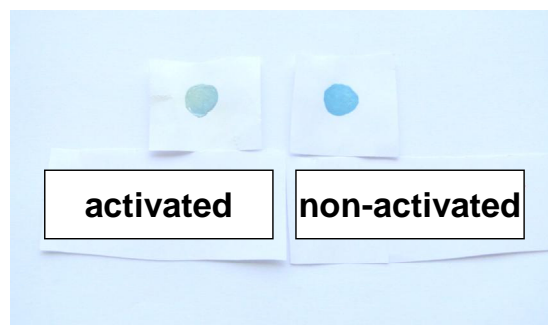
Figure 4. Comparison between activated and non-activated

It can be observed that during irradiation, the ink dot was partially discolored. The base paper became yellowish as compared with the white color of the non-irradiated piece of paper.

After actiavtion, the decolored ink dot was kept in air (22^0C, humidity 35%) and the recolorisation occured after 5 days (Figure 5). Moreover, on the ink dot, small spots having dark blue color appeared.
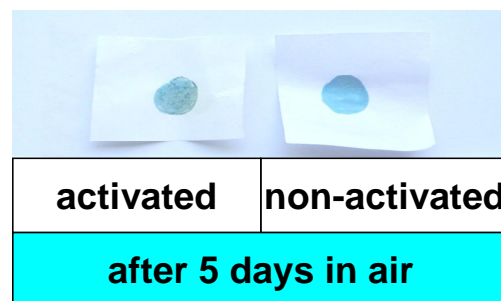
Figure 5. Recolorisation of nanosensor

3.2. Functionally of the nanosensor in vacuum packages

Figures 6 and 7 present the images of the vacuum packages after 5 and 8 days of storage. By analyzing Figure 6, it can be observed that the activated nanosensor from the damaged vacuum package have the same color as that in the non-activated one, thus suggesting the presence of oxygen in the damaged package. Moreover, a change in color of the summer salami during irradiation was observed, suggesting that the properties of the food sample were affected.

Similar changes of the color of nanosensor were observed also in Figure 7.

Figure 6. Macroscopic view of packages and nanosensors after 5 days of storage

Figure 7. Macroscopic view of packages and nanosensors after 8 days of storage

3.3. Principle of nanosensor activity

The activation of the TiO_2 with a UV light with energy higher than gap energy of TiO_2 (3.2.eV) induces tranfer of the electrons from the valence band (VB) into the conduction band (CB), thus leaving in the VB holes (reaction 1). The holes in the VB oxidize the glycerol molecules to glycerin aldehyde (reaction 2) and the electrons reduce the methylene blue molecules, having blue color (oxidized form) to its reduced form, white colored (reaction 3). In the presence of oxygen molecules, the reduced form of methylene blue is reoxidized, thus becoming blue again (reaction 4).

$$TiO_2 \rightarrow e^- + h^+ \qquad (1)$$

$$h^+ + glycerol \rightarrow glicerin\ aldehyde \qquad (2)$$

$$e^- + oxidized\ methylene\ blue\ (blue) \rightarrow$$
$$reduced\ methylene\ blue\ (white)\ (3)$$

$$reduced\ methylene\ blue\ (white) + 1/2\ O_2 + 2H^+ \rightarrow$$
$$oxidized\ methylene\ blue\ (blue) +$$
$$H_2O \qquad (4)$$
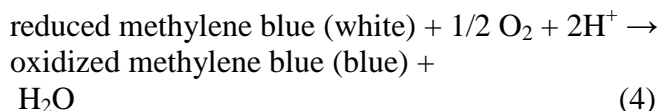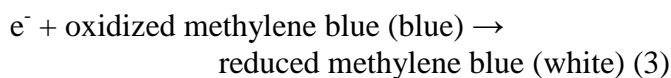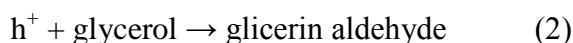
Thus, the damaging of the vacuum package and penetration of the oxygen into the package induces the colorisation of the ink dot. This is the indicatior of the damaging of the package.

4. Conclusions
- ✓ A nanosensor based on TiO_2, glycerol and methylene blue was succesfully prepared;
- ✓ The efficiency of the obtained nanosensor was demonstrated by decolorisation during the activation with UV light and by the fact that it became again blue, after 5 days of storage in air;
- ✓ The nanosensor in the unbroken vacuum packages kept its initial white color during the storage at 5 and 8 days
- ✓ The nanosensor in the broken vacuum packages became dark blue during the storage at 5 and 8 days, thus demonstrating the presence of oxygen

Our future aims are to develop procedures to enhance the techniques for the preparation of a more homogeneous ink.

6. References
1. Mills, A., Tommons, C., Bailey, R.T., Tedford, C., Crilly, P.J. (2008) UV-Activated Luminescence/Colourimetric O₂ Indicator, *Int. J. Photoenerg.*, doi:10.1155/2008/547301.
2. Hitchman, M.L. (1978) Measurement of Dissolved Oxygen, *John Wiley & Sons,* New York, NY, USA.
3. Valenty, S.J. (1978) Gas chromatographic determination of dissolved hydrogen and oxygen in photolysis of water, *Analytical Chem.*, 50 (4), pp. 669–671.
4. Mills, A., Hazafy, D., Lawrie, K. (2011) Novel photocatalyst-based colourimetric indicator for oxygen, *Catal. Today*, 161, pp. 59–63
5. Duncan, T.V. (2011) Applications of nanotechnology in food packaging and food safety: Barrier materials, antimicrobials and sensors, *J. Colloid Interface Sci.*, doi:10.1016/j.jcis.2011.07.017.
6. Valdés, M.G., Valdés González, A.C., García Calzón, J.A., Díaz-García, M.E. (2009) Analytical nanotechnology for food analysis, *Microchim. Acta*, 166, pp. 1–19.
7. Shtykov, S.N., Yu, T. (2008) Nanomaterials and Nanotechnologies in Chemical and Biochemical Sensors: Capabilities and Applications, *Russian J. General Chem.,* 78 (12), pp. 2521–2531.

PRODUCTION OF PROCESSED SPREAD CHEESE SUPPLEMENTED WITH PROBIOTIC BACTERIA AND STUDYING GROWTH AND VIABILITY UNDER DIFFERENT STORAGE CONDITIONS

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ABSTRACT

The aim of this study is to produce processed spread cheese supplemented with two strains of probiotic bacteria Lactobacillus *acidophilus LA 5, Bifid animalissubsplactisBB12* , and the evaluation of the growth and viability at manufacturing time and after 2,4,6,8 weeks of storage at refrigeration temperature and at room temperature. The data obtained showed that the total count of the two probiotic bacteria were 3.1×10^7cfug^{-1} and 3.2×10^7cfug^{-1} respectively at manufacturing , consequently the viability count of the two strains were 2.9×10^7 cfug^{-1} and 1.9×10^7cfug^{-1} respectively, at refrigerated temperature after eight weeks of storage. These numbers are higher than recommended ones which the positive impact on the consumer's health. Results of sensory evaluation by the consumers with the results of sensory evaluation coincided by panelists, but there were no significant differences between supplemented processed spread cheese with probiotic and non-supplemented, and two types of cheese got the acceptance of the consumers and the panelists and emphasized the possibility of the production of functional processed cheese with a good sensory properties, supplemented by probiotics bacteria and without a change in the sensory qualities or a change in its chemical composition when stored refrigerated. The study showed that processed spread cheese is suitable and appropriate environment for the growth and survivability of *Lact acidophilus AL5, Bifid.animalissubsplactisBB12*, and thus the bacteria is transferred to the consumer with higher numbers than the recommended and that leads to positive health effect on the consumer.

Keywords: *Functional food, Lactobacillus acidophilus LA5, Bifid.animalissubsplactis BB12*.

1. Introduction

Foods that contain probiotic bacteria are classified as functional foods [1], it is a food that gives a positive impact on consumer's health in addition to their nutritional value. Probiotics are defined as "live microorganisms which when administered in adequate numbers confer a health benefit on the host" [2]. For probiotics to offer health benefits, they must remain viable in food products above a threshold level (at least, 10^6colony forming unit/gram (cfu·g^{-1}), at a universally agreed minimum level) until the time of consumption [3], and consume food containing them daily at a rate of 100 g [4]. The probiotics beneficially affect the human health by improving the balance of the intestinal microflora and improving mucosal defenses against pathogenic bacteria. Additional health benefits include enhanced immune response, reduction of serum cholesterol, reduction and prevent ion of diarrhea, vitamin synthesis, anticarcinogenic activity, improve digestion of lactose, antibacterial activity and have an important role in prevention of gastritis caused by *Helicobacter pylori* [5, 6, 7]. Microbial probiotics must be safe and free of any useful characteristics, resistant to the acidity of the stomach, bile salts, able to reach the intestines, and able to epithelial cell adhesion of the digestive tract and multiply to form colonies at the expense of harmful germs [8, 9]. Probiotic bacteria belong to a group lactic acid. The bifidobacterium and lactobacillus genus were the most commonly used in foods because of their safety over a long history, which is also found as part of the intestinal microflora [10], while the most popular species of the two genes *Bifid, animalissubsplactisBb12*. and *Lact. acidophilus LA5 bacteria* [11]. The *Bifid.animalissubsplactisBB12* bacteria are used in dairy products and baby food widely around the world, with good ability to carry oxygen

because it grows well in milk, milk based foods, and these qualities facilitate growth in commercial products in circumstances that are anaerobic [12]. Probiotic bacteria are available in the form of pills or capsules or powder added to some types of food and One of the most common foods where probiotics bacteria were added are fermented dairy products and milk[13], as well as they have been added to the different types of cheese, which is considered reasonable and appropriate environment for the growth of probiotics bacteria more than yogurt and fermented dairy products, it has also the potential pH higher and a relatively high content of fat , total solids which provide them with protection when passed through the digestive system and the top ability of regulatory buffering capacity[14]. The addition of probiotics bacteria to food is successful if used probiotics bacteria are resistant to manufacturing and storage conditions without carrying viability and if they prepared before consumption which is higher than the recommended number to give a positive health impact, and if there were no adverse effects on the product in terms of sensory and chemical properties [15]. Michael *et al*. [16] has tried to produce cheddar cheese that contains different types and strains of probiotics bacteria, showed that the *lact. casei, lact.paracaei, lact. bifid, rhamnosus, spp* bacteria remained prepared above the recommended level 10 cfu·g^{-1}to give positive health impact after 32 weeks of storage, while the *L.acidophillus* bacteria was less than the recommended level. In another study a number of strains of have been added of *Lactobacillus* bacteria in the manufacturing of cheddar cheese and the results of the study showed that *Lact. Paracasei* bacteria remained alive and effective and higher than the recommended levels after 8 months of storage [17].Also *Lact. Acidophilus, Bifid spp* bacteria have been added to the white cheese saved in brine and was prepared after 90 days of storage close to the numbers recommended to give a positive impact health [18]. *Bifid. Bifidum* bacteria was added to cheddar cheese and remained prepared after 24 weeks of storage 2.0×10^{7}cfu·g^{-1} and without a change in flavor, texture and appearance [19]. In other *study Bifid. Spp, Lact. Casei, Lact. Acidophilus* bacteria

was added to Fresco cheese, this study showed that the number of those types of bacteria remained higher than the recommended numbers after 60 days of storage[20]. The aim of this study was the production of processed spread cheese, supplemented with two probiotics bacteria they are *Lact. acidophilus LA5* and *Bifid. Animalissubsplactis BB12*, to estimate the viability of these types under room temperature (20 – 25°C) and refrigerated conditions (7– 4°C) storage up to 8 weeks and evaluated the cheese in chemical, microbiological and sensory terms.

2. Materials and methods
2.1. Initially activate two strains of probiotics bacteria

There are two strains of probiotics bacteria that were activated to be used in this study: *Lact. acidophilus LA5, Bifid. animalissubsplactis BB12* (Hansen, Denmark). Through two phases: in the first used MRS Liquid media added L-cysteine - HCL by 0.05 as oxygen scavenger and nitrogen sources (sigma), then added 1 g of each bacterial strain and inoculated in anaerobic conditions at 37°C for 18 hr. Followed by the collection of biomass of bacteria developing using centrifuge speed of 8500rpm/ min. for a period of 10 minutes under 5°C, then twice washed biomass with sterile distilled water. The second phase, skimmed milk powder reconstituted by 12% and heated to 90°C /30 min. then cooled to 37 °C then added L-cysteine - HCL by 0.05% was inoculated with biomass of bacteria, under anaerobic conditions at 37°C/18 hr and then keeping refrigerated until used [20, 21].

2.2. Manufacturing steps for processed spread cheese

The cheese was manufactured in a dairy factory using a cooking device type Stephan (Germany) with 25 kg capacity rates, raw materials shown in Table 1, the percentage was reached through the experience and sensory evaluation by special sensory panel. According to the following steps [22]:

A. Weighing of basic materials and additions were carried as follows:1- Emulsifying salts: kasomel 2366, kasomel 2394 (Disodium

phosphate, Polysodium phosphate) (Euro Phose - Belgium); 2- White cheese was manufactured in a factory using good chemical and microbiological raw milk; 3- Kashkawan cheese, Cottage cheese, and salt, citric acid were purchased from the local market; 4-Whey powder, skimmed milk powder (France), potassium sorbet (Netherlands); 5- Water; **B.** Cutting and shredding different cheese; **C.** Putting all components inside double jacket cooker, heated using steam at 90°C for 5 min and stirring by knives spin at 1500-3000 rpm/ min; **E.** Cooling the mixture to a temperature of 45°C cooling processed took 11 min; **F.** Add L-cysteine – HCL with rate of 0.5 g/kg; **G.**Supplemented the product with probiotics bacteria: This was done through the addition of reconstituted powdered milk fortified with probiotics bacteria previously prepared to the mixture with the continuation of stirring for 2 minutes to get a homogeneous mass; **H.**Control sample was prepared in the same way, without adding the supplemented bacteria.; **I.**Packing: the Product was packaged in sealed glass containers; **J.** Cooling of the product; **K.**Storage: compatible product supplemented cheese and non-supplemented (control) each one divided to two parts, the first one stored at room temperature (20 - 25°C) and second one stored at refrigeration temperature (4 - 7°C) for 8 weeks , and analysis of microbial numbers , acidity and pH were taken once every two weeks .

2.3. Microbial analysis
2.3.1. Sample Preparation for microbial analysis:
primary dilution 10^{-1} was prepared by mixing 11 g of the sample with 99 ml of sterile 2% sodium citrate and other dilutions are prepared as needed with sterile peptone water (Scharlau Chemie Company - Spain) , added to dilutions 0.05% of L – cysteine – HCL [13, 19]. Pour plate technique using two Petri dishes for each dilution [23].

2.3.2. Microbial tests
Estimate of the number of probiotics bacteria in processed spread cheese: estimate the number of probiotics bacteria *Lact. acidophilus LA5, Bifid. animalissubsplactis BB12* in processed spread cheese fortified them immediately after

manufacturing and after 2, 4, 6, 8 weeks of storage at room temperature (20-25°C) and under refrigeration (4-7°C). Using Bifidus Selective Medium Agar, pH 6.8 [12] and added 116 mg/L of supplement BSM (a mixture of antibiotics inhibits the growth of other microbes) the colony color should be purple but when less nitrogen compounds in the middle colonial color has to be pink (according to the manufacturer's instructions Sigma).

The number of *Lact. acidophilus LA 5* bacteria was estimated using Rogosa SIA gar, pH 5.4 (Sigma) and added to it glacial acetic acid 1.32 ml /L [24]. The dishes were incubated at 37°C anaerobic conditions for 3 days using J. P. Selecta type incubator (Spain) to provide anaerobic conditions, incubator was equipped with a pump to oxygen suction then pumped CO_2, N_2 gas from specific gas cylinders and also conducted movement. Examination, catalase testing, gram stain and gram stain for spores as confirmatory tests for probiotics bacteria [25].

General microbial tests: it was estimated that the total number of aerobic microorganisms, using Nutrient agar (ScharlauChemie co. Spain) Dishes were incubated under aerobic conditions at (37°C) for 48 hours and *Staphylococcus aureus* using Baird Parker media , then Added per 950 ml of this prepared environment 50 ml of Egg Yolk tellurite emulsion (ScharlauChemie co. Spain). Dishes were incubated under aerobic conditions at (37°C) for 48 hours, And the number of coliform bacteria as using Violet red yellow agar (VRBA) (ScharlauChemie co. Spain) dishes were incubated at (37°C) for 24 hours and the number of yeast cells as using Potato dextrose agar and added to it 10 ml of tartaric acid /L (ScharlauChemie co. Spain). Dishes were incubated at (25°C) for 5 days. Also been detected *salmonella* bacteria by mixing 25 grams of the sample with 225 ml of buffer peptone water Dishes were incubated at (37°C) for 24 hours and then used Tetrathionate and Selenite cystien Dishes were incubated at (37°C) for 24 hours and then used Lysine desoxycholatexylose agar (ScharlauChemie co. Spain) were Incubated at 37°C for 24 hours. Also estimated the number of *Escherichia coli* bacteria using the most likely

counting method (MPN) using three tubes per ease (10, 10, 10)   Using liquid environment Lauryl Tryptose (ScharlauChemie co. Spain) [23].

2.3.3. Chemical analysis

Chemical analysis was conducted for the two types of processed cheese supplemented and non-supplemented terms were estimated proportion of the total dry matter and ash, acidity and salt ratio using the Moore method, protein and fat and pH [26].

2.3.4. Sensory evaluation

Sensory tests were divided and made into two groups. The first group includes two tests, in the first test three specialized people in tasting processed cheese, in the second test 12 people of specialists in the field of food and sensory evaluation and French specifications relating to the sensory characteristics of processed cheese were adopted, divided sensory properties to taste and smell with (0- 10) marks, texture (0-5) marks, color and appearance (0 – 3) marks, The total of all signs of assessing sensory properties 18 marks [27]. The second set of tests sensory satisfaction and to conduct the hedonic scale, which varied between one degree (not like very much), the slightest sensory evaluation to 9 degrees (like very much), the highest degrees of sensory evaluation. The test group was a random sample of 60 consumers of different age and sex [28].

2.3.5. Statistical analysis

A statistical analysis conducted of the data using the arithmetic means and standard deviations and T-test to investigate the existence of significant difference between the averages studied using the SPSS program.

3. Results and discussion
3.1. Chemical results

The chemical composition of supplemented (with probiotics bacteria) and non-supplemented processed spread cheese as a control are recorded in Table 2. The data shows that the percentage of dry matter of supplemented processed spread cheese was 39.50%, while for the control was 40.20%, these ratios are within the permissible

limits of the Jordanian Standard No. 182 of 1997 [29]. As regards percentage of fat of dry matter was 53.16% for supplemented cheese and 51.90% for control, these ratios are within the permissible limits of the Jordanian Standard [29].

The other components, protein 12.6% and 12.66%, ash 3.8% and 3.73%, NaCl 1.56% and 1.75%, acidity 1.24 and 1.16, pH 5.52 and 5.75 for supplemented and non-supplemented processed spread cheese (control), respectively (Table 2).

3.2. Effect refrigerated storage (4–7°C) on the survivability of probiotics bacteria.

Table 3 shows the number of *Bifid. animalissubsplactis BB12*, *Lact. acidophilus LA 5*, titratable acidity and pH in supplement processed spread cheese during cold storage (4 – 7°C) of up to 8 weeks , data indicate that the number *Bifid. animalissubsplactis Bb12*, at fresh time of storage (immediately after manufacturing) was 3.2×10^7cfu·g^{-1}then gradually reduced in its numbers and reached 2.9×10^7cfu g^{-1}after 8 weeks of storage, which is not significantly (p > 0.05) different in the decline in numbers between the beginning and the end of storage . These results were consistent with the results obtained by Krajewska–Kamińska*et al*. [30], While, for the *Lact. acidophilus LA5* bacteria data in table 2 indicate that the number of the bacteria immediately after manufacturing was 3.1×10^7cfu·g^{-1} it was significantly (p ≤ 0.05) reduced in numbers with the progress of the storage period at the end (of 8 weeks) to 1.9×107 cfu·g^{-1}. This may be due to the complicated dietary requirements of that this bacteria need or the unsuitability of storage temperature   [31]. However, the number of each of the two types of *Lact. acidophilus LA5*   and *Bifid. animalissubsplactis BB12*, bacteria remained higher than the recommended numbers to give a positive impact on consumer's health and those results confirmed that processed spread cheese is a good way for the transfer of probiotics bacteria to the consumer in higher   numbers more than the recommended numbers.

Table 1. Proportion of ingredients used in processed spread cheese manufacturing

Ingredient	Proportion, %
Desalt white cheese	37.2
Kashkawan cheese	9.3
Cottage cheese	9.3
Pasteurized cream	18.6
Whey powder	3.25
Emulsifiers salts	2.3
Table salt	0.93
Citric acid	0.23
Potassium Sorbet	0.23
Water	18.6

Table 2. Chemical composition of supplement and non-supplement processed spread cheese

Processed spread cheese	Total DM** %	Fat % of DM	Protein %	Ash%	NaCl %	Acidity %	pH
Supplement	39.5±.25	53.16±.75	12.60±.20	3.8±.04	1.56±.02	1.24±.04	5.52±.007
Non-supplement (control)	40.21 ±.25	51.90 ±1.44	12.66 ±0.32	3.73 ±0.05	1.75 ±0.050	1.16 ±0.05	5.75 ±0.05

* Values are the average of three replicates; **DM, Dry mater

Table 3. Viability number of *Bifid. animalissubsplactis BB12* and *Lact. acidophilus LA5,* acidity 9%) and pH in processed spread cheese under refrigeration temperature for different storage time

Storage time (week)	*Bifid. animalissubsplactis BB12* cfu·g^{-1}	*Lact. acidophilus LA-5* cfu·g^{-1}	Acidity %	pH
0	3.2×10^{7a}	3.1×10^{7a}	1.04^a	5.65a
2	3.1×10^{7a}	2.9×10^{7a}	1.10^a	5.55^a
4	3.1×10^{7a}	2.7×10^{7ab}	1.21^a	5.33^a
6	3.0×10^{7a}	2.5×10^{7ab}	1.32^a	5.23^a
8	$2.9. \times 10^{7a}$	1.9×10^{7b}	1.43^a	5.15^a

* Values are the average of two replicates; Different superscript letters in the same column indicate a significant (p ≤ 0.05) difference according to Duncan's test.

Table 4. Number of *Bifid. animalissubsplactis BB12*, *Lact. acidophilus LA5,* acidity percent and pH for supplement processed spread cheese under 20-25C° of storage

Storage time (week)	*Bifid. animalissubsplactis BB12* cfu·g^{-1}	*Lact. acidophilus LA5* cfu·g^{-1}	Acidity %	pH
0	$3.2 \times 10^{7\,b}$	$3.1 \times 10^{7\,b}$	1.04^c	5.65^a
2	1.0×10^{8a}	1.0×10^{8a}	1.25^c	5.15^a
4	3.0×10^{7b}	6×10^{7b}	1.7^b	4.40^b
6	2.5×10^{5c}	4.9×10^{7b}	2.2^a	4.21^b
8	$1.2 \times 10^{5\,c}$	$4.5 \times 10^{6\,c}$	2.29^a	4.1^b

- Values are the average of two replicates; Different superscript letters in the same column indicate a significant (p ≤ 0.05) difference according to Duncan's test.

Table 5. General microbial analysis for supplement processed spread cheese by *Bifid. animalissubsplactis BB12* and *Lact. acidophilus LA5*

	Storage time (week)	
	Zero	**Eight**
Total aerobic count (cfu g^{-1})	$2\times 10^{3\,a}$	$5\times10^{2\,b}$
Total coliform bacteria (cfu g^{-1})	0	0
Total yeast and mould(cfu g^{-1})	0	0
Staph. aureus(cfu g^{-1})	0	0
E. coli(cfu g^{-1})	0	0
Salmonella(cfu g^{-1})	0	0

* Values are the average of three replicates; Different superscript letters in the same rows indicate a significant (p ≤ 0.05) difference according to Duncan's test.

Table 6. Panelists sensory evaluation of supplement and non-supplement processed cheese

Product type / Sensory attributes (score)	Supplement	Non-supplement
Flavor (0-10)	7.20 [a] ±1.05	6.91[a] ±1.73
Texture (0-5)	3.66[a] ±0.65	3.75 [a]±0.91
Color and appearance(0-3)	2.75[a] ±0.45	2.64 [a] ±0.48
Total (18)	13.61[a] ±1.28	13.30 [a]±2.36

* Values are the average of 12 panelists; Different superscript letters in the same rows indicate a significant (p ≤ 0.05) difference according to Duncan's test.

Table 7. Consumers sensory evaluation of supplement and non-supplement processed cheese

Hedonic scale*	Supplement	Non-supplement
9	2	5
8	7	10
7	31	24
6	9	11
5	9	7
4	2	2
3	0	1
2	0	0
1	0	0
Mean	6.61	6.75
Std.Deviation	1.09	1.3

*Scale:9=like very much, 8=like much, 7=like moderately, 6=slightly like, 5=nether like nor dislike, 4=slightly dislike, 3=dislike moderately, 2=dislike much, 1=dislike very much
Number of consumer 60 persons

According to Flimelová et al. [32] various reports published that the count of probiotic cells in a foodstuff should be approximately 10^6cfug^{-1}, and in order to obtain a beneficial effect, a daily ingestion of 10^8–10^9 cfu for each portion is recommended. Obtained results indicate that all samples achieved the bacterial counts exceeded the value of 10^6 cfu·g^{-1} during the whole period of storage. The titratable acidity of the cheese at manufacturing time (zero time) was 1.04% and gradually increased with the time of storage up to 1.43% at 8 weeks of storage were confirmed by pH values which ranged from 5.15 to 5.65 with no significant difference (Table 3).

3.3. Effect of room temperature storage (20 – 25°C) on the survivability of probiotics bacteria.

The number of *Bifid. animalissubsplactis BB12* was increased till 2 weeks of storage at (20 – 25°C) reached to 1.0×10^8 cfu cfug^{-1} then reduced significantly (p ≤ 0.05) to reach 1.2×10^5 cfug^{-1} at the end of storage (8 weeks) as it shown in Table 4, while the number of *Lact. acidophilus LA5* was increased till 6 weeks of storage reached 4.9×10^7 cfug^{-1} then reduced significantly (p ≤ 0.05) and reached 4.5×10^6 cfug^{-1} at the end of storage. Reduction of the probiotics count may be related to intensive production of lactic acid. Kabeir et al. [33] reported that the viable populations of *Bifidobacterium* and *Lactobacillus* tend to decline in fermented soymilk held at 25°C, due to accumulation of acids and low tolerance of some probiotics to the acidic environment. The titratable acidity of the cheese at manufacturing time (zero time) was 1.04% and gradually increased with time of storage and reached 1.43% at 8 weeks of storage was confirmed by pH values ranged from 4.1 to 5.65 with significant deference (Table 4). This result obtained that processed spread cheese supplemented with probiotics bacteria is not fit for storage room temperature unless used after a short period (less than two weeks). As well as results of the general microbial analysis (Table 5) showed that the total aerobic count immediately after manufacturing was 2×10^3 cfug^{-1} and at 8 weeks of cold storage the number had dropped to 5×10^2 cfug^{-1}, the results showed that the product was free from yeasts and molds, coliform bacteria, *Staphylococcus aureus* and *salmonella*. The decline in the total count and the product free from pathogenic microbes immediately after the manufacturing might be due to efficient heat treatment and the good general health conditions during processing and packaging, while the decline in their numbers at the end of a period of storage may be due to the use of L-cysteine-HCL which captured oxygen and thus provided an anaerobic environment [34], or due to the production of antibacterial (antibiotic) like *Bacteriocins* produced by probiotics bacteria [35]. Same results by Krajewska–Kamińska et al. [30] obtained that the total bacteria count were decreased during storage of cheese supplemented with bacteria displaying probiotic properties.

3.4. Sensorial evaluation:

In terms of sensorial analysis two sensorial evaluations were conducted one by the panelists and the other by consumers. Tables 6 and 7 show the results of panelists sensory evaluation of processed spread cheese supplemented with probiotic and the non supplemented, where the supplemented cheese had the higher values of total scores (13.61) more than the non-supplemented cheese (13.30). These results showed no significant (P >0.05) differences between supplemented processed spread cheese and non-supplemented, which means that the addition of probiotics bacteria did not occur any adverse effects on the sensory characteristics of the product which is compatible and confirms the success of the consolidation processed, as well as the results suggest that the supplemented processed spread cheese with probiotic bacteria gained acceptance by the specialists. Those results coincided with the findings by Shah [36]. Karimi et al. [3] reported that many investigations indicate that adding probiotic bacteria to cheese in a suitable culture composition and formulation does not significantly or considerably change the flavor and/or other sensory characteristics of the final product compared to the control. While, the sensory evaluated by the consumers the data obtained in (Table 7) based on the hedonic scale (degree of

admiration), the results show that 40 consumers acceptance of the processed spread cheese with score ranged between like moderately to like very much, but for the non- supplemented processed spread cheese the acceptance of 39 consumers fell between like moderately to like very much. The results also showed no significant (P>0.05) differences between the two types of cheese, those results mean that the addition of probiotics bacteria *Lact. Acidophilus LA5. Bifid.Animalissubsplactis BB12* to processed spread cheese were accepted by consumers within score that ranged from slightly like to like moderately with mean score 6.61 compared with non-supplemented cheese mean score 6.75.

4. Conclusions

Results of the present study showed that processed spread cheese supplemented with probiotic bacteria can be manufactured , and the numbers of those bacteria remained higher than the recommended numbers which gives a positive impact on consumer's health when cheese is stored at refrigeration temperature until 8 weeks and up to 4 weeks when stored at room temperature, but the acceptably for the cheese storage at room temperature remained edible until 2 weeks and became unacceptable in terms of sensory qualities after two weeks due reduction in pH and water separation. Results of sensory evaluation by the consumers with the results of sensory evaluation coincided by panelists ,but there were no significant differences between supplemented processed spread cheese with probiotic and non-supplemented, and two types of cheese got the acceptance of the consumers and the panelists and emphasized the possibility of the production of functional processed cheese with a good sensory properties, supplemented by probiotics bacteria and without a change in the sensory qualities or a change in its chemical composition when stored refrigerated. The results of the study showed that processed spread cheese suitable and appropriate environment for the growth and survivability of probiotics bacteria *Lact acidophilus AL5, Bifid, animalissubsplactis BB12*. The following is a good way to transfer these bacteria to the consumer where its number remained higher than the recommended numbers to give a positive health effect for the consumer, after 8 weeks of cold storage and that it is possible to get processed cheese with functional health benefits.

5. References:

1. Ross, R., Fitzerald, G., Collins, K., Stanton, C. (2002).Cheese delivering biocultures probiotic cheese. *Aust Journal of Dairy Tech*, 57, 71-78.
2. Food and Agriculture Organization &World Health Organization (FAO/WHO) (2001). Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria,http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf.
3. Karimi R., Sohrabvandi, S., Mortazavian A. (2012). Review article: Sensory characteristics of probiotic cheese. *Compr Rev Food Sci*F, 11, 438-452.
4. Salminen,S.(2001). Humanstudies on probiotics: aspects of scientific documentation. *Scand J Nutr*, 45, 8-12.
5. Frece, J., Kos, B., Beganović, J., Vuković, S., Šušković, J. (2005). In vivo testing of functional properties of three selected probiotic strains. *World J Microb Biot*, 21, 8–9, 1401–1408.
6. Mortazavian, A., Sohrabvandi, S., Mousavi, S. Reinheimer, J.(2006). Combined effects of temperature-related variables on the viability of probiotic micro-organisms in yogurt. *Aust J Dairy Technol,* 61, 248–52.
7. Karimi, R., Mortazavian, A., Cruz, A. (2011). Viability of probiotic microorganisms in cheese during production and storage: a review. *Dairy Sci Technol*, 91, 283–308.
8. Kaizu, M., Sasaki, H.,Nakajama, H.,& Suzuki, Y. (1993). Effect of antioxidative lactic acid bacteria on rat fed a diet deficient in vitamin E. *J Dairy Sci*, 76, 2493-2499.
9. Mikelsaar, M., Mändar, R., Sepp, E., Annuk, H. (2004). Human Lactic Acid Microflora and Its Role in the Welfare of the Host.Salminen, S., Wright, A., Ouwehand, A. (Ed.) Lactic Acid Bacteria Microbiological and Functional Aspects (3[rd]ed). New York: Marcel Dekker, Inc
10. Saarela, M., Mogensen, G., Fonden, R., Matto, J. Mattila-Sandholm, T. (2000). Probiotic bacteria:

safety, functional and technological properties. *J Biotechnol*, 84: 197-215.

11. Allgeyer, L., Miller, M., Lee, S.(2010). Sensory and microbiological quality of yogurt drinks with prebiotics and probiotics, *J Dairy Sci*., 93, 4471–4479.

12. Aguilar, G., Dawson, H.,Restrepo, M., Andrews, K., Vinyard, B.. Joseph,F. (2008). Detection of *Bifido bacterium animali ssubs Lactis Bb12* in the intestine after feeding of sow and their piglets. *Appl. Environ. Microbiol*, 74, 20, 6338-6347.

13. Vinderola, C., Prosello, W., Ghinerto, D. Reinheimer, J. (2000). Viabilityof probiotic (*bifidobacterium, Lactobacillus acidophilus, Lactobacillus casei*) and nonprobiotic microflora in Argentinian Fresco Cheese. *J Dairy Sci*, 83, 1905-1911.

14. Stanton, C., Gardiner, G., Lyanch, P., Collins, J., Fitzgerald, G., Ross, R., (1998). Probiotic cheese. *Int Dairy J,* 8, 491-496.

15. Corbo, M., Albenz, O., DeAngelis, M., Sevi, A. Gobbetti, M. (2001). Microbiological and Biochemical properties of Canestrato Hard Cheese Supplemented with *Bifidobacterium. J Dairy Sci*, 84.551-561.

16. Michael, P., Pathy, K., Lai, T. (2006). Viability of Commercial probiotic culture (*Lactobacillus acidophilus, Bifidobacterium Spp*, *Lactobacillus Casei L-paracasei and L- rhamnosus*) in Cheddar Cheese, *Int. J Food Microbiol*, 108, 226-280.

17. Gardiner, G., Ross, R., Collins, J., Fitzgerald, G., Stanton,C. (1998). Development of a probiotic Chedder cheese containing human-derived *Lactobacillus paracasei strain. Appl. Environ Microbiol*, 6, 2192-2199.

18. Yilmaztekin , M . BH, O., Atasoy, F. (2004). Survival *of Lactobacillus acidohilus LA-5* and *Bifido bacterium bifidum BB-12* in white-brined cheese. *Int J Food Sci Nutr*, 55, 1, 53- 60.

19. Dinakar, P., Mistry, V. (1994). Growth and viability of *Bifido bacterium bifidum* in Cheddar Cheese. *J Dairy Sci,* 77, 2854-2864.

20. Nighswonger, B., Brashears, M. Gilliland, S. (1996). Viability of (*Lactobacillus acidophilus, Lactobacillus Casei)* in fermented milk products during refrigeration. *J Dairy Sci*, 79, 212-219.

21. Ostlie, H., Helland, M., Narvhus, J. (2003). Growth and metabolism of selected strain of probiotic bacteria in milk. *Int J Food Microbiol*, 87, 17-27.

22. Caric, M . (1993). Processed cheese product, In P. Fox, (Ed.), Cheese: chemistry, physics and microbiology .(pp 476-505),New York, An Aspen Publication.

23. Yousef, A., Carlstrom, C. (2003). Food Microbiology: A Laboratory Manual, John Wiley and Sons, Inc., Hoboken, NJ.

24. Tharmaraj, R, Shah, R. (2003). Selective enumeration *L-deibrueckii ssp bulgaricus Streptococcus thermophilus, L- acidophilus, Bifidobacterium , L-casei L-rhamnosus and propionibacteria. J Dairy Sci*, 86, 2288-2296.

25. Charteris, W., Kelly, P., Morelli, Collins, K. (1998). Ingredient selection for probiotic microorganisms in dairy food *.Int J Dairy Technol*, 44- 56.

26. AOAC., Association of Official Analytical Chemists, (2000). Official Methods of Analysis 18[th]Ed.Margland: AOAC international.

27. Afnor, (1993). Controle de la qualite des produits alimentaires. Lait et produits laitiers. Analyse physic − chimic. Partie 4 − Analyse sensorielle (norms francaises).

28. Larmond, E. (1982). Laboratory Methods for Sensory Evaluation of Food.Publication 1637. Food Research Institute. Research Branch Canada Department of Agriculture, Ottawa, Ontario.

29. JISM (1997). Milk and milk products − Processed cheese and spreadable processed cheese, Technical Regulation, Jordan.

30. Krajewska − Kamińska, E., Śmietana, Z. Bohdziewicz, K. (2007). Production and Characteristics of Twarog Cheeses containing strains of probiotic bacteria. *Pol J Food Nutr Sci*., 57, 4B, 325-328

31. Phillips, M., Kailasapathy, K., Tran, L. (2006). Viability of commercial probiotic cultures (*L. acidophilus, Bifidobacterium* sp.,*L. casei, L. paracasei* and *L. rhamnosus*) in cheddar cheese. *Int J Food Microbiol*, 108: 276–280.

32. Flimelová, E., Kňazovická, V., Čanigová, M., Benczová, E. (2013). Changes in quality of fresh cheese using dressing with and without probiotic culture during storage. *Acta Univ Agric Fac Agron,* 86, 1, 51-57.

33. Kabeir, B, Yazid, A., Hakim, M., Khahatan A.,Shaborin A., Mustafa, S. (2009). Survival of *Bifido bacterium pseudocatenulatum G4* during the storage of fermented peanut milk (PM) and skim milk (SM) products. *African Journal of Food Science*, 3, 6,150-155.

34. Gobbetti, M., Corsetti, A., Smacchi, E., Zocchetti, A., Angelis, M. (1998). Production of Crescenza cheese by incorporation of bifidobacteria. *J Dairy Sci,* 81, 37-47.

35. Shah, N. (1999). Probiotic bacteria: Antimicrobial and antimutagenic properties. *Probiotica,* 6, 1-3.

36. Effat, B., Mabrouk, A., Sadek , Z., Hussein, G., Magdoub, M., (2012). Production of novel functional white soft cheese, *JMBFS*, 1, 5, 1259-1278.

STUDY THE INFLUENCE OF WORK PARAMETERS ON THE LEACHEABILITY OF MINERAL ELEMENTS INTO THE COFFEE INFUSION

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ABSTRACT

The paper studies the influence of work parameters expressed as brewing time, temperature of water, particle sizes and contact time on the releasing of mineral elements (Ca, Mg, Zn, Cu, Fe and Pb) in coffee infusion. The higher extractability was obtained in the first 10 minutes for infusion made of coffee samples with particles in range of 125 μm – 500 mm and hot water for Ca (33.02 mg/100g) while in case of diffusion made of coffee beans (diameter > 2 mm) in contact with cold water no lead was noticed after 60 minutes.

Keywords: coffee infusion, mineral elements

1. Introduction

Coffee is one of the most popular drinks accross the world and it consumption followed an ascendent trend due the icentive taste induced by caffeine and pleasant aroma resulting from roasting. As previous papers reported each ground coffee has unique mineral composition depending by the variety, origin, soil characteristic, harvesting and processing conditions [1-5].

Beyond the caffeine, the daily coffee intake contributes with some amounts of mineral elements coming from coffee beans. The level of minerals element in coffee infusion is influenced by the work parameters. The aim of the present investigations was to analyze the influence of work parameters (particles size, brewing time, and water temperature) on the total concentrations of Ca, Mg, Cu, Zn, Fe and Pb released into infusions of Arabica coffee and to assess the differences between theirs leacheability.

2.Materials and methods

Arabica coffee was purchased from the local market in Baia Mare (Romania). Samples of randomly selected beans were separated and the defective (black, immature) and non-defective beans were manually separated order to perform the analysis.The initial characterization of coffee considered the next parameters: water content, water-soluble fraction, beans density, water-soluble fraction, pH, total acidity, total lipids and minerals.

2.1. Water content

In order to express the humidity of initial coffee beans the water content of each sample was determined according to gravimetric method. A coffee beans sample accuracy weighted was dried to constant mass at $105^{\circ}C$ and the water content was calculated according to Equation 1:

$$\text{Water content}(\%) = \frac{m_1 - m_2}{m_1} \cdot 100 \qquad (1)$$

where:

m_1 – initial weight of coffee sample, g
m_2 – constant weight of coffee sample, g

2.2 Beans density

Beans density was evaluated as the ratio between the weight of the 100 beans sample and the bean volumes (considered equally with the water displacement volume) [6].

2.3. Water-soluble fraction

The analysis of water-soluble fraction was carried out by immersing of 2 g of milled coffee (0.5 mm) in 200 ml boiled water and kept under mechanical stirring for 1 h. After this period the solution was filtered and replenished to 500 ml with distilled water. A 50 ml aliquot is evaporated until constant mass weight ($105 \pm 2^{\circ}C$).

The water-soluble fraction is calculated according to Equation 2:

$$\text{Water} - \text{soluble fraction, } \% = \frac{m_1 \cdot 10}{m_2} \cdot 100 \quad (2)$$

where:

m$_1$- the weight of dried matter, g
m$_2$ – the weight of coffee sample, g
10 – volumic correlation factor, adim.

2.4 pH of coffee

The pH of coffee was potentiometrically measured (Inolab pH 730) in the infusion resulted by treating of 3g of milled coffee (0.5 mm) in 50 ml of boiled water after 1 h of stationary at room temperature (20°C).

2.5. Total acidity of coffee beans

The acids from 10 g of milled coffee were extracted in 30 ml ethanol (80%) during 16 h under continuous stirring. An aliquot of 5 ml of filtered extract is titrated against NaOH 0.1N using phenolphthalein as indicator. The titratable acidity (expressed as Thörner grades ^{o}T) was calculated using the equation:

$$A(^oT) = 2V \quad (3)$$

where:

V – the equivalence volume of NaOH 0.1N used for titration, ml;

2.6. Total lipids content

The AOAC No 920.39B method [7] as published in the Velp Scientifica-SER 148 Operating Manual, was used to perform the analysis of total fat. A dried sample with a weight of 5g, accurately established, was sieved at 0.5 mm and was hydrolyzed in the presence of 5 mL ethanol 95% (v/v) and 25 mL HCl 8N in a water bath held at 70-80°C for 40 minutes to extract the lipids occluded or bonded to other components. The hydrolyzed sample was dried at 105°C until the constant weight and then was subject to fat extraction at 60°C for 90 minutes using petroleum ether [8]. The extract was dried at 105°C for 30 minutes, cooled and weighted. The result was calculated according to Equation 4 and expressed as total lipids g/100 g of dried sample.

$$\text{Lipids content, g/100 g of dried sample} = \frac{m_1}{m_2} \cdot 100 \quad (4)$$

where:

m$_1$ – the weight of total lipid, g
m$_2$ – the weight of coffee sample, g

2.7. Mineral analysis

The initial mineral composition of coffee beans was measured by weighting of 5.0 ($\pm$0.0001) g of dry coffee to quartz crucibles and ashed in an electric furnace at 540 °C with gradual increase in temperature. Next, the ash was dissolved in 15 ml of HNO$_3$ 65% (Suprapur® Merck) and replenished to 25 ml with distilled water.

After drying to constant weight and milling of coffee beans, three granulometric classes were selected: coffee beans (> 2 mm), 250 µm – 2 mm and 125 µm – 500 µm.

The coffee infusions were obtained by dissolving of 5 ($\pm$0.0001) g of coffee samples (coffee beans and milled coffee) in 100 ml of distillated water at 20, 50 and 100^{o}C respectively and stirred. During one hour, at each 10 minutes, the resulted infusion was separated from settled grounds by filtration through dense filter paper, acidulated with HNO$_3$ and submitted to mineral analysis.

In total, 60 samples (dry coffee and infusions) were analysed for macro- and microelements by atomic adsorbtion spectrometry (Perkin Elmer, AAnalyst 800). Three replicas of each sample were prepared for analysis mineral, so in total of 180 subsamples were processed against calibration standard solutions and respective procedural blanks.

2.8. Reproducibility of results

The results are presented as means± relative standard deviation (RSD%). All RSD values were statistically tested using STATISTICA 7.0 program and the Gaussian distribution of the experimental results subsequently obtained suggests those results are not significantly affected by the work errors.

3. Results and discussion

The investigated physical-chemical attributes of coffee samples are listed in Table 1 while the influence of work parameters over the solubilization efficiency of mineral elements are displayed in Figures 1-17.

Table 1. Physical-chemical attributes of coffee beans

Parameter	Average (x^- ±RSD %)
Water content, %	3.3948±0.6
Beans density, g/cm^3	0.7305±0.3
Water-soluble fraction, %	11.627±0.2
pH	5.83±0.3
Total acidity, ^{0}T	4.67±0.1
Total lipids content g/100 g of dry weight	4.6965±1.2
Mineral elements mg/100g of dry matter Ca	129.66±1.3
Mg	13.894±0.6
Cu	0.825±1.2
Zn	0.82±0.7
Fe	2.418±1.1
Pb	0.423±1.2

As can be seen the work parameters strongly influence the leaching of mineral elements from coffee beans. Thus, *the prolongation of contact time* between water and coffee sample resulted in increasing of concentration of released minerals. The infusion obtained after 60 minutes by using water at 100°C and samples with sizes in range of 250 µm – 2 mm has concentrations of Ca higher by 1.31 times, of Mg by 1.03 times, of Cu by 2.13 times, of Zn by 1.53 times, of Fe 1.37 times and Pb by 48.5 times.

Reducing the *particle sizes* results in the increasing of solubilization degree due to the increase of specific area of particles. By changing the samples granulometry from coffee beans (> 2 mm) to advanced milled coffee (125 µm – 500 µm) the minerals extractability increased in case of Ca by 2.49 times, of Mg by 2.08 times, of Cu by 4.3 times, of Zn by 1.12 times, of Fe by 3.53 times, even after a short brewing time (10 minutes) and

under the medium temperature of water (50°C). Low concentration of Pb was obtained in value of 0.143 mg/100g, explained due to the low solubility in cold water of its compounds.

The *temperature* of distilled water played also an important role in the mineral releasing in infusion. After 60 minutes of water-coffee samples contact, in case of the lowest granulometry investigated (125 µm – 500 µm), low concentrations of mineral can be noticed at 20^{0}C, namely: 30.362 mg/100 g of Ca, 6.015 mg/100g of Mg, 0.39 mg/100g Cu, 0.282 mg/100 g Zn, 0.481 mg/100g of Fe and 0.11 mg/100g of Pb. The concentrations of solubilized minerals resulted in diffusion after hot water (100°C) was used increased by 1.27 times in case of Ca, by 1.03 for Mg, by 1.5 times in case of Cu, by 1.76 times for Zn and 1.18 times for Fe. Moreover, the presence of lead in the diffusion was notice in a concentration of 0.396 mg/100 g.

As Figures 1-17 indicate the highest solubilization rate is obtaining after 10 minutes, the range time also being reported by the work of Stelmach et al., (2013) [9]. In case of Ca, in the first 10 minutes of brewing under hot water (100°C) the solubilization rate is 11.64 % in case of coffee beans, 24.74% in case of samples with particle sizes in range of 250 µm – 2 mm and 25.46% in case of particle sizes in range of 125 µm – 500 µm. After another 50 minutes, the solubilization rate became 17.55% in case of coffee beans, 32.45% in case of samples with particle sizes in range of 250 µm – 2 mm and 34.38% in case of particle sizes in range of 125 µm– 500 µm. Considering the lowest granulometry of coffee sample (125 µm – 500 µm), 100^{0}C the temperature of water and 10 minutes of brewing, the rank of minerals element released in the infusion is as the next row displays: Ca (33.02 mg/100g) > Mg (6.015 mg/100g) > Cu (0.586 mg/100g) > Fe (0.456 mg/100g) > Zn (0.369 mg/100g) > Pb (0.243 mg/100g) indicating that the metals with the higher extractability are Ca and Mg, as a consequence either of unbond forms, a less stronger bonds of these metals with the caffeine or other alkaloids presented in the coffee matrix or higher solubility forms of these metals.
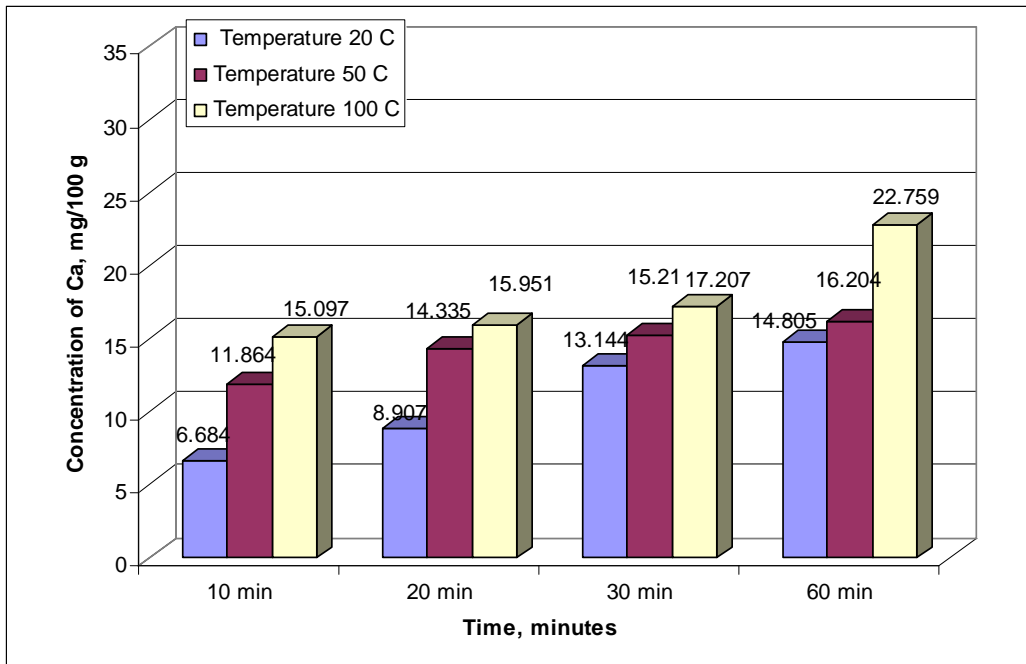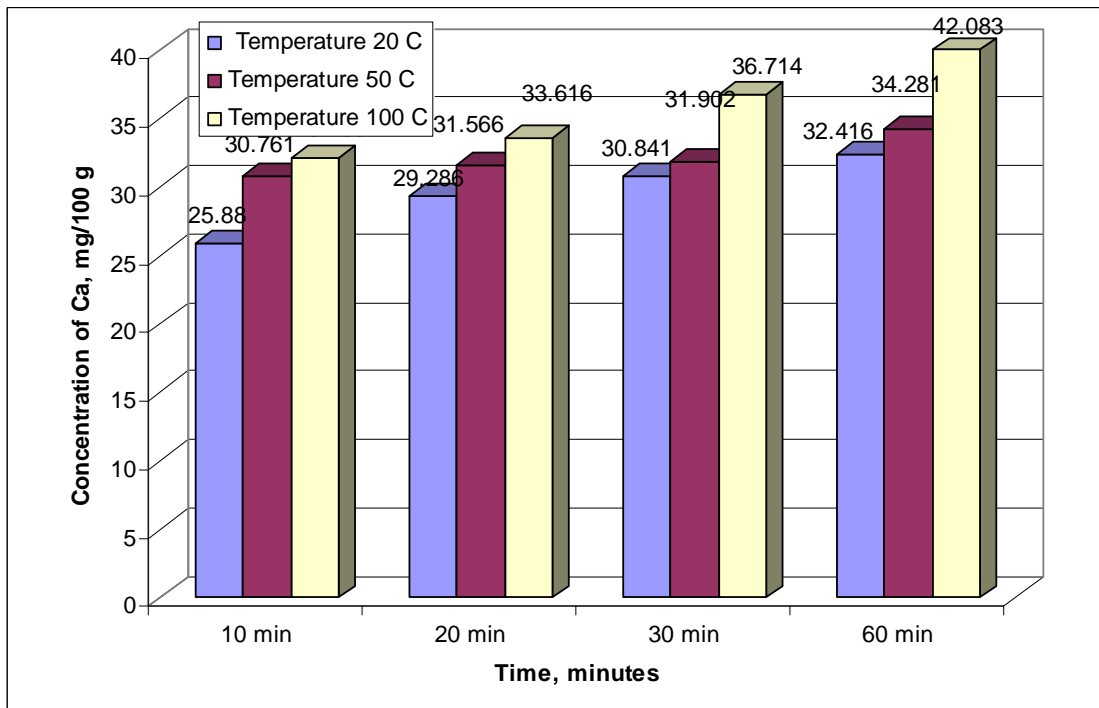
Figure 1. Releasing of Ca from coffee beans

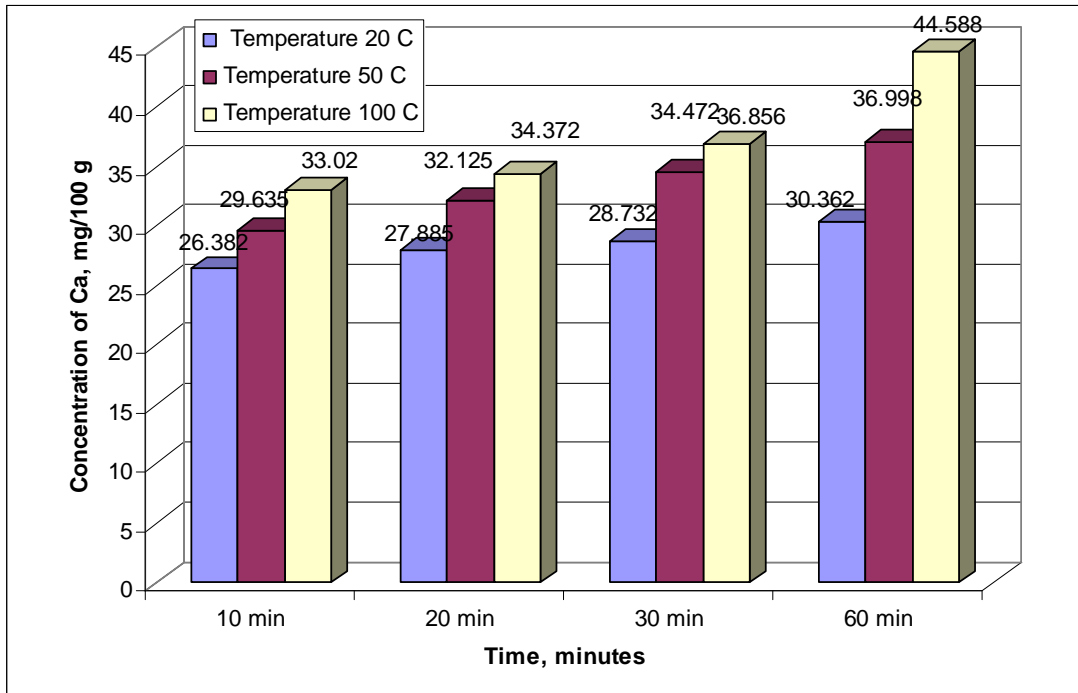**Figure 2.** Releasing of Ca from milled coffee with particle sizes in range of 250 μm – 2 mm

Figure 3. Releasing of Ca from milled coffee with particle sizes in range of 125 μm – 500 μm

Figure 4. Releasing of Mg from coffee beans

27

Figure 5. Releasing of Mg from milled coffee with particle sizes in range of 250 μm – 2 mm

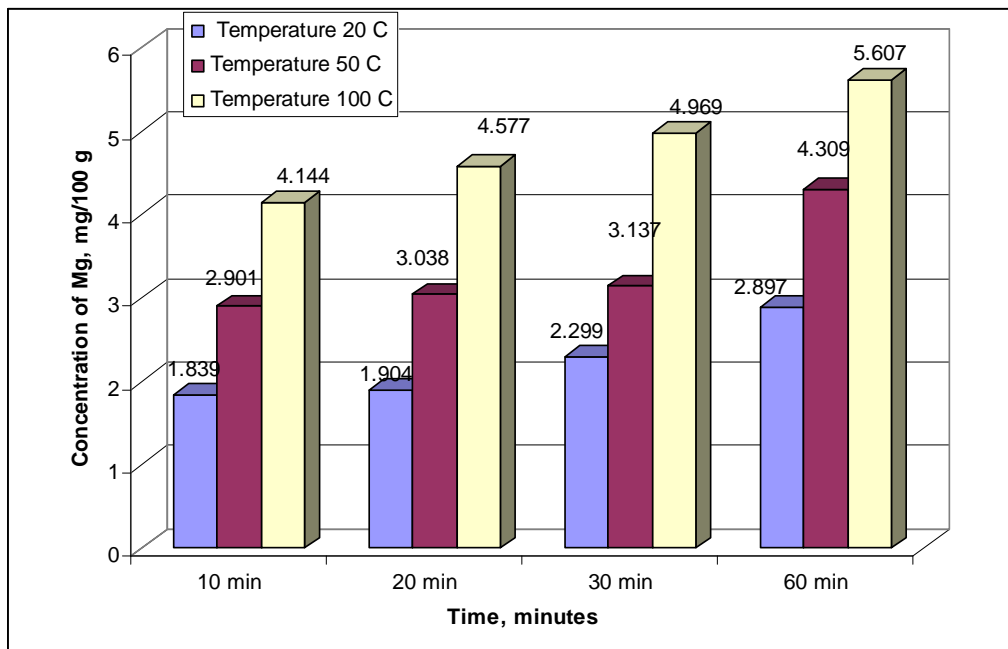**Figure 6.** Releasing of Mg from milled coffee with particle sizes in range of 125 μm – 500 μm

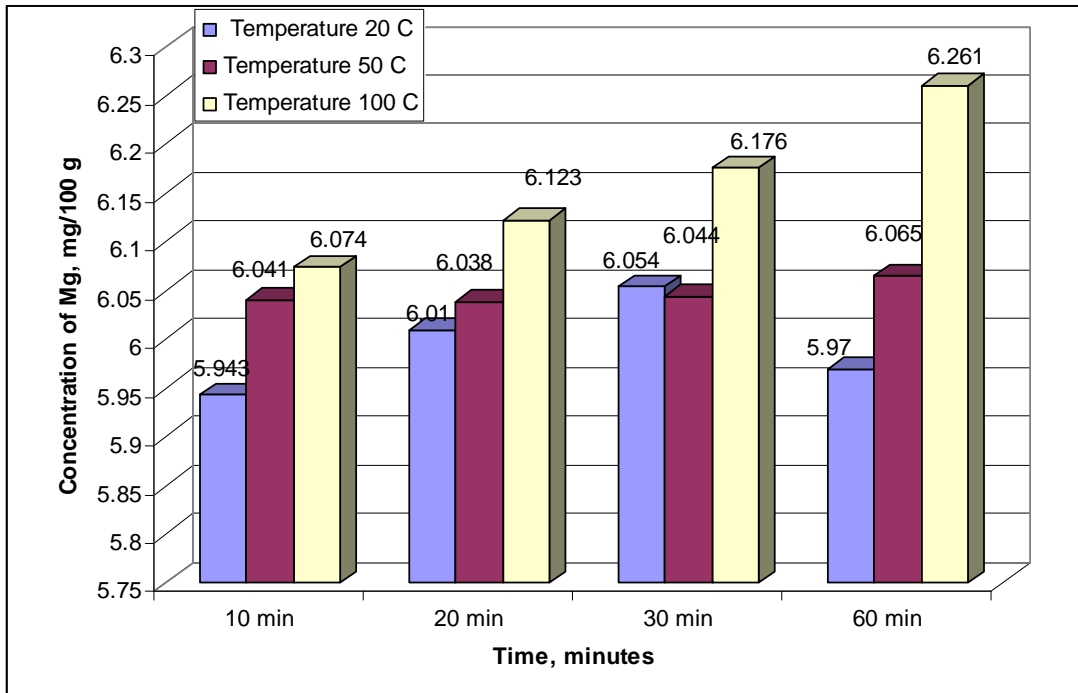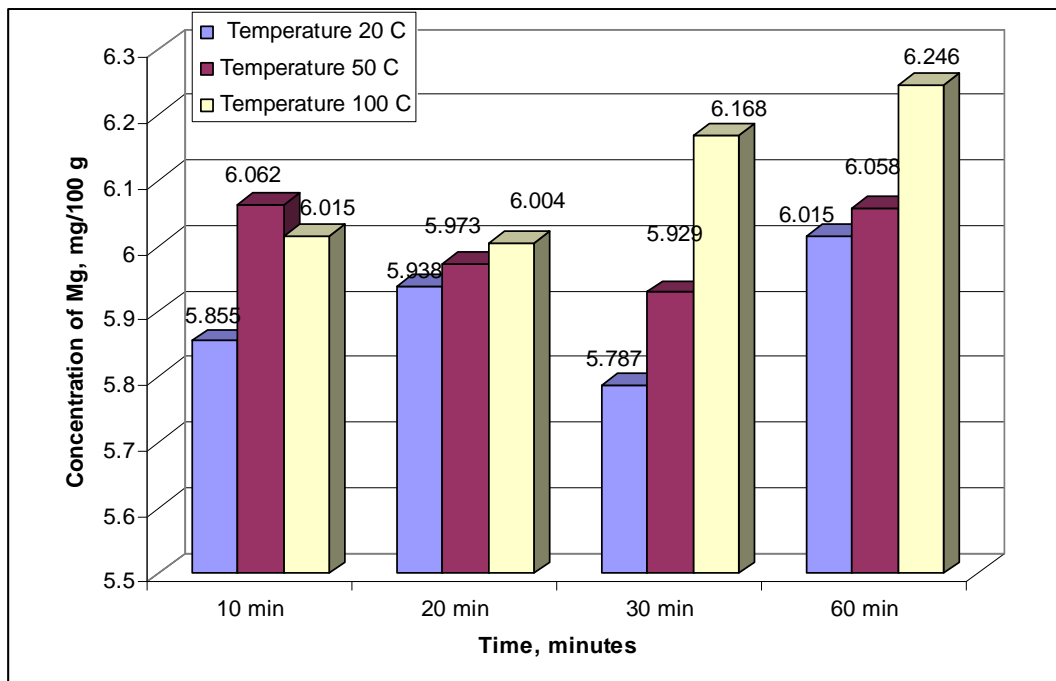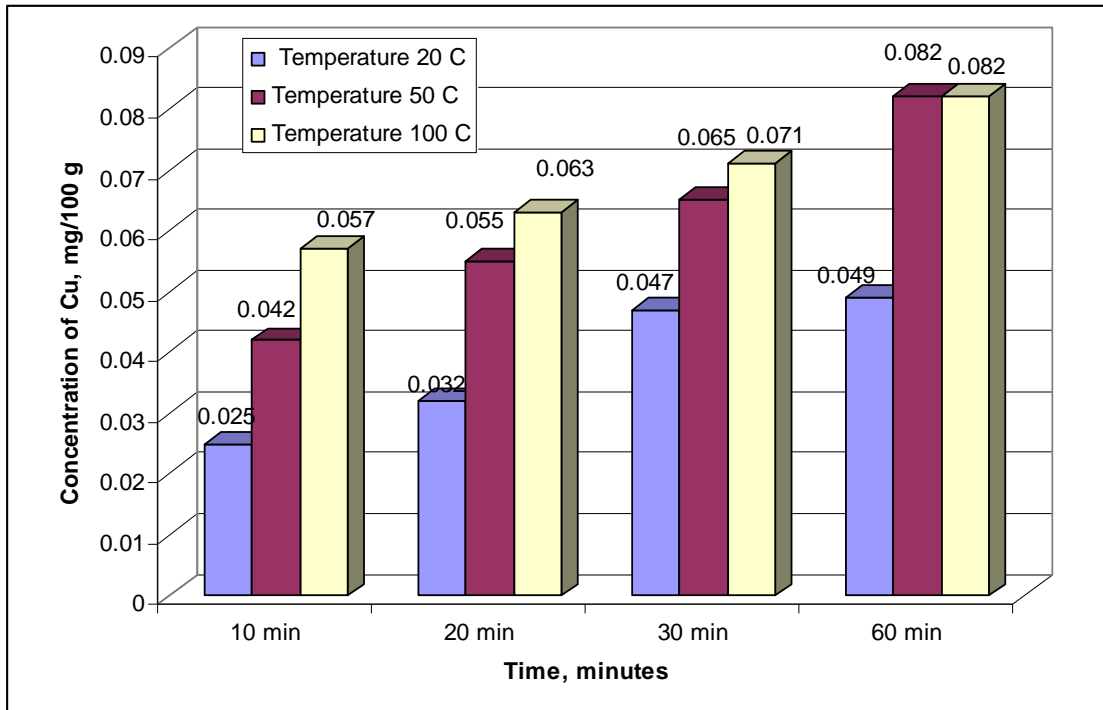**Figure 7.** Releasing of Cu from coffee beans

Figure 8. Releasing of Cu from milled coffee with particle sizes in range of 250 μm – 2 mm

Figure 9. Releasing of Cu from milled coffee with particle sizes in range of 125 μm – 500 μm

Figure 10. Releasing of Zn from coffee beans

Figure 11. Releasing of Cu from milled coffee with particle sizes in range of 250 μm – 2 mm
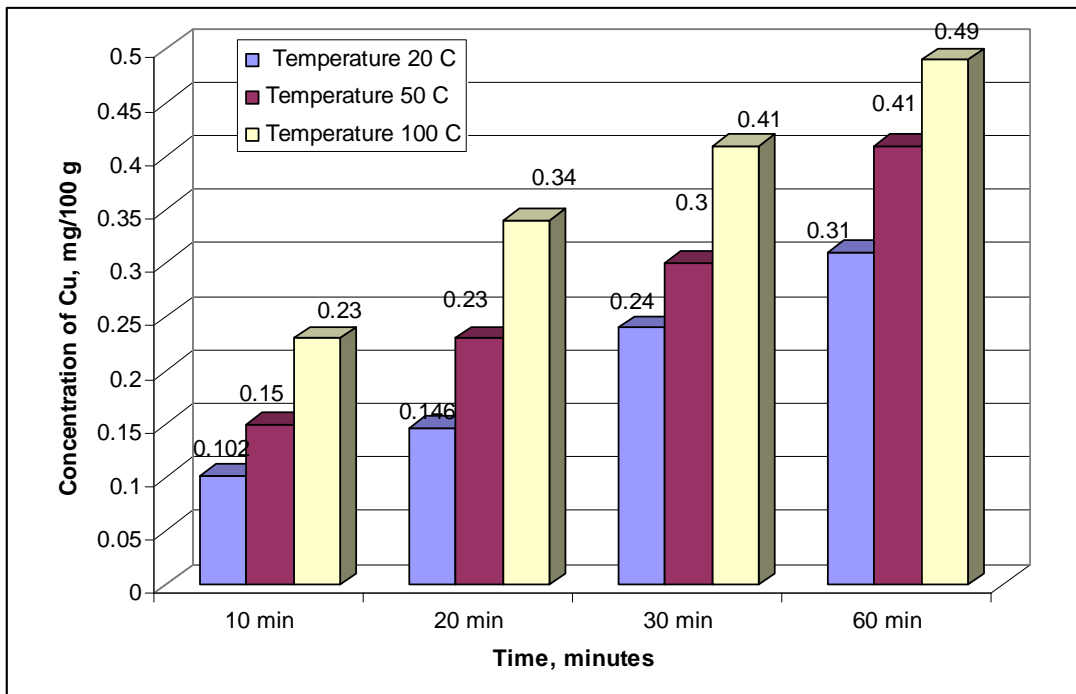

Figure 12. Releasing of Cu from milled coffee with particle sizes in range of 125 μm – 500 μm

Figure 13. Releasing of Fe from coffee beans

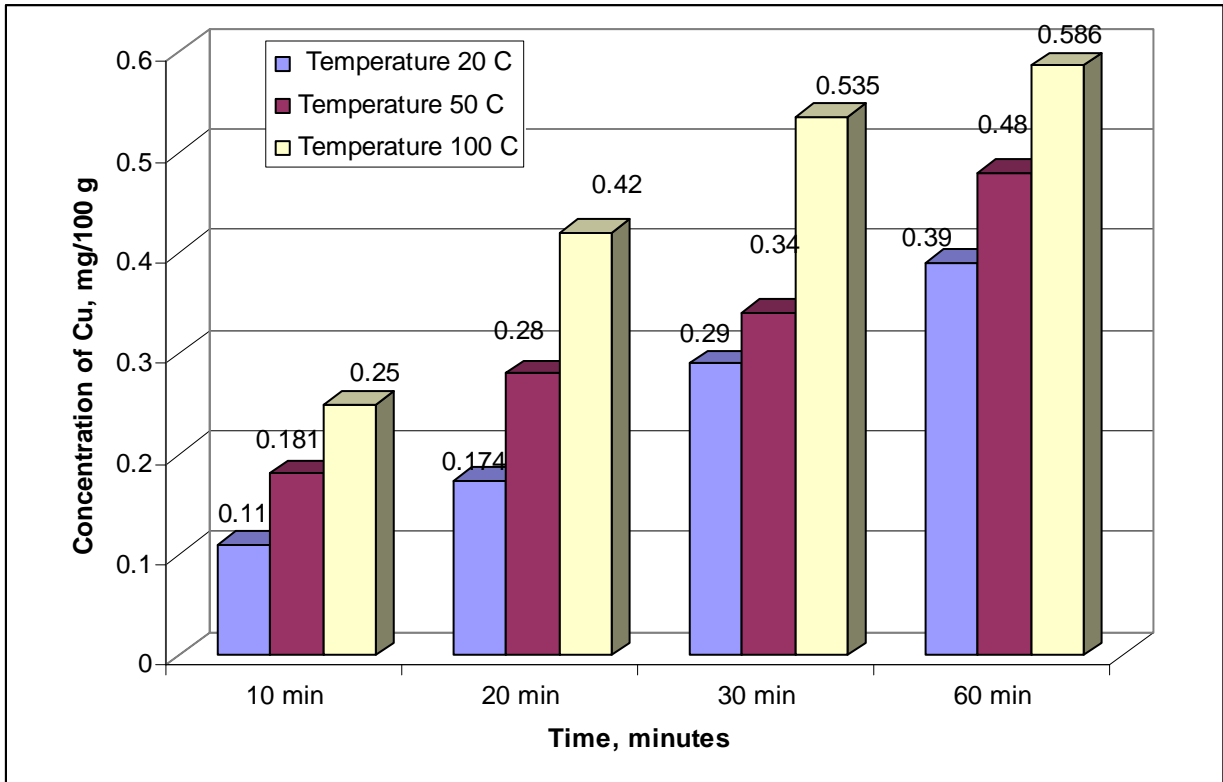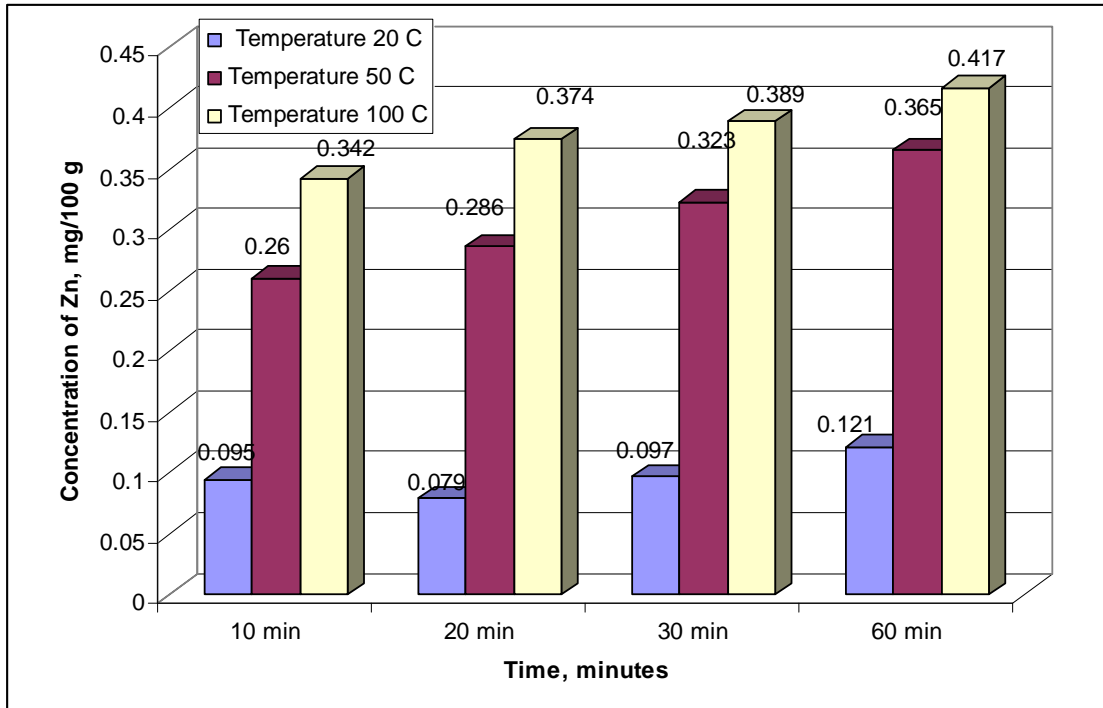**Figure 14.** Releasing of Fe from milled coffee with particle sizes in range of 250 μm – 2 mm

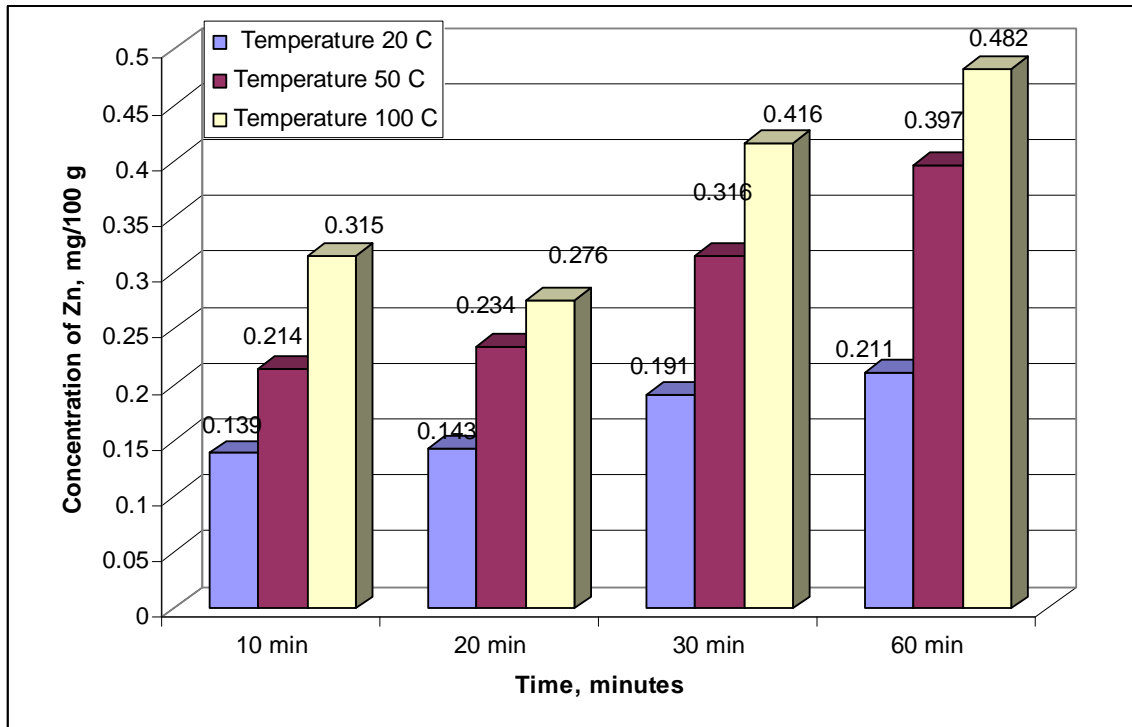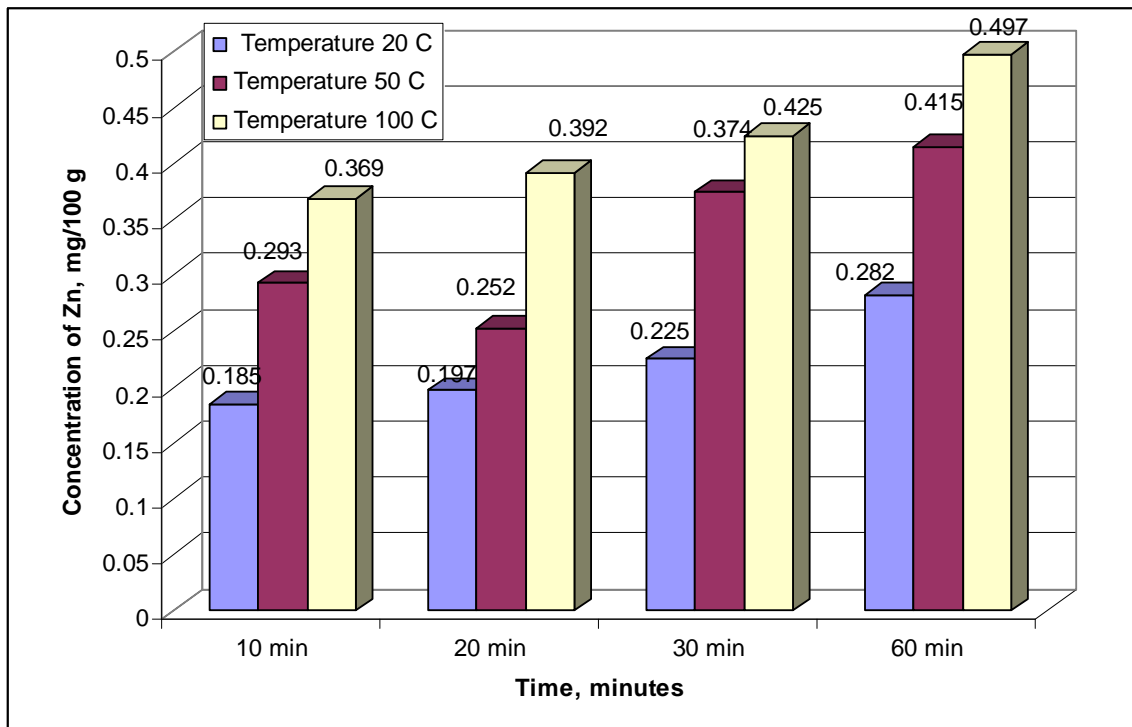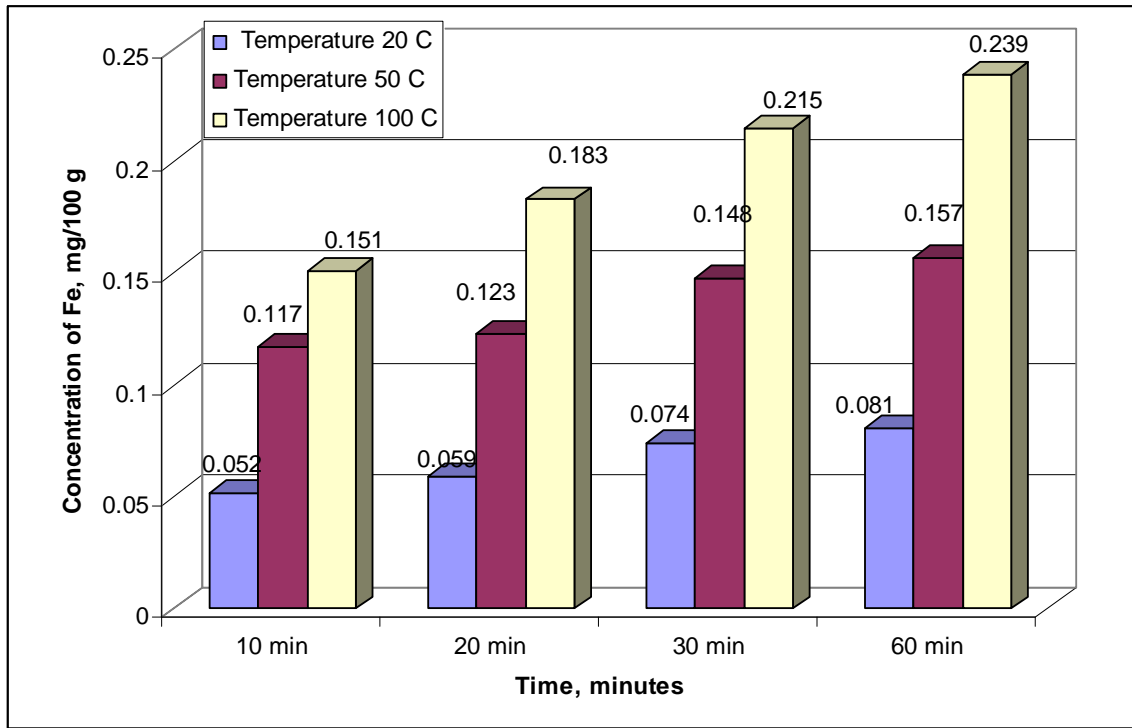**Figure 15.** Releasing of Fe from milled coffee with particle sizes in range of 125 μm – 500 mm
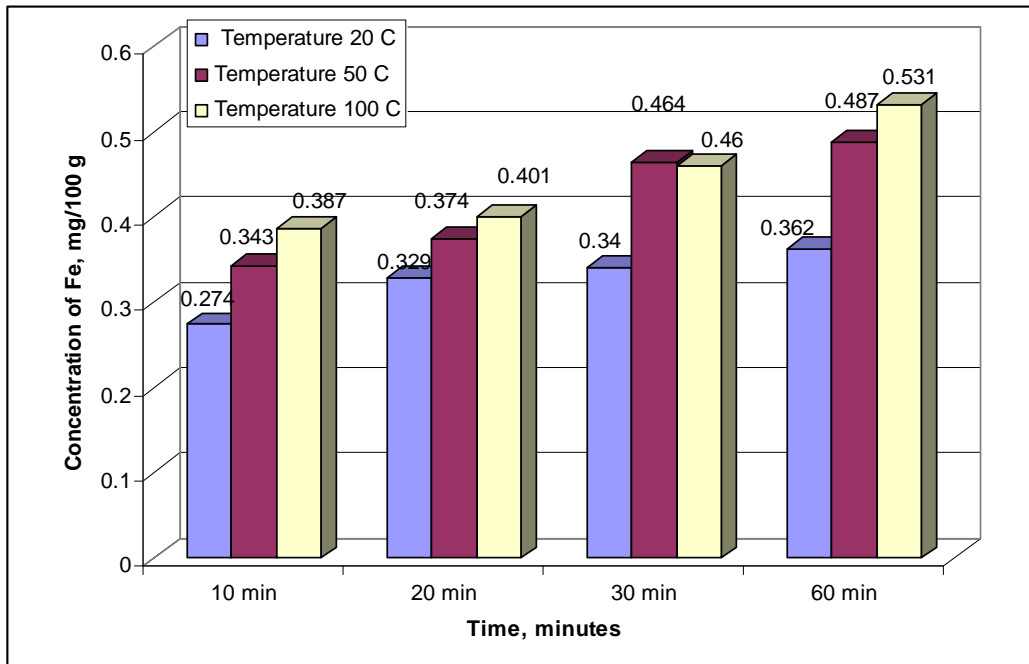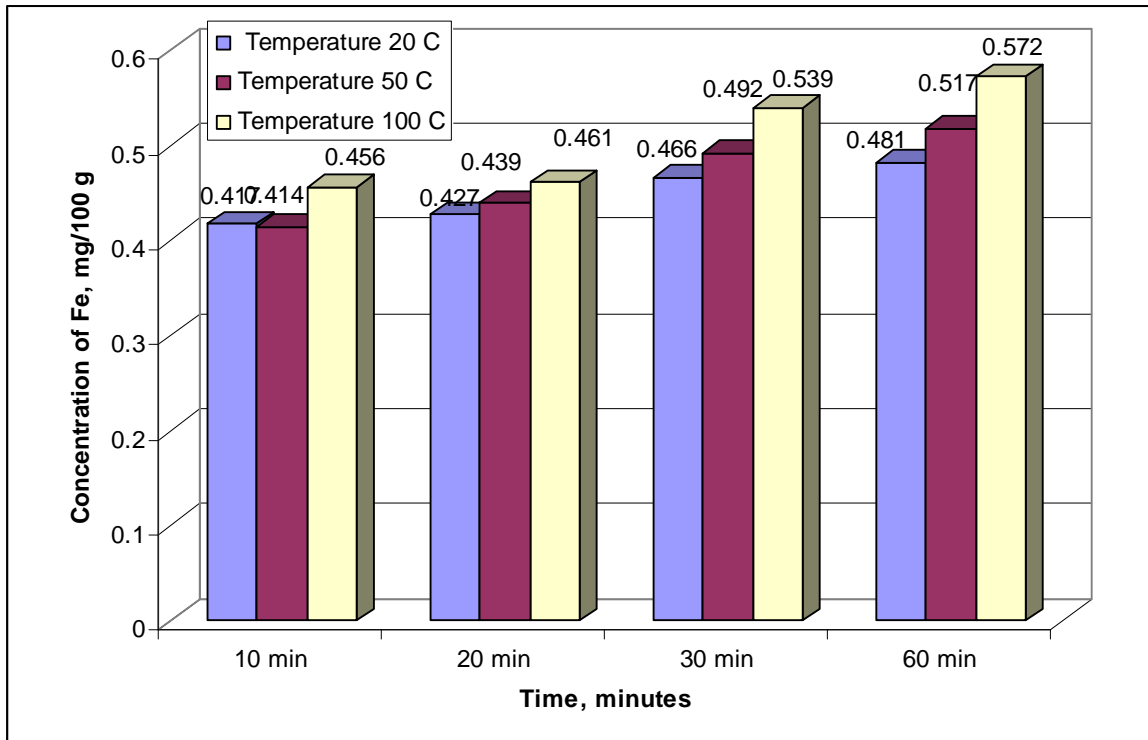

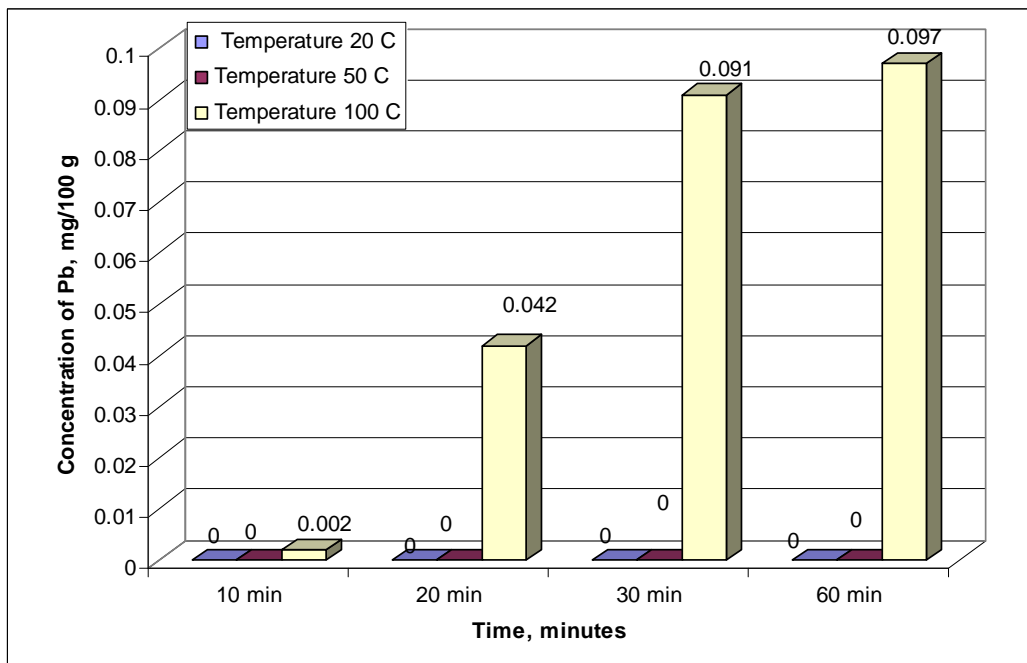**Figure 16.** Releasing of Pb from milled coffee with particle sizes in range of 250 μm – 2 mm
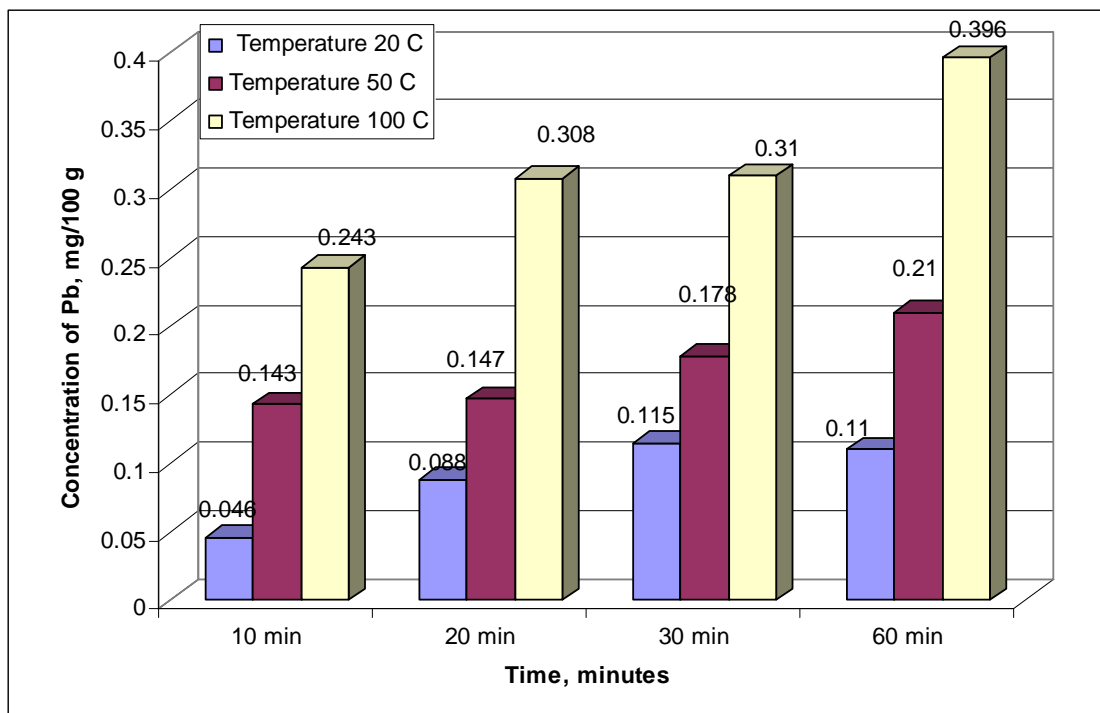
Figure 17. Releasing of Pb from milled coffee with particle sizes in range of 125 µm – 500 µm

In opposition, the lowest solubilization degree can be noticed for Zn and Pb, probably due to the formation of strong complexes of ions with the compounds of the coffee matrix as other authors reported [2, 10]. The first three positions of the rank are in a good agreement with the results of E. Stelmach et al. (2013) [9] even our values indicate lower mineral concentrations. Good agreement could be reported between our results and those published by [2, 11, 12, 13] which reported values in range of $(0.4–1.4) \times 10^3$ µg/g for Ca, $(5–108) \times 10^3$ µg/g for Fe, $(3.1–9.5) \times 10^3$ µg/g for Mg and $(4.1–2.9) \times 10^3$ µg/g for Zn.

4.Conclusions

In this work was established that the work conditions influence the releasing efficiency of mineral elements (Ca, Mg, Zn, Cu, Fe and Pb) in resulting infusion. Generally, the increasing in water temperature, brewing time and the reducing the coffee particle sizes resulted in the increasing of the minerals concentrations especially Ca, Mg and Pb. With one exception (Pb), the highest releasing rate of mineral elements was noticed after 10 minutes of brewing. In case of Pb, no extraction was observed in case of coffee beans and cold of water, the reducing of particles sizes, increasing the water temperature and brewing time having favourable effect over the extraction.

5. References

1. Anderson, K. A., & Smith, B. W. (2002). Chemical profiling to differentiate geographical growing origins of coffee. *J. Agr. Food Chem.*, 50, 2068–2075.
2. Ashu, R., Chandravanshi, B. S. (2011). Concentration levels of metals in commercially available Ethiopian roasted coffee powders and their infusions. *Bulletin of the Chemical Society of Ethiopia*, 25, 11–24.
3. dos Santos, J. S., dos Santos, M. L. P., & Conti, M. M. (2010). Comparative study

of metal contents in Brazilian coffees cultivated by conventional and organic agriculture applying principal component analysis. *Journal of the Brazilian Chemical Society*, 21, 1468–1476.

4. dos Santos, J. S., dos Santos, M. L. P., Conti, M. M., dos Santos, S. N., & de Oliveira, E. (2009). Evaluation of some metals in Brazilian coffees cultivated during the process of conversion from conventional to organic agriculture. *Food Chem*, 115, 1405–1410.

5. Filho, V. R. M., Polito, W. L., & Neto, J. A. G. (2007). Comparative studies of the sample decomposition of green and roasted coffee for determination of nutrients and data exploratory analysis. *Journal of the Brazilian Chemistry Society*, 18, 47–53.

6. Franca, A.S., Oliveira, L.S., Mendonça, J.C.F., Silva, X.A., 2005. Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chem*,90 (1–2), 89–94

7. AOAC Official methods of analysis, methods 920.39.B and 945.18-B.

8. Velp Scientifica – UDK 127 Manual, Method 945.18-B; SER 148 Operating Manual.

9. Stelmach, E., Pohl, P., Szymczycha-Madeja, A. (2013). The suitability of the simplified method of the analysis of coffee infusions on the content of Ca, Cu, Fe, Mg, Mn and Zn and the study of the effect of preparation conditions on the leachability of elements into the coffee brew, *Food Chem*, 141, 1956–1961.

10. Santos, E. E., Lauria, D. C., & Port da Silveira, C. L. (2004). Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. *Sci Total Environ,* 327, 69–79.

11. Oliveira, M., Casal, S., Morais, S., Alves, C., Dias, F., Ramos, S., et al. (2012). Intra- and interspecific mineral composition variability of commercial coffees and coffee substitutes. Contribution to mineral intake. *Food Chem*, 130, 702–709.

12. Santos, E. E., Lauria, D. C., & Port da Silveira, C. L. (2004). Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. *Sci Total Environ*, 327, 69–79.

13. Suseela, B., Bhalke, S., Vinod Kumar, A., Tripathi, R. M., & Sastry, V. N. (2001). Daily intake of trace metals through coffee consumption in India. *Food Addit Contam A*, 18, 115–120.

NITRATE AND NITRITE CONTENT IN VARIOUS TYPES OF CUCUMBERS AND OPTIONS TO REDUCE IT

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ABSTRACT

The goals of the experiments detailed below were, on one hand, to determine the nitrate and nitrite content in supermarket (greenhouse) cucumbers as compared to the cucumbers collected from farms located in two villages, Coltau and Sisesti, in Maramures County, and on the other hand to assess to what extent such content could be reduced, in order to minimise the negative impact on human health.

Keywords: *nitrate content in vegetables, nitrite content in vegetables, cucumbers*

1. Introduction

Consumers could often be exposed to the negative effects of various compounds inherently found in vegetables and fruits. In many cases, the sources of danger, as well as the ways to mitigate, if not to eliminate, those compounds' toxic effects, are already known.

Nitrates and nitrites have a dual origin in food. They are naturally present in plants, and especially vegetables, which are able to accumulate such compounds from the soil. But nitrates and nitrites are also food additives, added to maintain the pink colour of meat and related preparations, and for preservation purposes (cooked meat, cheese). Micro-organisms determine, by reductase, the reduction of nitrate to nitrite, a more toxic compound. The formation of methemo-globinemy [1] is the direct outcome, while the formation of nitrosamines, known for their carcinogenic and mutagenic action, is the indirect effect of such toxicity [2-4].

The European Food Safety Authority recommends that the intake of nitrates found in vegetables should be reduced [5-7]. However, the content of other nutritional components essential to the human body (vitamin C, minerals) is reduced as the content of nitrate decreases.

Some of the ways to reduce the content of nitrates in food include:
- Cooking in water, without consuming the liquid [8, 9];
- Peeling fruits and vegetables; potatoes contain nitrates in the shell and adjacent areas [10]; by peeling them the content of nitrates could decrease with up to 30%.
- Preheating potatoes before cutting them, this meaning 20% less nitrates [10].
- Reheating culinary processed vegetables does not increase the nitrate content.
- The consumption of vegetables grown in open spaces [11, 12], and not in greenhouses (without direct access to sunlight).

Nitrates are usually present in vegetables as a consequence of soil fertilization with nitrogen-based fertilizers [13, 14]. Some pesticides used in agriculture can also increase the nitrogen content in plants.

Bacteria such as *Nitrobacter* determine, in the presence of oxygen, the transformation of nitrites in nitrogen. While less toxic for the aquatic creatures, nitrates are indispensable nutrients for plants.

Nitrates and nitrites are natural components of the organic matter of soil. The mineralization of nitrogen occurs especially because of the microorganisms present in soil. In countries with temperate climate, this process is most intensive during summer.

The transformation of nitrates and nitrites takes mainly place in roots and leaves. The amount of nitrogen present in the plant at any time is the difference between the quantity of nitrogen absorbed and the quantity used in the genesis of proteins. Any factors capable of interfering with the absorption process, or at any point of the metabolic chain transforming the amine nitrogen and nitrate nitrogen in nitrogen proteins, could affect the amount of free nitrate in the plant.

The accumulation of nitrogen can be amplified by such other factors as the existence in plants of small amounts of nitrate reductase (as occurs in families *Chenopodiaceae* vegetables - spinach, beets, radishes, etc. - and *Umbelliferae* - carrot, dill, parsley, etc.)[15, 16],poor sunlight (for example, greenhouse plants, as there is not enough energy for reactions) [17, 18], deficiency in trace elements participating in the reduction of enzyme activity (for example, insufficient molybdenum or other growth-stimulating minerals).

When nitrogen fertilizers are added, some species, such as lettuce, onions, carrots, cabbage [19], cauliflower, beets and cucumber grow faster, but accumulate a higher quantity of nitrates and nitrites as well. Generally the land used to grow vegetables is more fertile, because of the repeated application of organic fertilizers.

Nitrates themselves are not very toxic; they do not accumulate as they enter in the human body (simultaneously with the intake of water, of vegetables or with processed meat), the micro-organisms in the intestinal flora converting them into nitrites. A person with a normal diet could tolerate about 100 mg nitrate and 3 mg nitrite daily. After ingesting nitrates in large amounts, the body reduces then to nitrites, which are 100 times more toxic than nitrates. Nitrates are converted into nitrites in the human intestine, under the influence of the intestinal micro-flora.

The higher the quantity of nitrates applied on soil, the higher becomes the content of nitrates in the crops grown on that soil. The mineral and the organic fertilizers are both sources of pollution. The amount of nitrate accumulated in plants depends not only on the quantity of nitrogen fertilizer used, but also on the time at which the vegetables are reaped (according to the ability to accumulate the nitrate and nitrite morning hours for some species, evening hours for others, at overripe stage for others, etc.), on the seeding time and on the lighting conditions [20]. The content of nitrate is, for instance, higher in shaded areas or where the density of crops is higher.

Some of the recommendations in order to reduce the amount of nitrate include:
- To supplement the nutrition of vegetables with nitrate-free fertilizers, or with amino-nitrogenous fertilizers.
- To refrain from adding nitrogen fertilizers during the last 1-1.5 months before harvest. The longer the time elapsed between the most recent application of fertilizers and the harvest time, the smaller will be the quantity of nitrates still present in the plant.
- To water the plants regularly, in order to reduce the accumulation of nitrates generated by the repeated application of fertilizers.
- To harvest vegetables at the right time, when the nitrates content is small (as they are converted into proteins and other components during growth) up to maturity, but the quantity of nitrates becomes much higher in overripe fruits.
- To prefer open air – gardening over greenhouses, as the diminished intensity of

light inside a greenhouse is a stimulus for nitrites accumulation.

- To boil vegetables and fruits, as approximately 50% of the nitrate content is thus removed.
- To peel fruits and vegetables, as by doing so the nitrate content is reduced by 15-20%.
- To soak the potatoes, the carrots, the beets and the cabbage in water for one hour, as the nitrate content thus decreases by 25-30%.
- To boilthe potatoes for 30-40 minutes, as thus up to 80% of the content of nitrate is removed (as well as 70% for carrot and cabbage, and40% for beet) [21].
- To prepare pickled or marinated vegetables, in which the nitrate content is reduced to 50%; during the first days of pickling, when the synthesis process of nitrate and nitrite is very intensive, the consumption of such vegetables is not recommended [22].

2. Materials and methods

Cucumbers from three sources, namely the type of long cucumber purchased from a supermarket, and respectively garden cucumbers from two farms in Maramures County (Sisesti and Coltau), two of the local communities known for "clean" agriculture, based on open-fields gardening, supported with organic fertilizers, and locally-produced seeds, allowing plants to evolve at their natural growth rate) were analysed in terms of nitrate and nitrite content.

On cucumbers from each source, three determinations for nitrate and nitrite content were made, namely for each peel (Figure 1a), for each pulp (Figure 1b), and for each cucumber as a whole (Figure1c).

a   **b**   **c**
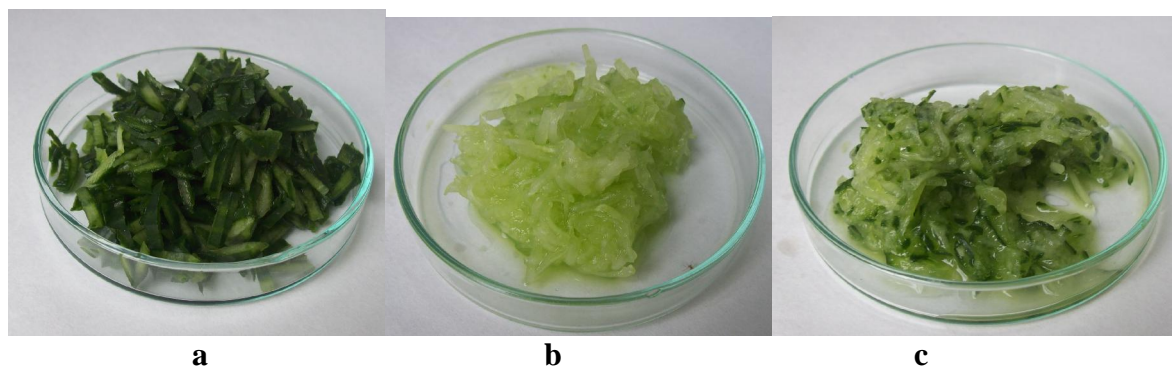
Figure 1. Shredded samples: a – cucumber peel, b – cucumber pulp, c –whole cucumber

In order to determine the content of nitrate and of nitrite, samples were prepared as described following.

For each sample, 10g of plant tissue were placed into a 200 ml beaker. Next, a 5 mL disodium-tetraborate solution (50g $Na_2B_4O_7 \cdot 10H_2O/1L$) and 100 mL hot water(~80°C) were added in the beaker. The beaker with this solution was placed into a boiling water bath for 15 minutes. The beaker was removed from the water bath, and 2 mL potassium hexa-cyanoferrateII (106g $K_4Fe(CN)_6 \cdot 3H_2O/1$ L) were added and the solution was shaken. Then, 2 mL zinc acetate (220g $Zn(CH_3COO)_2 \cdot 2H_2O$ + 30 mL glacial acetic acid/1 L) were added to the solution, which was shaken again. This solution was allowed to cool down to room temperature, and it was transferred to a 200 mL volumetric flask. The beaker was rinsed with deionized water and the rinse water was poured into the flask. The flask was filled up to the200 mL mark with deionized water. This solution was then filtered through a cellulose nitrate filter, to obtain a clear liquid, named the filtrate sample. Three of such filtrates were produced, and each of the three filtrate samples was separated into a replication for nitrate analysis and a replication for nitrite analysis.

a) *The determination of nitrite* was done by measuring the intensity of the pink color of the compound formed by nitric diazotization of sulphanilic acid with the nitrite aqueous extract of the sample, together with alpha-naphthyl-amine. The content of nitrite was calculated using a calibration curve.

(1)

(2)
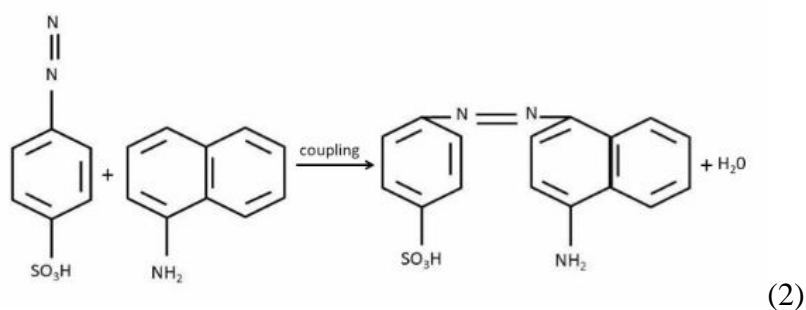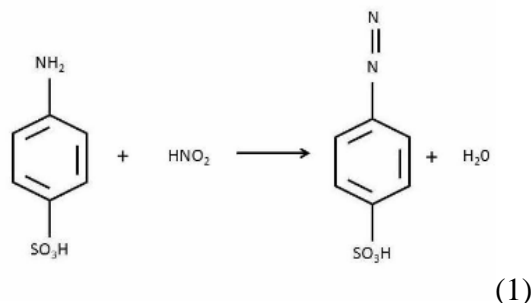
The three replications for nitrite (NO_2^-) concentration wereanalysed as follows. First, 10 mL of the extract filtrate sample were pipetted into a 50 mL volumetric flask. Next, 20 mL of deionized water were added. Then, 5 mL of solution I (6 g de sulphanilic acid - $NH_2C_6H_4SO_3H \cdot 2H_2O$ + 200 mL glacial acetic acid +400 mL distilled water + 200 mL sodium chloride solution 10%, filled to the 1000 mL mark with deionized water)and 5mLof solution II(0,3 g α-naphthyl-amine hydrochloride - $C_{10}H_7NH_2 \square HCl$ + 100 mL distilled water + 200 mL glacial acetic acid, filled to the 1L mark with deionized water) were added. The solution was allowed to stand still at room temperature for 20 minutes while being protected from light. The absorbance of the solution was measured within15 minutes using a spectrophotometer Perkin Elmer UV/VIS Spectrometer Lambda 35 at a wavelength of520 nm. The spectrophotometer monitor reading was recorded and used to determine the mass of nitrite from the calibration graph. Then, the nitrite ion (NO_2^-) concentrations were calculated from the following equation:

Nitrite (mg/kg) = $(m_1 \times 200) / (V_1 \times m_0)$ (1)

where:

m_0 = initial mass of the plant tissue for the test portion (g);

m_1 = mass of nitrite ion (NO_2^-) read from calibration graph (g);

V_1=volume of filtrate sample taken (mL).

A blank test was done to zero the spectrophotometer before analysing each set of three replications. For the blank test, the procedure described above was followed, in order to determine the nitrite content (NO_2^-), but the 10 mL of filtrate sample were replaced with 10 mL of deionized water.

A calibration graph was established for the spectrophotometer for nitrite readings before starting the effective research work. To do this, we first weighed 0.1 g of sodium nitrite($NaNO_2$), which was then dissolved into 1 L of deionized water. Next, 10 mL of this solution were pipetted into another1 L volumetric flask and filled to the 1L mark with deionized water, which gave a standard

sodium nitrite solution (0.001 mg NO_2^-/mL or 1 mg/L or 1 ppm).

Then several, pre-established concentrations were prepared ($2x10^{-4}$,$1x10^{-3}$,$2x10^{-3}$,$1x10^{-2}$,$2x10^{-2}$,1×10^{-1},2×10^{-1}, 4×10^{-1}, 8×10^{-1}, 1.0 ppm) of the sodium nitrite solution, by diluting the standard solution (1 ppm) with deionized water. The procedure described earlier for nitrite analysis was applied to get readings for each concentration from the spectrophotometer. From these results, was plotted a graph with the mass (lg) of nitrite in the calibration solutions as the graph abscissae, and the corresponding values of absorbance, measured by the spectrophotometer, as the graph ordinate.

b) *The determination of nitrate* was done by measuring the intensity of the orange color of the compound formed by the nitrate aqueous extract of the sample mixed with brucine reagent. The content of nitrate was calculated using a calibration graph.

The three replications for nitrate (NO_3^-) concentration were analysed as follows. First, 1 mL of the extract filtrate sample was mixed with 0.1 mL urea (saturated aqueous solution) and 1 ml of a sulphuric acid-phosphoric acid mixture (equal volumes of concentrated sulphuric acid, d = 1.84 g/mL, and ortho-phosphoric acid, 85%) in a test tube. The solution was allowed to stand still at room temperature for 5 minutes and then cooled to 10°C.

In parallel with the above procedure, two other reference samples were prepared. First, this entailed the preparation of a reagent reference sample, in which distilled water is introduced instead of the 1 mL of the extract filtrate sample (as in the paragraph above). Second, this referred to prepare an extract filtrate reference sample, in which 1 mL of the extract filtrate sample was introduced.

While the tubes were cooled, 1 mL of brucine reagent was added in each tube (10 g brucine + 80 mL ethylic alcohol 95% + 3 mL distilled water filled to the 100 mL mark

with ethylic alcohol 95%),except the extract filtrate referenc esample, in which 1 mL ethanol 95% were addedand9 ml mixture of H_2SO_4 and H_3PO_4were added in each tube.

All tubes were placed at the same time in a boiling water bath and were kept for 2 minutes. Then, the tubes were placed immediately in a cold water bath (10°C) in which were maintained for 3-5 minutes.

Extinctions were read from a Perkin Elmer UV/VIS Spectrometer Lambda 35 at a wavelength of 420 nm against distilled water as a reference solution.

For the standard solutions, the readings on the spectrophotometer were corrected with the readings for the reagent reference sample.

For the sample solutions, the readings on the spectrophotometer were corrected with both the readings for the reagent reference sample and the filtrate reference sample.

The spectrophotometer monitor reading was recorded and used to determine the mass of nitrite from the calibration curve.

Stock solution was obtained using sodium nitrate (1 g) filled to the 1 L mark with deionized water. Standard solutions were obtained from 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 mL stock solution filled to the 100 mL mark with deionized water. Standard solutions were treated as above.

3. Experimental

Greenhouse cucumbers (namely purchased from a supermarket) were analysed, in order to establish what changes occur during storage in terms of nitrates and nitrites content. Such cucumbers were stored for two weeks starting the date of purchase in a refrigerator (4-6 C), and then subjected to the analysis of nitrates and nitrites.

The capacity of lemon juice to reduce the nitrate and nitrite content of grated cucumber pulp was also investigated. A proportion of 60 mL juice (one lemon) at 300 g cucumber was used in this respect.

After 20 and respectively 40 minutes of cucumber exposure to lemon juice, the mix of cucumber and lemon juice was filtered and the nitrite and nitrate content in pulp was analysed.

4. Results and discussion

Figures 1 and 2 include the concluding details about the content of nitrate and of nitrite, expressed in mg/kg of fresh sample forthe three types of cucumbers analysed.

Figures 3 and 4 reveal the results about the nitrate and nitrite content in the supermarket cucumber, stored for two weeks starting the date of purchase in a refrigerator, as compared with the initial values.

Figure1. Nitrate content in supermarket cucumbersas compared to cucumbers from Sisesti and Coltau

Figure 2. Nitrite content in supermarket cucumbers as compared to cucumbers from Sisesti and Coltau

Figure 3. Nitrate content in supermarket cucumbers after 2 weeks of refrigeration

Figure 4. Nitrite content in supermarket cucumbers after 2 weeks of refrigeration

The decrease of nitrate and nitrite in the supermarket cucumber after treatment with lemon juice was expressed as percent change over the initial values. The changes, after 20 minutes of exposure, and respectively after 40 minutes of exposure in lemon juice, are those presented in Figure 5.

Content decrease (%) in the pulp of supermarket
cucumbers treated with lemon juice

□ after 40 min with juice ■ after 20 min with juice
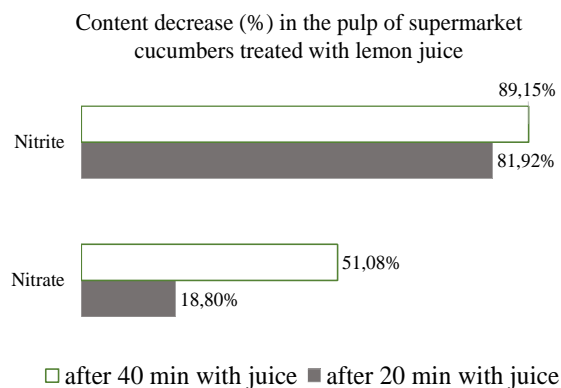
Figure 5. Decrease (% over initial content) of the nitrate and nitrite content in the pulp of supermarket cucumbers after treatment (20 minutes, and respectively 40 minutes) with lemon juice.

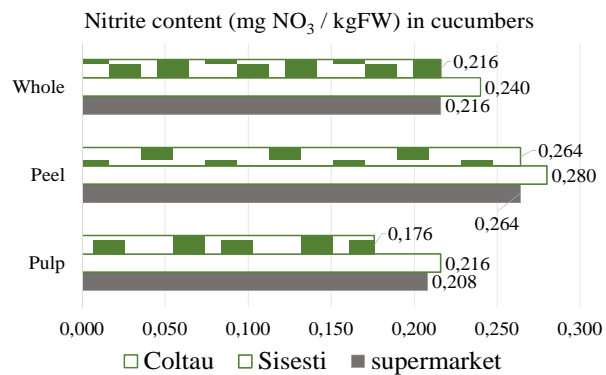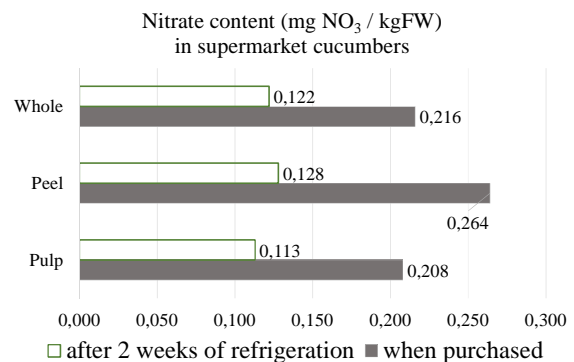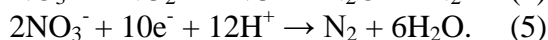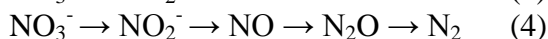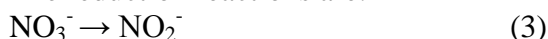The quantity of nitrates is highest in supermarket cucumbers (the Palermo – type), as comes out of experimental results. The way in which nitrates and nitrites accumulate in vegetables depends on the seeding conditions (in greenhouses/open air), on the type of fertiliser selected (organic/mineral), on the cucumber variety cultivated (cornichon/ half-long/long) and on other factors. The nitrates content in the cornichon cucumbers cultivated in open air in two villages is within the maximum threshold acceptable (of 200 ppm for cucumbers) pursuant to Order No. 293/640/2001-1/2002 of Romania's Ministry of Agriculture, regarding the quality and safety requirements for fresh vegetables and fruits for human use.

The content of nitrates and nitrites decreases in time, especially in the peel of cucumbers, because of enzyme and bacteria activities.

Bacteria could reduce the nitrite to nitrogen oxide or ammonium. A series of conversion mechanisms occur to convert nitrites in NO, including the enzymatic reduction by xantyne-oxidoreductase, nitrite-reductase, andby NO-synthetase (NOS), as well as by non-enzymatic acidic disproportioning reactions.

The reduction reactions are:

$$NO_3^- \rightarrow NO_2^- \qquad (3)$$
$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (4)$$
$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O. \qquad (5)$$

The significant decrease in the content of nitrites in cucumbers exposed to lemon juice occurs because of the change in pH toward the acidic range, given the presence of the citric acid in the juice of lemon.

The regulatory framework in force does not provide for a specific maximum acceptable level of nitrites, but according to the literature, this is advisable to be less than 1-5 ppm.

The content of nitrites is below that threshold in all the varieties of cucumbers selected for analysis.

5. Conclusions

The content of nitrogen compounds in vegetables depends on the content of nitrogen in soil, on the genetic potential of the plant and on the cultivation and processing methods used.

The content of nitrites was in all cases within the generally accepted 1-5 ppm threshold.

The accumulation of nitrogen compounds in plants is smaller by using organic fertilizers then by using mineral fertilizers. This happens because the organic compounds are not available from the beginning in forms accessible to plants. The micro-organisms in soil mineralize the organic compounds, thus the assimilation of nitrogen, required for the protein-genesis of the plant, occurring at a slower speed.

By applying processing methods conducive to lower acidity, the content of nitrates, and especially the content of nitrites, potentially more harmful for the human health, decreases.

6. Acknowledgements

7. References

1. Chan, T.Y.K.(2011), Vegetable-borne nitrate and nitrite and the risk of methaemoglobinaemia, *Toxicology Letters*, 200, 107–108.

2. Ralt, D. (2009), Does NO metabolism play a role in the effects of vegetables in health? Nitric oxide formation via the reduction of nitrites and nitrates, *Medical Hypotheses*,73, 794–796.

3. Bondonno, C.P., Yang, X., Croft, K.D., Considine, M.J., Ward, N.C., Rich, L., Puddey, I.B., Swinny, E., Mubarak, A., Hodgson, J.M. (2012), Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: a randomized controlled trial, *Free Radical Biology&Medicine*, 52,95–102.

4. Sindelar, J.J., Milkowski, A.L.(2012), Human safety controversies surrounding nitrate and nitrite in the diet, *Nitric Oxide*, 26, 259–266.

5. van Velzen, A.G., Sips, A.J.A.M., Schothorst, R.C., Lambers, A.C., Meulenbelt, J. (2008), The oral bioavailability of nitrate from nitrate-rich vegetables in humans, *Toxicology Letters*,181, 177–181.

6. Iammarino, M., Di Taranto, A., Cristino, M. (2013), Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: Results of five years of official controls and monitoring, *Food Chem*,140, 763–771.

7. Hsu, J., Arcot, J., Lee, N.A. (2009), Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians, *Food Chem*, 115, 334–33.

8. Prasad, S., Chetty, A.A. (2008), Nitrate-N determination in leafy vegetables: Study of the effects of cooking and freezing, *Food Chemistry*,106, 772–780.

9. Chetty, A.A., Prasad, S. (2009), Flow injection analysis of nitrate-N determination in root vegetables: Study of the effects of cooking, *Food Chem*,116, 561–566.

10. Rytel, E. (2012), Changes in glycoalkaloid and nitrate content in potatoes during dehydrated dice processing, *Food Control*, 25, 349-354.

11. Jaworska, G. (2005), Nitrates, nitrites, and oxalates in products of spinach and New Zealand spinach. Effect of technological measures and storage time on the level of nitrates, nitrites, and oxalates in frozen and canned products of spinach and New Zealand spinach, *Food Chemistry*,93, 395–401.

12. Chang, A.C., Yang, T.Y., Riskowski, G.L. (2013), Changes in nitrate and nitrite concentrations over 24 h for sweet basil and scallions, *Food Chemistry*,136, 955–960.

13. Citak, S., Sonmez, S. (2010), Effects of conventional and organic fertilization on spinach (*Spina ceaoleracea L.*) growth, yield, vitamin C and nitrate concentration during two successive seasons, *ScientiaHorticulturae*,126, 415–420.

14. Elwan, M.W.M., Abd El-Hamed, K.E. (2011), Influence of nitrogen form, growing season and sulfur fertilization on yield and the content of nitrate and vitamin C of broccoli, *Scientia Horticulturae*,127, 181–187.

15. Xiao-Yu, Y., Wangxiu-Feng, Wei Min, Yang Feng-Juan, Shi Qing-Hua, (2010), Changes of Nitrate Reductase Activity in Cucumber Seedlings in Response to Nitrate Stress, *Agricultural Sciences in China*, 9(2): 216-222.

16. Ping-Mei Yan, Wen-Tong Xue, Sze-Sze Tan, Hui Zhang, Xiao-Hui Chang, (2008), Effect of inoculating lactic acid bacteria starter cultures on the nitrite concentration of fermenting Chinese paocai, *Food Control*,19, 50–55.

17. Ierna, A. (2009), Influence of harvest date on nitrate contents of three potato varieties for off-season production, *Journal of Food Composition and Analysis*,22, 551–555.

18. Jaworska, I. (2005), Content of nitrates, nitrites, and oxalates in New Zealand spinach, *Food Chem*, 89, 235–242.

19. Tang, Y., Sun, X., Hu, C., Tan, Q., Zhao, X. (2013), Genotypic differences in nitrate uptake, translocation and assimilation of two Chinese cabbage cultivars [*Brassica campestris* L. ssp.*Chinensis* (L.)], *Plant Physiology and Biochemistry*,70,14-20.

20. Chang, A.C., Yang, T.Y., Riskowski, G.L. (2013), Ascorbic acid, nitrate, and nitrite concentration relationship to the 24 hour

light/dark cycle for spinach grown in different conditions, *Food Chem*, 138, 382–388.

21. Leszczynska, T., Filipiak-Florkiewicz, A., Cieslik, E., Sikora, E., Pisulewski, P.M. (2009). Effects of some processing methods on nitrate and nitrite changes in cruciferous vegetables, *J Food Compos Anal*,22, 315–321.

22. Hou, J.C., Jiang, C.G., Long, Z.C. (2013), Nitrite level of pickled vegetables in Northeast China, *Food Control*,29, 7-10.

SENSORY AND PHYSICAL ATTRIBUTES OF SUGAR SNAPS COOKIES AS A SOURCE OF PROTEIN

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ABSTRACT

Protein-energy malnutrition problems are arises in entire world due to current economic crunch and food prices to the highest level. Therefore, economically cheap and high quality food products development is imperative. The present study was aim at standardization of protein level in cookies for assessing consumer acceptance and for achieving optimal accuracy. In the present study we produced yeast as single cell protein (SCP) on agricultural wastes peels (potato). The resultant protein biomass contained sufficient proteins and were biologically evaluated (*in-vivo,* published). Later on cookies were fortified with SCP and then cookies were evaluated on sensory and physical basis. Trained sensory evaluation judges score the SCP cookies up to 6% SCP acceptable score. These protein rich cookies may be used to combat protein malnutrition in economically poor countries. It can be concluded from the present exploration that SCP derived from yeast *Saccharomyces cerevisiae* can be efficiently produced using appropriate carbon source such as potato peels which are economical cheap and available easily in Pakistan. Furthermore, fluctuation in meat price and availability may expose population to protein in-security and therefore, protein fortified cookies might be useful to secure population from protein malnutrition.

Keywords: Quality attributes sensory evaluation, product development, protein rich cookies, Single Cell Protein

1. Introduction

Sensory evaluation of the food products is very sophisticated method of assessing quality of food on sensory basis. Since, advancement in food industry and food fortification of various products, spurred the need of evaluating desired products to achieve market based goals and consumers acceptability. The consumer demand is increasing for bakery products with high nutritional, nutraceutical [1] and acceptable sensory properties. Global information system enables consumers' knowledge about food and consumers expectation for safe and high sensory quality food products. Therefore, consumers' preferences of food must be regarded by food manufacturers and other retailers. Sensory attributes like appearance is very crucial parameters which consumers at once choose before to purchase a food products and similarly, taste become the second important attribute during eating. Therefore, food industry should consider sensory-related information of the products in order to increase consumers' confidence and marketing. Sensory attributes changes with respect to nutrients level in the product. This indicates importance of level optimizationof specific

nutrient or important substances in fortification process or enrichment [2] and thus consumer's acceptability becomes point of concern. Majority of the fortification programme includes wheat flour fortification due to its high consumption in various products and its advantage of having enzyme proteinase. This enzyme acts on protein sources, liberating amino acid of the protein source to the bread or cookies and thus, this free amino acid contribute to the flavor[3], [4], [5] an another important attributes of sensory science. Among sensory evaluated fortified products in the market include Soy-ogi and Soy-moinmoin, made from cowpea and soy flour (CPSF) blends), Bambara groundnut, ogi [6], yackwa [7], Teff flour [1]. The objective of the present study was to determine physico-chemical and sensorial characteristics of three enriched cookies with various SCP concentration.

2. Materials and methods
2.1. Product (cookies) development

Single cell protein was used to develop protein fortified cookies to improve protein quantity and quality of finished products. Based on

some preliminary trials, wheat flour was fortified with SCP at 2, 4 and 6 % level and these fortified samples along with control were used for cookie preparation and evaluation (Appendix I).

2.1.1.Preparation of cookies

Cookies were prepared with some modifications according to method [8] from all the treatments. The ingredients for cookies preparation were accurately weighed according to recipe; flour=200g, sugar=100g, shortening (vegetable ghee) =100g, baking powder=3g, eggs=1 in No., water as per requirement (10-25mL). Mixing of ingredients was carried out in Hobart Mixer (Model N-50, Hobart Corp. Troy, Ohio, USA). Creaming of shortening and sugar was done, followed by the addition of eggs. The process continued till foaming occurred. The flour and baking powder were added to the foamy mass and mixed to a homogeneous mixture. The water was added according to requirement to obtain batter of optimum consistency. The batter was then rolled and cut with the help of a cookie cutter. The cookies were then placed on baking trays at a proper distance and baked at 425 °F in the baking oven for 12-15 minutes. After baking, the cookies were cooled to room temperature and packed in polythene bags for further analysis.

2.2. Analysis of cookies

The cookies were analyzed for physical analysis and evaluated for sensory acceptability as discussed below:

2.2.1.Physical analysis

The cookies were analyzed for width, thickness and spread factor according to their respective methods as described in [8]. The diameter of cookies was measured by placing six cookies horizontally (edge to edge) and rotated at 90° angles for duplicate reading. The process was repeated for triplicate analysis. The thickness of cookies was measured by placing six cookies on one another. The readings thus obtained were divided by six to get values for single cookie. The spread factor was calculated according to the following formula:

$$\text{Spread Factor} = \frac{\text{Diameter}}{\text{Thickness}} \text{Correction factor} \quad (1)$$

where:

Correction factor- Factor at constant atmospheric pressure is 1.0 in this case.

2.2.2. Sensory evaluation

Cookies were subjected to sensory evaluation by a trained taste panel consisting of 15 judges on a 15-cm unstructured line scale perform as described [9]. For this purpose judges were contacted through personnel contacts, e-mails and fliers (Appendix II). The panelists were selected, who frequently used to eat cookies and were asked to sign the consent form (Appendix III) to participate in the organoleptic evaluation. The judges were asked to fill out additional questionnaire form. The judges were trained for the evaluation of cookies. The cookies were prepared one day before the evaluation. On the day of evaluation the cookie samples were presented in trays with white background. The description of the sensory attributes for the evaluation of sugar-snap-cookies is given in (Appendix IV). The panelists were given sensory evaluation performance in which all the attributes were presented (Appendix V). They were also provided water to clean their mouth before proceeding to next sample. All the evaluations were conducted at room temperature on the same day in the Department of Food and Nutrition, University of Veterinary and Animal Science, Lahore. The samples were presented in random order and panelists were asked to rate their acceptance by marking a cross on the line for all the parameters. The data thus obtained was converted to numeric scores using metric scale.

2.3. Statistical analysis

The data thus collected was analyzed statistically through analysis of variance technique by using Cohort Costat-2003 software under completely randomized design [10]. Means were compared for significance of difference with the Duncan Multiple Range Test [11]. The level of significance was defined as *P≤0.05*.

3. Results and discussion
3.1. Physical characteristics

Different physical parameters of SCP fortified cookies are presented in Table 1. The results depicted significant differences in cookies diameter, thickness and spread factor with SCP augmentation. Statistically significant (P≤0.05) difference was found for diameter between the groups upto 4% SCP addition. However, further addition of SCP at 6% produced no significant changes in diameter. Similarly, thickness of the cookies was found to be statistically significant (P≤0.05) at 6 % SCP addition. As far as spread factor is concerned, SCP addition at 4% and 6% in cookies were found significantly different.

3.2. Sensory evaluation SCP-fortified cookies

Analysis of variance for sensory evaluation scores of SCP-fortified cookies based on color, aroma, taste, crispiness and overall acceptability shows non-significant difference for color, taste and overall acceptability. However, aroma and crispiness showed significant (P≤0.05) difference. Mean score for all attributes is presented in Table 2. The color analysis showed highest 11.84±1.76value for control while; the lowest (10.79±2.18) color value was noted for 6 % SCP fortified cookies. Similarly, in aroma panelist gave (12.03±1.31) score to 2 % SCP fortified cookies while, the lowest score (10.19±1.76) was given to 6 % SCP fortified cookies. Regarding taste of the cookies, more score (11.59±1.55) was given to 2 % SCP fortified cookies while, the lowest (9.78±1.68) score was given to 6 % SCP fortified cookies. Similarly, the highest (11.79±1.01) crispiness score was given to control cookies while, the lowest (9.66±1.20) crispiness score was given to 6 % SCP fortified cookies. There was significant difference (P≤0.05) among crispiness treatments. Mean sensory score for overall acceptability is presented in Table 2. The highest score value (12.05±2.24) was given to 2 % SCP fortified cookies while, the lowest (10.55±1.96)

acceptability was given to 6 % SCP fortified cookies.

3.3. Physical characteristics of single cell protein-fortified cookies

The finding of the present study for thickness of the cookies (control, 2 %) was in collaboration with value (0.79-0.86 cm) reported [1]. Spread factor was decreased gradually when concentration of SCP was increased. The decreasing in spread factor may be due to strong peptide bond of SCP with wheat flour, which is also, reported by [1]. Increasing the level of single cell protein, diameter of the cookies becomes short which may be due to strong peptide bonds of the protein concentrate keeping the wheat flour surface tight. Other researchers are also in favor of the same phenomena [12], [13]. Reduced spread factor of fortified biscuits could be attributed to the fact that composite flours apparently form aggregates with increased numbers of hydrophilic sites available that compete for the limited free water in biscuit dough [14].

3.4. Sensory evaluation scores of single cell protein-fortified cookies

All the values for different treatments were statistically non-significant which confirms that SCP addition to the cookies does not alter the color. The finding of the present study is in line with value (12.8±1.4 %) reported [15] for defatted maize germ (DMG) fortified cakes. However, the maximum scores for 2 % fortified cookies exceed then the value reported (Nutrend, 9.00 ± 0.00 %) by [16]. It can be concluded that addition of 6% SCP fortified cookies might be used for fortification of the food. Beyond this level color and texture of the cookies was deteriorated. The same finding has been quoted [17]. Increasing the concentration of SCP to the cookies, taste score reduces. This is possibly due to flavonoid contents having bitter taste in SCP [1]. It is concluded that SCP addition improved sensory and physical attributes of finished products.

Table 1. Physical characteristics of SCP-fortified cookies

Treatments	Diameter (cm)	Thickness (cm)	Spread factor
T_0	4.24 ± 0.02^a	0.99 ± 0.02^b	43.01 ± 0.61^a
T_2	4.19 ± 0.02^b	0.99 ± 0.01^b	42.11 ± 0.49^a
T_4	4.12 ± 0.01^c	1.01 ± 0.01^b	40.94 ± 0.20^b
T_6	4.09 ± 0.05^c	1.04 ± 0.03^a	39.19 ± 0.66^c

Means sharing the same letter in a column are not significantly different ($P\geq0.05$).
T_0= 100% All-purpose wheat flour; T_2 = 2% Single cell protein + 98% All- purpose wheat flour; T_4= 4% Single cell protein + 96% All- purpose wheat flour; T_6= 6% Single cell protein + 94% All- purpose wheat flour

Table 2. Sensory evaluation scores of SCP-fortified cookies

Treatments	Color	Aroma	Taste	Crispiness	Overall acceptability
T_0	11.84 ± 1.76^a	10.99 ± 2.00^{ab}	11.05 ± 1.99^{ab}	11.79 ± 1.01^a	11.62 ± 1.54^a
T_2	11.89 ± 1.83^a	12.03 ± 1.31^a	11.59 ± 1.55^a	11.65 ± 1.70^{ab}	12.05 ± 2.24^a
T_4	10.73 ± 2.31^a	11.16 ± 1.87^{ab}	10.85 ± 1.70^{ab}	10.68 ± 1.42^b	10.88 ± 1.89^a
T_6	10.79 ± 2.18^a	10.19 ± 1.76^b	9.78 ± 1.68^b	9.66 ± 1.20^c	10.55 ± 1.96^a

Means sharing the same letter in a column are not significantly different ($P\geq0.05$)
T_0= 100% All-purpose wheat flour; T_2= 2% Single cell protein + 98% All- purpose wheat flour; T_4= 4% Single cell protein + 96% All- purpose wheat flour; T_6= 6% Single cell protein + 94% All- purpose wheat flour

Appendix I. Various Levels of single cell protein used for wheat flour fortification for cookie production

Treatments	All-purpose wheat flour (%)	Single cell protein (%)
T_0	100	-
T_2	98	02
T_4	96	04
T_6	94	06

Appendix II. Flyer for sensory evaluation

Participate in Sensory Evaluation of Protein Fortified Cookies!
SPEND 20 MINUTES FOR TASTING AND FILLING OUT

A QUESTIONNAIRE

We will give you a treat (cold drinks & snacks) for participating!
 At **Sensory Lab, 1ˢᵗ Floor**
 Department of *Food Science&Human Nutrition*

For any questions call (042-99211449-50 x 283) or email Dr.Muhammad Nasir(nasir@uvas.edu.pk); Umar Bacha (bacha.umar474@gmail.com) in the Department of Food Science and Human Nutrition, UVAS, Lahore

Appendix III. Consent Form: Sensory evaluation of SCP fortified sugar-snap cookies

Dear Participant:

The researchers in Department of Food Science&Human Nutrition, University of veterinary and Animal Science are investigating consumer perceptions of cookies made with added Single Cell Protein (SCP) powder. We would like you to take about 30 minutes (including the time you spend reading this letter) to help us evaluate SCP Cookies. We are asking for volunteers, 18 years or older. ***If you have a known food allergy or sensitivity to*** <u>**yeast protein**</u>***,*** <u>**eggs**</u>***,*** <u>**wheat flour**</u>***, please do not volunteer for this study.***

If you meet the above requirements, we would like you to look at, taste and answer questions related to the product quality. If you agree to taste these and provide your evaluation based on the survey questionnaire, please sign the consent form below.

If you believe there is a potential of an **allergic reaction upon sniffing and tasting**, notify the on-site sensory evaluation coordinator and/or principle investigator, immediately. You will be released from participating in this study. Your response is anonymous and we have no way to connect you, as an individual, to this completed survey form. However, we do depend upon you taking the time to honestly respond to the questionnaire. You are free not to answer any question you choose, but please try to answer every question. If you have any questions during your reading this consent form, or during or after your participation, please do not hesitate to contact the on-site sensory evaluation researcher and/or the Principal Investigator. Feel free to contact Dr. Muhammad Nasir (nasir@uvas.edu.pk, 0321-7660521, 042-9211449-50/283) or Umar Bacha (0300-3934533 bacha.umar474@gmail.com) for any inquiry you might have regarding participation in our study.

PLEASE NOTE UPON YOUR SIGNING THIS CONSENT FORM, YOU VOLUNTARILY AGREE TO PARTICIPATE IN OUR STUDY. YOUR SIGNATURE INDICATES YOU HAVE READ THE INFORMATION PROVIDED ABOVE AND THAT YOU HAVE HAD AN ADEQUATE OPPORTUNITY TO DISCUSS THIS STUDY WITH THE PRINCIPLE INVESTIGATOR AND HAVE HAD ALL YOUR QUESTIONS ANSWERED TO YOUR SATISFACTION. YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM WITH YOUR SIGNATURE FOR YOUR RECORDS UPON YOUR REQUEST.

SIGNATURE _____ DATE_____

Appendix IV. Description of sensory attributes for the evaluation of sugar-snap-cookies

Attributes	Description of Attributes
Color	Intensity of color, typical of sugar-snap cookies
Flavor	Degree of flavor intensity associated with typical of sugar-snap cookies
Taste	Intensity of perceived taste typical of sugar-snap cookies
Crispiness:	Crispiness judged by breaking with hand and chewing in mouth typical of sugar-snap cookies
Overall quality	Overall impression of the cookies based on all attributes

Appendix V. Performa for sensory evaluation of sugar-snap cookies

Time. ……………．   Date……………..

Instructions

1. *Read carefully the description of individual attributes*
2. *Make inter-comparison of the samples & rate your acceptance by putting cross (X) on lines for all the samples*
3. *Before proceeding to the next sample, rinse your mouth with water*
4. *Don't disturb the order of the samples*

Color: Intensity of color, typical of sugar-snap cookies

Not at all intense   Very intense

673: ————————————————————————————

692: ————————————————————————————

658: ————————————————————————————

851: ————————————————————————————

Flavor:Degree of flavor intensity associated with typical of sugar-snap cookies

Not at all intense   Very intense

673: ————————————————————————————

692: ————————————————————————————

658: ————————————————————————————

851: ————————————————————————————

Taste:Intensity of perceived taste typical of sugar-snap cookies

Not at all intense   Very intense

673: ————————————————————————————

692: ————————————————————————————

658: ————————————————————————————

851: ————————————————————————————

Crispiness: Crispiness judged by breaking with hand and chewing in mouth typical of sugar-snap cookies

Not at all crispy   Very crispy

673: ————————————————————————————

692: ————————————————————————————

658: ————————————————————————————

851: ————————————————————————————

Overall quality: **Overall impression of the cookies based on all attributes**

Highly unacceptable   Very acceptable

673: ————————————————————————————

692: ————————————————————————————

658: ————————————————————————————

851: ————————————————————————————

Any Additional Comments ► ►	**673**	**692**	**658**	**851**

4. References

1. Baljeet, S.Y., Ritika, B.Y., Roshan, L.Y. (2010).Studies on functional properties and incorporation of buckwheat flour for biscuit making. *Int Food Research J*, 17, 1067-1076.

2. Sirivicha, S., Kamel, B., Kramer, A. (2007). The effect of protein fortification on the acceptability and rheological properties of cookie dough and its final product. *J Food Quality*, 4, 137-154.

3. Thiele, C., Ganzle, M.G. & Vogel, R.F. (2002). Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavor. *Cereal Chem,* 79, 45-51.

4. Gobbetti, M., Simonetti, M.S., Rossi, J., Cossignani, L., Corsetti, A. & Damiani, P. (1994). Free D- and Lamino acid evolution during sourdough fermentation and baking. *J Food Sci,* 59, 881-884.

5. Collar, C., Mascaros, A.F. &Benedito, C.D.B. (1992). Amino acid metabolism by yeast and lactic acid bacteria during bread dough fermentation. *J Food Sci*, 57, 1423-1427.

6. Ijarotimi, O. S. (2008). Protein and hematological evaluations of infant formulated from cooking banana fruits (Musa spp, ABB genome) and fermented Bambara groundnut (*Vigna subterranean L. Verdc*) seeds. Nutrition Research and Practice, 2, 165-170.

7. Lee, A.K., rennand, C.P. (2005). Physico-chemical, textural and sensory properties of a fried cookie system containing soy protein isolate. *Int J Food Sci Tech,* 40, 501–508.

8. AACC. (2000). American Association of Cereal Chemists. Approved methods of AACC international, 10th Ed. The American Association of Cereal Chemists, Inc. St. Paul MN, USA.

9. Meilgaard, M.C., Civille, G.V., & Carr, B.T. (4th Ed.). (2007). Sensory Evaluation Techniques. CRC Press, INC, 448.

10. Steel, R.G.D., Torrie, J.H. and Dickey, D.A. (3rd Ed.). (1997). Principles and procedures of statistics: A Biometrial Approach. Mc. Graw Hill Book Co. Inc., New York, USA, 481.

11. Duncan, D. B. (1955). Multiple ranges and multiple F test. *Biometrics*, 11, 1-42.

12. Singh, R., Singh, G. and Chauhan, G.S. (1996): Effect of incorporation of defatted soya flour on the quality of biscuits. *J Food Sci Tech*, 33, 355-357.

13. Hooda, S., Jood, S. (2005). Organoleptic and nutritional evaluation of wheat biscuits supplemented with untreated and treated fenugreek flour. *Food Chemistry*, 90, 427-435.

14. McWatters, K.M. (1978). Cookie baking properties of defatted peanut, soybean and field pea flours in baking powder biscuits. *Cereal Chemistry*, 55, 853-863.

15. Nasir, M. (2009). Characterization of maize germ from various hybrids and use of its components for value added baked products. PhD Thesis, University of Agricultural. Faisalabad, Pakistan.

16. Ijarotimi, O.S., Temitope, O.M. & Stephen, O.B. (2009). Chemical, functional properties of roasted bambara groundnut (Vigna subterranean L. Verdc) and cooking banana (Musa Spp., ABB genome) weaning diet. *African Journal of Food Science*, 3, 139-146.

17. Mohammed, M.I.O., Mustafa, A.I. & Osman, G.A.O. (2009). Evaluation of wheat breads supplemented with Teff (*Eragrostistef* (ZUCC.) Trotter) Grain flour. *Aust J Crop Sci*, 3, 207-212.

TEXTURIZATION OF DEFATTED SOYBEAN PROTEIN: OPTIMIZATION BY RESPONSE SURFACE METHODOLOGY AND STUDY OF FUNCTIONAL PROPERTIES

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ABSTRACT

The objective of study was to texturize and evaluate the nutritional and functional properties of defatted soybean meal used as byproducts of the oil extraction. From RSM generated model optimum conditions were 19.84 percent feed moisture content, 300 rpm screw speed and $180^{o}C$ barrel temperature, under these experimental conditions, functional properties of texturized protein flour was 63.48 % fat absorption capacity (FAC), 2.13 ml/g water holding capacity (WHC), 3.58 % water solubility index (WSI), 2.89g/g water absorption index (WAI), 0.2155g/ml bulk density (BD) and 18.859 % foaming capacity (FC). The texturized soybean defatted meal contained 2.70 % moisture, 52.86 % protein, 2.26 % fat and 3.29 % fiber. To ensure that the texturized defatted meal could be utilized for food applications, some functional properties such as FAC, WHC, WAI, BD, PD and FC were evaluated. Texturized defatted soybean protein free from antinutritional factors such as trypsin inhibitor. Thus it can be used in food formulations.

Keywords: *extrusion, soybean meal, texturization, response surface methodology.*

1. Introduction

The soybean (*Glycine max*) is a species of legume widely grown in India for its edible bean which has numerous uses. The plant is classed as an oilseed rather than pulse by the UN Food and Agricultural Organization (FAO). Food systems include components of food variability, access and utilization which underpin food security [1]. The expanding world population has resulted to a greater pressure for the consumption of plant products in foods with aesthetic and organoleptic appeal, consequently resulting in a great emphasis on the need for food ingredients with multiple functional properties. The meal is usually used as an animal feed; a smaller percentage is further processed into food ingredients including soy flour, concentrates and isolates and textured protein. These are soy protein products used as food ingredients because of their multiple functional properties. Functional properties have been defined as "those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption" [2]. Texturization means the development of a physical structure that when eaten provide a sensation of eating meat [3]. Textured soy protein product was obtained by thermoplastic extrusion process [4]. Extrusion or steam texturized products were obtained using flour, concentrate or isolated protein [3]. These products known as textured vegetable protein could simulate meat fibre structures [5]. Extrusion texturization is one of the most economic means of converting vegetable proteins into textured products with a wide range of applications as meat supplements or extenders.

Functionality has been defined as any property of a food ingredient that has an important impact on its use. Functional properties affect processing applications, food quality, acceptance and use in formulating food products [6]. The objective of the present study was to obtain a texturized protein from defatted soybean meal by extrusion, optimize the process using surface response methodology and evaluate the functional properties of the product produced.

2.Materials and Methods
2.1. Raw material

The soybean (*SL 744)* used in this study was procured from Directorate of Seeds, Punjab Agricultural University, Ludhiana, India.

2.2. Oil Extraction

The soybean was cleaned and defatted using laboratory oil expeller. The meal was dried and milled into grits using Super Mill (Perten Instruments, Sweeden). After that, the sample was sieved using mesh screen to separate out the large particles of the seed coat.

2.3. Extrusion process for soybean

Texturization of soybean was carried out by using Clextral BC 21 twin screw extruder (Clextral, Firminy, France). The operating conditions were 14-20 %t feed moisture, 300-500 rpm screw speed and 120-180°C barrel temperature. Texturized proteins was milled into flour using cyclotec mill (Newport Scientific, Australia) and packed in suitable packaging material for further study.

2.4. Experimental design

Central composite design was to optimize the process. Extrusion process variables (feed moisture content, screw speed and temperature) were coded to the level of -1, 0, +1 such that one factor at a time of experimental design was as Table 1 presents [7].

Table 1.Experimental design

Extrusion parameters	**-1.682**	**-1**	**0**	**+1**	**+1.682**
Moisture content (%)	11.954	14	17	20	22.046
Screw speed (rpm)	231.800	300	400	500	568.200
Barrel temperature (^{o}C)	99.54	120	150	180	200.460

2.5.Functional properties texturized defatted soybean meal

2.5.1. Water absorption index (WAI), Water solubility index (WSI) and fat absorption capacities (FAC)

Water absorption index (WAI) was measured according to the Stojceska et al.,(2008) [8] method. First, 1g of defatted flour was placed in a previously weighed 50 ml centrifuge tube.

Then, 10 ml of distilled water was added and stirred homogeneously with a glass rod and centrifuged at 3000 rpm for 10 min at room temperature (22°C) using a Model T-8BL Laby^{TM} centrifuge (Laby Laboratory Instruments, Ambala Cantt, India). The residue was weighed together with the centrifuge tube. The WAI values were expressed as gram of water absorbed/gram of defatted flour. The supernatant was transferred to previously weighed dish which put in hot air oven for evaporation of water. The residue was weighed. A similar method was used to measure fat absorption capacity (FAC), although a 0.5 g sample was used in this case [9].

$$WAI\ (g/g) = \frac{\text{Weight of residue}}{\text{Dry weight of residue}} \quad (1)$$

$$WSI(\%) = \frac{\text{Weight of dry matter in supernatant}}{\text{Dry weight of sample}} 100 \quad (2)$$

$$FAC(\%) = \frac{\text{Weight of absorbed fat}}{\text{Weight of sample}} 100 \quad (3)$$

2.5.2. Foaming capacity

One gram of defatted flour was dissolved in 100 ml of distilled water. Then the suspensions were whipped at a low speed in a blender for 1 min at room temperature (22°C). The resulting foam was poured into a 100 ml cylinder. Total foam volume was recorded and foam capacity was expressed as the percent increase in volume. To determine foam stability (FS), foam volume was recorded 30 min after whipping and calculated according to the method proposed by Kabirullah & Wills, (1982) [10].

$$FS = \frac{\text{Foam volume after 30 minutes}}{\text{Initial foam volume}} 100 \quad (4)$$

2.5.3. Water holding capacity (WHC)

Five gram of defatted flour was placed in a previously weighed 50 ml centrifuge tube. Then,

10 ml of distilled water was added and stirred homogeneously with a glass rod and centrifuged at 2000 rpm for 10 min at room temperature (22°C). The supernatant was decanted and the residue was weighed together with the centrifuge tube [11].

$$WHC\,(mL/g) = \frac{(weight\ of\ tube + sediment) - (weight\ of\ tube + 5.0)}{5}$$
(5)

2.5.4. Bulk density (BD)

The Bulk densities (g/ml) of defatted flour were determined by volumetric method. The volume of the expanded sample was measured by using a 25 ml graduated cylinder and gently tapped for 5 times. The volume of 10 g randomized samples was measured for each test. The ratio of sample weight and the replaced volume in the cylinder was calculated as bulk density (w/v) [12].

$$WHC\,(mL/g) = \frac{Weight\ of\ sample}{Volume\ displaced\ by\ sample}$$
(6)

2.5.5. Chemical composition

The moisture, fat, fiber and protein content were measured using AOAC (2000) methods [11].

3. Results and discussion
3.1. Chemical composition

The moisture, fat, fibre and protein content were 2.56 %, 2.26 %, 3.29 % and 52.86 %, respectively.

3.2. Functional properties

Fitting the models: The experimental values for responses (FAC, WHC, WSI, WAI, BD and FC) ranged from 54.57 to 77.28 %, 1.20 to 2.21 ml/g, 2.51 to 5.25 %, 2.10 to 3.32 g/g, 0.21 to 0.34 g/ml and 10.05 to 21.81 %, respectively, under different conditions of feed moisture content, screw speed and temperature. The fitness and adequacy of the model was judged by the coefficient of determination R^2. The significance of each coefficient was determined using F-test and *p*-value. The F-values for all response models were greater than the tabulated F-value indicating the adequacy of the models to predict various responses at different levels of ingredients.

3.3. Analysis of response surface

3.3.1. Fat absorption capacity

The quadratic model obtained from regression analysis for fat absorption capacity (FAC) in terms of coded levels of the variables was developed as follows.

Equation in Terms of Coded Factors:
$$FAC = + 91.73 - 1.57 \times A - 3.34 \times B - 6.17 \times C + 0.49 \times A \times B + 4.41 \times A \times C + 4.69 \times B \times C - 0.66 \times A^2 - 5.28 \times B^2 - 1.95 \times C^2$$
(7)

The significance of coefficient of fitted quadratic model was evaluated by using F- test and P- value. The analysis of variance (ANOVA) for FAC of quadratic model is given in Table 3. The Model F-value of 630.57 implies that model was significant. Values of "Prob > F" less than 0.05 indicated model terms were significant. In this case A, B, C, AB, AC, BC, A^2, B^2, C^2 were significant model terms. Values greater than 0.10 indicate that model terms were not significant. The "Lack of Fit F-value" of 0.39 implies that it was not significant relative to the pure error. There was a 84.03 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non significant lack of fit was good to fit. The P-value for Lack of fit 0.84 implies that it was not significant. The value of R^2 was found to be 0.99. Regression analysis results (Table 3) showed that the significant negative influence of moisture, screw speed and temperature (P<0.05) was recorded. There was a significant interaction of feed moisture with screw speed, feed moisture with barrel temperature and screw speed with barrel temperature (P<0.01) on FAC. The FAC for defatted flour varied from 71.11 to 104.42 % (Table 2). It was predicted from regressions analysis (Table 3) that during extrusion-cooking, lower moisture contents increase FAC of protein flour (Figure 1a). The increase in FAC with increasing screw speed was consistent.

**Table 2.** Effect of Extrusion condition on product responses (n=3)

Run	Extrusion conditions			Responses					
	Moisture content (%)	Screw speed (rpm)	Barrel temperature (^{0}C)	FAC (%)	WHC (ml/g)	WSI (%)	WAI (g/g)	BD (g/ml)	FC (%)
1	14	300	120	65.82	1.2	2.76	2.1	0.25	13.12
2	20	300	120	77.28	2.01	4.01	3.1	0.34	14
3	14	500	120	69.45	1.92	3.27	3.15	0.212	22.6
4	20	500	120	71.72	2.15	4.45	3.32	0.307	16.2
5	14	300	180	68.2	1.96	2.51	2.73	0.28	10.25
6	20	300	180	63.79	2.11	3.65	2.87	0.21	19
7	14	500	180	69.4	2.045	2.87	3.045	0.25	12.23
8	20	500	180	54.57	1.69	3.45	2.67	0.239	13.21
9	17	400	150	58.69	1.88	4.89	2.98	0.29	20.61
10	17	400	150	58.76	1.75	4.92	2.7	0.271	21.3
11	17	400	150	58.68	1.81	4.75	3.05	0.28	20.91
12	17	400	150	58.2	1.9	4.85	2.75	0.27	21.81
13	17	400	150	58.51	1.89	4.95	2.82	0.25	20.3
14	17	400	150	58.35	1.98	4.82	2.835	0.3	20.72
15	11.95	400	150	67.32	1.61	3.48	2.628	0.21	14.56
16	22.05	400	150	65.34	1.97	5.25	2.75	0.27	15.45
17	17	231.8	150	69.68	1.75	3.1	2.8	0.242	17.3
18	17	568.2	150	65.66	1.9	3.61	3.317	0.233	21.1
19	17	400	99.54	72.46	2.1	2.95	3.21	0.32	14
20	17	400	200.46	60.88	2.21	2.19	2.915	0.26	10.05

Table 3. Coefficient of estimate for functional properties

| Factors	FAC (%)	WHC (ml/g)	WSI (%)	WAI (g/g)	BD (g/ml)	FC (%)
Intercept of Model	58.53	1.870	14.86	2.860	0.2800	20.94
A: Moisture content	-0.65	0.110	0.52	0.083	0.0150	0.42
B: Screw speed	-1.22	0.057	0.14	0.170	-0.0064	1.04
C: Barrel temperature	-3.50	0.052	-0.24	-0.062	-0.0170	-1.31
AB (Moisture content x Screw speed)	-2.45	-0.140	-0.08	-0.170	0.0080	-1.88
AC (Moisture content x Barrel temperature)	-4.12	-0.160	-0.08	-0.180	-0.0330	1.91
BC (Screw speed x Barrel temperature)	-.076	-0.150	-0.09	-0.140	0.0088	-1.94
A^2 (Moisture content)^2	2.78	-0.036	-0.17	-0.073	-0.0120	-2.10
B^2 (Screw speed)^2	3.26	-0.024	-0.53	0.057	-0.0130	-0.62
C^2 (Barrel temperature)^2	2.90	0.093	-0.83	0.059	0.0060	-3.15
P-Value for lack of fit	0.38	0.86	0.37	0.69	0.78	0.86
R^2	0.99	0.95	0.99	0.90	0.90	0.99
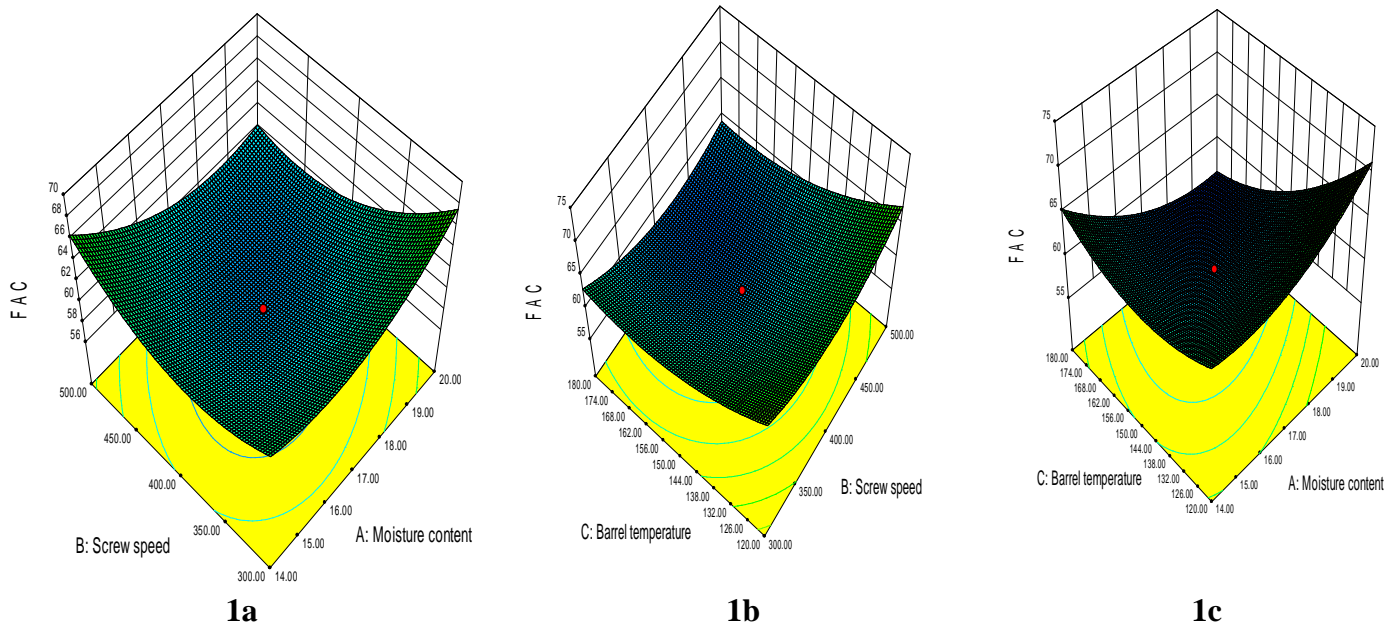
Figure 1. The effect of Moisture, Screw speed and Temperature on FAC (fat absorption capacity**)**
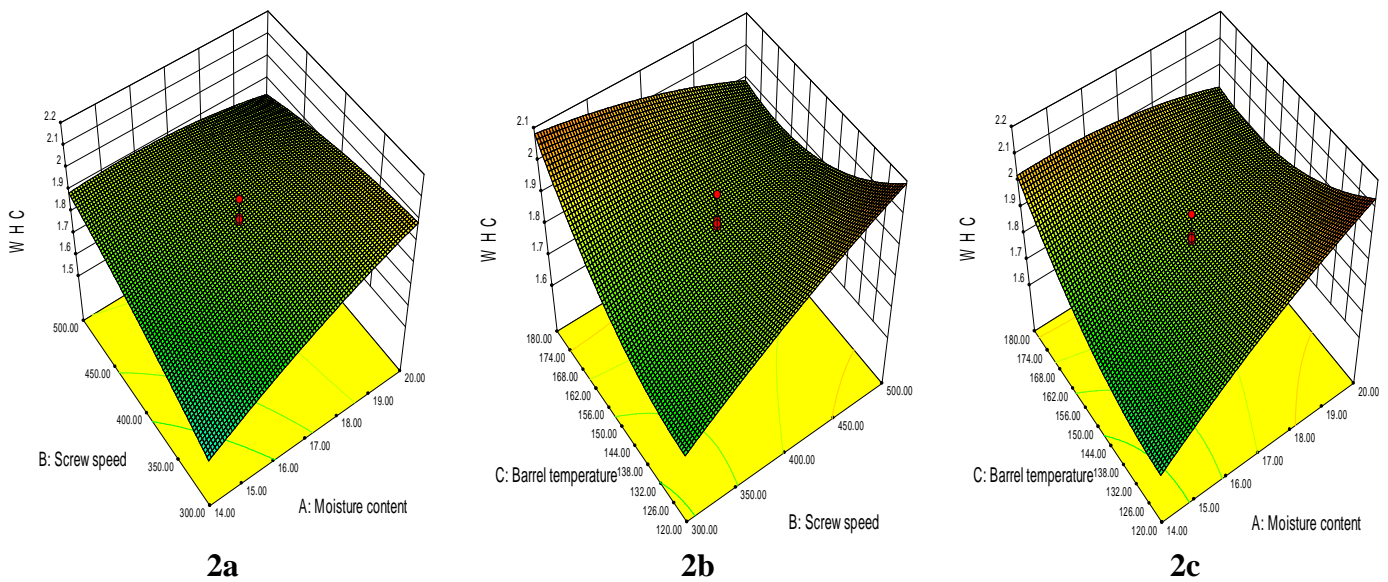
Figure 2. The effect of Moisture, Screw speed and Temperature on WHC (water holding capacity)
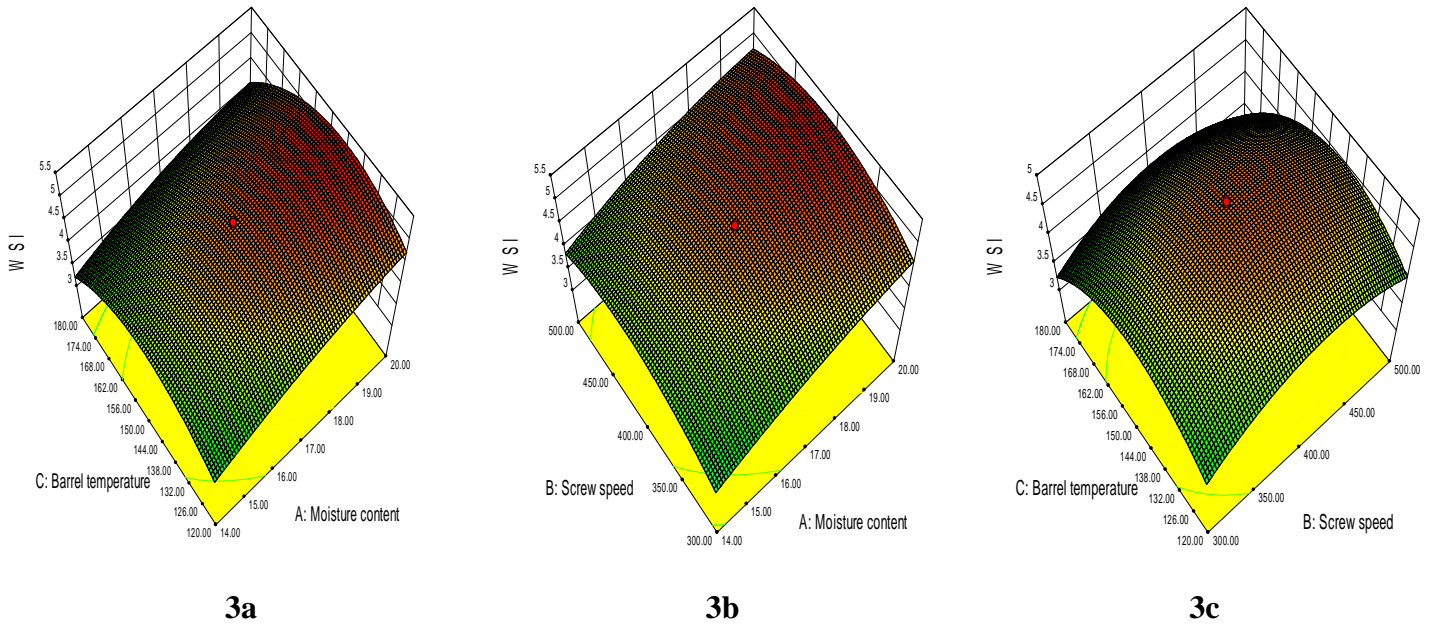
Figure 3. The effect of Moisture, Screw speed and Temperature on WSI (water solubility index)
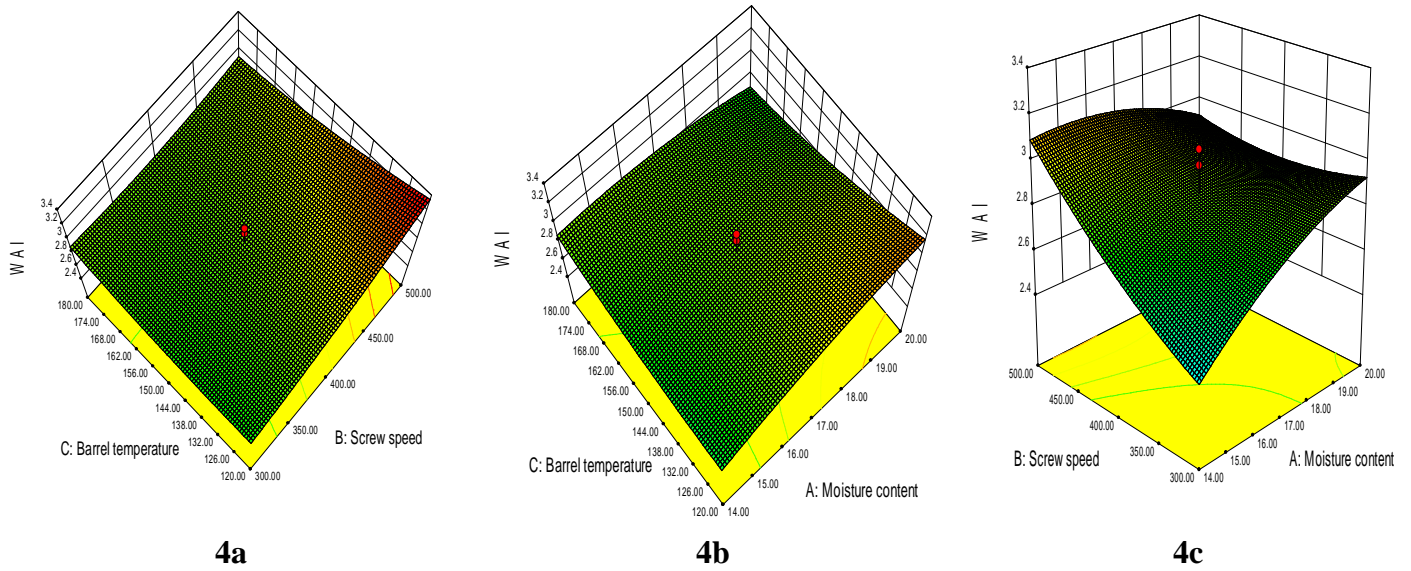
Figure 4. The effect of Moisture, Screw speed and Temperature on WAI (water absorption index)
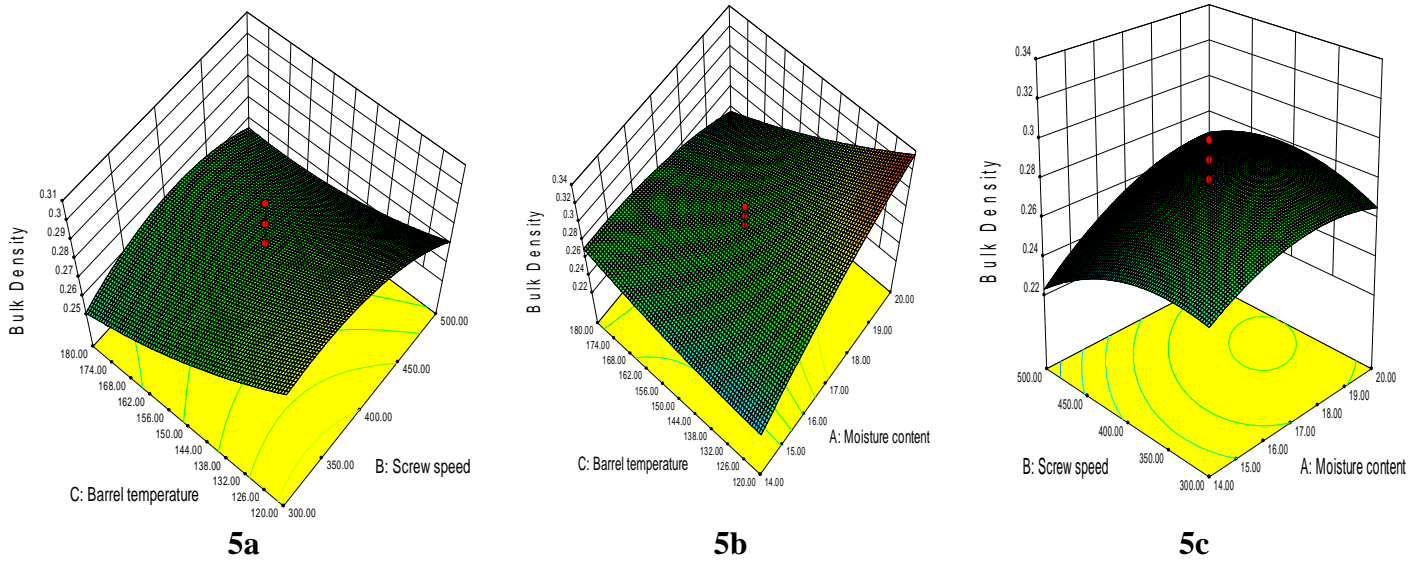
Figure 5. The effect of Moisture, Screw speed and Temperature on BD (bulk density)
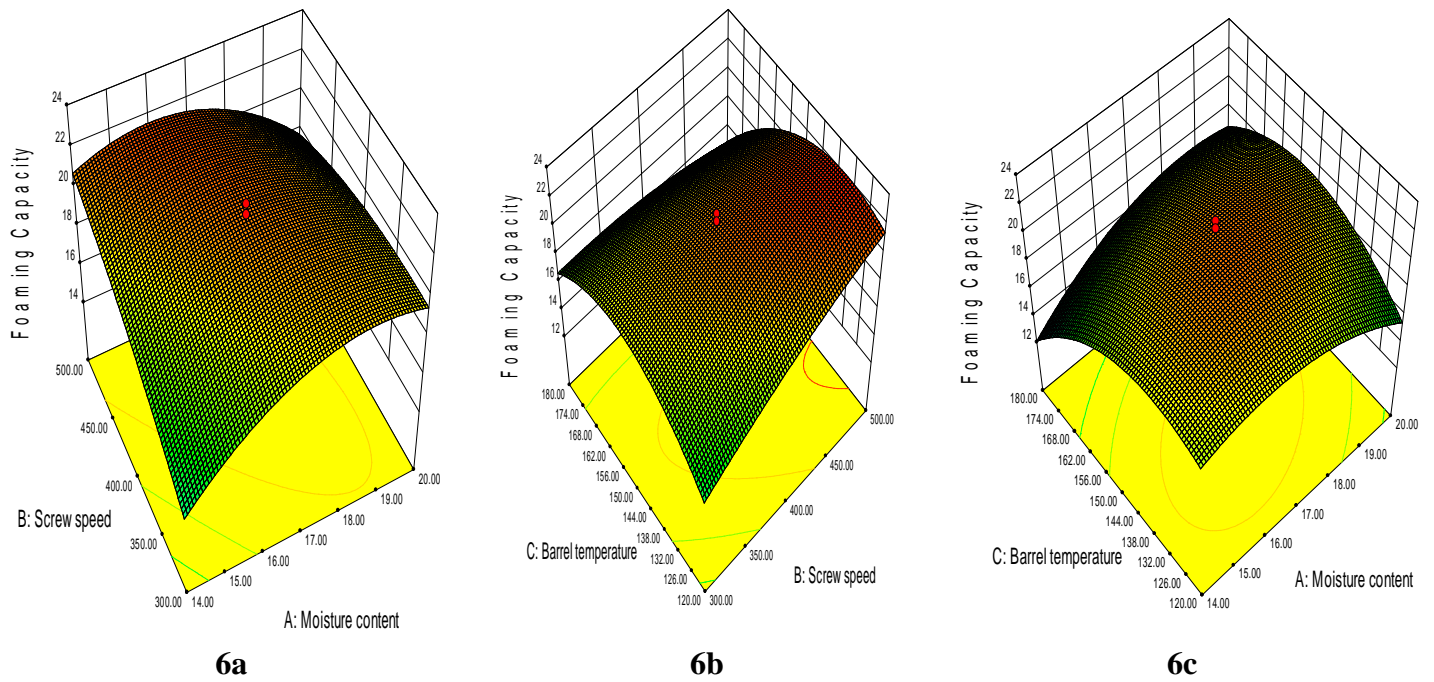
Figure 6. The effect of Moisture, Screw speed and Temperature on FC (Foaming capacity)

Defatting increased the protein solubility and water and oil absorption capacity of the meals. Water holding capacity of protein is very important as it affects the texture, juiciness and taste of food products and in particular the shelf-life of bakery products. The capacity of protein to absorb water and oil is determined by its polar and non polar amino acids composition, respectively [13]. FAC of the flaxseed protein concentrate was higher than that of amaranth protein concentrate [14].

3.3.2. Water holding capacity

Water holding capacity is the ability to retain water against gravity and includes bound water, hydrodynamic water, capillary water and physically entrapped water [15]. The quadratic model obtained from regression analysis for water holding capacity (WHC) in terms of coded levels of the variables was developed as follows.

Equation in Terms of Coded Factors:

$$WHC = + 2.13 - 0.050 \times A - 0.090 \times B - 0.15 \times C + 0.073 \times A \times B + 0.27 \times A \times C + 0.18 \times B \times C + 0.013 \times A^2 - 0.14 \times B^2 - 0.026 \times C^2 \qquad (8)$$

The Model F-value of 56.19 implies that model was significant. The Model F-value of 45.19 implies that model was significant. Values of "Prob > F" less than 0.05 indicated that model terms were significant. In this case A, B, C, AB, AC, BC, B^2 were significant model terms. Values greater than 0.10 indicated that model terms were not significant. The "Lack of Fit F-value" of 1.07 implies that it was not significant relative to the pure error. Non significant lack of fit was good to fit. The P-value for Lack of fit 0.47 implies that it was not significant. The value of R^2 was found to be 0.97. Regression analysis results (Table 3) showed that the significant negative linear influence of moisture, screw speed and barrel temperature ($P<0.05$) was recorded. There was significant interaction of feed moisture with screw speed, feed moisture with barrel temperature and screw speed with barrel temperature ($P<0.01$) on WHC. The WHC varied from 1.59 to 2.87 ml/g protein flour (Table 2). It was predicted from regressions analysis that during extrusion cooking, lower moisture contents increased WHC of protein flour (Figure 2a). Water holding capacity of protein is very important as it affects the texture, juiciness, and taste of food products and in particular the shelf-life of bakery products. The capacity of protein to absorb water was determined by its polar and non polar amino acids composition, respectively [13]. The amount of water associated to proteins was closely related with its amino acids profile and increased with the number of charged residues [16], conformation, hydrophobicity, pH, temperature, ionic strength and protein concentration [17].

3.3.3. Water solubility index

WSI is often used as an indicator of degradation of molecular components [18]. WSI measures the amount of soluble components released from the starch after extrusion. High WSI was an in vitro indicator of good starch digestibility as it implies that extent of gelatinization and dextrinization [19]. It can also measure the degree of starch conversion during extrusion, which corresponds to the amount of soluble polysaccharide released from the starch granule [20]. The quadratic model obtained from regression analysis for water solubility index (WSI) in terms of coded levels of the variables was developed as follows.

Equation in Terms of Coded Factors:

$$WSI = + 3.89 + 0.065 \times A - 0.051 \times B - 0.29 \times C - 0.23 \times A \times B + 0.61 \times A \times C + 0.012 \times B \times C + 0.14 \times A^2 - 0.57 \times B^2 + 0.034 \times C^2 \qquad (9)$$

The significance of coefficient of fitted quadratic model was evaluated by using F-test and P-value. The Model F-value of 95.68 implies that model was significant. Values of "Prob > F" less than 0.05 indicated that model terms were significant. In this case A, C, AB, AC, A^2, B^2 were significant model terms. Values greater than 0.10 indicated that model terms were not significant. The "Lack of Fit F-value" of 2.25 implies that Lack of Fit was not significant relative to the pure error. There was a 19.76 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit was good to fit the model. There was significant interaction of

feed moisture with screw speed and feed moisture with barrel temperature (P<0.01) on WSI (Table 3). The WSI varied from 2.10 to 4.48 % protein flour. It was predicted from regressions analysis (Table 3) that during extrusion-cooking, higher moisture contents increase WSI of protein flour (Figure 3a). The high mechanical shear caused breakdown of macromolecules to small molecules with higher solubility. The increase in WSI with increasing screw speed was consistent with the results reported by other researchers [21]. Also increasing temperature would increase the degree of starch gelatinization that could increase the amount of soluble starch resulting in an increase in WSI [20]. WSI increased with increasing feed moisture and decreasing barrel temperature and screw speed.

3.3.4. Water absorption index

High WAI is an in vitro indicator of good starch digestibility as it implies the extent of gelatinization and dextrinization [19]. The WAI measures the amount of water absorbed by starch and could be used as an index of gelatinization. WAI depends on the availability of hydrophilic groups that bind water molecules. The quadratic model obtained from regression analysis for water absorption index in terms of coded levels of the variables was developed as fallows.

Equation in Terms of Coded Factors:

$$WAI = + 3.07 + 0.033 \times A - 0.090 \times B - 0.13 \times C - 0.059 \times A \times B + 0.16 \times A \times C + 0.063 \times B \times C - 0.039 \times A^2 - 0.12 \times B^2 + 0.0001589 \times C^2 \qquad (10)$$

The significance of coefficient of fitted quadratic model was evaluated by using F- test and P- value. The Model F-value of 32.77 implies that model was significant. Values of "Prob > F" less than 0.05 indicated that model terms were significant. In this case A, B, C, AB, AC, BC, A^2, B^2 were significant model terms. Values greater than 0.10 indicated that model terms were not significant. The "Lack of Fit F-value" of 3.75 implies that there was a 8.65 % chance that a "Lack of Fit F-value" this large could occur due to noise. Regression analysis results showed that

screw speed and temperature had significant negative and moisture had positive linear (P<0.001) effect and significant quadratic effect on WAI. Interaction of feed moisture with screw speed and screw speed with barrel temperature had significant influence (P<0.01). The WAI of protein flour ranged from 2.59 to 3.27 g/g (Table 2) increased in moisture content reduces the water absorption index. Moisture content, acting as a plasticizer during extrusion cooking, reduces the degradation of starch granules, this result in an increased capacity for water absorption [22]. WAI was higher for lower screw speed and lower temperature as shown in response surface plot as shown in (Figures 4b and 4c). It could be expected that more undamaged polymer chains and a greater availability of hydrophilic groups, which could bind more water resulted in higher values of WAI under low shear conditions with lower screw speed [23]. Higher Temperature increases degradation and dextrinization of starch. WAI decreases with increasing temperature if dextrinization or starch melting prevails over the gelatinization phenomenon [24].

3.3.5. Bulk density

Bulk density is a very important parameter in the production of texturized products. Density is a measure of how much expansion has occurred as a result of extrusion. The heat developed during extrusion can increase the temperature of the moisture above the boiling point so that when the extrudate exits from the die, a part of the moisture would quickly flash off as steam and result in an expanded structure with large alveoli and low density. On the other hand, if not enough heat was generated to flash-off enough of the moisture (either through low process temperature or high feed moisture), less expansion occurs resulting in a high bulk density product with collapsed cells which usually disintegrates on cooling. The quadratic model obtained from regression analysis for bulk density (BD) in terms of coded levels of the variables was developed as follows.

Equation in Terms of Coded Factors:

Bulk Density = + 0.35 + 0.009272× A + 0.030×B 0.033 × C + 0.0015 x A × B + 0.011 × A × C + 0.011 × B × C - 0.018 × A^2 − 0.00163 × B^2+ 0.005614 × C^2 (11)

The significance of coefficient of fitted quadratic model was evaluated by using F- test and P- value. The Model F-value of 18.41 implies that model was significant. Values of "Prob > F" less than 0.05 indicated that model terms were significant. In this case A, B, C, A^2 were significant model terms. Values greater than 0.10 indicated that model terms were not significant. If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Lack of Fit F-value" of 1.41 implies that it was not significant relative to the pure error. There was a 35.81 % chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit was good to fit the model. The bulk density of protein flour ranged from 0.189 to 0.359 g/ml (Table 2). Regression analyses (Table 3) indicated that bulk density decreased with decrease in moisture (Figure 5a).The high dependence of bulk density and expansion on feed moisture would reflect its influence on elasticity characteristics of the starch based material. Increased feed moisture content during extrusion might reduce the elasticity of the dough through plasticization of the melt, resulting in reduced SME and therefore reduced gelatinization, decreasing the expansion and increasing the density of extrudate. Bulk density low at high screw speed (Figure 5b). It was observed that an increase in screw speed resulted in an extrudate with lower density. Higher screw speeds may be expected to lower melt viscosity of the mix increasing the elasticity of the dough, resulting in a reduction in the density of the extrudate [20]. It was depicted that Bulk density increased with decrease in moisture as higher water content produced extrudates denser than those produced with low water content similar results were reported by Patil et al. (1990) [25] in study of effect of extrusion condition on the soya rice extrudates and by Koksel et al. (2004) [26] effect of extrusion Variables on waxy hull less barley.

3.3.6. Foaming capacity

Foams were gaseous droplets encapsulated by a liquid film containing soluble surfactant protein resulting in reduced interfacial tension between gas and water. The quadratic model obtained from regression analysis for foaming capacity (FC) in terms of coded levels of the variables was developed as follows.

Equation in Terms of Coded Factors:

Foaming Capacity = + 14.88 - 0.065 × A - 0.64 × B + 0.026 × C - 1.54 × A × B - 0.75 × A × C + 4.10 × B × C - 1.43 × A^2 - 0.31 × B^2 0.61 ×C^2 (12)

The significance of coefficient of fitted quadratic model was evaluated by using F- test and P- value. The Model F-value of 54.33 implies that model was significant. Values of "Prob > F" less than 0.05 indicated that model terms were significant. In this case B, AB, AC, BC, A^2, C^2 were significant model terms. Values greater than 0.10 indicated that model terms were not significant. The "Lack of Fit F-value" of 2.00 implies that it was not significant relative to the pure error. Non-significant lack of fit was good to fit the model term. The P-value for Lack of fit 0.2328 implies that lack of fit was not significant. The value of R^2 was found to be 0.98. Interaction (P<0.05) of feed moisture with screw speed, feed moisture with barrel temperature and screw speed with barrel temperature were found significant. The foaming capacity of protein flour ranged from 6.20 % to 19.92 % (Table 2). Regression analyses (Table 3) indicated that foaming capacity decreased with increase in moisture (Figure 6a). Foaming capacity was low at high screw speed (Figure 6b).

4. Conclusions

It was concluded that functional properties of the defatted soybean meal protein has a great potential to serve as an excellent source of edible protein, owing to its high water and fat absorbing capacity. It also had higher Water holding

capacity, foaming capacity and bulk density. Defatted meal due to its high content in protein has the potential to replace other protein source. Texturized defatted soybean protein flour exhibit satisfactory functional properties, and therefore have a bright prospect for application in the food industry. Although soybean is mainly used for its oil, the data suggest that the seeds, a byproduct, could provide protein for feed and food. Such uses could significantly increase the economic value of this crop.

5. References

1. Gregory, P.J., Ingram, J.S.I., Brklacich, M. (2005). Philosophical Transactions of the Royal Society, 360, 2139-2148.

2. Kinsella, J.E. (1976). Functional properties of food proteins: a review. *Cr Rev Food Sci Nutr,* 7, 219-280.

3. Berk, Z. (1992). Technology of production of edible flours and protein products from soybeans. *FAO Agricultural Services Bulletin* No. 97. Food Agriculture Organisation of the United Nations, Rome.

4. US Soybean Export Council. 2010. Soy protein. Available at http://www.ussec. org/ resources / soyprotein.html Assessed 17/09/2010.

5. Anon. 2008. Soy processing Available at http://dspace.dial.pipex.com/town/park/gfm11/ soya.shtml Assessed 17/09/2010.

6. Mahajan, A., Dua, S. (2002). Salts and pH induced changes in functional properties of amaranth (Amaranthus tricolor L.) seed meal. *Cereal Chem,* 79, 834-837.

7. Myers, R.H. (1971). Response surface methodology. 1st edition. Boston, Mass: Allyn and Bacon, pp. 247.

8. Stojceska, V., Ainsworth, P., Plunkett, A. & Ibanoglu, S. (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chem* ,114, 226-232.

9. Lin, M.J.Y., Humbert, E.S. & Sosulski, F.W. (1974). Functional properties of sunflower meal product. *J Food Sci* 39, 368-370.

10. Kabirullah, M. & Wills, R.B.H. (1983). Characterization of sunflower protein. *J Agric Food Chem*, 31, 953-956.

11. AOAC. (2000). Official methods of Analysis.Association of Official Analytical Chemists, Guthersburg, Maryland, USA.17th edition.

12. Pan, Z., Zhang, S. & Jane, J. (1998). Effects of extrusion variables and chemicals on the properties of starch based binders and processing conditions. *Cereal Chem,* 75, 541-546.

13. Sathe, S. K., Salunkhe, D.K. (1981). Functional properties of the Great Northern bean proteins: emulsion, foaming, viscosity, and gelation properties. *J Food Sci,* 46, 71-81.

14. De Luquez, N., Fernandez, S., Arellano, M,. Mucciarelli, S. (1997). Concentrado proteico de Amaranthus mantegazzianus: Caracterización físico-químico-biológica. *Archivos Latinoamericanos de Nutrición* 47, 359-361.

15. Moure, A., Sineiro, J., Domınguez, H. & Parajo, J.C. (2006). Functionality of oilseed protein products: A review. *Food Res Int,* 39, 945-963.

16. Kuntz, I.D.Jr. & Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Ad Protein Chem*, 28, 239-345.

17. Damodaran, S. (1997). Food proteins: An overview. In: S. Damodaran & A. Paraf (ed.). *Food proteins and their application.* New York: Marcel Dekker.

18. Kirby, A.R., Ollett, A.L., Parker, R. & Smith, A.C. (1988). An experimental study of screw configuration effects in the twin-screw extrusion-cooking of maize grits. *J Food Eng,* 8, 247-272.

19. Guha, M., Ali, S.Z. & Bhattacharya, S. (1997). Twin-screw extrusion of rice flour without a die: Effect of barrel temperature and screw speed on extrusion and extrudate characteristics. *J Food Eng,* 32, 251-267.

20. Ding, Q.B., Ainsworth, P., Tucker, G. & Marson, H. (2005). The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice based expanded snacks. *J Food Eng,* 66, 283-289.

21. Dogan, H. & Karwe, M.V. (2003). Physicochemical properties of quinoa extrudates. *Food Sci Tech Int Madrid,* 9, 101-114.

22. Hagenimana, A., Ding, X. & Fang, T. (2006). Evaluation of rice flour modified by extrusion cooking. *J Cereal Sci,* 43, 38-46.

23. Jin, Z., Hsieh, F. & Huff, H.E. (1995). Effects of soy fiber, salt, sugar, and screw speed on physical properties and microstructure of cornmeal extrudate. *J Cereal Sci,* 22, 185-194.

24. Altan, A., Mccarthy, K.L., & Maskan, M. (2009). Effect of Extrusion Cooking on Functional Properties and in vitro Starch Digestibility of Barley-Based Extrudates from Fruit and Vegetable By-Products. *J Food Sci,* 74, E77-E86.

25. Patil, R.T., Singh, D.S. & Tribelhorn, R.E. (1990). Effect of processing conditions on extrusion cooking of soy-rice blend with a dry extrusion cooker. *J Food Sci Tech,* 27, 376-378.

26. Koksel, H., Ryu, G.H., Basman, A. & Demiralp, H. (2004). Effects of extrusion variables on the properties of waxy hull less barley extrudates. *Nahrung Food,* 48, 19-24.

SYNTHETIC MILK: A THREAT TO INDIAN DAIRY INDUSTRY

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ABSTRACT

Synthetic milk is not milk but an artificial imitation of natural milk with a high degree of adulteration to increase the volume of milk and thereby the profit. Main components of synthetic milk are water, pulverized detergent or soap, sodium hydroxide, vegetable oil, salt and urea. Most of these components such as urea, neutralizers and detergents are very harmful to human health. Presently the preparation of synthetic milk is practiced at village level but it is steadily spreading to urban areas in various Indian states.

Keywords: Synthetic milk, adulteration, urea, health, testing methods

1. Introduction

India is the world's top milk producer with annual production of about 127 million tons (NDDB, 2013). But the phenomenon of synthetic milk makes this achievement worthless. Liquid milk is an essential nutritional food for infants as well as the aged. Adulteration of natural milk with a chemically synthesized milky liquid (synthetic milk) is a matter of serious concern. The dairy industry employs various tests such as determination of fat and total solids by chemical or physical analyses; estimation of sediment by forcing milk through filter pads and noting the residue left; determination of bacterial count, determination of freezing point etc. However, most of these measurements are expensive and time consuming, as the milk samples need to be taken to the laboratory for testing [1]. Synthetic milk is an excellent imitation of natural milk. Milk fat is mimicked by vegetable oil; the nitrogen component in milk is mimicked by urea; detergents are added to make it frothy [2, 3]. This mixture is so expertly prepared that the specific gravity of the concocted milk is the same as natural buffalo milk. This mixture is then mixed with natural milk in varying proportions. Such milk can be processed into ''value added'' products which bring in a substantial profit. Recent Indian Council of Medical research (ICMR) report has suggested that such adulterated items have a cancerous effect on the human system and can lead to gradual impairment of the body.

2. Natural and Synthetic Milk

Natural milk may be defined as the whole, fresh, clean, lacteal secretion obtained by the complete milking of one or more healthy milch animals, excluding that obtained within 15 days before or 5 days after calving or such periods as may be necessary to render the milk practically colostrum free, and containing the minimum prescribed percentages of milk fat and milk solids not fat [4]. Synthetic milk is not milk but it is entirely a different component with a high degree of adulteration to increase the volume of milk and ultimately the margin of profit. Generally it is a mixture of water, pulverized detergent or soap, sodium hydroxide, vegetable oil, salt and urea. The simplicity and rapidity with which milk can be adulterated always tempted the unscrupulous milk vendors to indulge in fraudulent practices and adulterate the milk. The ever-rising greed has given way to the development of a new type of adulterated milk known as synthetic milk. Similar to genuine milk production, the practice of preparing the synthetic milk too starts at the village level. The places notorious for the production of synthetic milk include parts of Rajasthan, Haryana, and Uttar Pradesh in India. Slowly but steadily the practice is spreading to other parts of India [5].

3. Differences between natural and synthetic milk

The most common differences in physical and chemical properties of natural and synthetic

milk are tabulated as Table 1. Synthetic milk when rubbed on the palm, foam formation noticed while natural milk when rubbed on the palm, it doesn't form foam. There is great difference in pH of both milks. Synthetic milk (pH 10-11) is highly alkaline in nature, while natural milk (pH 6.6-6.8) is somewhat neutral in nature. There is no change in color on heating in case of natural milk, while synthetic milk turns yellow on boiling. Natural milk remains white on storage but synthetic milk turns pale yellow after sometime. Nonetheless fats and solid not fat percentages are equal in both [5].

4. Components of Synthetic Milk

i) Water is a medium component used in the preparation of synthetic milk. All other components are mixed in water medium to get equivalent consistency and appearance like natural milk.**ii) Cane sugar** is added in synthetic milk to adjust the sweetness of milk and it is also added to mask the sour taste developed due to the acidity in stored milk. Existing chemical test for the detection of cane sugar in milk could detect as low as 0.1% cane sugar as adulterant [1]. **iii) Starch** is added in synthetic as well as natural milk to adjust and or to increase the consistency and viscosity. The existing test for the detection of starch in milk by iodine reagent was able to detect as low as 0.1% of starch as adulterant [1].**iv) Urea** is a source of nitrogen, thus, it is generally added in synthetic milk to increase its nitrogen content and hence the level of the protein in milk. Dimethyl amino benzaldehyde (DMAB) test for the detection of the urea in milk detects 0.1% urea as adulterant in milk. Natural milk also contains urea, so which also gives a faint yellow color when analyzed with DMAB test. The test, though rapid has one drawback that it shows color in control milk sample also. Thus, at lower level of urea adulteration, one cannot detect whether the positive test for urea is due to inherently present urea in animal milk or added urea in natural/synthetic milk.**v) Glucose** is also added in synthetic milk to increase sweetness. The existing test for the detection of glucose in milk could detect 0.5%. **vi) Neutralizers** are also added in synthetic milk to mask acidity. Milk turns acidic

when it is stored for a prolonged time. Upon storage for a long time, the lactose in milk is converted to lactic acid by the growth of bacteria. Such milk clots easily upon boiling and become unfit for consumption. Addition of neutralizers masks the developed acidity in milk. The existing Rosalic acid test for the detection of neutralizers in milk could detect as low as 0.1% sodium carbonate. **vii) Detergents** are added to make the milk frothy like natural milk. The existing test for the detection of detergents in milk could detect as low as 0.1% of adulterant.

5. Effect of Synthetic Milk Components on Human Health

i) Water is the chief adulterant used in milk. It not only decreases the density of milk but also diluted the desirable nutritional effect of all natural milk nutrients. If the milk used in the adulteration is contaminated it will lead to the harmful diseases like cholera, typhoid, shigella, polio, meningitis, and hepatitis A and E [6]. These are mainly caused by waterborne pathogens like protozoa, viruses and or bacteria, many of them are intestinal parasites [7]. Contaminated water based microbial diseases, their causes and symptoms are enlisted in Table 2. **ii) Cane sugar** imparts sweetness in synthetic milk. As such cane sugar does not have any ill effect on human health because it is used in very small quantity to imitate the natural milk sweetness. But poor quality of sugar in synthetic or adulterated milk can cause decreased whole someness of milk. Lactose found in natural milk does not contribute to diabetes but cane sugar does. **iii) Urea** is a organic water soluble molecule composed of carbon, nitrogen, oxygen, and hydrogen of chemical formula $CO(NH_2)_2$ and is also known as carbamide [8]**.** Urea is commonly added to increase the milk solid not fat (SNF) content or its total nitrogen content. Although, urea is naturally found in milk and contributes to the non-protein nitrogen (NPN) normally found in milk. When urea is added in milk with low fat and SNF, the milk appears thick and concentrated giving a feeling of rich milk and become poisonous (hazard toxic) due to the presence of excess urea [8, 9].

Table 1. Differences in physical and chemical properties of natural and synthetic milk

Properties	Synthetic Milk	Natural Milk
Physical		
Color	White	White
Odor	When freshly prepared, the mixture has a 'soapy' smell, which disappears on overnight storage at 4°C.	Not distinctive
Taste	Bitter	Palatable
Density	1.025-1.035	1.025-1.035
Storage	Turns yellowish after sometime	Curdling but no change in color
Texture	Give soapy feel when rubbed	No soapiness
Boiling	Becomes yellow	No change
Chemical		
Fat	4.5%	4.5%
pH	Highly alkaline, 10.5	Slightly acidic, 6.4-6.8
Urea test	Intense yellow color	Faint yellow color
Urea concentration	14 mg/ml	0.2-0.7 mg/ml
Sugar test (Resorcinol)	Positive	Negative
Neutralizers test (Rosalic acid)	Positive	Negative

Table 2. Microbial diseases and their symptoms from contaminated water

Disease	Microbial Agent	Symptoms
Amoebiasis	Entamoeba histolytica	Abdominal discomfort, weight loss, diarrhea, fever
Cryptosporidiosis	Cryptosporidium parvum	Loss of appetite, nausea, bloating, flu-like symptoms
Hymenolepiasis	Hymenolepis nana	Abdominal pain, anorexia, nervous manifestation
Botulism	Clostridium botulinum	Vomiting, diarrhea, double vision and respiratory failure
Cholera	Vibrio cholerae	diarrhea, nausea, cramps, nosebleed, rapid pulse, vomiting, and hypovolemic shock
E. coli infection	E. coli	Dehydration
Dysentery	Shigella and Salmonella	Blood in feces and vomiting of blood
Salmonellosis	Salmonella	Diarrhea, fever, vomiting and abdominal cramps.
Typhoid	Salmonella typhi	Fever, profuse sweating, diarrhea, spleen and lever enlargement
Hepatitis A	Hepatitis A Virus	Fatigue, fever, abdominal pain, nausea, diarrhea, weight loss, itching, jaundice and depression.
Polio	Poliovirus	Delirium, headache, fever, and occasional seizures, and spastic paralysis.

A recent Indian Council of Medical research (ICMR) report has suggested that urea adulterated item has a cancerous effect on the human system. **iv)Detergents** are added in synthetic milk preparation to develop froth and their emulsification action. Most of the detergent contains dioxane which carcinogenic in nature and can cause cancer on consumption. In addition to dioxane, other toxic ingredients present in detergents are sodium lauryl sulfate (SLS), nonylphenol ethoxylate, and phosphates [10]. Results from various studies shows that sodium lauryl sulfate in any form causes eye and skin irritation, organ toxicity, neurotoxicity, developmental and reproductive toxicity, endocrine disruption, mutations and cancer. Nonylphenol ethoxylate has been shown to cause kidney and liver damage, disrupted growth and metabolic development, decreased testicular growth and sperm count, and increased mortality. Phosphates, the main cleaning ingredients in detergents and household cleaners, can cause nausea, diarrhea and skin irritations. All these hazards makes detergents toxic and the synthetic milk containing these detergents become unfit for consumption [10]. **v) Neutralizers** are added to mask the developed acidity or bitter taste in synthetic milk. Among these neutralizers the most common is sodium hydroxide which can be very harmful if ingested. Its ingestion may result in a burning sensation, abdominal pain, shock or collapse [11]. Sodium carbonate also referred as soda ash or washing soda can cause severe health hazards if ingested. Sodium carbonate on ingestion may cause irritation along the digestive tract or stomach linings and may cause vomiting. Its ingestion may also cause diarrhea which may further result in frequent, loose bowel movements [12].

6. Conclusions

Synthetic milk is composed of the components such as urea, detergents and neutralizers which are very harmful or toxic in nature. Other components used in synthetic milk such as water, sugar and starch do not have severe health problems but their poor quality (food or microbial) may cause health problems. Regular intake of synthetic milk in place of natural cow/buffalo milk not only causes serious health problems but can also makes consumers deficient in the nutrients which are obtained from natural milk. There should be formation and implementation of strict food laws to restrict the preparation and distribution of synthetic milk or milk adulterated with harmful components.

References
1. Mudgil, D., (2008). Detection of adulteration in milk. MSc Thesis, India.
2. Paradkar, M. M., Singhal, R. S. & Kulkarni, P. R. (2000). An approach to the detection of synthetic milk in dairy milk: 1. Detection of urea. *Int J Dairy Tech*, 53, 87–91.
3. Paradkar, M. M., Singhal, R. S. & Kulkarni, P. R. (2008). An approach to the detection of synthetic milk in dairy milk: 4. Effect of the addition of synthetic milk on the flow behavior of pure cow milk. *Int J Dairy Tech*, 54, 36–37.
4. Sukumar, D., (1980). Outlines of Dairy Technology, Oxford University Press, India.
5. Bansal, P. & Bansal, N. (1997). Synthetic milk-Genesis, current status and options. *Curr Sci*, 73, 904-905.
6. Dziuban, E. J., Liang, J. L., Craun, G. F., Hill, V., Yu, P. A., Painter, J., Moore, M. R., Calderon, R. L., Roy, S. L. & Beach, M. J. (2006) Surveillance for Waterborne Disease and Outbreaks Associated with Recreational Water-United States, 2003–2004". MMWR Surveill Summ, 55, 1–30.
7. Nwachcuku, N. & Gerba, C. P. (2004) Emerging waterborne pathogens: can we kill them all? *Curr Opin Biotech,* 15, 175–180.
8. Ferguson, J. D. (2000). Milk Urea Nitrogen., Center for Animal Health and Productivity., New Bolton Center., 382 West Street Road, Kennett Square, PA 19348-1692, USA.
9. Renny, E. F., Daniel, D. K., Krastanov, A. I. & Zachariahl, C. A. R., Enzyme based sensor for detection of urea in milk. *Biotechnol & Biotechnol Eq*, 19**,** 198-201.

10. http://fdnmanager.com/laundry-detergents-pose-serious-health-risks/
11. http://www-group.slac. stanford.edu /esh/ eshmanual/references/hazmatGuideSodiumHydr oxi de. pdf

SENSORY, PHYSICOCHEMICAL AND ANTIMICROBIAL EVALUATION OF JAMS MADE FROM INDIGENOUS FRUIT PEELS

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ABSTRACT

Fruit peel waste are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies. The fruit peels are rich in nutrients and contain many phytochemicals; they can be efficiently used as drugs or as food supplements. The present study was carried out with the objective of producing jams from peels of indigenous fruits like orange, pineapple, pomegranate and banana and to assess the sensory, physiochemical and antimicrobial properties. Fruit peel pectin was extracted from four different indigenous fruits like pineapple (*Ananas comosus* L.*),* orange (*Citrus sinensis* L.), pomegranate (*Punica granatum* L.) and banana (*Musa balbisiana* Colla) and processed to make jams. Total soluble solids, acidity, pH, and moisture were analyzed by the standard methods of AOAC. Sensory evaluation was conducted using a five point hedonic scale. The antimicrobial potency of the peel jams was studied using disk inhibition method. Results indicated that the mean Brix was 68.5, while pH ranged from 4.4 – 5.9, this would hinder microbial growth and maintain keeping quality of Jams. Pineapple peel jam was most acceptable by the panel. Pomegranate peel jam should highest antimicrobial activity against Shigella. Utilization of fruit peels will improve the nutritional status, broaden the food base, raise standards of living and provide opportunities for income generation.

Keywords: *Orange, Pineapple, Pomegranate, Banana, Shigella*

1. Introduction

Food industry produces large volumes of wastes, both solids and liquid, resulting from the production, preparation and consumption of food. These wastes lead to increasing waste disposal and can pose severe pollution problems and represent a loss of valuable biomass and nutrients [1]. Waste utilization in fruits and vegetable processing industries is the one of the important and challengeable job around the world. It is anticipated that the discarded fruits as well as weight materials could be utilized for further industrial purposes [2]. Considering the challenges in the area of food industry, efforts are to be made to optimize processing technologies to minimize the amount of waste. Processing of fruits produces two types of waste - a solid waste of peel/skin, seeds, stones and a liquid waste of juice and washes waters. In some fruits the discarded portion can be very high (e.g. mango 30-50%, banana 20%, pineapple 40-50% and orange 30-50%). Fruit peels are rich in health-promoting flavonoids and aroma compounds [3]. Possible products that can be considered from fruit waste are as candied peel, oils, pectin, reformed fruit pieces, enzymes, wine and vinegar [4]. Each of the above uses for fruit

waste requires: a good knowledge of the potential market for products and of the quality standards required, a careful assessment of the economics of production, a certain amount of additional production knowledge, a certain amount of additional capital investment in equipment, a fairly large amount of waste to make utilisation worthwhile. Besides, it finds numerous applications in pharmaceutical preparations, pastes and cosmetics. All these combined efforts of fruit waste minimization during the production process, environmentally friendly preservation of the fruit peel, and utilization of fruit waste by-products would substantially reduce the amount of fruit waste, as well as boost the environmental aspect of fruit processing industry [5]. Fruit peel waste are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies. There is always an increased attention in bringing useful products from waste materials and fruit wastes are no exceptions [6]. The fruit peels are rich in nutrients and contain many phytochemicals; they can be efficiently used as drugs or as food supplements too. Since there is an increase in the number of antibiotic resistance pathogens, there is always a search of an

alternative drug that is regarded as safe. Fruit peels if proved to have antibacterial activity; they can be also used in same food industry which generates large peel wastes as a food preservative [7]. Thus the present study was carried out with the objective of producing jams from peels of indigenous fruits like orange, pineapple, pomegranate and banana and to assess the sensory, physiochemical and antimicrobial properties.

2. Materials and methods
2.1. Formulation of Jams from Fruit Peel
Fruit peel pectin was extracted from four different indigenous fruits like pineapple (*Ananas comosus* L.), orange (*Citrus sinensis* L.), pomegranate (*Punica granatum* L.) and banana (*Musa balbisiana* Colla) and processed to make jams. Ripe fruits were selected from local market and cleaned. Peels were removed from the fruit, soaked overnight in clean water and ground using grinder with the soaked water. Ground peels were cooked with sugar in a high flame. When sugar dissolved completely, citric acid was added and stirred. The jam is ready when bubbles form at the sides of the vessels. The hot jam was poured in clean, dry, wide-mouthed jars or bottles and cooled well. Total soluble solids, acidity, pH, and moisture were analyzed by the standard methods of AOAC [8].

2.2. Total Soluble Solids
The jam was weighed into the tarred beaker to the nearest 0.01 mg, a suitable quantity (up to 40 gm.) of the sample and added 100 – 150 ml of distilled water. The contents were heated with the beaker to boiling and allowed to boil gently for 2-3 minutes, stirring with a glass rod. The contents were cooled and mixed thoroughly. After 20 minutes weighed to the nearest 0.01gm, then filter through a fluted filter paper or a Buchner funnels into a dry vessel. The filtrate was used for the determination of direct reading of the soluble solids content on the refract meter [9].

2.3. Titratable acidity
About 1 g of the sample was taken, diluted to 20ml with distilled water titrate with 0.1 N NaOH using 0.3 phenolphthalein for each 100 mL

of the solution to pink end point persisting for 30 seconds [10]. The report acidity was as ml.0.1 N NaOH per 100g. Titrable acidity can be calculated as in equation:

$$Ta = \frac{B \cdot 0.1 \cdot 0.064}{W}100 \qquad (1)$$

where: Ta is titrable acidity, ^{o}T; B is reading burette, mL; W is weight of sample, g

2.4. *pH value*
The pH value of the sample was measured with a digital glass electrode pH meter (CD 175 E) at room temperature, which was calibrated prior to sample pH measurement using buffer solutions of pH value 4.0 and 7.0 [9].

2.5. *Moisture*
The gravimetric method by AOAC [10] was used for this analysis. Exactly 2ml of the sample was measured each into 2 previously weighed moisture crucible. The crucibles and samples were allowed to dry in a hot air electric oven at 105ºc for 2 hours at the end of the time; the crucibles were carefully removed and kept to cool in a desiccator. The crucibles and the samples were re-weighed and put back into the oven for further drying; cooling and weighting were done respectively until a constant was obtained.

$$M = \frac{W_2 - W_3}{W_2 - W_1}100 \qquad (2)$$

where: M - moisture content, %; W_1 - weight of empty containers, g; W_2 - weight of container and sample before drying, g; W_3 - weight of the container and sample after drying, g

2.6. *Sensory Evaluation of the Jams*
Sensory evaluation was conducted by thirty panel members using a five point hedonic scale to evaluate the organoleptic properties like appearance, color, taste, texture, flavour and acceptability of fruit peel jams.

2.7. *Assessment of antimicrobial activity*
The antimicrobial potency of the orange peel jam, pineapple peel jam, pomegranate peel jam and banana peel jam was studied using disk

inhibition method. In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacteria and fungi to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvents (negative controls) 100% pure jam (100mg/ml) is placed on the plates. After incubation at 37˚C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks, streptomycin and Amphotericin. Interpretation of inhibition zones of test cultures was adopted from Johnson and Case [11]. Diameter zone of inhibition of 10 or less indicates test product being resistant to test organism, diameter zone of inhibition of 11 to 15 indicates test product being intermediate resistance to test organism, diameter zone of inhibition of 16 or more indicates test product being susceptible resistance to test organism.

2.8 *Statistical Analysis*

All the experiments were done in triplicates. The data obtained were statistically analyzed using SPSS Version 17; Chicago, Inc. Sensory evaluation was analyzed using one-way ANOVA followed by Duncan's Multiple Range Test. The type I error rate was a P value of <0.05, was considered statistically significant for all statistical tests conducted.

3. Results and discussion
3.1 *Physicochemical properties of fruit peel jams*

The physicochemical properties of the fruit peel jams are presented in Table 1. Physicochemical characteristics indicate that the highest Brix was observed in orange peel jam (85) and the lowest was in pomegranate peel jam (57). The sugar (Brix) present in jam comprises natural and added sugar and is an important preservative. The proportion of sugar to fruit varies according to the type of fruit and its ripeness, but a rough starting point is equal weight of each [12]. Orange peel jam had the highest (0.47%) and pomegranate and banana peel jam had the lowest (0.16%) value for acidity. The highest and the lowest pH values were recorded in banana peel jam (5.94) and

pomegranate peel jam (4.48) respectively. Variation in acidity among different fruits might be due to the activity of citric acid glyoxalase during ripening process which leads to the degradation of citric acid [13].Moisture content was found to be highest in pineapple peel jam (62.6%) and lowest in banana peel jam (31.9%). Moisture has a great impact on the shelf life of products. The Brix value of 68.5 + 0.71 and pH 3.44 in the jam conforms to values recommended for jam to hinder microbial growth and maintain keeping quality [14].

3.2 *Sensory Evaluation*

Sensory evaluation of fruit peel jam is presented in Figure 1, ANOVA indicates that there is a significant variation in the sensory attributes of fruit peel jams (p<0.001). The appearance, color, taste, texture, flavours and overall acceptability of pineapple jam was significantly higher compared to the other jams. The yellow colored shell of pineapple fruits was found to be relatively rich in carotenes and flavones, while the green leaves contained in addition to chlorophyll even larger concentrations of flavones, carotenes and xanthophylls than were found in the fruit flesh [15]. HRGC-MS analysis of flavour of fresh-cut pineapple fruit revealed the known prevalence of esters, with methyl 2-methylbutanoate, methyl 3-(methylthio)-propanoate, methyl butanoate, methyl hexanoate, ethyl hexanoate and ethyl 3-(methylthio)-propanoate, as well as 2,5-dimethyl-4-methoxy-3(2H)-furanone (mesifurane) and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) as major constituents [16].

Pineapple waste is a by-product of the pineapple processing industry and it consists of residual pulp, peel and skin. These wastes can cause environmental pollution problems if not utilized [17]. Pineapple is mainly valued for its pleasant taste and flavor. Pineapple fruit is a good source of Bromelain, a digestive enzyme with biological functions i.e., a non toxic compound have a number of potential therapeutic applications, including treatment of trauma, inflammation, autoimmune diseases, enhancement of immune response, and malignant disorders [18, 19].

**Table 1.** Physicochemical characters of Fruit Peel Jams

Sample	Total Soluble Solids (ºBrix)	Acidity (%)	pH	Moisture (%)
Pineapple Peel Jam	77	0.25	4.91	62.6
Orange Peel Jam	75	0.47	5.64	32.25
Pomegranate Peel Jam	65	0.16	4.48	43.23
Banana Peel Jam	57	0.16	5.94	31.9

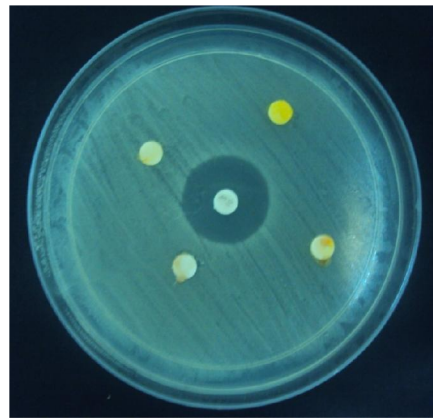**Table 2.** Antimicrobial Activity of Fruit Peel Jams

Organism	Fruit Peel Jams				Control
	Pineapple	Orange	Pomegranate	Banana	
	Zone of Inhibition (mm)				
Escherichia coli	6mm	6mm	6mm	6mm	24
Staphylococcus aureus	6mm	6mm	6mm	6mm	24
Salmonella typhi	6mm	6mm	6mm	6mm	16
Shigella	6mm	6mm	**12mm**	6mm	24
Candida albicans	6mm	6mm	6mm	6mm	20
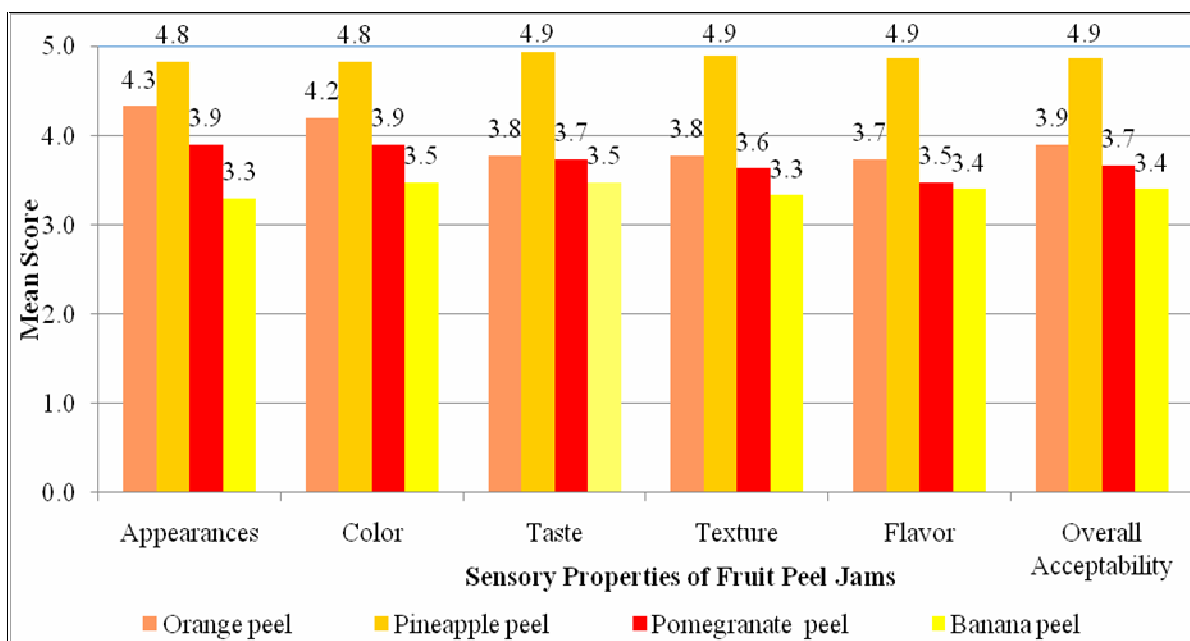
Figure 1. Sensory Evaluation of Fruit Peel Jams
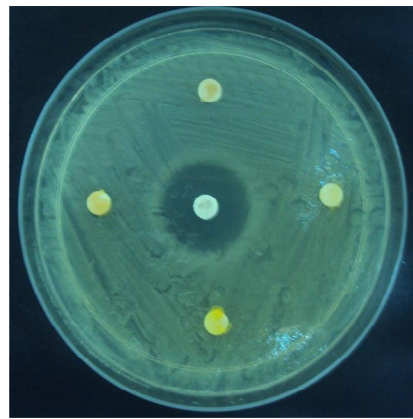
Escherichia coli

Staphylococcus aureus
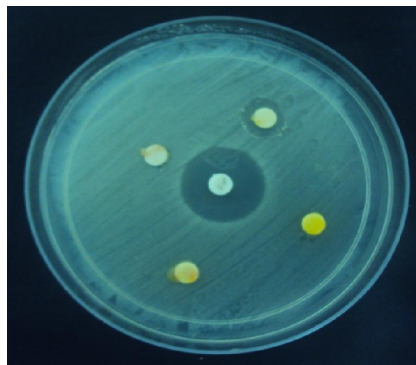
Shigella
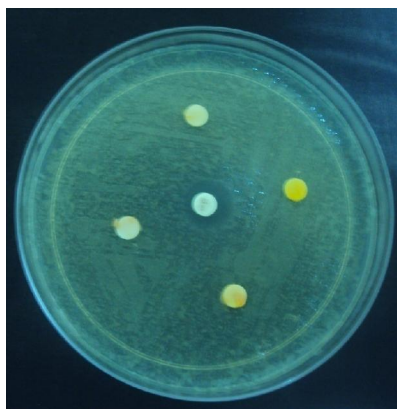
Salmonella typhi
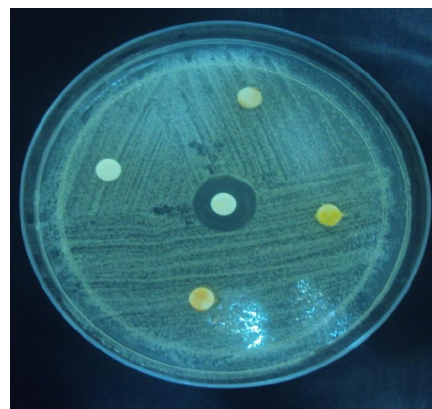
Candida albicans

Figure 2. Zone of inhibition produced by Fruit Peel Jams against Test microorganisms using Agar Well Diffusion Method

3.3 *Antimicrobial Activity of Fruit Peel Jams*

As seen in Table 2 and Figure 2, antimicrobial activity of jams reveals that the zone of inhibition produced by pomegranate (12mm) against Shigella exhibited an intermediate resistance (11-15mm) against the test organism. Valeria and coworkers [20] assessed in vitro antimicrobial activity of Aqueous Extracts from pomegranate peel (*Punica granatum L.*) against clinical isolates of *Shigella* and found that pomegranate peel showed highest inhibition against *Shigella*. Phytochemical analysis revealed the presence of active inhibitors in the peel including phenolics and flavonoids. The activity was related to the higher content (46%) of total phenolic compounds. Many other researchers also found that pomegranate peel extract proved to be the potent antibacterial agent against *Shigella flexneri*. The antibacterial activity of the pomegranate peel extracts might be due to the presence of various phytochemicals such as phenolic punicalagins, gallic acids, catechin, quercetin and retin [21, 22].

4. Conclusion

Results indicate that overall acceptability of pineapple peel jam was significantly higher compared to the other fruit peel jams. Pomegranate peel jams exhibited antimicrobial activity against *Shigella.* Pomegranate peel extracts have great potential as antibacterial compounds against *Shigella*, and they can be used in the treatment of infectious diseases caused by resistant microbes. Indigenous fruits form a significant part of the diets and livelihoods of most rural Indian people. In India, processing and utilization of indigenous fruits at both household and commercial levels has been very limited. There are numerous ways of utilizing and processing indigenous fruits at household and industry level. Utilization of fruit peels will improve the nutritional status, broaden the food base, raise standards of living and provide opportunities for income generation.

5. References

1. Vasso O, Winfried. R, (2007) Utilization of By-Products and Treatment of Waste in the Food Industry, Integrating Food Science and Engineering Knowledge Into the Food Chain, Vol. 3**,** Springer.
2. Atul Upadhyay, Jeevan P. Lama, Shinkichi Tawata, (2010) Utilization of Pineapple Waste: A Review. *J Food Sci Tech*, 6, 10.
3. Paranthaman,R., R. Vidyalakshmi, S. Murugesh and K. Singaravadivel (2009) Effects of Fungal Co-Culture for the Biosynthesis of Tannase and Gallic Acid from Grape Wastes under Solid State Fermentation Global. *J Biotech Biochem*, 4 (1): 29-36.
4. Singh M., (2007) Extraction of pectin from kinnow peels. *Int J Environ Stud*, 64, 3.
5. Pap N, Pongrácz E, Myllykoski L & Keiski R. (2004) Waste minimization and utilization in the food industry: Processing of arctic berries, and extraction of valuable compounds from juice- processing by- products. In: Pongrácz E. (ed.): Proceedings of the Waste Minimization and Resources Use Optimization Conference. June, University of Oulu, Finland. Oulu University Press: Oulu. 159-168.
6. Apsara Madhav and P. B. Pushpalatha, (2002) Quality Degradation Of Jellies Prepared Using Pectin Extracted From Fruit Wastes. *J Tropical Agri*, 40, 31-34
7. Kumar, K. Ashok Narayani, M. Subanthini, A. Jayakumar, M. (2011) Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels -Utilization of Fruit Waste International. *J Eng Sci Tech*, 3, 6, 5414.
8. AOAC (1990). Association of Official Analytical Chemist. Methods of Analysis. Washington, DC.
9. Ranganna S (1986) *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*. 2nd ed. Tata McGraw Hill Publishing Company Ltd., New Delhi, India. p.12.
10. A.O.A.C (2000), Official method 942.15 Acidity (Titratable) of fruit products with AOAC official method 920. 17th Edition, Washington, DC.
11. Johnson, T. & Case, C. (1995). Laboratory Experiments in Microbiology. Brief Edition,

4th ed. Redwood City, CA: Benjamin/ Cummings Publishing Co.

12. Eke-Ejiofor. J, Owuno. F. (2013) The Physico-Chemical and Sensory Properties of Jackfruit (Artocarpus Heterophilus) Jam. *Int J Nutri Food Sci*. 2, 3, 149-152.

10. Rathore H.A., Masud T. Sammi S. and Soomro AH. (2007) Effect of storage on physiochemical composition and sensory properties of mango variety Dosehar. *Pak.J. Nutrition*, 6, 143-148.

11. Aina JO and AA Adesina (1999) Suitability of Frozen Indigenous Tropical Fruits for Jam Processing. *Adv Food Sci*, 2, 15 – 18

12. Magistad, O. C. (1995). Carotene and Xanthophyll in Pineapples. *Plant Physiol*, vol. 10. 187-191.

13. Elss, S., Preston, C., Hertzig, C., Heckel, F., Richling, E. and Schreier, P. (2005). Aroma profiles of pineapple fruit (Ananas comosus [L.] Merr.) and pineapple products. *LWT - Food Sci Tech*, 38 (3), 263-274.

14. Rajalakshmi Madurai, Sheila John and Bhavani I.L.G. (2010) Study on preparation, nutrient analysis and shelf life of biovinegar and its formulations. Biosci Biotech Rese Asia, 7(2).

15. Maurer, H.R. (2001) Bromelain: biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences*, 58(9), 1234-1245.

16. Orsini, Roger A. (2006) Bromelain. *Plastic and Reconstructive Surgery*, 118(7), 1640-1644.

17. Valeria I. Ruiz Parra, Cristina Gaudioso, Marta Cecilia and Clara Silva, (2011). Evaluation of the Antimicrobial Activity of Aqueous Pomegranate (*Punica granatum L.*) extract against *Shigella*. *Res J Biol Sci*, 6, 205-212.

18. Janani J and Estherlydia D (2013) Antimicrobial Activities Of *Punica Granatum* Extracts Against Oral Microorganisms. *Int. J. Pharm Tech Res*, 5(3).

19. Vijayanand S, Hemapriya J (2011). In vitro antibacterial efficacy of peel and seed extracts of *Punica granatum L.* against selected bacterial strains. *Int. J. Microbiolog. Res.* 1(4), 231-234.

MILES OF A FOOD BASKET

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ABSTRACT

"Food miles" is a term that is used to draw attention to the ecological cost of how far food travels from producers to consumers. Transportation is an important factor in evaluating the environmental impact of food. The concept of "food miles" presents an argument to buy goods which have traveled the shortest distance from farm to table, and to discriminate against long-haul transportation, especially air-freighted goods. The long-distance transport of food is associated with additional emissions due to increased transportation coupled with greater packaging, as well as negative impacts on local rural communities, and a disconnection between the public and local farming. Furthermore, "food miles" encapsulates the carbon emission that may have a profound effect on climate change. In light of growing concern over the speed and scale of climate change, the concept of "food miles" has captured public attention and apparently is changing some consumers' behavior, although only around one-third of shoppers know of the concept. This paper presents an overview on food miles with a focus on its carbon foot print.

Keywords*: Food miles, transportation, carbon emission, climate change, carbon foot print.*

1. Introduction

'Food miles' is a relatively recent issue which has arisen over food transportation. A simple definition of this concept would be: 'the number of miles (kilometres) a product has to be transported from the farmer/grower to various stages of production until it reaches the supermarket and finally the plate of the consumer [1].

Figure 1. Food miles

It has been born out of concern for the environment, especially in regard to greenhouse gas emissions such as carbon dioxide and the global warming. The argument is that the longer the transport distance (food miles),more energy is consumed, more fossil fuels are burned and consequently more greenhouse gases are released into the air, which cause global warming [1].

The term "food miles" was first used by a UK report in 1994 to highlight the environmental and social impacts caused by the increasing distances that food was travelling. It has been widely adopted by local food movements and actively used to promote the widespread growth of initiatives such as farmers' markets [2].

Regardless of whether the term is a valid measure, "food miles" is being used more and more by buyers, retailers and consumers while making purchasing decisions. It's even beginning to appear in government policies. The implications for exporters is a potential reduction in access to overseas markets due to perceived higher costs, carbon taxes based on freight tonnage, and higher landing fees. Interestingly,

"food miles" is commonly used as a surrogate measure for greenhouse gas (GHG) emissions from food sources [2].

Figure 2. Miles of a food basket
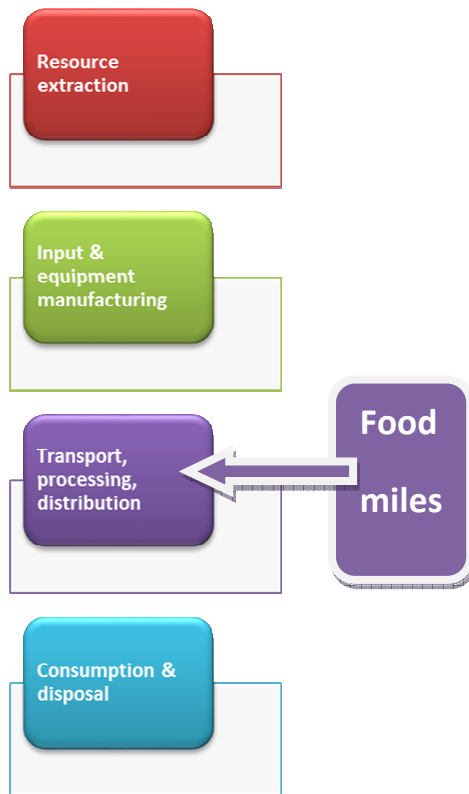
Figure 3. Life cycle of food and the context of food miles [3]

2. Food Distribution systems

The three food distribution systems are represented in Figure 4.

Communal Farm and Garden
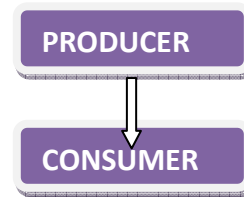
Farmers Market
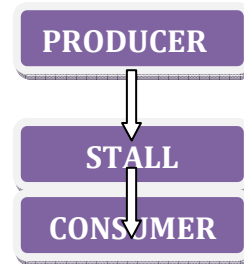
Supermarket
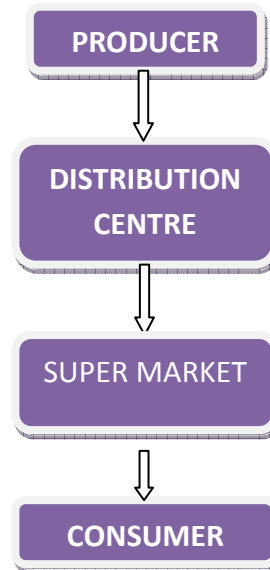
Figure 4. Travel steps in food distribution systems [4]

3. Food: The weary traveler

The days of only eating what is in season have long passed and people expect to consume the same foods all year round. This necessitates importing foods that are out of season.

It is processed food that particularly clocks up the miles, with ingredients that have been sourced from all over the world. The country of origin is usually in tiny print on the

back or underneath but most people don't take the time to read all the detail on the labels.

Figure 5. Food: The weary traveler

For instance, 'Made in Australia' doesn't mean buying a totally Australian product either. In fact, many 'Australian made' foods are made from entirely imported ingredients. These days, people are extremely time poor and increasingly reliant on packaged and convenience foods, which clock up food miles and greenhouse emissions from all the packaging and processing. Organic food also does a lot of travelling and up to 70% of food in organic stores is imported [4].

4. Causes for the increase of food miles

The four most striking changes in the food production and supply that have greatly increased food transport are as follows [5]:

It is possible to calculate the number of food miles for value-added products. The simplest calculation can be done when there is only one location where the products are produced and only one location where the products are sold. The food miles in this situation would equal the distance between the production location and the sales location [6].
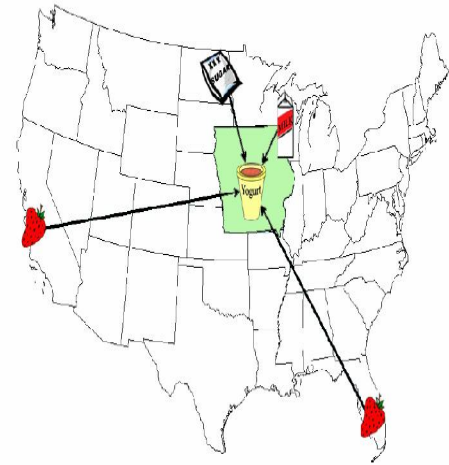
Figure 6. Food miles for a multiple ingredients food product

The calculation gets slightly more complicated when there is more than one production or sales location. In this case, the food miles can be calculated using a Weighted Average Source Distance (WASD) formula. The formula gives an average distance based on the amount of the product transported from each production location to each sales outlet [6]. The formula for WASD is:

$$WASD = \frac{\Sigma\,(m(k)*d(k))}{\Sigma m(k)}$$

where: k = different locations of the production origin, m = amount consumed from each location of consumption origin, d = distance from the locations of production origin to the point of consumption.

5. Calculation of Food miles

Figure 7. Miles traveled by produce to Reach Chicago Market**.**
(Source: Leopold Center for Sustainable Agriculture)

Information for this chart is based on the weighted average source distance, a single distance figure that combines information on distances from production source to consumption or purchase endpoint. Distances from production origin to Chicago were estimated by using a city located in the center of each state as the production origin, and then calculating a one-way road distance to Chicago using the Internet site Mapquest (mapquest.com). [7]

6. Food miles a cause for concern [1]
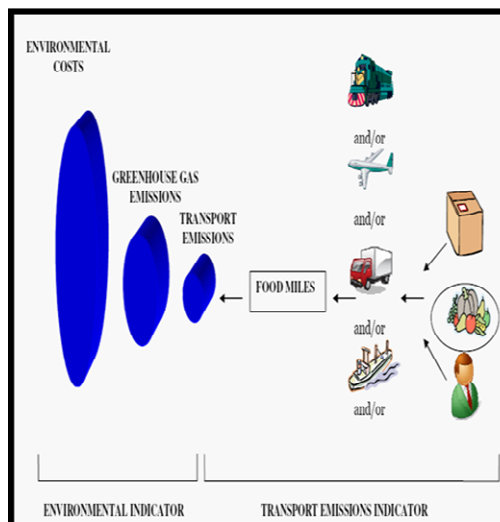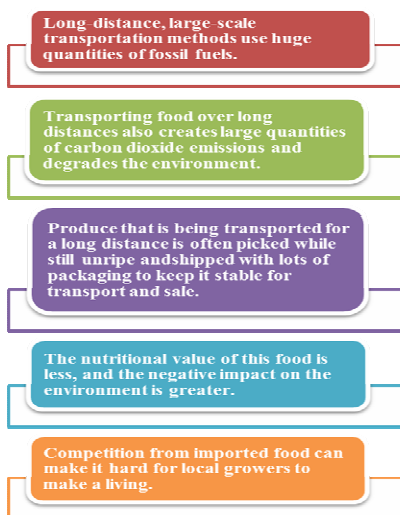
Figure 8. Food miles as an indicator – a critical evaluation of limitations

7. Food miles and carbon footprint

Food miles refer only to the transport aspect of food products which is a narrowly defined system and only a small contribution to the overall carbon footprint of the product. A 'carbon footprint' is a measure of the greenhouse gas emissions associated with an activity, group of activities or a product [8].Helen Smith defined carbon footprint as a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or is accumulated over the life stages of a product [3].


Figure 9. Carbon foot print

A wider system boundary incorporating the entire life cycle from its production,

distribution, consumption and disposal is a more holistic way to determine the carbon intensity of food.

Figure 10. Carbon Footprint on Earth

A carbon footprint analysis can evaluate any of the life stages in the food production but to ensure a holistic understanding all stages should be evaluated, similarly to a Life Cycle Assessment [3]. 'Carbon footprint' has become a widely used term and concept in the public debate on responsibility and abatement action against the threat of global climate change. It had a tremendous increase in public appearance over the last few months and years and is now a buzzword widely used across the media, the government and in the business world [9].
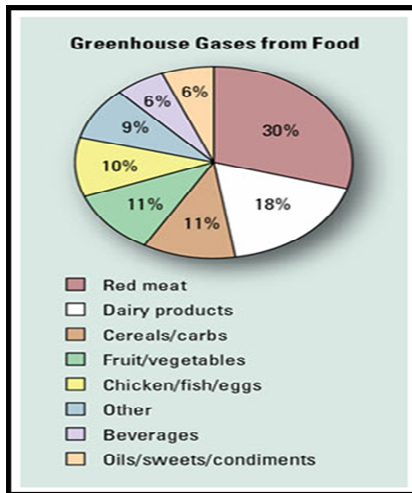

Figure 11. Greenhouse gases from foods [10]

The food miles concept is used as a metaphor to contrast the differing spatial nature of local and global food supply and distribution systems. However food miles are a small part contained within a carbon footprint .It is essential that carbon footprinting incorporates life cycle thinking in order to identify carbon hotspots in the life cycle of food. The carbon footprint is a vital tool to provide climate relevant information to enable consumer behavioural adjustment.

However a carbon footprint is only one environmental indicator solely focused on climate change and this weakness becomes the strength of a holistic Life Cycle Assessment which utilises several environmental indicators. Nonetheless whether it is food miles, carbon footprint or any other environmental indicator undertaken in scientific studies they are all necessary steps towards enabling achievement of sustainability and ensuring global food security [3].
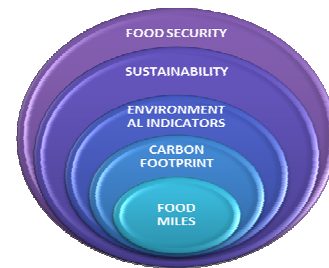
Figure 12. The wider context of food miles and carbon footprint [3]

8. Food miles: complexities and trade-offs

The relationship of food transport to overall sustainability is complex. Transport of food has significant direct environmental, economic and social impacts. Therefore, in like for like systems, where food supply chains are identical except for transport distance, reducing food transport will improve sustainability. However, differences between food supply systems often involve trade-offs between various environmental, social and economic effects. These must be taken into account when designing an indicator to measure the impacts of food transport, and when formulating associated policies. Some of the main issues are summarised below [11].

Figure 14. Food Transport
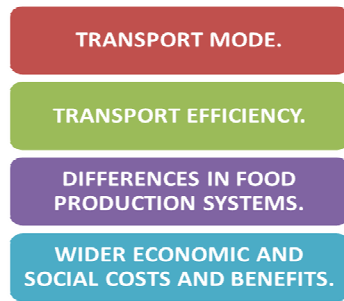
8.1 Transport mode:

The impacts of food transport are highly dependent on the transport mode. Air transport has a very high climate change impact per tone carried, whereas sea transport is relatively efficient. Transport by HGV (Heavy Goods Vehicle) accounts for most of the infrastructure (road maintenance), noise and air pollution costs, yet shopping for food by car accounts for a high proportion of the congestion and accident impacts [11].
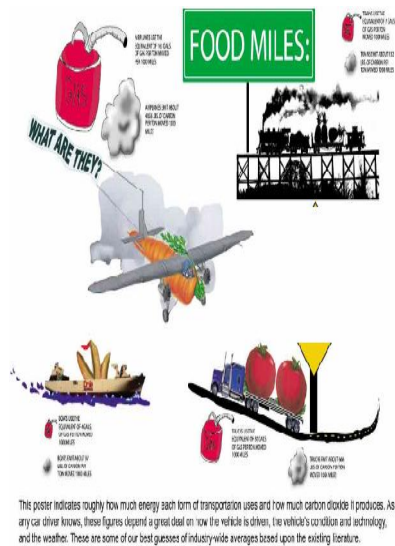

Figure 13. Modes of transportation

8.2 Transport efficiency

There is a trade-off between transport distance, vehicle size and transport efficiency. The current dominant system of food supply in the UK involves large HGVs traveling long distances between suppliers and shops via centralised distribution centres.

However, this system enables very efficient loading of vehicles, which reduces the impacts per tonne of food. More local sourcing can greatly reduce the distance travelled by food, but the reduction in transport impacts may be offset to some extent by the use of smaller vehicles or lower load factors [11].

8.3 Differences in food production systems

The impact of food transport can be offset to some extent if food imported to an area has been produced more sustainably than the food available locally. For example, a case study showed that it can be more sustainable (at least in energy efficiency terms) to import tomatoes from Spain than to produce them in heated greenhouses in the UK outside the summer months. Another case study showed that it can be more sustainable to import organic food into the UK than to grow non-organic food in the UK. However, this was only true if the food was imported by sea, or for very short distances by road [11].

8.4 Wider economic and social costs and benefits

The term "food miles" has come to signify more than the transport of food and the direct physical impacts of this transport. A number of other economic and social issues are bound up in the food miles debate. It is clear that transport and trade of food has the potential to lead to economic and social benefits, for example through economic gains for both developed and developing nations, reduced prices for consumers and increased consumer choice. However, the

realisation of these benefits depends on a number of complex political, social and economic factors, such as global trade rules and trends in commodity markets. At the individual level, food trade and the consolidation of food supply chains can lead to both winners and losers [11].

9. The long and short of food miles

Recent decades have seen a flourishing of the local foods movement, and thankfully so – the environmental and social benefits of eating close to home are many [12].

The average meal travels 4,200 miles en route to the store, and this journey is powered almost entirely by fossil fuels. But of those food miles, only a quarter are "final delivery" from farm or factory to retailer, while three quarters are delivery of inputs like fertilizer or raw ingredients. 6 Food groups vary in their average travel distances from a low of fruit (at 1,265 miles) to a high of red meat (at 13,273 miles) [12].
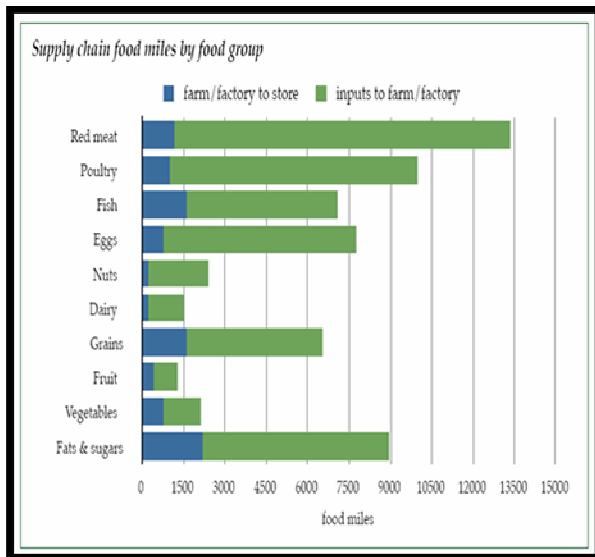
Figure 15. The long and short of food miles [12]

10. Reducing Food miles for a greener life

The underlying concern of the food miles 'debate' is that transporting food requires energy, which (predominantly) comes from fossil fuels, and the burning of fossil fuels releases carbon dioxide into the atmosphere which is a cause of global warming [13]. Proponents of reducing food miles often suggest that buying local food will reduce the amount of energy involved in the transportation process, as food sourced locally travels shorter distances.

For consumers, convenience and cost are often driving factors when purchasing food. Consumers who are reducing their food mile footprint: [7]

The choices consumers make, however, can have a great deal of influence on the direction in which food system is headed. Reducing the energy intensiveness of food has

several economic, social and environmental benefits [7].

The benefits that could be realised by reducing food miles are as follows: [7]

- Lower transport costs
- Shorter, faster reacting supply chains
- Improved customer service
- Satisfying increasing customer demand for local produce.
- Increased shelf life
- Closer connection between producer and end use

12. Outlook

Food miles are a growing cause of concern due to the greenhouse gas emissions released through the transportation of foods and rightly so, as food miles consume a considerable amount of energy. Other important issues include the mode of transportation,the production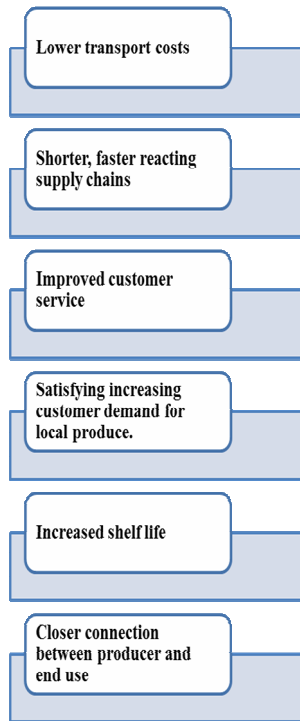 method, and packaging considerations, as well as personal dietary choices. Growing consumer interest in local and regional foods is creating new marketing opportunities and new possibilities for partnerships with agricultural producers. Each consumer's food decision provides an opportunity to make a difference (large or small) in the way energy is used and greenhouse gases are emitted.

Local and organic foods may or may not be good indicators of low climate impact, although they do support the health of the communities. When it comes to climate change, a stronger focus on food life cycle energy use is the key to a smaller footprint.

References

1.Saunders, C. (2006). Food Miles– Comparative Energy/Emissions Performance of New Zealand's Agriculture Industry, ISSN 1170-7682.
2.Scholz, Al. (2011) Food miles: What does it mean for farmers , The farm journalist.
3.Smith, H. (2010). Food for thought: Food miles and carbon footprint of a food basket in the Northern Rivers region. .
4. Hay, J. (2010). *Food: The Weary Traveller*, 10.
5. Mae-Wan Ho, Gala, R. (2005). Food miles and Sustainability, ISIS Report.
6. Bruch, M.L. (2003). Marketing and the Food Miles Concept, Agricultural extension service.
7.Hill, H. (2008). NCAT Research Specialist, Food Miles: Background and Marketing.

8.Edinburgh Centre for Carbon Management *What is a Carbon Footprint,* The Swedish Forest Industries Federation and Timcon, 2007

9. Wiedmann, T., Min, J. (208). *A Definition of 'Carbon Footprint',* ISAUK Research Report.

10.Whitty, J. (2008). Food Miles & Your Carbon Footprint.

11.Final Report produced for DEFRA, The Validity of Food Miles as an Indicator of Sustainable Development (2005).

12.The American Carbon Foodprint, Brighter Planet (2010).

13.The fallacy of food miles, Agro-ecological investment management.

Book review

FOOD PACKAGING – PRINCIPLES AND PRACTICE – 3rd **Ed. By Gordon L. Robertson**
CRC Press, Taylor & Francis Group, New York 2013, 703 pages, ISBN 978-1-4398-6241-4

This substantial book present a comprehensive work and accessible discussion in food packaging domain including information from the fields of chemistry, microbiology, food technology, engineering, packaging principles and their application. In twenty two chapters readers can discover information about main types and proprieties of packaging materials : plastic polymers, biobased and biodegradable packaging materials, thermoplastic polymers, paper and paper based packaging materials, metal packaging materials, glass packaging materials, printing processes, deteriorative reaction in foods, aseptic packaging of foods, active and intelligent packaging, packaging for flesh foods, for horticulture products, for dairy products, packaging of cereals, snack foods, package of beverages, legislative and safety aspects of food packaging.

Plastic packages present classification of polymers, chemical process of polymerization and chemical structure of different type of plastics. Each type of plastics is described at the level of monomers, each substance which compose the plastic compound is presented at level of chemical structure and physical proprieties. An important part is reserved for additives in plastics a new technology used in food packages.

Traditional food packaging, except paper products, are made from nonrenewable materials. Modern technology try to use new materials obtain from biomass and biodegradable resources named bioplastics. The advantages of bioplastics over traditional petrochemical polymeric packaging consists: bioplastics can be consumed with packages product, contribute to the reduction of environmental pollution, enhance the organoleptic properties of food packaged, they can function as antioxidant and antimicrobial agents. There are presented a variety of polysaccharides, proteins, lipids, isolated from plants or animals with active role in production of bioplastics.

Optical and mechanical properties of thermoplastic are described with mathematic formulas and lot of suggestive graphics. The reader can understand each physic property by lot of information from tables and graphics. Processing and converting of thermoplastic polymers shows modern methods of obtaining polymers package: extrusion, calendering, coating and laminating, blending, vapor deposition, nanocomposites, orientation of polymer films, microperforation. All processes to obtain thermoplastic polymers are shown in intuitive schemes which allow understanding of technology.

Paper and paper base packaging materials are presented start from production of pulp, than chemical processes used in manufacture of paper, conversion of paper in paper products packaging. Four metals are commonly used for packaging foods: steel, aluminum, tin and chromium. All of this are used in manufacture of tinplate, aluminum cans, protective coatings, foils and containers. Chemical structure of metal packages are susceptibility to corrosion. Corrosion are accelerate by acidity, pH, sulfur compounds, nitrates, phosphates, effects of processing and storage,

Glass packaging materials have an adequate coverage including glass composition and structure, physical properties (mechanical, optical, thermal) of glass, manufacture technology and glass container design.

A special part is covered by materials use in label manufacturing. Are presented printing techniques: gravure, lithography, digital, ink-jet electrophotography, type of ink used in food packaging classified by type

of packaging and label classification. An important part of book are dedicated to deteriorative reaction record in foods. There are presents enzymatic reactions, sensory, nutritional, chemical degradation, physical changes, biological changes with microbiological and macrobiological modification. All of the transformations are followed by food preservation and processing.

A lot of recent developments in food packaging are described in aseptic packaging of foods, packaging of microwavable foods and active and intelligent packaging present useful information in active packaging : gas absorber, ethanol emitters and moisture absorbers and intelligent packaging by product quality sensors : freshness indicators, temperature-time indicators, biosensors. Modified atmosphere packaging providing information about gases used in Map, methods to create MA conditions, application of MAP in food technology.

Next five chapters covering packaging by group of foods depends the technology. Each chapter described packaging of flesh food – sensory characteristics of red meat, microbiology, lipid oxidation , vacuum techniques to package of fresh meat, MAP in fresh meat, packaging of poultry, seafood and cooked meats; packaging of horticulture products : metabolic process in post-harvest phases, MAP use on fresh fruits and vegetables, other packagers techniques used on horticulture products; package in dairy products: problems appear on pasteurized and UHT milk, package of dairy fermented products, fat dairy products, cheese and powder milk packages; packaging of cereals, snack foods and confectionery; packaging of beverage : package of water, soda, coffee, tea, juice, beer and wine in glass, plastics, metals and laminated cartons. For each product author represent the chemical composition, manufactured technology, main transformation during the preserve, most know packaging types and new materials can be used with best results in food packing.

Last chapters covers legislative and safety aspects of packaging. Author givea us a complete overview about legislation from United States and Europe. A safety pack need to not change the human health, not change the composition of food in unacceptable way, not change taste, texture or odor of food, shall be manufactured by according of ISO 22000, HACCP and GMP system. Food packaging and sustainability present the possibility to recycling packages in accord with protection of environment. Waste manage options cover the waste management and technologies to recycle paper, steel, aluminum, glass and plastics packaging.

All over the book offer a comprehensive treaty of packaging. Food packaging is a science who gives information from a "several disciplines including chemistry, biology, microbiology, food science and engineering." An important part is allocated for recycling, reuse and recovery of food package in special for environment protection. Each chapter contains 25-85 references from 1965 to present. A lot of figures and tables come to help the reader to understand all of the information. One omission at the wine packaging, no aspects about wood packaging and phenomena that occur in wine during the age period. Overall this book is highly recommended for scientists, technologists, students, and all of the specialized worker required education in area of food technology. All of the knowledge present in this book can be used in: marketing, package design, food processing, food distribution and food preservation.

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Book review

HANDBOOK OF ASEPTIC PROCESSING AND PACKAGING – 2nd **Ed. (2012)**
Jairus R.D. David, Ralph H. Graves, Thomas Zsemplenski
CRC Press, Taylor & Francis Group, 372 pages, ISBN: 978-1-4398-0719-4

The importance of food packaging is well known due to continuous knowledge increasing of both food producers and consumers in the field of food quality and safety.

The **HANDBOOK OF ASEPTIC PROCESSING AND PACKAGING** highlights some aspects related to the aseptic processing and packaging in the food and beverage industry. Based on the extensive experience of the authors in the processing, marketing, business, quality assurance, research and development completed by hundreds of citations from books, papers published in rank journals or presented to symposiums and patent literature, Handbook of Aseptic Processing and Packaging provides a state-of-the-art guide to understand the past, present and future of aseptic packages. The book is organized in 15 Chapters and 2 Appendics.

Chapter 1 has an historical perspective by covering the aspects regarding the Past, Present and Future of Aseptic Processing and Packaging. The invention of Dr. C. Olin Ball (1936) who organized the first aseptic line of the Heat/hold-Fill/hold-Cool (HFC) and the installation of Dr. W.M. Martin's Dole (1951) aseptic canner for milk and milk-based low-acid products in metal cans are considered the start points of aseptic processing and packaging area which became one of the most dynamic and profitable sector in the USA food and beverage market. The future of aseptic processing is strongly connected with the innovative thermal (flow microwave heating, radio frequency ohmic/electric resistance heating) and nonthermal processes (pulsed electric field treatment, irradiation, sonication, manothermosonication), with the new techniques of real time and postprocess measurement and monitoring to all segments of aseptic processing, all designed to extend the shelf life of food.

Chapter 2 encompasses the history and evolution in USA of aseptic processing and packaging by presenting the leading personalities in the field. Early pioneering researching work of C.Olin Ball, William McKinley Martin Roy Graves, Jack Stambaugh which led to first commercial aseptic plant, to the real fresh company and to the first aseptic form-fill-seal-packages are briefly described.

Aspects regarding the US market for aseptic package are approached in the **Chapter 3**. The activity in the commercialization of aseptic processing and packaging showed an upward trend in the late of 1960s and early 1970s when the Dole Scanning System was used for food processor and Tetra Pak Swedish Company introduced the first laminated paper-aluminium foil-plastic container to USA. The market grew with each new developed aseptic packaging: metal can, bag-in-box, paperboard, plastic cup, pouch and plastic bottle.

Chapter 4 is dedicated to aseptic processing equipment and systems. Equipments and utilities employed in the development of an aseptic processing system, which fulfilled the basic requirements of aseptic processing, namely blending vessel, balance surge, formulation of product, timing pump (rotary pump, high-pressure pump, Marlen reciprocating piston pump), heat exchangers for sterilization of food (steam injection, plate heat, tubular heat, scraped surface, ohmic heating, microwave heating), several components (deaerator, tanks, barrier seals, valves, homogenizers, clan-in-place systems) and utilities (water preparation and sterilization, water heating/cooler/refrigeration, steam producers, filters, sterilizing agents) are presented and extensively discussed.

A wide variety of aseptic packaging systems is described in **Chapter 5**: Aseptic filling and packaging equipment. The first description, dedicated to Dole aseptic canning system which has been used in commercial applications since 1960s and whose main components are: can sterilizing unit, filling section, lid sterilizer and sealer is followed by the presentation of aseptic bag-in-box (invented by William Scholle in the late of 1950s), aseptic paperboard fillers (Tetra Pack, SIG Combibloc), aseptic plastic cups (Bosch and Erca, OYSTAR Hassia, Erca, Gasti, Hamba and Ampack Ammann, Benco and Metal Box), coffee creamers, aseptic pouches (Bosch, DuPont/Liqui-Box and Inpaco, Fres-co, OYSTAR Hassia, Cryovac), aseptic plastic bottle fillers (Ampack Ammann, Bosch, Krones, OYSTAR Hamba, Procomac, Serac, Shibuya Kogyo, Sidel/Tatre Laval) and Stork.

The chapter on Aseptic packaging materials and sterilants (**Chapter 6**) has updated information on the materials used in aseptic packaging production and the requirements that they should fulfilled to keep the food products in a safe and acceptable condition for its expected shelf life. Environment considerations in terms of recoverable and nonrecoverable energy component of various materials used in packaging are also approached.

Chapter 7 on Aseptic bulk packaging discusses the packaging of larger quantities of food for industrial and commercial uses in aseptic flexible packaging. The evolution from the initial rigid containers up to wide range of aseptic preformed bag packaging with different sizes was generated by the Scholle's vision. Different aseptic bulk containers and aseptic ocean liner transportation and storage are presented.

Chapter 8 on Regulations for aseptic processing and packaging of food consolidates the information on this subject by discussing the European regulations versus USA and by detailing of some USA state and federal regulations.

The chapter on Validation and establishment of aseptic processing and packaging operations (**Chapter 9**) provides current information regarding the efficiency demonstration of each process/equipment involved in aseptic process. Steps to be followed, namely decisional process, equipment selection, process schematic and instrument diagrams, postinstallation review, equipment testing, microbiological validation, factors that influence the aseptic process are detailed discussed.

Chapter 10 goes through the Aseptic processing operations and discuss the strongly connections established between food processing and packaging, starting with the presterilization of system and packaging prior to food processing or packaging filler and finishing with loss of sterility, cleaning and control of aseptic systems and packaging.

The principles that describe thermal processing and the equations that quantify them, processes optimization, comparison of different processing methods, some advantages of aseptic processing and definitions of main terms are subjects of the **Chapter 11** on Thermal processing and optimization. Issues about thermal vulnerability of food nutritional, qualitative and sensorial parameters are included, a special attention being paid to enzymes, C vitamin and microorganisms.

Chapter 12 on Quality assurance and food protection for aseptically processed and packaged food naturally continues the previous chapter by developing aspects concerning the quality assurance programs for aseptically processed and packaged food. The Dole Canner, Tetra Brik and Conoffast aseptic fillers are used to illustrate the principles of quality assurance and food safety due to their diversity and complexity. Three

stages are considered essential in quality assurance: (i) Preprocess assurance by purchasing of raw ingredients that certain meet physical, chemical and microbiological specifications not only proven by the supplier certification but also by cross-checking and rejection of those which fail the standards is the first step in quality assurance of final food product; (ii) In-process assurance, a complex program involving control of batching, thermal processing and aseptic packaging operations also requires considerable material, financial and human efforts to meet the quality standards; (iii) Postprocess assurance, includes data analysis and review, testing the final food products, distribution, handling and storage of food. All aspects concerning the food quality and safety (potential hazards, critical control points, specifications, monitoring procedures, policy for corrective actions for deviations, keeping the records and procedure for checking) are integrated in Hazard analysis critical control point (HACCP) program.

Complexity of processing systems is directly related to foreseeable failures. An analytical technique that combines the technology and experience of people in problems identifying and their solving is subject of **Chapter 13** on Failure mode and effect analysis, and spoilage troubleshooting. Seven different types of failure modes and their effect in term of microbial profile, starting from incoming of raw ingredients and ending with seal integrity were identified by the author and mean to either prevent or control their occurrence.

The chapter on Aseptic Packaging of Particulate Food (**Chapter 14**) addressed to particulate food of which heterogeneous structure present a series of challenges related to processing equipments, not only because of the thermal treatment of large solid pieces in a liquid media of varying viscosity, but also with the transport and packaging of such suspensions of solids. Important consideration from industrial perspective concerning the equipments design (heat exchangers and novel heating technologies, transport of liquids with particulates, validation of aseptic processes with particulates, achieving conservative thermal characteristics of carrier particle enclosure, thermal adjustments for advanced heating applications time-temperature history measurements) are extensively presented accompanied by explanatory graphical material.

The final chapter (**Chapter 15**) on Industry research and development, and management needs and challenges emphasizes the needs for industrial capturing of ongoing research findings both in basic and applied research, leading to coherent integration of thermal sterilization methods coupled with innovative no thermal methods. Briefly presentation of alternative processes, the experimental results that recommend them in aseptic packaging and processing industry is also included. Financial aspects related to current aseptic packaging equipment in terms of capital costs, management and administrative challenges, trends for future are shortly discussed.

The **Appendix A** referees to aseptic filler profiles while **Appendix B** presents aseptic contract packers in the USA.

The book's clear structure, suggestive chapter headings, concise and updated information (2012), logical approach of scientific issues and practical aspects make the book a excellent tool for both specialists in food industry (students, professors, engineers, marketing) and consumers with interest in aseptic processing and packaging of food.

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