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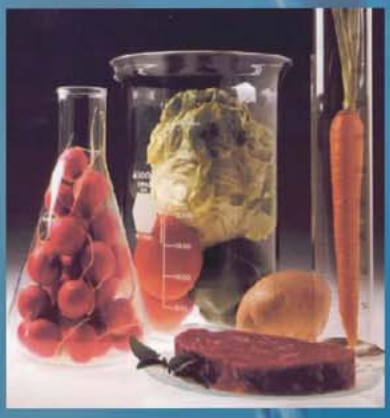

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Effect of Chia Seed on Physicochemical and Sensory Characteristics of Common Carp Restructured as Functional Food

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Abstract: Physicochemical and sensory characteristics of restructured meat of common carp (*Cyprinus carpio*) fortified with 0-8 g/100 g of chia seed flour (CSF) was evaluated. It had a higher nutritional value (higher fibre content and protein retention] ($p < 0.05$) and better cooking characteristics (higher cooking yield and moisture retention) ($p < 0.05$) than the control. The colour (a*, b*) increased; lightness and whiteness index decrease ($p < 0.05$). Hardness increase ($p < 0.05$) occurred because of CSF addition. Differential scanning calorimetry showed that fibre fortification did not interfere with the thermal transitions of the restructured meat. No significant differences were detected with the preference test scores of 4% or 8% CSF compared with the control. Restructured (4%-8% CSF) had a higher content of fibre and fat, which could be linoleic and linolenic acid, and an increase in the content of protein compared with those of commercial products, among had 1.62 and 2.25 mg AGE/g. Therefore, the restructured properties of common carp were governed by CSF addition.

Key words: Protein gel, common carp, chia seeds, restructured meat, physicochemical properties.

1. Introduction

Obesity is a chronic disorder with multiple causes that may affect an individual in isolation or act collectively at the population level. Virtually all obese people develop symptoms of chronic disease by the age of 40, and the majority will require medical intervention for obesity-related disease before they are 60 [1]; therefore, obesity is now regarded as a growing epidemic around the world. According to the World Health Organization [2] one billion adults are overweight, and more than 300 million people are obese. Without a population-level, multisectoral and multidisciplinary approach to curb the problem, this figure will surpass 1.5 billion by 2015. Altogether, there are more than 42 million children under five who are overweight globally.

Since the early 1980s, in Mexico, the odds of being overweight and obese have tripled: 39.05% of the population is overweight, and 32.15% is obese, which equates to seven out of 10 Mexicans between the ages of 30 and 60 years [3]. According to United Nations International Children's Emergency Fund [4], the country is first in the world for childhood obesity, second for adults after the United States, and first in the case of women [2].

Among the causes of these diseases is the intake of energy-dense foods rich in fats, salt and carbohydrates and low in vitamins, minerals and fibre, coupled with a decline in physical activity and a sedentary rhythm of

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life. Obesity and overweight are preventable by performing regular physical activity, balancing the energy content consumed, limiting the intake of sugars and total fat and increasing the consumption of fruits, vegetables, legumes, whole grains and nuts [2]; all these actions translate into a higher intake of fibre.

Studies show that increased consumption of foods rich in insoluble fibre is strongly associated with reduced diabetes [5] and, in turn, comorbid cardiovascular disease complications. In this regard, there are various food products contributing to the dietary required amount or a portion of the necessary fibre. A food product's functionality regarding dietary fibre may have several benefits, such as adjuvant texture, an increased volume of products low in sugar, fat substitutes, added colour and natural antioxidant activity [6]. In addition to contributing to the improvement of the textural features, a fibre-providing food product can improve the sensory appeal and shelf life of food, due to its ability to retain water and form gels and to mimic fat, texturing and thickening effects [7]. Examples of these soluble fibres derived from grains and the fractions of various fruit are pectins [8], beta-glucans, beet cellulose fibre [9], polydextrose [10], etc. Dietary fibre linked with soy proteins by their functional properties has been widely utilised in various branches of the food industry, including the meat industry [11]. Potato skins, a byproduct of the industry of potatoes shells, are rich in fibre and also have been used as a source of dietary fibre in breadmaking [12]. The seeds of *Salvia hispanica* L., better known as chia, are a pseudocereal rich in soluble and insoluble fibre, and they contain 25% to 35% polyunsaturated fatty acids, antioxidants, such as cinnamic, chlorogenic and caffeic acid, and the flavonoids myricetin, quercetin and kaempferol [13]. Thus, it is an excellent ingredient for dieters because it has beneficial effects, such as reducing blood cholesterol and blood glucose and modifying insulinaemic responses, as well as changes in the function of the intestine and antioxidant activity [13]. Several authors [14-17] have added various types of fibre, such as wheat to hake and mackerel, dietary fibre wheat to surimi giant squid, pea fibre to surimi, carrageenan and komjac carrageenan-flour in bass, Solka-Floc (cellulose fibre) in surimi pollock (*Alaska pollock*) and powdered cellulose dietary fibre to obtain restructured meat based on seafood or aquaculture species. Among these, the common carp is a species underutilised around the world [18], but it presents significant nutritional characteristics. So far, there have not been any reports on the use of the chia seed as a source of fibre for the production of restructured meat from this species, so the use of these two products could be an alternative for consumption, taking advantage of a fishing product that is infrequently marketed because of its size, due to its content of thorns or the abundance of large fish that are already processed and contribute to health. Therefore, the aim of this study was to evaluate the effect of chia flour (CSF) (*Salvia hispanica* L.) on physicochemical and sensory characteristics of developed restructured meat of common carp (*Cyprinus carpio*) as a functional food based.

2. Materials and Methods

2.1 Samples

Ten carp (*Cyprinus carpio*) with weights of 1.5 kg were obtained from the San Luis Mextepec business community, State of Mexico, Mexico, and they were transported to the laboratory under refrigeration at 5 °C in high-density polyethylene bags (HDPB). Afterward, they were washed, eviscerated and stored at 4 °C until further use. Chia seeds were purchased from the central supply of Toluca, Mexico, and they were ground to achieve the texture of flour, after which the chia flour was stored in HDPB.

2.2 Physicochemical Analysis

2.2.1 Water-holding Capacity (WHC)

The evaluation of WHC was described according to

Dublán et al. [19]. Five grams of common carp muscle were homogenised with 8 mL of 0.6-M NaCl. The homogenate was placed in an ice bath and stirred with a glass rod for 1 min. The tubes were left on ice for 30 min, stirred again for 1 min and centrifuged at 8,000 × g for 15 min. The supernatant volume was measured. WHC was reported by the difference as millilitres of 0.6-M NaCl held/100 g of muscle. All determinations were performed in triplicate.

2.2.2 pH

pH values were determined using a Hanna Instruments potentiometer (pH 210, Italia Srl). Ten grams of muscle were ground in 90 mL distilled water for 1 min. After filtration of the mixture, the pH value was determined [20].

2.2.3 Titratable Acidity

The titratable acidity was determined by the A.O.A.C. [21], Part 942.15 method.

2.2.4 Colour

The colour of the common carp muscle, CSF and restructured meat was determined using a Chroma Meter CR-400 colorimeter, according to the CIELAB model. We obtained the values of L*, a* and b* as estimates of the luminosity (L*) on a scale from 0 to 100 and indicators of red-green (a*) and yellow-blue (b*). These measurements were used to calculate the whiteness of the gels according to the equation:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}.$$

From the coordinates, the hue (H*) and chroma (C*) were calculated as follows [22]:

$$\text{Hue} = \tan^{-1} b^*/a^*$$

$$\text{Chroma} = (a^2 + b^2)^{1/2}.$$

2.3 Proximal Analysis

Protein was analysed by the Kjeldahl method, the ether extract by the Soxhlet method [23], the moisture by A.O.A.C.14,003 [21], ash by the method of calcination in a muffle furnace at 500 °C [21], and the neutral detergent fibre by A.O.A.C. [24] using an ANKOM mark 200 Fiber Analyzer (Ankom Technology Corp., Fairport, NY).

2.3.1 Myofibrillar Protein (MP)

MP was prepared from the common carp muscle according to Ngapo et al. [25], with slight modifications. One hundred grams of common carp muscle were homogenised by blending for 10 min with ice-cold distilled water 1:1:1 (w/w/v) and then were magnetically stirred for 10 min in an ice bath. The myofibril suspension was filtered through two layers of cheesecloth to remove connective tissues, stirred and filtered twice. The muscle homogenate was centrifuged at 3,000 × g at 4 °C for 25 min, and the supernatant was discarded. Part of the myofibril pellet was placed into a capped glass and stored to immediately begin gel formation. The protein concentration of the myofibril pellet was determined using the biuret method.

2.3.2 Preparation of the Protein Mixture: Common Carp-Chia Seed Flour (CSF)

For each 100 g of myofibrillar protein extracted from the carp, various percentages of CSF, including 0%, 1%, 4% and 8%, were added. The samples were mixed until incorporation and subsequent gelation.

2.3.3 Gelation of Proteins

The gelation of proteins was performed according to Klettner [26]: 30 g of the mixture (carp-chia) with the four different concentrations of flour chia were added to bottles with an internal diameter of 25 mm and a height of 50 mm. The vials were placed into a shaking water bath and gradually heated at a rate of 1 °C min^{-1} until reaching an internal temperature of 80 °C to induce gelation [27]. The vials were subsequently removed from the water bath and cooled in an ice bath at a temperature below 4 °C.

2.3.4 Cooking Loss% of the Restructured Common Carp

The cooking loss of the restructured meat was determined according to Estevez et al. [28]. Fifteen grams of the mixture [carp-chia] for each of the concentrations (0%, 1%, 4% and 8%) were placed in previously weighed test tubes. The tubes were subjected to a heat treatment for 20 min at 80 °C. Water

and the exuded fluids were separated and discarded, and the tubes with the sample were weighed again. The process yield is given by the weight before and after the heat treatment of each sample in the tube.

2.3.5 Differential Scanning Calorimetry (DSC)

The samples weighed between 10 and 15 mg in a capsule that was placed into the sample holder to be subjected to heating; additionally, a reference air capsule was used. Then, the samples were scanned using a heating rate of 10 °C/min at an energy flow of 0.1 to 0.2 mcal/sec. A differential scanning calorimeter (DSC) from Mettler Toledo, which was calibrated between 10 and 100 °C, was used, and the endotherm areas were calculated. The measurement method for the determination of the thermograms is based on that described by Schubring [29].

2.3.6 Quantification of Phenolic Compounds

For samples Folin Ciocalteu method which involves placing 100 µL of extract in test tubes previously covered with foil, add 650 µL of purified water in each tube was applied also added a 375 µl 1 N solution of Folin-Ciocalteu and 1,875 µL of sodium carbonate solution 20%. It was allowed to react for 2 h in the dark. After this time the absorbance was measured in a spectrophotometer at 750 nm [31]. All this was done in triplicate and results are expressed as mg gallic acid/g, based on a standard curve prepared with this reagent.

2.3.7 Consumer Test

The consumer evaluation consisted of 53 untrained judges, including 33 males and 15 females, ranging in age from 18 to 23 years. The AMSA [30] recommends a consumer panel size of at least 50 individuals. The panellists were untrained students recruited from the campus of the University Autonomous of Mexico State. All were already involved in fish meat preference/acceptability tests and were regular consumers of fish meat. The restructured (burger shape) from the muscles of *Cyprinus carpio* were cooked with salt or spices and were boiled in individual bags of HDPB to a final internal temperature of 80 °C. The cooking temperature was monitored by an iron/constantan thermocouple placed in the geometric centre of each restructured sample. After boiling, the burgers were immediately cut into equal sizes and coded with a three-digit random number. The burger samples from the four common carp samples with 0%, 1%, 4% and 8% were given to the panellists in a predetermined, balanced order and were evaluated in a preference-ranking test. The panellists were asked to rank the samples in order of preference, with 1 being the most preferred and 3 being the least preferred. The evaluation took place in individual booths in a sensory testing laboratory under controlled conditions. Between each sample, the panellists were instructed to rinse their mouths with water served at room temperature.

2.4 Statistical Analysis

The data were subjected to analysis of variance and Tukey multiple-range tests ($p < 0.05$), using SPSS 8.0 for Windows software (SPSS 1997).

3. Results and Discussion

3.1 pH and Acidity in the Common Carp Muscle

The pH was within the range designated by Huss et al. [31], which indicates that it is a fresh product for processing, with a value of 6.49 ± 0.06; additionally, established that marine and aquatic species should be in the range of 6.3 to 6.9, and that the pH ranges from 6.6-7.5 for decomposing fish and is 7.5 for more decomposed fish and that the average pH of a carp is 6.21; the acidity has a value of 0.038 ± 0.008 because lactic acid, generated in anoxic conditions from glycogen, is the main factor lowering the post-mortem pH in the fish muscles. However, the values for the samples used were within the parameters established for freshness by Huss et al. [31].

3.2 Water-Holding Capacity of Common Carp Muscle

The WHC values represent the percentage of water retained in each meat sample after centrifugation. In

this sense, the muscle of *Cyprinus carpio* had a value of 63.75%. Cardoso and Mendes [32] have reported values of WHC for various species, such as ostrich at 40.34%, beef at 37.30%-37.40%, squid at 75% to 85% and *Argyrosomus regius* at 69.5%, respectively, to indicate that it is fresh raw material. The difference between the values for WHC could be due to the chemical composition, origin and state of maturity of each species. Taking into account the values of pH and acidity, the common carp can be considered a species with good quality parameters for processing.

3.3 Common Carp Muscle Colour

The colour of carp muscle has a dark tone because the blood present in it makes a low L (Table 1) (39.36 ± 0.080) with respect to that of other species. Examples have been reported, such as L = 57.8 for catfish fillets [33]; L = 54.4 for Atlantic halibut fillets, as observed by Roth et al. [34]. In contrast, for carp species, Sequeira et al. [35] observed L = 41.7. This variation in the brightness is due to the species, the origin and the type of habitat present. Additionally, there are variations in a* and b* (5.28 ± 0.11 and 4.81 ± 0.04, respectively), depending on the species. The differences can be related to (a) the fishing season, which in turn can be correlated with the physiological stage of the specimens (mature vs. youth); (b) sex, as it is well established that females are bigger in mantle length than males; and/or c) different fishing areas.

3.4 Proximal Composition of Common Carp (Cyprinus Carpio)

The chemical-composition data indicate that the fresh carp has high protein content (24.01 ± 0.30) and a low fat content (2.43 ± 0.26). The moisture and ash contents were 79.48 ± 0.37 and 0.45 ± 0.01, respectively. This proximal composition may vary according to the species. For example, for tuna (*Thunnus alalunga*), the water, protein and lipid contents were 71-72.2, 25.2-28.1 and 0.61-4.1, respectively, and for salmon (*Salmosalar*), they were 67-77, 21.5-22.3 and 0.3-15.9 [36]. The common carp in this study has a composition below that of tuna protein and above those of other species; additionally, the fat content is between of those of several species. It should be noted that, according to Mráz et al. [37], the lipids of the common carp are mainly composed of a high content of omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), making it possible to say that this type of meat provides these types of compounds.

3.5 Proximate Analysis and Colour of CSF

In this study, chia flour presented protein levels (23.31 ± 0.12) that are within the parameters outlined in the literature, ranging from 20.01%-35.32% [38, 39]. The values obtained for lipids (34.45 ± 0.07) also correspond to the range reported in the literature; ranging from 29.56-34.88 g/100 g. Luna et al. [39] and Segura et al. [38] have reported values of 27.57, 32.84, 23.81 and 23.35 g/100 g, according to various extraction methods. Finally, the ash was 7.24 g/100 g, coinciding with the result of Capitani et al. [40]; however, content of 6.51 g/100 g and 4.58 g/100 g was reported by Luna et al. [39]. The chia used in this study is produced within the parameters reported by the

Table 1 Colour (L*, a* and b*), chroma, tone, hue and IW of muscle common carp and chia seed flour (*Salvia hispanica* L.) (CSF).

Property	*Common Carp Muscle*	*Salvia hispanica* L.
Lightness (L*)	39.36 ± 0.080	36.45 ± 1.187
Redness (a*)	5.282 ± 0.102	3.49 ± 0.082
Yellowness (b*)	4.805 ± 0.046	16.285 ± 0.315
Chroma (C*)	5.22 ± 0.02	10.96 ± 0.29
Tone	1.15 ± 0.009	1.50 ± 0.37
Hue (H*)	220.59 ± 0.14	256.54 ± 0.57
IW	38.94 ± 0.084	34.069 ± 1.084

literature. The difference observed in the values may be due to the region, temperature, rainfall and months of growth and the extraction methods [40]. The colours obtained in CSF show that the IW and L* (34.06 ± 1.08 and 36.45 ± 1.18, respectively) are low, due to the dark colour that presents the CSF. a* (3.49 ± 0.08) has a tendency to red and b* (16.28 ± 0.31) to yellow, so that the combination has a tendency to brown.

3.6 Gellification of Common Carp Muscle

The restructured sample obtained was a surimi based on common carp: irreversible heat-induced protein denaturation or a protein endothermic transition is necessary for the initiation of surimi gelation because sarcoplasmic proteins are removed during the surimi manufacturing. The hardness obtained for the common carp muscle proteins restructured after the gelation process was 5.67 ± 0.41 N, which is a low hardness value, compared with those of other species such as sea bass (*Sea bass*) with a hardness of 10.3 N. For Alaska pollock surimi gels, Tabiloand Barbosa [41] found a hardness of 13.15 N. Compared with the values for seafood-product species, the hardness of the restructured carp sample is higher (1 N), which may indicate that the species under study could be used for the production of restructured and surimi products. Several authors have performed the integration of adjuvants to obtain surimi or restructured products, providing specific functionality and structural or nutritional contributions [7, 42].

3.7 Effect of CSF on the Hardness of Restructured Common Carp

The results of gel strength are shown in Table 2. Adding CSF at 1, 4 and 8% showed a significant difference ($p < 0.05$) in this parameter with respect to the control (0% w/w CSF). This could indicate that a greater concentration of CSF increases the hardness ($p < 0.05$). Similar results were observed by Debusca et al. [7] when cellulose fibre (4% and 8%) was added. This could be due to the crosslinking reactions of CSF-protein, CSF-CSF and protein-protein, which would require more force and energy to break down the gel system. Park et al. [43] reported a similar result that, as the level of the added potato starch increased, the breaking strength of the thermal gel of salted squid paste increased, and the starch-reinforced gel became firm and less elastic.

3.8 Cooking Loss% of the Restructured Common Carp

The changes in the cooking loss of restructured CSF spiked with (0%, 1%, 4% and 8%) are shown in Table 2, showing a significant difference ($p < 0.05$) in the cooking loss between the flour-added and the control groups (0% w/w CSF). A low cooking performance was observed for the control, and the highest yield was observed for the sample with 8% (Fig. 1). The results indicate that the different concentrations of CSF influence the yield because it can prevent water loss during cooking ($p < 0.05$) (Table 2), which provides a protective effect on the product stability with respect to that of the control, thus increasing the concentration of CSF and decreasing the content of free water, suggesting that the water-retention capacity of the restructured gels increased with the addition of CSF; this result coincides with that reported by Yang et al. [42] when 0%, 2%, 4%, 6% and 8% of rice starch was added for the preparation of gels with proteins of grass carp. Additionally, the high water-absorbing ability or the hydrophilic group interacting with free water may have altered the bound water, which was not easily extracted. The same mechanism could work in the case of CSF. The statistical analysis shows an inverse linear relationship between the percent yield and the hardness (positive) and with the content of free water (negative), which could predict the effect of the addition of various concentrations of CSF.

3.9 Colour of the Restructured Common Carp

In Table 3, the tristimulus values L*, a* and b* are

Table 2 Percentage cooking loss, water loss and hardness at various CSF concentrations.

Restructured with CSF	% Cooking loss	Water loss	Hardness
0%	62.521 ± 0.495[a]	5.63 ± 0.11[b]	5.67 ± 0.41[a]
1%	70.797 ± 0.386[b]	4.43 ± 0.40[b]	6.3 ± 0.60[b]
4%	87.376 ± 1.866[c]	2.16 ± 0.90[a]	7.76 ± 0.28[b]
8%	91.155 ± 0.651[d]	1.40 ± 0-17[a]	9.69 ± 1.09[c]

Means in the same column with different letters are significantly different ($p < 0.05$).

Fig. 1 Restructured gels at various CSF concentration 0%, 1%, 4% and 8%.

shown. The whiteness index was significantly reduced ($p < 0.05$), increasing the concentration of CSF, which could be due to the low value of CSF L* (L* = 36.45), which, when combined with the carp protein, caused a decrease in colour; the IW decrease is correlated with a decrease in L* ($p < 0.05$). Similar results were reported by Debusca et al. [7], by increasing the concentration of cellulose fibres in Alaskan pollock surimi; by Xiong et al. [44], who observed a decrease in IW by adding konjac glucomannan. Thus, the IW is correlated with a decrease in L*. A reduction in b* of 1% is correlated with a decrease in the IW* because b* gives a yellow colour, which contributes to the effect of the whiteness of the product; however, by adding up to 1% of CSF, b* increases ($p < 0.05$), retaining a decrease in IW ($p < 0.05$). a* decreased significantly ($p < 0.05$) by adding 1% CSF, indicating that the product becomes darker, tending to a brown colour, but with increasing concentration chia above 1%, a* increased significantly ($p < 0.05$), indicating a slight darkening in the product obtained, that took on a dark-brown coloration. This could be a disadvantage for the product, but the colour obtained resembles an integral-type product (Fig. 1). The results of the whiteness obtained in this study and other studies [14, 7] show different values of L*, a* and b* because of the meat species and the type of fibre used; however, all of these studies show similar trends in IW and L* when different types of fibre were added. The chroma (C*) increased slightly with the addition of 1% CSF, which was significant ($p < 0.05$). From the point of view of colour, CSF could be added to restructured burger with no significant modification in this parameter at other concentrations. The hue [H*] decreased with the addition of CSF ($p < 0.05$) at various concentrations, but it slightly increased when additives were added, and no significant differences were observed between the control and these samples.

3.10 Differential Scanning Calorimetry (DSC)

Heat-induced irreversible protein denaturation or a protein endothermic transition is necessary for the initiation of surimi gelation. Because sarcoplasmic proteins are removed during surimi manufacturing, the proteins present in the restructured gel in the present study were mainly the myofibrillar proteins actin and myosin. DSC was employed to determine if fibre has an effect on the endothermic transition of the restructured gel. Fig. 2 shows that fibre does not interfere with heat-induced protein denaturation, a prerequisite for restructured products such as surimi gelation. Several authors [7, 14] have reported that fibre

Table 3 Colour, chroma, hue and whiteness index restructured gel at various CSF concentrations.

Restructured		L*	a*	b*	Croma	Hue	IW
Without additives	0%	79.30 ± 0.72^{f}	1.79 ± 0.35^{b}	10.63 ± 0.08^{cd}	259.40 ± 0.36^{a}	15.46 ± 0.30^{d}	70.82 ± 0.13^{f}
	1%	73.36 ± 0.83^{e}	0.47 ± 0.10^{a}	8.59 ± 0.30^{a}	263.94 ± 0.79^{c}	12.74 ± 0.13^{b}	63.85 ± 0.50^{e}
	4%	67.58 ± 0.20^{d}	1.36 ± 0.11^{ab}	9.78 ± 0.16^{b}	260.35 ± 1.08^{ab}	12.96 ± 0.25^{b}	58.17 ± 0.44^{d}
	8%	64.08 ± 0.64^{cd}	1.74 ± 0.31^{b}	10.16 ± 0.575^{bc}	260.64 ± 0.85^{ab}	13.80 ± 0.15^{bc}	51.77 ± 0.30^{b}
With additives*	*8%	57.14 ± 1.52^{a}	3.31 ± 1.11^{c}	10.83 ± 0.20^{d}	258.92 ± 0.92^{a}	14.61 ± 0.64^{cd}	48.59 ± 0.97^{a}
	*4%	59.08 ± 2.45^{ab}	1.82 ± 0.61^{b}	11.69 ± 0.19^{e}	262.24 ± 1.04^{bc}	14.98 ± 0.96^{d}	52.48 ± 01.07^{b}

* (onion, garlic, salt, black pepper, parsley and dry chile).

Means in the same column with different letters are significantly different ($p < 0.05$).

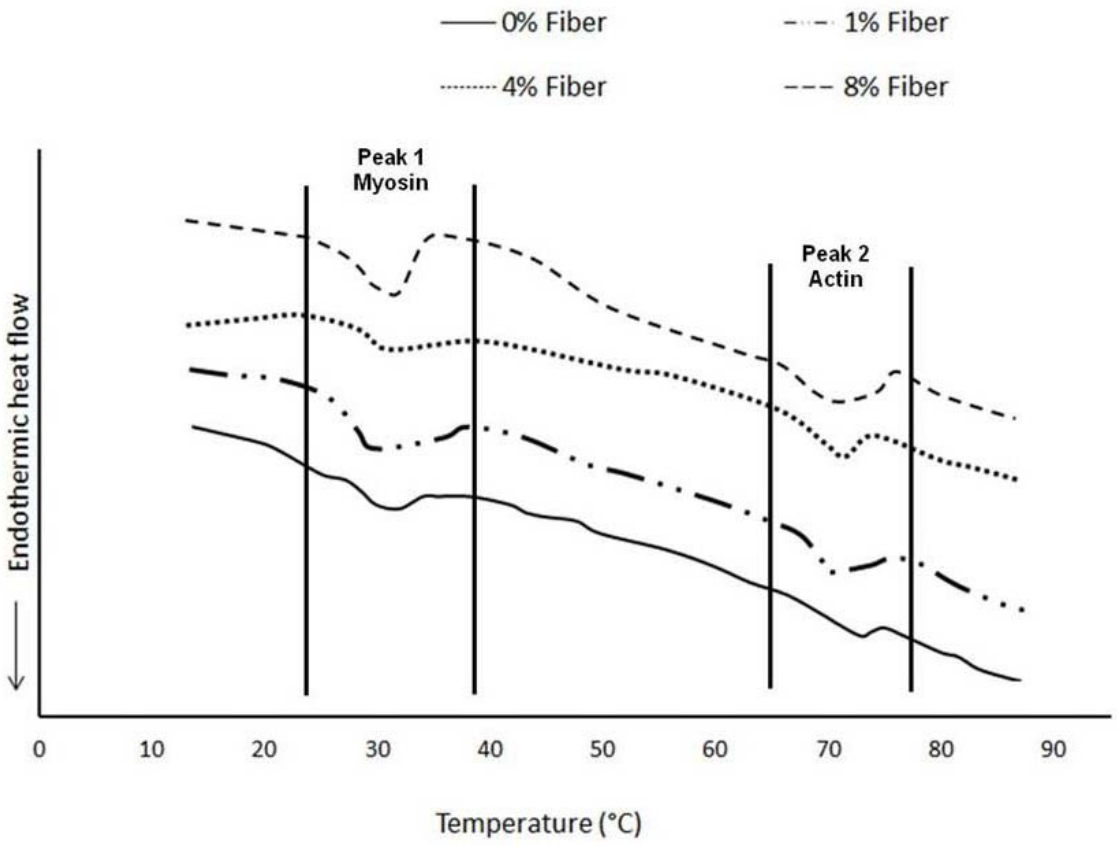

Fig. 2 Differential scanning calorimetry (DSC) thermogram of restructured gel with different levels of added CSF.

is chemically inert and does not participate in protein denaturation. The fibre does not interfere with the thermal transition/denaturation of products such as surimi myosin or actin, and it improved the textural properties.

3.11 Proximal Analysis of Restructured Gel at Various CSF Concentrations

After the proximal-analysis results were observed for each of the samples (Table 4), there were significant difference ($p < 0.05$) in the protein of each restructured from 0% to 8% of CSF. With the two restructured samples (4% and 8%), burger products were developed, and they were compared with commercial products (Table 5). The products obtained in this study contain a higher percentage of protein than commercial products and also an increase in the dietary fibre and fat. The increase of fat in the burgers with 4% and 8% CSF could be because chia seed oil has approximately 250-390 g/kg fresh matter (FM) [45]. The fatty acids (FA) of chia oil are highly unsaturated, and their main components are linoleic

Table 4 Proximal analysis of the restructured gel at various CSF concentration.

Gel	% Protein	% Lipid	% Fibre	% Moisture	% Ash
0%	16.83 ± 0.06^{d}	2.43 ± 0.26^{a}	0.88 ± 0.086^{a}	79.38 ± 0.37^{d}	0.45 ± 0.01^{a}
1%	16.41 ± 0.17^{c}	5.54 ± 1.11^{b}	5.88 ± 0.10^{b}	71.54 ± 0.56^{c}	0.62 ± 0.06^{b}
4%	13.25 ± 0.03^{b}	11.06 ± 0.63^{c}	7.09 ± 0.036^{c}	67.84 ± 0.72^{b}	0.74 ± 0.02^{c}
8%	12.32 ± 0.34^{a}	14.21 ± 0.56^{d}	10.91 ± 0.034^{d}	61.48 ± 0.71^{a}	1.06 ± 0.08^{d}

Means in the same column with different letters are significantly different ($p < 0.05$).

Table 5 Comparison of proximal analysis of restructured samples, obtained with commercial products.

Gel	%Protein	%Lipid	%Fibre	%Moisture	%Ash	mg AGE/g
[1]Commercial sausage	11.77 ± 0.06^{c}	11.76 ± 0.14^{e}	0.06 ± 0.03^{a}	72.94 ± 0.11^{de}	3.47 ± 0.07^{g}	-
[2]Commercial sausage	10.65 ± 0.07^{b}	10.39 ± 0.09^{c}	2.39 ± 0.07^{c}	73.28 ± 0.14^{e}	3.28 ± 0.08^{fg}	-
[3]Commercial ham	11.93 ± 0.07^{c}	1.87 ± 0.07^{a}	-	82.86 ± 0.22^{g}	3.34 ± 0.13^{g}	-
[4]Commercial ham	12.87 ± 0.09^{e}	2.23 ± 0.07^{a}	0.79 ± 0.01^{b}	81.01 ± 0.25^{f}	3.11 ± 0.11^{f}	-
[5]Commercial burger	7.26 ± 0.06^{a}	12.97 ± 0.14^{f}	4.63 ± 0.12^{d}	72.96 ± 0.19^{de}	2.18 ± 0.04^{e}	-
[6]Commercial burger	13.34 ± 0.1^{f}	17.53 ± 0.35^{h}	0.07 ± 0.02^{a}	68.91 ± 0.34^{c}	0.01 ± 0.01^{a}	-
[7]Restructured common carp [*Cyprinus carpio*]	16.83 ± 0.06^{g}	2.43 ± 0.26^{b}	7.82 ± 0.46^{f}	72.46 ± 0.37^{d}	0.46 ± 0.01^{a}	-
[8]Burger 4% CSF	13.25 ± 0.03^{f}	11.06 ± 0.63^{d}	7.09 ± 1.56^{e}	67.84 ± 0.72^{b}	0.75 ± 0.02^{c}	1.62 ± 0.08^{a}
[9]Burger 8% CSF	12.32 ± 0.34^{d}	14.21 ± 0.56^{g}	10.91 ± 1.04^{g}	61.48 ± 0.71^{a}	1.07 ± 0.08^{d}	2.25 ± 0.05^{b}

1, 2, 3, 4 turkey; 5 chicken; 6 soy protein: beef; 7, 8 and 9 restructured gel with 0, 4 and 8 CSF %, respectively.

Table 6 Preferences for the restructured gel expressed as rank sums and preference %.

Restructured	Rank sums	Preference means for groups	Preference (%)
Most preferred 8% CSF	94	1.77^{a}	49^{a}
4% CSF	98	1.84^{a}	51^{a}
Least preferred 0% CSF	124	2.33^{b}	-

Means in the same column with different letters are significantly different ($p < 0.05$).

(LA, C18: 2n-6, 188 g/kg of the total FA) and linolenic acid (ALA, C18: 3n-3; 641 g/kg of the total FA). The meal is high in protein and fibre, and it can be used as animal and human food [46]. Furthermore, several authors have reported that rat diets that included chia have induced a dramatic decrease in triglycerides and an increase in HDL cholesterol; additionally, Brissette et al. [47] observed in clinical data that the consumption of *Salvia hispanica* L. seeds may increase satiety and aid weight loss in type 2 diabetes mellitus (T2DM). Thus, they may be useful for body-weight regulation in overweight/obese individuals with type 2 diabetes mellitus (T2DM). The results of total phenolics content (TPC) presented in Table 5 indicated that TPC were higher in both products prepared with 8% and 4% of CSF (2.25 mg/g GAE and 1.62 mg/g GAE) respectively, compared to the commercial products. The consumption of this type can not only be restructured for alternative uses of underutilised aquaculture products such as common carp, but it can also be supplemented with chia seeds, which may be consumed in a normal diet to produce the aforementioned effects.

3.12 Consumer Tests

The results of the consumer tests are summarised in Table 6. The preference test indicated that the restructured samples with 4% chia seed flour were the most preferred (rank sum = 98), followed by the restructured samples with 8% chia seed flour (rank sum = 94) and those with 0% chia seed flour (rank sum = 124). The data analysis found significant differences between product ranks ($p < 0.05$). In this case, the consumers were capable of significantly

differentiating between the restructured meats from the 0% CSF groups that reached the highest rank sum. Because the meat from the 0% CSF group was the least preferred, this suggests that the two samples were essentially identical in terms of preference. According to this result, the preference among both samples showed no significant difference. This could be used as the basis for the development of a restructured meat as a functional food type.

4. Conclusions

This study demonstrated that dietary fibre from CSF has positive effect on physicochemical and sensory characteristics of common carp restructured and can be used to fortify this kind of products based on aquaculture or marine species, so, populations who have an insufficient dietary fibre intake, with this healthful and beneficial product could cover part of it. The fortification of restructured meat with the dietary fibre contained in CSF up to 4 g/100 g improved the hardness, cooking yield and fibre content, maintaining a similar protein content to that of commercial products. DSC showed that CSF did not interfere with the thermal transitions of the restructured proteins. The colour properties were affected by the fortification, resulting in a wholemeal colour product. The scores for preferences in the tested groups were significantly higher than those for the control samples. These results are promising for the future implications of manufacturing and marketing of restructured gel from aquaculture or seafood, which are untapped species fortified with dietary fibre that have possible health benefits. Although the results are encouraging, an assessment of the storage stability is recommended.

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Alginate Encapsulation as a Preservation Method of Pitaya Fruit Juice (*Stenocereus* spp.)

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Abstract: Alginate is a widely used polymer matrix in food industry since it allows formation of spherical, soft, and strong membranes adequate for encapsulation of a large amount of products, including food. The flow rate of alginate solutions and the permeability of the capsules were evaluated within an acidic-low acidic pH range and different alginate concentrations. In solutions adjusted at different pH (3.0 to 7.0) with concentrations of alginate of 0.8, 1.0, and 1.2% w/v, flow rates at 20 °C were 6.95 to 10.00, 4.54-5.35, and 2.60-2.80 mL s^{-1}, respectively. Permeability of the capsules was evaluated in terms of the diffusion of H^+ions (expressed as pH) and soluble solids (°Brix). Meanwhile both diffusions were minor at 4.0 < pH < 7.0 and were significantly superior at more acidic pH ($P < 0.05$), alginate concentration did not present significant effect. Yellow, purple, and red juices from *Stenocereus* spp. fruits (pitayas) were encapsulated using 1.0% of alginate and stored with isotonic solution (3 mL g^{-1}) at 4 °C in the dark. The capsules were spherical with diameter between 4.59 and 470 mm, weight from 82.60 to 97.50 mg, and volume of 0.075-0.098 mL. Pigment (total betalains content) diffusion reached equilibrium at 24 h of storage, at which point retentions of total betalains in the yellow, purple, and red capsules were 87.79, 96.13, and 85.13%, respectively. Also, changes in the color of the capsules were observed during storage.

Key words: *Stenocereus*, pitaya, betalains, alginate encapsulation, color stability.

1. Introduction

The *Stenocereus* genus (pitaya) is a plant native to America with some species endemic to Mexico [1]. The fruits from pitaya may have yellow, red or purple pulp [2], whose color is due to the presence and concentration of betalains, water-soluble pigments with antioxidant properties and positive effects on human health [3]. According to their chemical structure, betalains are divided into two groups: yellowish betaxanthins, and reddish betacyanins [4].

The production of pitaya in Mexico has increased more than 200% in the last 10 years [5]. The fruits are locally consumed fresh and their juices have great sensorial acceptance due to their pulp color and high acidity, which confers a pleasant sweet-sour taste [2]. Nevertheless, the fruit shelf-life is no longer than 6 days [6] and new methods of preservation of pitaya fruit, pulp or juice are required in order to extend product shelf-life, for instance encapsulation [7].

Encapsulation is a technology of packaging solid or liquid materials, which are covered with semipermeable, spherical, and strong membranes [8] that provide enhanced stability under unfavorable environmental conditions [9]. This technology can be used for many applications in food industry: to stabilize the core material, control the oxidative reaction, provide controlled or sustained release, mask flavors, colors or odors, extend shelf life or preserve active compounds against loss, such as fatty acids [10], pigments [11], phenolic compounds [12], and

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microorganisms [13] among others.

The use of natural polymers in techniques of food encapsulation is advantageous over the use of synthetic ones due to their biocompatibility. Equally important is that natural polysaccharides are available in their raw form from natural sources. Furthermore, they are biodegradable, inexpensive, and friendly with the environment [14]. Several matrixes, such as maltodextrin, starch, gelatin, whey protein, caseinate, Arabic gum, chitosan, or alginate [15] are mainly used, applying different encapsulation technologies. Alginate encapsulation is an ionic gelation method and is one of the most widely used in food conservation due to its biocompatibility and low toxicity [16]; it also allows preserving ready-to-eat food for long storage periods in adequate conditions [17].

Alginic acid is extracted from seaweeds and is composed of units of mannuronic (M) and guloronic (G) acids in different proportions, depending on the source and growth conditions. These units are present in block copolymers (MM, GG, and MG). The binding of divalent cations and subsequent gel formation depend on the arrangement and relation of the blocks [18]. The GG blocks exhibit preferential binding sites for divalent counter-ions (for instance Ca^{2+}, Ba^{2+}, Fe^{2+}, or Sr^{2+}), and the bound ions interact with other blocks to form links that subsequently form gel structures; on the other hand, MM and MG blocks provide flexibility to the structure. Interfacial polymerization is instantaneous when sodium alginate solution is added to a calcium solution, with calcium alginate precipitation followed by a gradual gelation of the interior of the capsule as Ca^{2+} permeate trough the alginate system [17]. The characteristics of the gel can be manipulated by modifying manufacturing conditions: temperature, pH, ion concentration or alginate concentration.

The flow rate of aqueous alginate solutions and permeability of alginate capsules were analyzed as a function of the alginate concentration and pH of the aqueous solution. In addition, encapsulation of pitaya juices was performed to evaluate its preservation during storage.

2. Materials

2.1 Chemicals

Food-grade sodium alginate, calcium carbonate, citric acid, sodium chloride, and sucrose (Mexico).

2.2. Plant Material

Yellow fruits (pitayas) from *Stenocereus pruinosus*, purple, and red pitayas from *Stenocereus stellatus* were collected from Santiago Tonahuiztla, Puebla, Mexico, during harvesting season in 2013.

3. Methods

3.1 Alginate Solutions Encapsulation

Sodium alginate was dissolved in water (adjusted at pH 3, 3.5, 4, 5, 6, and 7 with citric acid) at 3 different concentrations (0.8, 1.0, and 1.2% w/v) at 60 °C. Using a separation funnel, alginate solutions were dropped at 20 °C into a 1.0% w/v calcium chloride solution without agitation, where capsules were kept for one minute. After that, capsules were washed with water and then storaged in aqueous medium. Flow rate of alginate solutions and permeability of alginate capsules were measured.

3.2. Flow Rate of Alginate Solutions

Flow rate was determined using samples of alginate solutions at different concentrations and pH; 100 mL samples were placed into a separation funnel opened to a quarter of the total aperture and total flow time was measured at 20 °C. The results were expressed as mL of solution per second (mL s-1).

3.3. Permeability of Alginate Capsules

Capsules were elaborated according to 3.1. section and then storaged with distilled water as packing liquid with a solution/capsule ratio of 2 mL g-1 during 0, 1, 2, 3, 4, 5, 6, 7, 10, 17, 21, and 24 d at 4 °C in the

dark.

Capsules were drained and packing liquid was measured to H^+, expressed as pH (Denver Instrument, UB-10, USA) and total soluble solids (Boeco, VBR32, Germany), expressed as °Brix.

3.4 Fruit Selection and Juice Preparation

Undamaged yellow, purple, and red pitayas without spines were packed in Food Saver ® plastic bags of 500 g to 800 g whole fruit under vacuum, and stored at -20 °C until encapsulation. Prior to encapsulation, samples were placed at 20 °C for 1 day, and peel. Using a juice extractor (Nutri-Max, Mexico), seeds were removed from the pulp, which was sieved through 710 μm mesh, and then centrifuged (Velocity 14R, Dynamica Scientific, UK) at 10,576 × g for 20 min at 20 °C. Juices were analyzed for pH, total betalains, and color parameters.

3.5. Alginate Encapsulation of Pitaya Fruit Juices

Sodium alginate was mixed with pitaya juice (1.0% w/v) until homogenization at 60 °C. Using a separation funnel, homogenized pulp with sodium alginate was dropped at 20 °C into a 1.0% w/v calcium chloride solution without agitation, where capsules were kept for one minute. After that, capsules were washed with water and then storage with isotonic solution (0.1 M sodium chloride, 0.3 M sucrose) as packing liquid with a solution/capsule ratio of 3 mL g-1 during 0, 1, 2, 4, 6, 24, 48, 72, 96, and 120 h at 4 °C in the dark.

For analysis, capsules were drained and their color was determined, the packing liquid was used to measure betalain content.

3.6 Quantification of Betalains

Total betalains (the sum of betacyanin and betaxanthin) content were quantified using a UV-Vis spectrophotometer [19]. Pigment concentrations were calculated using Eq. (1).

$$B = \frac{(A \times DF \times W)}{(\varepsilon \times \mathrm{L})} \times 1{,}000 \quad (1)$$

Where B is betaxanthin and betacyanin content (μg mL-1 or μg g-1) using indicaxanthin and betanin respectively as reference. A is absorbance (483 nm for betaxanthin and 538 nm for betacyanin), DF is dilution factor, W is molecular weight: 550 g mol-1 for betanin, and 308 g mol-1 for indicaxanthin); ε represents the molar extinction coefficient (60,000 L mol-1 cm-1 for betanin and 48,000 for indicaxanthin), and L is path length (1 cm).

A mass balance was performed to determinate betalain retention in capsules. At each time, betalain content in capsules was calculated by the difference between initial content in capsules and pigment content in the isotonic solution.

3.7 Color

Chromatic parameters (L*, a*, and b*) were measured at 0, 24, 48, 92, 96, and 120 h using a colorimeter (Hunter Lab, Color Flex EZ, USA). Samples of drained capsules were placed in a quartz cell with a measurement area of 5 cm (2 in) in diameter. The illuminant used was D65, and the standard observer angle was 10° against a white background. With a* and b* values, Hue angle (H°) and Chroma (C*) were calculated using Eqs. (2) and (3), respectively.

$$H° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

3.8 Statistical Analysis

Results are reported as mean ± standard deviations (n = 3). Means were compared by analysis of variance and Tukey's comparison test ($P < 0.05$). Data were analyzed using Minitab 16 (Minitab Inc., USA).

4. Results and Discussion

4.1. Flow Rate of Alginate Solutions

The mechanical and physicochemical properties of alginate structures are affected by the composition and the formation process [20]. Regardless pH values,

changes in the consistence and in the flow rate of solutions were observed with different alginate concentrations (Table 1): the higher alginate concentration in the solution, the greater viscosity or flow resistance [21].

Aqueous alginate solutions present shear-thinning behavior and are non-Newtonian fluids [16]. When the alginate is in solution it behaves as pseudoplastic and as Newtonian fluids at concentrations of 2.5% and 0.5%, respectively [22], but there is not a well-defined characterization of the fluids at concentrations in between.

On the other hand, flow rate (or thickness) for the 0.8% alginate solution peaked at pH of 6. Moreover, the maximum resistance to flow was reached at pH 3.5 for solutions formulated with 1.0 and 1.2% of alginate. It has been reported that greater thickness of alginate solutions is reached at 3 < pH < 4 [23]; in contrast, some authors reported that the resistance to flow increases at pH 5 and it is unstable above 11 [22].

4.2. Permeability of the Capsules

The permeability and other properties of alginate gel structures (such as viscosity, strength, molecular weight, among others) are directly influenced by several process conditions, in particular alginate concentration, pH, temperature, alginate composition, and availability of calcium ions [24, 21].

Regardless alginate concentration, with capsules of pH < 3.5 solutions diffusion of H^+ ions through membranes was increased ($P < 0.05$) after 24 d of storage among different encapsulated solutions. Thus, pH of liquid packing (water) was diminished over time. Indeed, pH decreased from 7.5 to 4.5, 4.3, and 4.1 after 24 d in liquid packing of capsules manufactured with solution at pH = 3.0 and 0.8 (Fig. 1A), 1.0 (Fig. 1B) and 1.2% (Fig. 1C) of alginate, respectively. Solutions with less H^+ diffusion were those encapsulated at 4.0 < pH < 7.0 for all alginate concentrations assayed ($P < 0.05$). Alginate films in aqueous medium are fully permeable due to their high porosity and large amount of water in the gel structure [14].

Similarly to diffusion of H^+ ions, acidic pH values (< 4.0) increased soluble solids diffusion from capsules to the medium and 4.0 < pH < 7.0 showed less °Brix in liquid packing.

Therefore, low acidic pH grants superior capsule integrality over time. Indeed, in 0.8% alginate capsules the minimal diffusion of solids was observed with pH = 6.0; however, statistical differences were no significant ($P < 0.05$) after 24 d of storage among pH range tested (Fig. 2A).

Furthermore, pH 4.0 and 6.0 showed fewer soluble solids permeability than other pH values assayed ($P < 0.05$) for 1.0% alginate capsules (Fig. 2B). Moreover, in those manufactured with 1.2% of alginate, pH = 7.0 guaranteed physical stability of capsules over time, even though there were no significant statistical differences ($P < 0.05$) among conditions evaluated (Fig. 2C).

4.3. Alginate Encapsulation of Pitaya Juices

Pitaya juice (4.05 < pH < 4.87) adequately formed

Table 1 Flow rates of solutions at several pH values (3-7) and different alginate concentrations (0.8%, 1.0%, and 1.2%).

	Flow rates of alginate solutions (mL s^{-1})		
pH	0.8%	1.0%	1.2%
3	6.95 ± 0.08^{d}	4.54 ± 0.40^{b}	2.68 ± 0.03^{b}
3.5	9.01 ± 0.28^{bc}	5.35 ± 0.08^{a}	2.80 ± 0.02^{a}
4	9.01 ± 0.16^{bc}	5.29 ± 0.13^{a}	2.79 ± 0.04^{a}
5	8.77 ± 0.10^{c}	4.76 ± 0.08^{b}	2.67 ± 0.05^{b}
6	10.00 ± 0.11^{a}	5.00 ± 0.06^{ab}	2.70 ± 0.02^{b}
7	9.29 ± 0.10^{b}	4.59 ± 0.06^{b}	2.79 ± 0.02^{a}

Different letters represent significant differences between pH values ($P < 0.05$).

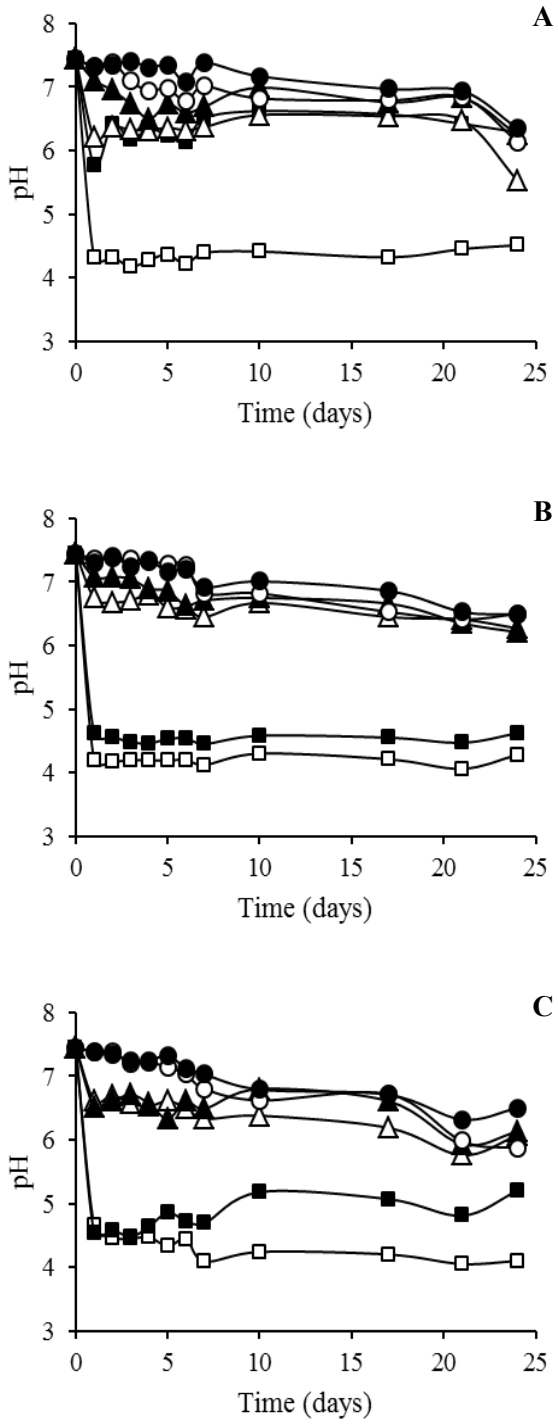

Fig. 1 Diffusion of H^+ions from capsules to medium expressed as pH. Capsules were prior adjusted at pH 3 (□), 3.5 (■), 4 (Δ), 5 (▲), 6 (○), and 7 (●). Capsules were manufactured with 0.8% (A), 1.0% (B), 1.2% (C) of alginate.

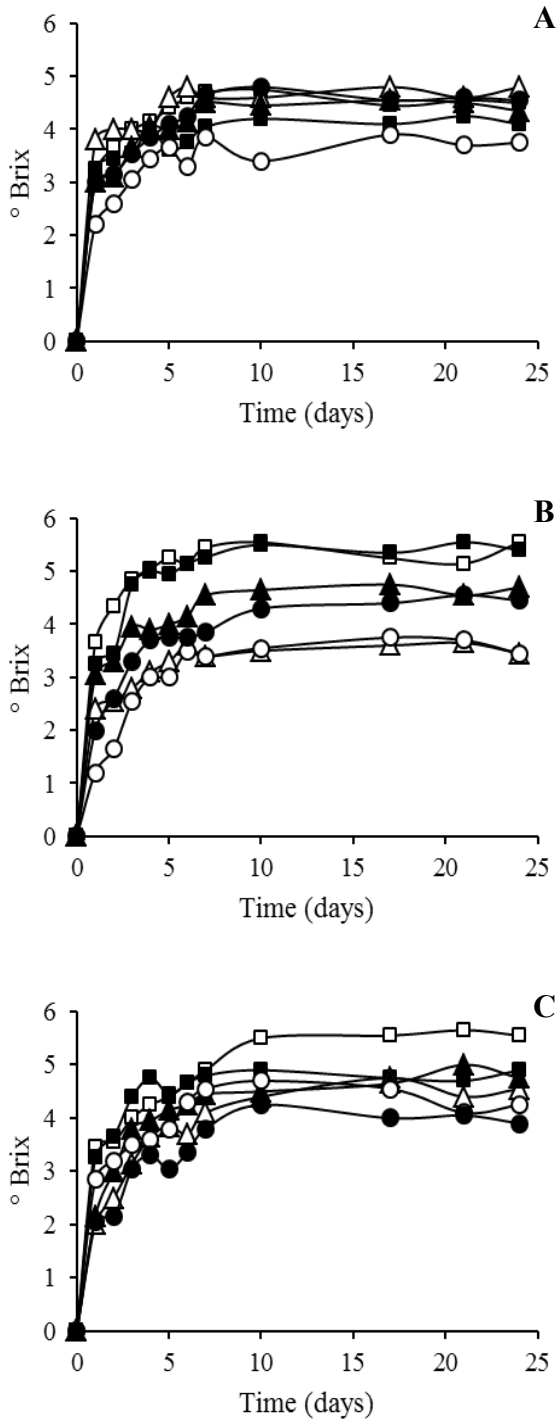

Fig. 2 Diffusion of soluble solids from capsules to medium expressed as °Brix. Capsules were prior adjusted at pH 3 (□), 3.5 (■), 4 (Δ), 5 (▲), 6 (○), and 7 (●). Capsules were manufactured with 0.8% (A), 1.0% (B), and 1.2% (C) of alginate.

capsules (Fig. 3) using 1.0% of sodium alginate as polymer matrix. The capsules presented a spherical shape with similar diameter, weight, and volume among fruits (Table 2).

Equilibrium of pigment diffusion from the capsules to the media was reached within 24 h with retention of total betalains in the capsules of 87.79 ± 0.06, 96.13 ± 0.05, and 85.13 ± 0.12% in the yellow, purple, and red juice, respectively; mentioned retentions were maintained for 120 h without significant differences (Fig. 4).

Pigment diffusion affected the chromatic parameters of the capsules during storage (Table 3). Changes in L*values suggested that capsules presented brighter colors at the end of the experiment.

On the other hand, H° indicated changes in the tone of the product along with more saturated colors (C* values) with storage.

(a) (b) (c)

Fig. 3 Alginate capsules of yellow (A), purple (B), and red (C) pitaya juice.

Table 2 Physical characterization of juice capsules.

Pitaya capsules	Diameter (mm)	Weight (mg)	Volume (mL)
Yellow	4.61 ± 0.19	82.60 ± 0.75	0.075 ± 0.01
Purple	4.59 ± 0.14	83.10 ± 7.56	0.080 ± 0.00
Red	4.70 ± 0.16	97.50 ± 0.35	0.098 ± 0.01

Presented results are the mean ± S.D. of 3 replicates.

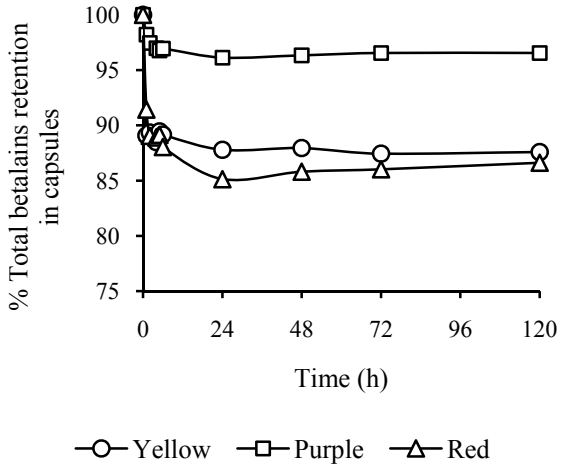

Fig. 4 Retention of total betalains in capsules of pitaya juices preserved using isotonic solution during 120 h of storage period at 4 °C.

Table 3 Chromatic parameters of alginate capsules at 0, 24 and 120 h of storage at 4 °C.

Capsules	Time (h)	L*	H°	C*
Yellow	0	23.96 ± 0.42	37.52 ± 0.17	54.49 ± 1.07
	24	28.44 ± 0.56	45.42 ± 0.37	65.48 ± 0.85
	120	29.04 ± 0.35	45.17 ± 1.46	66.57 ± 1.01
Purple	0	22.05 ± 0.09	21.93 ± 0.39	49.98 ± 0.42
	24	25.39 ± 0.44	17.73 ± 0.13	54.83 ± 0.35
	120	25.82 ± 0.84	17.24 ± 0.44	56.20 ± 1.65
Red	0	17.89 ± 0.03	27.90 ± 0.01	45.82 ± 0.08
	24	20.22 ± 0.82	33.39 ± 0.58	58.24 ± 1.45
	120	20.27 ± 1.10	33.11 ± 1.40	58.63 ± 2.90

Presented results are the mean ± S.D. of 3 replicates.

In products pigmented with betalains, similar color changes have been reported [25, 26] during storage or stability tests, and they are directly related with the pigment stability, which is influenced by its concentration, temperature, presence of light, water activity, among others [4], conditions that should be controlled if a good color stability are desired.

5. Conclusions

In this paper it was demonstrated that alginate encapsulation is an adequate method to preserve food under controlled conditions.

Physical properties of the capsules are determined by the alginate concentration and pH used during process. Importantly, it was found that pH plays an important role in capsules stability during storage. Particularly, pH between 4.0 and 7.0 allows obtaining uniform and strong membranes with minimal diffusion of H^+ and soluble solids through the capsules.

Indeed, encapsulation of pitaya juices preserves betalain content in the capsules with good color stability during storage. Further sterilization after food encapsulation is recommendable in order to extend shelf life and to guarantee appropriate microbiological quality. This information may help to develop new products, and also increase commercialization and value of pitayas.

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Homemade versus Commercial Jarred Baby Foods with Regard to Nitrites and Nitrates Content

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Abstract: This paper presents results of nitrites and nitrates determination in two types of baby foods: commercial products in jars and their homemade conventional counterparts. Nitrites levels in all analyzed samples were below of the detection limit (< 0.9 mg/kg) of applied spectrophotometric method with Griess reagent. Nitrates contents in commercial products ranged: 9.1-38.1 mg/kg while in homemade baby foods levels between 26.6 mg/kg and 118.8 mg/kg were obtained. All the contents of nitrates were lower than the EU legislation maximum limit (200 mg/kg). Comparison of each type of commercial product with its homemade counterpart baby food evidenced significant differences ($p < 0.05$) in average nitrates levels in favor of the first type. Apart from determining and comparing the levels of nitrates in the baby food samples also risk assessment for an average 6-months old infant to nitrates exposure was conducted. The estimated nitrates intake with a typical portion of 200g of baby food ranged between 6% and 25.7% of acceptable daily intake for commercial and from 18.0% to 80.3% for homemade ones.

Key words: Nitrites, nitrates, baby food, food safety, weaning, infants.

1. Introduction

Optimal kind of complementary foods during the first year of life has always been common subject of discussion. Nutrition practices differ in many countries, however according to WHO weaning should start at the age of 6 months to cover infant's nutritional needs and get used to solids [1]. During this transition period children are particularly vulnerable. Dietary exposure to chemical hazards of an infant tends to be even three times that of an adult. Both composition and consumption pattern of baby foods are important. Complementary meals may be either self-prepared or bought as ready-to-eat products. Quality of commercial baby foods is regulated in EC guideline [2]. In turn, composition and preparation of homemade meals is parents' responsibility. Nutrition specialists provide recipes for complementary food, nevertheless there are no studies available how parents use them [3]. Commercial products are thought to be safe and nutritious alternatives instead of self-prepared meals. However, several harmful substances originating from the nature of the product's ingredients may be present. Consequently assessment of dietary exposure to contaminants in baby foods is needed to ensure healthy growth of infants.

Reports regarding serious effects for infants connected to nitrates and nitrites consumption with food are available. Nitrates and nitrites ions may cause many physiologic effects including hematological, cardiovascular and respiratory outcomes [4, 5]. Therefore, the interest in its determination in baby meals is of great importance because of its possible exposure route through different foodstuff prepared at home or bought as commercial baby food.

Current women's lifestyle, convenience and legislatively guaranteed quality of commercial baby foods encourage mothers to buy them to a large extent. Great percentage of women declares using this kind of products but when asked about preferences towards the kind of complementary food they rather show willingness to home prepared traditional meals. It reveals lack of their real conviction in weaning food choices and need of carrying research of the both

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types of meals. Mothers of infants are strongly interested in evidence, which could help to clear their doubts concerning choice of complementary baby foods [6]. Little available information is to confirm any opinion: about superiority of homemade over commercial baby foods or inverse.

Recent studies on nitrates and nitrites contents represent outcomes only commercial baby foods without taking into consideration home-prepared ones [7-10]. There is a lack of data concerning comparison of commercial and equivalent homemade weaning meals.

This research presents comparison of ready-to-eat baby foods in jars with their homemade counterparts on the basis of nitrates and nitrites content and assessment whether at the beginning of weaning period the use of any type of meal could be a cause of safety concern.

2. Materials and Methods

2.1 Selection of Material

The most responsible sources of infants' dietary intake of nitrates are vegetables and drinking water [11]. Vegetable and vegetable-meat products were researched. Homemade meals were prepared with components bought in popular groceries in western Poland—in Poznan city. Commercial baby foods were purchased in the same shops. Ready-to-eat foods in jars constituted five types of the most popular products indicated by mothers in previous study concerning their preferences towards complementary foods in jars [6]. Recipes of homemade meals derived from infant feeding guides. Selection of recipes aimed to remind composition of preferred jarred foods as much as possible. To the current knowledge, no studies are available on how caregivers use complementary meal recipes at home. The assumption was made to self cook the meals for assurance that they reflect chosen recipes of the greatest. Conducted study apparently shows a very realistic and most probably common case when decision to choose type of a meal is taken among easily accessible products without searching for any special variants of ingredients or preparation methods.

Shelf life of commercial baby foods was maximum 24 months. Nevertheless this long term usefulness should not affect nitrates content for the reason that products were previously intended to thermal preservation and tight seal. Home-prepared meals were made right before the research to restrain changes of nitrates contents during storage.

Table 1 presents composition of all the meals. Samples "C" (commercial) and "H" (homemade) indicated with the same number constitute each other's counterparts in nitrates content comparison.

2.2 Nitrites and Nitrates Determination Method

The spectrophotometric method according to the

Table 1 Composition of meals purposed for nitrates and nitrites content determination (C—commercial, H—homemade baby foods).

C	Composition of commercial baby foods (labels of jarred baby foods)	H	Composition of homemade baby foods (*www.parenting.pl*; infant nutrition guides)
C1	carrot 39.2%, water, potatoes 16%, rabbit meat without decoction 9%, corn starch, celery 2.5%, soybean oil	H1	3/4th of glass of chopped vegetables: carrot, potato, celery, 250 ml of water, 1 spoon of precooked rabbit meat without decoction, 1 spoon of butter, 2 spoons of corn cereal
C2	carrot, water, potatoes, turkey meat without decoction, peas, gluten-free wheat starch, pore, soybean oil	H2	1 middle-sized carrot, 1/2 middle-sized potato, small piece of parsley, 1 spoon of separately cooked turkey meat without decoction
C3	water, carrot 28%, potatoes 15.7%, rice 10.3%, chicken meat without decoction 9%, celery 1.8%, soybean oil	H3	One small carrot, one small potato, a piece of parsley, 250 mL of water, one spoon of separately cooked chicken meat without decoction, one spoon of soybean oil
C4	water, carrot 29%, potatoes 17%, gluten-free wheat starch, pore 2.5%, soybean oil	H4	3/4 glass of chopped vegetables: carrot, potato, parsley, celery, a spoon of butter, 250 mL of water
C5	potato 31%, spinach 30%, skimmed milk 29%, water, cream 3%, rapeseed oil 1.3%	H5	One middle-sized potato, 15 spinach leafs, one little spoon of fresh butter, 100 mL of water

methodology of AOAC was used. Results are expressed as mg/kg fresh weight of the sample. The analysis was performed following colorimetric procedure and measuring intensity of the color produced through the reaction between nitrites ions with Griess reagent. Nitrates were earlier reduced to nitrites with the use of powdered cadmium [12]. All the analyses were made in three replications.

2.3 Statistical Methods

Experimental data were subjected to one-factor analysis of variance (ANOVA) in order to determine statistical differences between mean values. Also the Games Howell's multiple comparison post-hoc tests were carried.

The statistical significance level was set at $p < 0.05$ (results with p values less than 0.05 were considered as statistically significant). Calculations were performed with statistical software package SPSS Statistics 14.0.

2.4Estimation of Infants' Dietary Exposure

To estimate infants' dietary exposure to nitrates and nitrites, mean values of their concentration in a meal portion were compared with acceptable daily intake for an average 6-months old baby of body weight (bw) 8 kilograms.

3. Results and Discussion

3.1 Concentration of Nitrates and Nitrites

Nitrites content in all the samples was below the detection limit of applied method (< 0.9 mg/kg). Consequently nitrites levels comparison was not possible. Although even if considering nitrites amount of 0.9 mg/kg in a meal, a 200-gram portion would contain 0.18 mg of nitrites. According to ADI of 0-0.07 mg/kg bw/day [13], a daily limit to an 8 kg baby amounts 0.56 mg of nitrites. Therefore according to legislation limits levels below 0.9 mg/kg consist of values below 32% of ADI and examined foods can be perceived as safe.

Average concentrations of nitrates in all samples are summarized in Fig. 1. Values obtained varied considerably according to the composition and type of the meal.

Nitrates contents in commercial products ranged from 9.1 mg/kg to 38.1 mg/kg. In homemade meals values extended from 26.6 mg/kg to 118.8 mg/kg being much more diversified than in jarred foods. Levels in all samples were lower than legislation limit of 200 mg/kg [14].

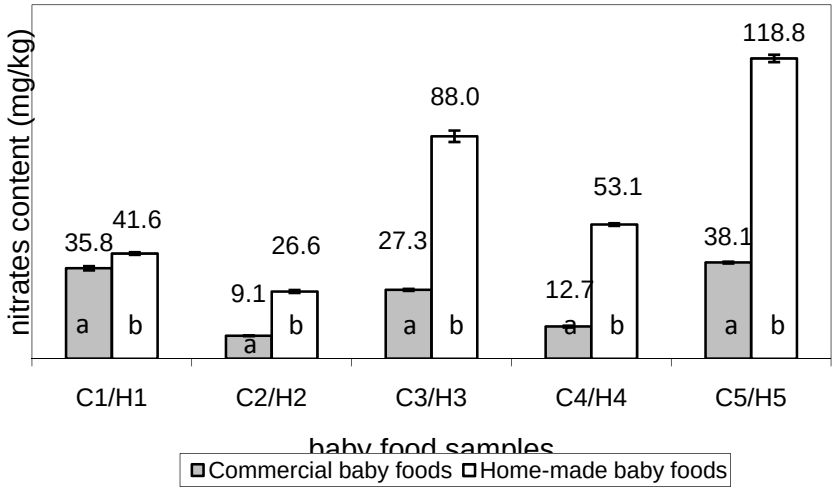

Fig. 1 Mean nitrates content results in examined baby foods (mg/kg).

Error bar represents standard deviation. Different lower-case letters (a and b) indicate significant differences (one-way ANOVA test $p < 0.05$) between commercial and homemade meals.

Both within commercial alike in homemade foods maximum contents of nitrates were found for the meal number 5 (potatoes and spinach). This is consistent with common observation that spinach is classified as one of the most nitrates-accumulating vegetable, causing health risks for infants [15]. Study's investigation revealed that homemade variant of spinach-potato dish contained approximately 3 times more nitrates than its commercial counterpart.

Comparison of nitrates contents revealed visible differences between both types of foods in favor of the industrially produced ones. The most significant difference was observed between jarred vegetable C4 meal and its homemade equivalent H4. In the latter one nitrates content of 53.1 mg/kg appeared to be 4-times higher than in its commercial counterpart: 12.7 mg/kg. In three other variants of: vegetable-turkey, vegetable-chicken and pure vegetable meals numbered respectively: 2, 3,5, differences ranged at least 3-times more contents in homemade counterparts. Values in vegetable-rabbit samples C1 and H1 did not differ considerably: amounted respectively 35.8 and 41.6 mg/kg.

Mean nitrates content for total commercial samples amounted 24.6 mg/kg, while in homemade equivalents: 65.6 mg/kg. Comparison of nitrates levels of commercial and homemade foods revealed statistically significant differences between the two groups of meals ($p < 0.05$).

Little data regarding nitrates content in homemade baby foods are available. Study carried by Murone et al. in 2005 referred to excess of nitrates in inappropriately stored meals [5]. Current research was carried for freshly-prepared foods therefore is not relevant to compare with the mentioned. This study indicated lower than other authors' concentrations of nitrates in commercial foods: 61-108 mg/kg [7, 8, 10].

Results obtained reveal that much attentionis paid to safety of commercial foods. Low values of nitrites and nitrates in those products prove proper selection of raw materials and maintenance of surveillance by authorities.

It is of great interest that the highest levels of nitrates in any investigated meal did not provide more than regulations' limit.

3.2 Evaluation of Dietary Exposure to Nitrates

Apart from comparing the concentration of nitrates with the maximum European legislation, significant assessment of potential health hazards for children was obtained by calculating nitrates intake deriving from a portion of a meal and comparing each amount with ADI set by international organizations: for nitrates 0-3.7 mg/kg bw/day (Opinion, 2008). According to the FAO/WHO data estimated daily nitrates intake limit for a baby of bw 8 kg amounts 29.6 mg (indicated as 100% of ADI on the upper axis in Fig. 2).

According to data of "DONALD" study children aged 6 months ingest daily about 142-203 g of commercial vegetable/meat foods [16]. In this analysis assumption of a 200 g portion was taken into consideration. Fig. 2 presents results of a 6-months old infant's exposure scenario to nitrates from investigated meals.

Although any of determined nitrates content wasn't near legislation limit, some alarming issues can be observed within obtained percentage values of ADI for homemade foods. In particular attention should be paid to results of vegetable-chicken H3 (59.5% ADI) and potato-spinach meal H5 (80.3% ADI). Results for the rest of homemade meals amounted 18%-35.9% of ADI. Values obtained for commercial products: 6%-25.7% did not approach high levels of ADI for considered child's age.

Ingestion none of a single 200 g portion of examined meal would have been cause of exceeding ADI. Nevertheless problem can occur when consuming more servings per day, which contain comparable to homemade meal of vegetable-chicken H3 and potato-spinach H5 quantities of nitrates. Daily intake of around 336 g of H3 and 250 g of H5 could

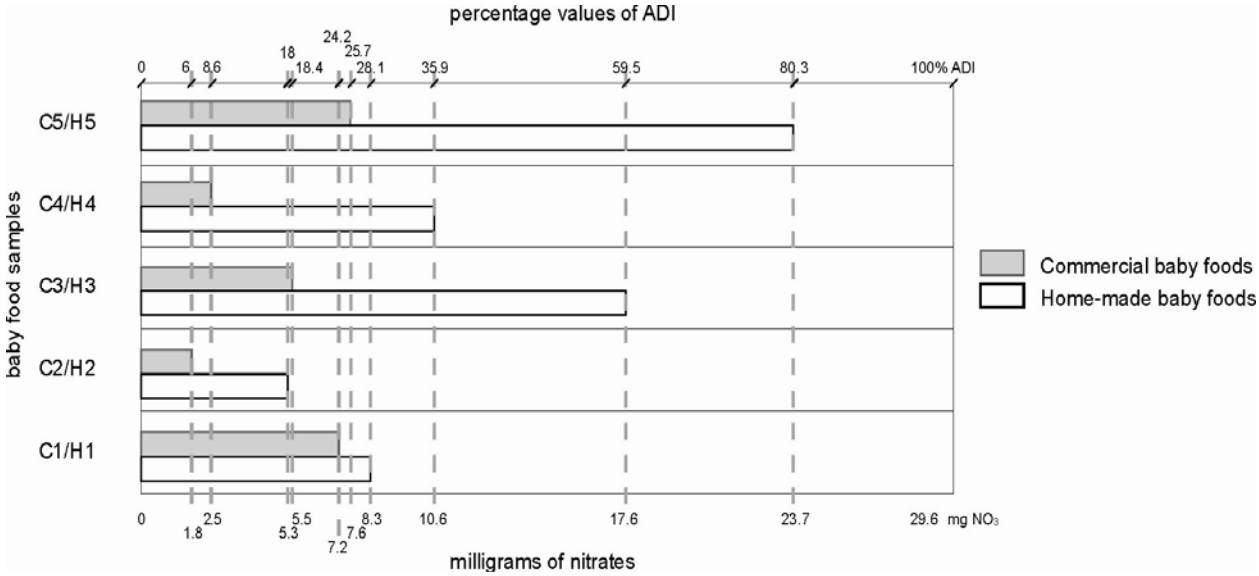

Fig. 2 Estimation of the nitrates intake through investigated baby foods consumption based on the mean concentrations found in the study.

Lower axis: mg of nitrates provided to an infant ingesting a 200 g serving of a meal.

Upper axis: results reflected as percentage values of ADI.

induce toxic effects in infant. Those estimated exposures are most likely to be of health concern. Total degree of risk depends not only on intake of these food products, but also overall dietary nitrates intake aside from other sources. Except vegetable also fruit-based and cereal foods have been reported as nitrates source. In case of non-breastfed infants-reconstitution of powdered milk with water may additionally raise nitrates content in their daily diet [17]. Fragility of infants requires special care about food choices destined for them. With long-term daily ingestion of high-nitrates foods the risk of mild to moderate methemoglobinemia would be increased.

Levels of nitrates found in homemade spinach meal reveal that its consumption should be carried with caution in little infants. In its relation to much more safe levels in equivalent commercial product, parents should consider frequency of feeding with homemade spinach meals. Levels of nitrates indicated in homemade vegetable-chicken meal are also disquieting.

4. Conclusion

Significant differences between commercial and homemade baby foods in nitrates levels were obtained. Investigated commercial foods appear to be less contaminated with nitrates when compared to their homemade counterparts. Evaluation of dietary exposure does not raise any doubts in potential danger. In turn: case of some homemade equivalents' nitrates contents, even though less than legislation limit appears to be alarming.

Study's data draw important conclusion: decisions about weaning foods should be based on the knowledge about critical points in the selection of homemade meals' ingredients. Findings imply that formulation of infant's diet only on homemade foods at the beginning of weaning period could impose danger in exceeding nitrates levels. This issue merits further research involving homemade foods composed of ingredients originating from different sources and with a comprehensive and well-defined sample to analyze the complete weaning diet of infants and to enable discussion on factors contributing to the nitrates contents in the weaning diet in more depth.

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The Effect of N-Fertilization on *Rosmarinus Officinalis L.* (An Upright Variety) Yield in Central Greece

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Abstract: The effect of three different N-fertilization levels (N1: 625, N2: 385 and N3: 770 kg ha^{-1}; where in case of N1 was used the 3-6-10+3MgO+30% OM and in cases of N2-N3 the 26-0-0 fertilizers) on fresh and dry weight of the perennial *Rosmarinus officinalis* (upright cultivar) was investigated during the 2nd year after establishment at the Experimental Farm of the Technological Educational Institute of Thessaly in Greece (TEI; Larissa plain) in 2015. It is well documented that the crop reaches its potential yield on the third year of cultivation and continues producing biomass for as long as eight years. Complete weather data (air temperature, radiation, air humidity, precipitation) were recorded hourly in an automatic meteorological station, which was installed to the experimental farm of TEI. Upon harvest (November 3rd 2015), the crop reached a maximum fresh yield of 11.67 tons per hectare and dry yield of 4.3, respectively. The average fresh weight was 8.2, 8.4 and 8.9 t ha^{-1} and the dry weight were 2.6, 3.1 and 3.2 t ha^{-1} for the N1, N2 and N3 levels, respectively. Furthermore the higher moisture content was observed in the case of N1 level (68%). Therefore, the above data show that rosemary cultivation could be a promising alternative crop, especially in case of the consideration that average selling price of dry rosemary in Greece is 3.5 € kg^{-1} and the average gross income exceeds the amount of 10,000 € ha^{-1}.

Key words: *Rosmarinus officinalis L.*, fertilization, upright variety, fresh yield, dry yield.

1. Introduction

Rosemary is an evergreen perennial shrub, which belongs to the class *Magnoliopsida*, subclass *Lamiales*, family of *Lamiaceae* or *Labiate* (*Labiatae*) which comprises up to 200 genera and about 3,500 species, and it is naturally found in all of the coastal regions of the Mediterranean Sea [1] and the genus *Rosmarinus*, *officinalis* species. The plant botanically characterized from its square stems, while leaves are opposite in pairs and arranged crosswise. Most important of all is the fragrant smell.

Generally rosemary is a plant which grows in areas where there is mild hot and cold climate. A feature of the plant is that it can be grown in lowland areas with an elevation of up to 600 meters [2]. *Rosmarinus officinalis* L., has been widely cultivated since antiquity as herb and garden plant, and also for its essential oil [3].

The red light affects the morphology of rosemary, the phenology as well as the essential oil. More specifically, the quantity and quality of the essential oil of rosemary which was grown in greenhouse conditions, affected by the wavelength of red light (660 nm) and dark-red (730 nm) which was applied [4]. Photoperiod is a very important factor, who irrelevant to the formation of the plant metabolic mechanism, from the production of photosynthetic carbon to determine the path that leads either to the class selection (terpenoids, phenylpropanoids) or in group selection (monoterpenes, sesquiterpenes, etc.) [5].

The temperature seems to be an important factor determining both the composition of the essential oil and the content of plant essential oil. The effects of temperature and moisture in growth, development and morphology of plants cannot easily be studied separately as it is closely related and interdependent

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environmental factors. The sum of the amount of the four main components represents the phenolic path and appears to be affected by how hot the climate is [6]. The number of flowers and their total weight was greatest at low than at high temperatures [7]. Plants adapted to hot and dry climates environments develop small leaves, wrapped and covered with a dense coat to resist the water loss from the surface [8].

Irrigation must be done depending on the precipitation of the selected region. Thus, in areas with low rainfall where plants get strong root system is necessary supplemental irrigation. In contrast, in areas with rainfall (over 500 mm) and in case that the plants have fully installed, the crop can continue to develop with minimal irrigation at critical points. Specifically, the lack of water and the water stress in rosemary reduces plant growth in contrast to the proportion of the essential oil which is increased [9].

Rosemary is cultivated for green and dry drug and essential oil. The harvest period is determined by the desired product. Because the crop is perennial, harvest takes place from the second year of crop establishment and the plant goes into full production in the third year. The aroma of flowers diffused in the environment so attracted insects and as a result have better pollination and crossing of not self-pollinating plants [10].

This study was conducted in the main agricultural plain (Thessaly) to evaluate the effect of Nitrogen fertilization on the fresh and dry yield of Rosemary repens and upright (R*osmarinus officinalis*) in Greece.

2. Materials and Methods

Rosemary upright was cultivated at the experimental Farm of TEI of Thessaly. The planting took place on 09.04.2014 and the harvest during its second year after establishment at the start of the flowering period when the concentration of essential oils maximized [11]. The effect of three different N-fertilization levels (N1: 625, N2: 385 and N3: 770 kg ha^{-1}; where in case of N1 was used the 3-6-10+3MgO+30% OM and in cases of N2-N3 the 26-0-0 fertilizers) on fresh and dry weight of the perennial *Rosmarinus officinalis* (upright cultivar) was investigated during the 2nd year after establishment at the Experimental Farm of the Technological Educational Institute of Thessaly (TEI; Larissa plain) in 2015. The experiment had a randomized block design and each experimental piece had an area of 16 m^2 with 28 plants. The leaves and shoots after harvesting were dried in a dark place at room temperature. To obtain the essential oil of rosemary was used the method of distillation following the below procedure. In a 500 mL flask placed 10 gr of dry drug and supplement to the medium with water. Then the flask placed on the heating plate of the Clevenker device. The fresh, dry weight and the essential oil content data were analyzed using the GenStat 7th Edition statistical package.

3. Results and Discussion

3.1 Soil Analysis

The soil used for the rosemary cultivation was of low organic matter content and low salinity as it is presented in the following Table 1.

3.2 Plant Height

The average plant height (Fig. 1) during the first 15 days after planting was higher in N3 treatment.

In the coming 45 days, the average height showed no significant differences in the different fertilization levels ($p > 0.05$). At the 75th day from the planting, the average height of plants (Table 2) showed no statistically significant difference in relation to the fertilization levels ($p > 0.05$).

3.3 Fresh Yield

Into Fig. 2 is illustrated the average fresh weight of rosemary as it is affected from the three different used nitrogen levels and fertilizer types. There was not found any statistical significant difference (Table 3) between the used nitrogen levels. It was found that the use of the N1 type and level of fertilizer (625 kg ha^{-1}

Table 1 Chemical properties of the Rosemary field experiment.

Property	Soil depth (0-25) cm	Soil depth (25-50) cm
	Before transplanting	After harvest
Texture	Loam	Loam
pH (1part soil:5parts H_2O)	7.81 ± 0.16	7.82 ± 0.16
Electrical conductivity, extract(dSm^{-1}) (1part soil:5parts H_2O)	0.11 ± 0.01	0.10 ± 0.01
Organic matter (%)	0.93 ± 0.05	0.77 ± 0.04
N-inorganic (mg kg^{-1})	44.8 ± 4.07	41.3 ± 3.44
K-exchangeable (mg kg^{-1})	373.3 ± 7.45	314.5 ± 7.86
P –Olsen (mg kg^{-1})	13.1 ± 1.87	10.2 ± 1.46
$CaCO_3$ (%)	0.63 ± 0.07	1.04 ± 0.12

* Data represent average means and SE deviation. (n) = 4.

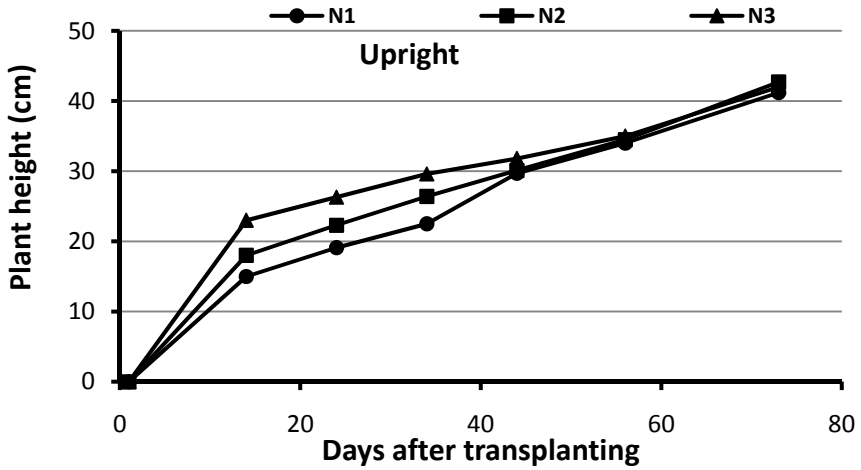

Fig. 1 Average height of *Rosmarinus officinalis* as affected of the three different nitrogen levels and fertilizer types.

Table 2 Average plant at the 75th day after transplanting.

Treatments	Rosemary
Fertilization levels	Plant height (cm)
N1	41.7 ± 2.09
N2	42.7 ± 2.25
N3	42.0 ± 2.17

* Data represent average means and SE deviation. (n) = 4.

from 3-6-10+3MgO+30% OM) which was almost the double quantity of N2 (385 kg ha^{-1} from 26-0-0) produced an average yield of 8.19 t ha^{-1}, yield almost the same with the produced in case of N2 level (8.36 t ha^{-1}). Moreover by increasing the quantity of the 26-0-0 to the double dose the average yield increased only by 0.54 t ha^{-1}.

3.4 Dry Yield

Fig. 3 illustrates the average dry weight of rosemary as it is affected from the three different nitrogen levels and fertilizer types. There was found no statistical difference (Table 3) for the examined factor. Verified the results of fresh weight, it was found that by increasing the quantity of the 26-0-0 to the double dose (N2 vs. N3) the average dry yield increased only by 0.15 t ha^{-1}. In case of the N1 the average dry yield was 2.64 t ha^{-1}.

Therefore, it could be assumed that N1 type is not delivering the expected results, and rosemary is a plant

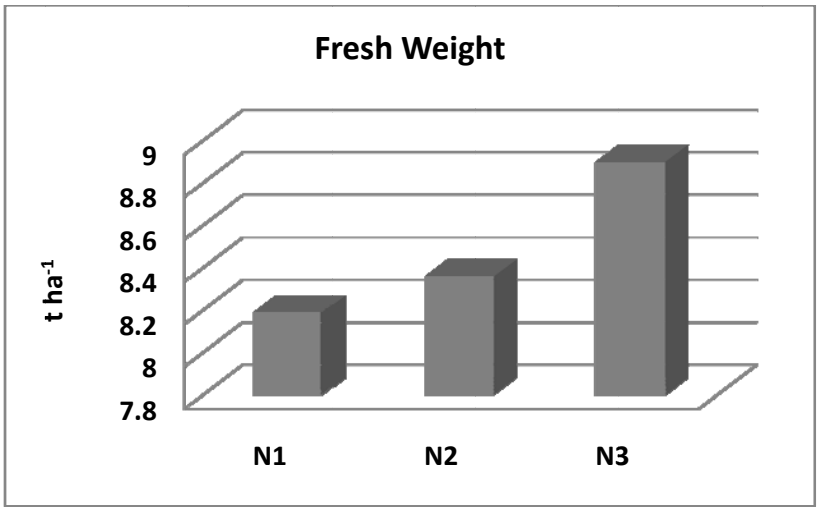

Fig. 2 Fresh weight of *Rosmarinus officinalis* as affected of the three different nitrogen levels and fertilizer types.

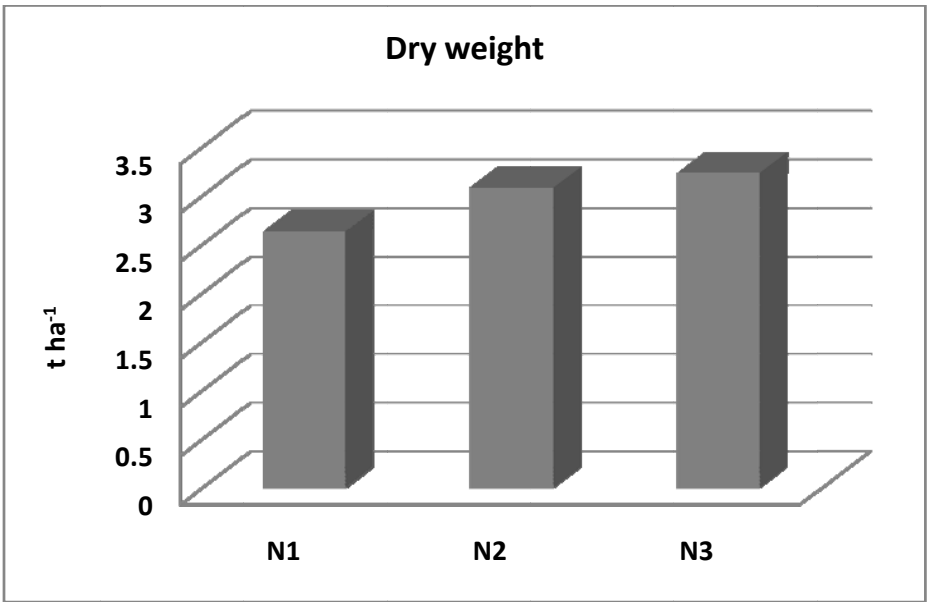

Fig. 3 Dry weight of *Rosmarinus officinalis* as affected of the three different nitrogen levels and fertilizer types.

Table 3 Fresh and dry weight of *Rosmarinus officinalis* under different nitrogen levels and fertilizer types.

Rosmarinus officinalis	Fresh weight (t ha^{-1})	Dry weight (t ha^{-1})
Nitrogen levels		
N1 (625 kg ha^{-1}; 3-6-10+3MgO+30% OM)	8.19	2.64
N2 (26-0-0; 385 kg ha^{-1})	8.36	3.09
N3 (26-0-0; 770 kg ha^{-1})	8.90	3.24
$LSD_{0.05}$	ns*	ns*
CV %	17.6	15.0

ns: non significant difference.

Table 4 Essential oil content of *Rosmarinus officinalis* under different nitrogen levels and fertilizer types.

Treatments	Essential oil (mL/10 gr)
Fertilization levels	
N1	0.1092 a
N2	0.1164 b
N3	0.1228 b
$LSD_{0,05}$	0.00668
CV (%)	12.3

* Dancan criterion: a, b.

of low nitrogen requirements, since by increasing the dose in double, the yield of the plant does not perform statistical significant differences.

In comparison with previous studies in six different locations [12] ranged between 0.55 t ha^{-1} to 0.82 t ha^{-1}, yield which is lower than the half of the produced in this study. In contrary, it was reported [13] a yield 7.82 t ha^{-1} during the first year after establishment and reached up to 22.12 t ha^{-1} for the year after. This high difference may best be explained by ecological conditions and different crop age. In a two year trial Sonmez [14] yielded 1.23-2.18 t ha^{-1} for two continuous years, which results are in agreement with those that were found. Furthermore the higher moisture content was observed in the case of N1 level and fertilizer type (68%).

3.5 Essential Oil Content

The essential oil production was counted in mL/10 gr dry drug. Table 4 shows the statistical analysis of the essential oil production, where it is clearly indicated that there is a statistically significant difference between the zero fertilization (N1) and the other two fertilization levels (N2 and N3).

4. Conclusions

The general conclusion that was found from this study is that *Rosmarinus officinalis* is a low nitrogen requirement crop, where by doubling the fertilization dose there was only observed constant voltage supremacy. The N-fertilization level of 385 kg ha^{-1} using the 26-0-0 fertilizer type produced an average dry yield of 3.09 t ha^{-1} setting this level as the best choice for farmers if someone will take in mind the fertilization costs. Finally, the unfertilized treatments of Rosemary produced significant higher amounts of essential oil, demonstrating the inverse effect of fertilization effect. Therefore, the above data show that rosemary cultivation could be a promising alternative crop, especially in case of the consideration that average selling price of dry rosemary in Greece is 3.5 € kg-1 and the average gross income exceeds the amount of 10,000 € ha^{-1}, but further investigation is necessary to be conducted as to be able to lead to safer conclusions.

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Antioxidant Activity of Peptides Obtained from Cotton Ground Oil-Cake Proteins

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Abstract: Antioxidant activity of the peptides derived from proteins of defatted cottonseed kernels and cotton ground oil-cake by their enzymatic hydrolysis with acidic (Asp. niger) and neutral proteinases (Bac. amyloliquefaciens) was studied. Antioxidant activity of the derived peptides depended on the used proteins and enzymes. The peptides derived by using of neutral proteinase possessed higher antioxidant activity, in comparison with the peptides derived by acidic proteinases.

Key words: Proteins, cotton ground oil-cake, hydrolysis, acid and neutral protease, peptides, antioxidant activity.

1. Introduction

Antioxidants are an important group of food additives that have the ability to protect against detrimental change of oxidizable nutrients and consequently they extend shelf-life of foods [1].

Various natural and synthetic preservatives and antioxidants are used for extending the storage period of end products. Great attention is also paid to the use of natural antioxidants based on polyphenols [2-5] and peptides derived from plant materials [6-8].

Some active peptide antioxidants and peptides that can utilize free radicals are identified in various hydrolysates of proteins such as ovalbumin [8], soybean protein [9], milk proteins such as α-laktatalbumin and β-lactoglobulin [10, 11] etc.

One study that aims at production of antioxidant peptides [12] focuses on the effect of various enzyme preparations on the enzymatic hydrolysis of defatted peanut kernels. Hydrolysates obtained by esperase possess higher antioxidant than the ones, produced with neutrase, pepsin, protease A and protease N. Antioxidant activity is measured kinetically, using linoleic acid. The molecular weight of the peptide derived with esperase ranged from 3 kDa to 5 kDa. Antioxidant activity was 3 times higher than that of ascorbic acid.

Cottonseed (*Gossypium*) is one of the important oilseed crops in Uzbekistan. The main by-product of the oil extraction process is cottonseed ground oil-cake, which has relatively high protein content of 35%-40%, making it an attractive and promising source of vegetable proteins. However, the presence of anti-nutritional compounds is a major drawback in the use of this bioresource as human food. Hence, it is usually used as an animal feed.

The aim of this work is a comparative study of the antioxidant activity of peptides derived from proteins of cotton ground oil-cake.

2. Materials and Methods

Neutral proteolytic enzyme preparation from *Bacillus amyloliquefaciens* (Neutrase, "Novozymes", Denmark) and acid proteolytic enzyme preparation from fungous—*Aspergillus niger* (Prolive PAC 30L "EnzymeBioProduct" Ltd Russia) were used. Proteins isolated from defatted cottonseed kernels and cotton ground oil-cake were used as substrate. Substrates were water-soluble and salt-soluble (10% NaCl).

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2.1 Enzymatic Hydrolysis of Proteins

1% solution of the corresponding protein in 0.1 M universal buffer, pH 4.2 (in the case of acid proteinase) and pH 7.0 (in the case of "neutrase") were prepared and a 0.1% proteinase solution was added. The mixture was stirred and kept for some time in a thermostat at 30 °C, then 2 mL TCA (trichloroacetic acid) was added to 2 mL of the sample in order to stop enzymatic reaction. Then the settled solution was passed through a paper filter and to 1 mL of filtrate 5 mL of 0.5 M solution of sodium carbonate was added. While stirring, 1 mL of working solution of Folin was added. The intensity of the blue coloring was measured with a photoelectric colorimeter at 670 nm against the control sample in a 10 mm cuvette [13] (GOST 20264.2-85, 1985). The content of hydrolysis products (*P*) was determined by a calibration curve, obtained using tyrosine.

2.2 Sample Preparation

5 mL fractions of the reaction mixture were collected at 0, 1st, 2nd, 4th, 6th, and 8th hour. Sample fractions were heated in a water bath for 5-10 min to inactivate the enzyme and passed through a paper filter.

The effect of peptides in the samples on the rate of oxidation of (+)-catechin was determined in model system, as well.

2.3 Measurement of Rate Oxidation of (+)-catechin

(+)-Catechin (4 mM) was used as an internal standard for quantification. It was dissolved in acetate buffer (0.1 M, pH 4.2) containing ethanol (20%, v). The rather high ethanol concentration was chosen in order to avoid microbial development during storage of the solutions. Ferrous chloride was added to give final iron concentrations of 10 mg/L. The content of hydrolysis products of protein in 10 mL incubation medium was 0.2 mL [14].

Browning of the solutions (40 °C) was estimated by measuring the increase in absorbance in a 10 mm cuvette at 440 nm using a KFK-2-YXL 4.2 photoelectric colorimeter (Russia).

3. Results and Discussion

It is known that the protein content of cotton seed core ranges between 25% and 38%. Thus, albumin and globulin is 90% of total amount of protein.

These proteins are hydrolyzed by acidic and neutral proteases with different rate.

Table 1 shows the comparison of hydrolysis rate of water-soluble and salt-soluble proteins isolated from defatted cottonseed kernels and cotton ground oil-cake.

The presented data show that the initial rate of hydrolysis of albumin and globulin of defatted cottonseed kernels with acidic proteinase was nearly the same. Enzymatic hydrolysis of albumin with neutral proteinase goes deeply than previous one and the hydrolysis rate was 0.4 mkmol/h, and for globulin it was 0.26 mkmol/h.

Heat treatment of cottonseed kernels promotes change of hydrolyzability of proteins. Thus, the rate of hydrolysis of albumin with acidic and neutral proteinase was decreased 8-9 times and it was 0.07 mkmol/h and 0.05 mkmol/h, respectively. Globulin

Table 1 The hydrolysis rate of proteins which extracted from defatted cottonseed kernels and cotton ground oil-cake (mkmol/h).

Enzyme	Protein source			
	Defatted cottonseed kernels		Cotton ground oil-cake	
	albumin	globulin	albumin	globulin
Acidic proteinase	0.58 ± 0.25	0.53 ± 0.023	0.4 ± 0.02	0.80 ± 0.03
Neutral proteinase	0.4 ± 0.02	0.26 ± 0.01	0.05 ± 0.003	0.62 ± 0.03

Composition of the reaction medium: protein content 1%, temperature 37 °C, pH-7.0 in the case of neutral proteinase and pH-2.8 in the case of acid proteinase.

Table 2 The effect of washing conditions of cotton ground oil-cake on hydrolysis of proteins.

The pH of water	The content of amino acids, mkmol/mL
3.5	0.7 ± 0.03
7.0	0.8 ± 0.03
9.0	1.2 ± 0.04

derived from cotton ground oil-cake became more hydrolyzable with acidic and neutral proteinases. The hydrolysis rate by acidic and neutral proteinases is 0.8 and 0.62 mkmol/h, respectively.

It should be noted, that at processing of cotton seeds according to conventional technology, there are many factors adversely affecting to catalytic properties of the enzymes.

We preliminary washed cotton ground oil-cake to remove accompanying substances with water of different pH values (Table 2).

The data presented show that washing of cotton ground oil-cake with hot water with pH 9.0 gives the best results. In this case this hydrolyzability of protein is increased 1.4-1.7 times in comparison with water at pH 3.5 and pH 7.0.

Fig. 1 shows the effect of sodium chloride ions on the activity of acidic and neutral proteinases in the hydrolysis of cotton ground oil-cake proteins.

Sodium chloride ions have a positive effect on the activity of acidic proteinases. The rate of hydrolysis of cotton ground oil-cake proteins at concentration of NaCl 3%, is increased by 1.3-1.4 times. At this concentration, neutral proteinase loses 50% of activity.

The products of hydrolysis of proteins isolated from cottonseed kernels and cotton ground oil-cake have different antioxidant properties. The antioxidant activity of peptides derived from cottonseed kernels and cotton ground oil-cake is shown in Fig. 2.

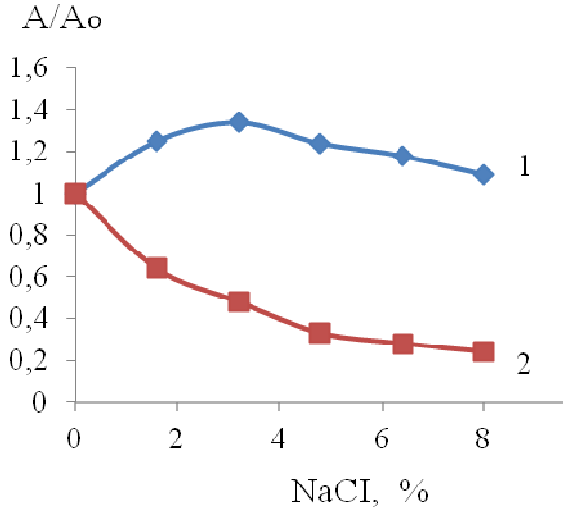

Fig. 1 The effect of sodium chloride on the activity of acidic (1) and neutral (2) proteinases in hydrolysis of cotton ground oil-cake proteins.

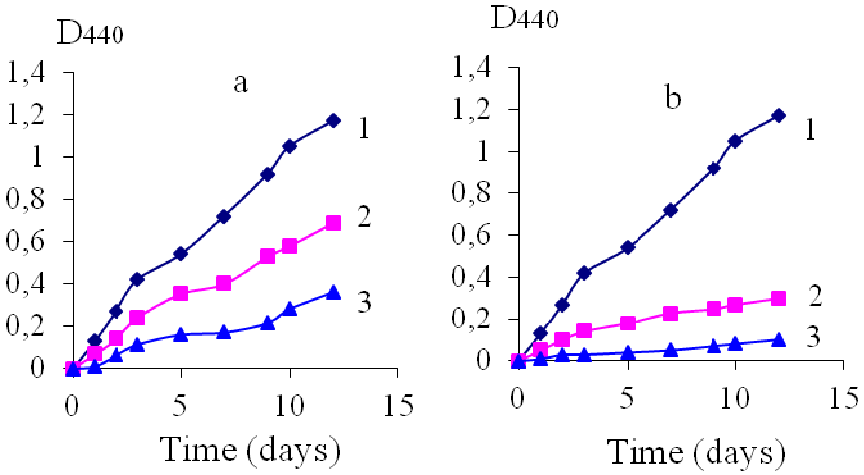

Fig. 2 The effect of peptides derived from globulin with acid and neutral proteases on the rate oxidation of (+)-catechin.
a—globulin from defatted cottonseed kernels; b—globulin from cotton ground oil-cake; 1—control; 2—peptides derived with acidic proteinase; 3—peptides derived with neutral protease.

The presented data show that the reduction of the oxidation rate (+)-catechin observed with all peptide samples. Significant difference can be seen in the case of peptides obtained by the globulin hydrolysis with various enzymes. The globulin hydrolysis products obtained with acidic proteinases have lover antioxidant activity and the (+)-catechin the oxidation rate in model systems decreases slightly (Fig. 2a, curve 2) as compared with control.

Peptides obtained by using neutral proteinase have the highest antioxidant activity (Fig. 2b, curve 3). The presented data show that reducing the oxidation rate of (+)-catechin in this case is much lower than if medium have peptides derived from globulin by using an acidic proteinase.

Thus, cotton ground oil-cake proteins can be used to obtain peptides with antioxidant properties.

4. Conclusion

Enzymatic hydrolysis of cottonseed proteins with acidic and neutral proteinases leads to obtaining peptides with different antioxidant properties. It was shown that antioxidant activity of peptides depends on a type of proteolytic enzyme. In the case of cotton ground oil-cake globulin, antioxidant activity of peptides, produced with neutral proteinases was by 2-3 times higher than the one of peptides derived with the acidic proteinase. In all cases, peptides, obtained by using acidic proteinase, reduced the oxidation rate of (+)-catechin a little.

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Contribution of Rupa Lake for Sustainable Food Security and Local Climate Change

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Abstract: Rupa Lake is small advancing eutrophic lake covering about 115 ha of the Lekhnath Municipality in Kaski district of western Nepal. The environment around the lake has been improved over a period of 10 years. Conservation practices were initiated by communities including the Rupa Lake Restoration and Fishery Cooperative (RLRFC). As a result, an abundance of non-timber Forest Products (NTFP) has increased considerably. At present, 49 NTFP are available in the lake basin. Some households (HH) i.e 10% has additional income from NTFP. It is an indirect source of food security for local people. They sell NTFP products to generate income. A few households have already started farming of NTFPs. Availability of fodder and fuel wood from community forest has significantly contributed to the livelihoods of people where as wild edible fruits and vegetables have become supplementary for food security. There is yearly food security for 57% of HH with 22% having surplus food. 5% of HH has food security for less than three months where as 19% HHs have food security for more than six months. However, livelihood and nutritional security have improved by fish farming in lake. This lake is most important for the local environment and also helps eco-tourism. The study found that 92% observed the climate change in the form of a rise in temperature (> 70% HHs); unpredictable rainfall (> 75% HHs); shifting rainfall (> 60% HHs); phonological changes (> 50%). It showed that the lake supports the restoration of natural water capacity, maintain local climate and sound environment by better natural resource management for an environment friendly ecosystem.

Key words: Community forest, livelihood, adoption, ecosystem, environment.

1. Introduction

Nepal is country facing food insecurity. Globally, Nepal ranks 145th out of 187 counties Human Development Index 2014 [1]. Annual population growth rate of the country is 2.2 percent. It is estimated that the country's population in 2025 will reach 40.5 million; and will face difficulty in fulfillment of food requirements [2]. Looking at this scenario, Nepal will face serious food insecurity in future as well.

Nepal holds ten wetlands of global significance that adds 0.025% of wetlands cover to the global target of Ramsar (250 million ha). This proportion is a quite high figure in a small country like Nepal which only share 0.1% its landmass in the global comparison. Now, Lake Cluster of Pokhara Valley is also included in Ramar site in 2016. Nine lakes are Phewa, Beganas, Rupa, Kamal Pokhari, Maidi, Khaste, Gunde, Neureni and Dipang. With this cluster includes 261.06 km^2 [3]. Among them Rupa Lake is one of them.

1.1 Wetland and Climate Change

Wetlands are proven as unique and the most productive ecosystem with 7 percent coverage in the world. They provide provisional services as freshwater for drinking, irrigation, fishery, as well as cultural services. Wetlands also support services as ecosystem and hydrological balance and mitigate environmental disasters and calamities including climate change impacts. These days, wetlands are considered as foundation of sustainable development and prosperity of the local community by eco-tourism. Similarly, wetland is considered as fertile lands for agriculture and is also important for food security of the people. Wetlands are rich in biological diversity.

Every lake in valley has its own significance in biodiversity, ecosystem services and functions. Lakes

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and catchment areas provide home to many threatened and endemic plants, birds and wetland dependent mammals [4].

The wetlands provide habitat for several species of wildlife and lie within various ecosystems of high-mountain and lowland plains in Nepal. The Nepali term for wetland is "*Simsar*" which means land with perennial source of water. Swampy rice fields, water logged areas and ponds are also understood as wetlands in Nepal.

In Nepal, wetlands are important resources in term of biological, hydrological, social, economic, religious and cultural values. Wetlands conservation in Nepal has a time series story that started from 1950s. So many wetlands are either in government forests or in public lands. With this journey, Nepal has made active wetlands conservation after its commitment in the Ramsar Convention (1971) followed by the establishment of Koshi Tappu Wildlife Reserves in 1978, the first Ramsar site in Nepal. Nepal consists of over 6,000 rivers and rivulets, 3,252 glaciers, 2,323 glacial Lakes, 23,000 ponds, 163 wetlands including 48 Lakes of ox-bow-type in Terai, and several tectonic Lakes in high and mid-mountains [5]. Nine wetlands are already designated under the Ramsar sites in Nepal having seven of these within the PA system.

Climate change affects green sectors more than other sectors of the economy. Agriculture production depends on nature and gets affected by the change in the climatic parameters such as extreme weather events. Study reported that expected changes in frequency, duration intensity and geographic distribution of rainfall and snowfall and increased frequency, duration and intensity of droughts [6]. Effects of climate change on agriculture are particularly sensitive as the agriculture produces food and provides the primary source of livelihood. Climate change is expected to influence crop and livestock production, hydrological balances, input supplies and other components of agricultural systems reports that climate change will affect all four dimensions of food security, namely food availability, access to food, stability of food supplies, and food. Climate change has also created risks to the food security of the large number of population [7].

The 1996 World Food Summit defined food security as "existing when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life" Commonly, the concept of food security is defined as including both physical and economic access to food that meets people's dietary needs as well as their food preferences [8].

1.2 Wetland and Food Security

Food security is one of the big problems in South Asia. About three quarters of the poor, about 800 million people, live in Asia, primarily in Bangladesh, China, India and Nepal. The largest population of people affected by potentially critical shortage of land is also in the Southeast Asia because of living in poverty [9].

Nepal is a country highly vulnerable to food insecurity and its impact in health, nutrition, livelihoods, and overall national security. The Food and Agricultural Organization of the UN considers Nepal which represents a low-level of food security country. Over 50 percent of all households in Nepal have food in sufficiency for even half the year [10]. Food deficiencies are most pronounced in hill and mountain-areas with 13 of 16 mountain and 21 of 39 hill districts having a severe food deficit. Nineteen of 24 districts in the mid and far-western regions of Nepal are in food deficit condition. Vulnerability to food insecurity is also on the rise because of climate change. Climate change is making more and more difficulties particularly in low land or disaster prone areas.

Domestic food production is insufficient to meet per capita caloric needs; Nepal has become a net importer of food. Reliance on imports has made the poor increasingly vulnerable to price shocks and has exacerbated food insecurity. The majority of Nepal's

populations are on small holder of farms for its livelihood. Many households operate on landholdings that are inadequate to produce enough annual food for survival. Certain families, particularly those from lower caste groups have landholdings in the *adhiya system* (share cropping on 50-50% basis to landowner and farmer); and are obliged to turn over a significant portion of their harvest to the wealthier or higher caste members holding the land. Nationally, 47 percent of the land owning HHs owned only 15 percent of the land with an average size of less than 0.5 ha, whereas the top 5 percent owned nearly 37 percent of land. Most *Dalits* (untouchable class) are landless.

Besides these weaknesses and challenges, Nepal has a rich natural resource with high biological value of wetlands that include high biodiversity of plant and animal genetic diversity. Similarly, fresh water originating from the Himalayas created a many wetlands with beautiful natural environment. These facts indicate that Nepal has a good prospect of diversifying and increasing agricultural production by wetland management and conservation for alleviating poverty and attaining sustainable livelihood. Most of the wetlands of Nepal are originated by Himalayas.

The direct uses of wetlands are fishing, food, medicine, agriculture, timber, Recreation & tourism transport and water supply. Indirect uses of wetlands are nutrient & sediment, flood control information and storm and erosion protection. The optional of wetland are Potential Future use (both direct & indirect), and Future value information. Non-use of wetland existing value is rich in biodiversity, good habitat, religious-cultural heritage and center of research and education in terms of landscape & aesthetic value. Wetland has spiritual value, unique ecosystem maintaining the integrity, source of genetic resources [11]. Despite these direct and indirect benefits wetlands are facing many problems. Some wetlands have site specific problems. Similarly, common problems are over extraction of resources such as food and NTFP products, drought, filled by sediments and sites, caused by flashfloods, natural calamities, felling the trees and vegetation in watershed areas, and encroachment of wetlands by people. Regardless to the number and sizes, the wetlands of Nepal are the persistent conventional sources to address livelihoods of many specially mountain communities including 10 percent of wetlands dependent ethnic communities like *Jangar, Tharu, Jalahari, Majhi, Satar, Mushar, Brahmu* and so on. Therefore, wetlands are important sources of food security to them.

Degradation of wetlands is due to expansion of agriculture and subsequent conversion of wetlands through drainage into rice fields, irrigation for enhancement of agricultural productivity; national, local and rural infrastructures. Similarly, overgrazing of livestock, over-fishing and associated disturbances, siltation due to degradation of the watershed areas, which are often trans-boundary in nature are the causes of depredation of wetlands. Also pollution of water due to industrial, urban agrochemicals and other types of pollutants including from trans-boundary sources leads to depreciation of water quality of wetlands [12].

As in the world, Nepal's wetlands are also degrading and which impact on regulating local micro-climate. It also helps maintaining the population of flora and fauna of biodiversity conservation. Further, it creates favorable environment for recreation, tourism promotion and environmental businesses through lake management. Wetlands in the Pokhara valley which are unprotected are even more at risk: from drainage, diversion, obstruction, siltation, encroachment, infrastructure development, land use changes, pollution and poison to kill fish resulting in a marked reduction in bird numbers and species diversity since the 1970s [13]. Among them, Rupa Lake has same problem.

Lake serves an important role in the hydrological cycle of the region. Rupa lake is the third biggest Lake of Pokhara valley. Similarly, it also plays a significant role in groundwater recharge, flood control and

sediment trapping. It is also important for evapotranspiration. Habitat of Rupa Lake and its catchment consists of open water, marsh and swamp areas, paddy fields and forests. It supports a number of floral and faunal species. This article is based on research of "Role of Wetland in Food Security and Livelihood of Local People in Rupa Lake Area in the Context of Climate Change". It also addressed the issues related to food security and environmental impact linked with contemporary issue of climate change in Rupa Lake Area of Kaski district.

2. Methodology

Rupa Lake is a small advancing eutrophic Lake that falls in 4 VDCs and wards 10 & 11 of Lekhnath Municipality in Kaski district of Nepal (28°08′39.72″ N and 84°06′29.29″ E). It covers an area of 115 ha with marshes and paddy field along its shores in its basin of 30 km^2. This study was carried out in 2012-13 in Kaski district at Rupa Lake area. Research was conducted by collecting both primary and secondary data.

The primary data were collected by field observation, group meeting, focus group meeting, semi-structured interview, stakeholder consultation and laboratory analysis of water quality. Meteorology data are referred from the Department of Hydrology and Metrology, Nepal. Secondary data were collected by review of existing data and information relevant to the area or topic (published and unpublished), like reports, census data, research findings, municipal and VDC statistics.

Household Food Insecurity Access Scale (HFIAS) tool was used for the measurement of food insecurity as suggested by Coates, J., Swindale, A., and Bilinsky, P. (2006) [14]. A set of nine questions was used as a research tool and the data analysis was done as per that tool. The HFIAS indicator categorizes households into four levels of household food insecurity (access) food secure, mild, moderately and severely food insecure.

The monthly meteorology data for the last 30 years were taken from Pokhara Airport station, which is the closest meteorological station to Rupa Lake. The meteorological data included minimum, maximum and average temperature, and rainfall. These data were computed to reveal the impact of climate change in terms of temperature region and rainfall in the study area. The result was also used to verify local people's experience/perception in this regard. Data analysis was done in SPSS and Microsoft Excel.

3. Results and Discussion

3.1 Climate Change: Meteorological Data and People's Experiences

Major two parameters were taken as temperature (maximum, minimum and average) and rainfall. Metrology data showed that the trend of temperature of average, maximum and minimum is increasing about 2 degree in 30 years. Maximum and minimum temperatures are also in increasing trend. However, rainfall pattern is oscillation. The research found that climate change in the form of rise in temperature (> 70% HHs); unpredictable rainfall (> 75% HHs); shifting rainfall (> 60% HHs); phonological changes (> 50%). Of the total sampled households, 92 percent shared their experiences regarding the impacts of climate on climatic parameters, wetland ecosystem, agriculture, food security and livelihoods. Rise in temperature, unpredictable rainfall, shift in rainfall pattern and phonological changes in plants were the indicators of climate change. It is evidenced that the metrological data and people's perception from questionnaire survey are similar in the context of climate change.

3.2 Climate Change: Agriculture and Livelihoods

Nepal is hit hard by climate change. Atmospheric temperature in Nepal is rising at a rate higher than the global average, with a 1.8 °C increase between 1975 and 2006, while precipitation has become increasingly unpredictable. It is similar in this research area too.

Furthermore, threats to biodiversity, deforestation, and increased frequency of extreme weather events have affected agricultural production and undermined the livelihoods of the rural poor [15]. Rain-fed farming system is major problem on the uplands above Rupa Lake wetland area. Some farmers in the study area responded that the climate change is visible as an impacts on agriculture, forestry and wetland, and therefore in their lifestyles and livelihoods. They remarked that the climate has changed their lifestyle and local farmers have adapted different coping strategies.

Nepalese fish farming practice has subsistence nature. Now, Rupa lake fish farming is based on professional and livelihood purpose to fulfill the demand of protein. Furthermore, intensive development and improvement is necessary to meet the demand of fisheries products, which is a source of low-priced but healthy animal protein supporting for food/nutritional security for the people and marginalized communities. Marginalized and lower middle class people cannot readily afford for meat; therefore wetland is the good source of protein supplement for them, particularly those residing in the wetland ecosystem. Keeping this reality into consideration, Rupa Lake Restoration and Fishery Cooperative is doing fish farming in Rupa Lake.

3.3 Climate Change: Food Security and Local Community

Majority of the households in the study area depended in agriculture for their livelihoods. The following impacts of climate change were realized in the context of food security system during the last 30 years in the study area (Table 1). Above 65 percent of the respondents stated that as an impact of climate change there has been decline in crop production. It means impact of climate change is negative in the study areas and creates the serious consequences on the level of food production and food security in the local level. Therefore climate change is crucial for livelihoods of local communities.

It means it has negative impact on food production that has direct relationship with food security. If climate change negatively affects the crop production, it creates serious consequences on the level of food/nutritional security. About 50% have food security for 9-12 months in the study areas. The Figure 1 shows the status of food security of the study area.

Livestock is one of the major components of Nepalese agriculture and food security. It includes poultry, cattle, buffalo, goat and pig farming. In the study area, majority of the households had similar kinds of the livestock. Ninety percent livestock contributed in the household food security as fertilizer in agriculture field and as cash during food shortage time. They are used to sale the livestock during food shortage. Most of the households had buffalo, goats and poultry as major livestock. People rely upon the nearby watershed and wetland area to meet the demand of food security. They were also relying upon various wetland products and forest resources to escape from the food insecurity. Moreover, people were willing to do bee keeping, coffee production and fish farming, poultry raising and cultivate *ayurvedic* (herbal) medicine as alternative source of income.

3.4 Adaptation Strategies of Climate Change for Food Security

Subsistence farmers have adopted different strategies to cope with climate change because of the

Table 1 Impacts of climate change in food security system during the last 30 years.

SN	Impact of climate change	Percent (%)	Remarks
1	Decline in food production	54	
2	Threats in livestock husbandry	15.7	
3	Increased vulnerability	4.3	People could not respond properly
4	Have no idea	26	

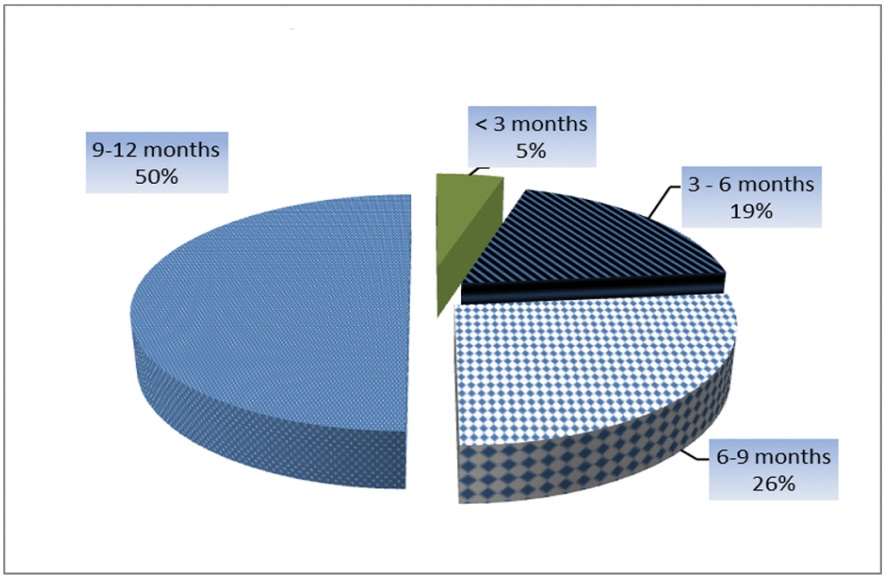

Fig. 1 Food security status in study area.

less food production in the study area. About 74 percent of households are aware about the effect of climate change and had adopted different strategies to resist against the effect of climate change. Out of the 74 percent of HHs, more than 50 percent of the household use chemical fertilizers and agriculture inputs to cope against the climate change and to increase the production yields. Only these coping strategies are not effective to resist against the climate change effect. All other strategies adopted against the climate change are very few and insignificant to resist the effect of climate change. Many households are unknown and do not adopt any types of strategies too. All these problems directly affect the production of these major crops. Mostly, subsistence farmers have adopted different strategies to cope with climate change because of the decline in food production. Most common strategies are use of chemical fertilizer/pesticide, hybrid seeds use of bio-gas and change in agriculture farming system. About 77, 52 and 50 percent have adopted chemical fertilizer/ pesticides, bio-gas and increased agriculture input such as compost & bio manure respectively as coping strategies for climate change. Most of them told that they were adopting mixed cropping; and were using short duration crops. Almost all of the respondents reported that they were using organic manure/compost and bio-pesticides whenever need arises.

Most of the farmers rely upon the rainfall for their agricultural production. Unpredictable rainfall and shifting pattern of rainfall affect the production of food. Besides agriculture, people are involved in different alternative source of income generation for livelihood. Vulnerable households for the food security often responded by skipping meals, reducing food intake, selling assets like livestock, temporary migration for employment, cross boarder migration and day labor.

Remittance has become the highest priority for alternative source of income in the respondents' household. 61.4 percent of the households informed that at least one household members are in abroad to earn money to secure food and livelihood. Using non-timber forest products for *ayurvedic* (herbal) medicine production is also alternative source of income for livelihood. Storage of food is also help in food security. All cereals storage technique is tradition. It means 10%-30% losses by rodents and pest. However, more than 80% practices vegetable drying when they have adequate vegetables. About 60% make pickles, *Mashura* (value added vegetable drying) and fermentation. They use these food items during

the vegetable scarcity time.

3.5 Food Security Assessment

The HFIAS indicator categorizes households into four levels of household food insecurity (access) food secure, and mild, moderately and severely food insecure as per methodology description. The categorization scheme is designed to ensure that a household's set of responses will place than in single, unique category. Table 2 shows that about five percent have severely food insecure in the study areas.

3.6 Climate Change and Wetland

Wetland also plays an important role for the regulation of climate change and the people residing nearby the wetland areas. However, long term climate change may affect the wetland areas and the resources, those who are dependent on different purposes.

Presently, Rupa Lake area is also in decreasing trend. Main cause of lake shirking is siltation problem. Previously, Rapa Lake is 135 hectares now it is 90 hectares based the area measurement. 10 years back the depth of the Lake was 10 meter and now it is only 4-6 meters [16]. But its height (volume of water) of the lake is extremely reduced. So the lake has big problem of siltation as per Lake Conservation Committee. Major cause of siltation is deforestation in upstream, development works, soil erosion and cultivation practices in upstream and erosion prone areas. It affects to downstream. These practices make the Nepalese wetlands in verge of extinction. Similar situation is found in Rupa Lake. If the conservation activities are not done by Rupa Lake Restoration and Fishery Cooperative, the Lake is going to extinct.

Climate change is slow in process of resources changes. It cannot be seen by direct observation but if we do in-dept study, it can be observed. Different resources such as drinking water, irrigation and domestic water supply, fish supply, timber, fiber, fuel wood and fodder supply are provided by lake in the study areas. It also provides some wild foods, medicinal plants and handicraft raw materials and so on. However, the impact of climate change affects all these resources of wetland. The study shows that only fish supply is increasing and remaining others resources are in declining rate such as aquatic flora and fauna by Wetland Inventory, Assessment and monitoring Tools [17]. Therefore, it is necessary to perform proper conservation plan and that can save them from being history. Table 3 shows climate change impact in resources changes during the last 30 years.

Table 2 Assessment of food security by HFIAS score method.

SN	Status of food security	%	Remarks
1	Food secured	57	From different sources of income
2	Mild food insecure	32	
3	Moderately food insecure	6	
4	Severely food insecure	5	

Table 3 Impact of climate change on wetland resources.

SN	Impact observed of climate change	Increased	Decreased	Indifferent
1	Core water area	-	98.5	1.5
2	Water quality in general	19.0	52.4	28.6
3	Water volume	-	98.5	1.5
4	Birds bio-diversity	8.3	83.3	8.3
5	Fish biodiversity	19.3	78.9	1.8
6	Herpetofauna	6	58.0	36.0
7	Phytoplankton density	4.1	59.2	36.7
8	Zooplankton density	4.2	60.4	35.4
9	Siltation	100		

3.7 Forest and Climate Change

Forest ecosystems play an important role in the global bio-geochemical cycles. Forests act both as sources and sinks of green house gases (GHGs), through which they exert significant influence on the earth's atmosphere climate. Forests can contribute to the mitigation of climate change.

Forest area is an important aspect to regulate the local climatic pattern too. In the study area, the present status of the forest as per the respondent's remarks is in average condition. Above 76.6% of the respondents tell the average condition of the forest. The study shows that the condition of the forest was bad before 30 years [18]. There was not any conservation effort in community forest at that time.

The surrounding forest in and around the wetland and watershed area of the lake are used for the various purposes by local people. Fuel wood and fodder are the mostly used products of the forest by the local people. Most of the fuel wood demand was met by the nearby community forest and few of them only use their private land to fulfill the need of the fuel wood. Above 98 percent fuel wood and 85 percent fodder are fulfilled by community forest.

At present, 81% of the respondents tell that they are not having any fuel wood problem. This also indicates the forest condition in the present situation is somehow good. People rely upon the forest for not only to meet the fuel wood demand but also they have access to various forest products like fodder, timber, NTFPs and grazing access too. Presently, 49 NTFP are available in the lake basin areas [16]. Some households earn additional income about 10 percent from NTFP. It is indirect source of food security by selling NTFP products. A few households already started farming of NTFPs. Availability of fodder and fuel wood from community forest has been significantly contributing to livelihoods where as wild edible fruits and vegetables have supplementary for food security [16].

Afforestation is considered as conservation of forest and it mitigates the climate effect of local climate. Therefore, Rupa cooperative is supporting and doing conservation activities such as awareness of conservation forest, NTFP collection and cultivation, afforestation programme, school awareness programme for conservation and plantation of trees in community forest to mitigate the climate change in and around the surrounding areas of Lake Basin. It helps to protect the beauty of lake and control the siltation to the lake. Still, people are dependent on the community forest for fuel wood, fodder, timber and medicinal plant. They are also conserving of community forest by plantation of new trees and managing the community forest by community.

3.8. Climate Change and Gender

Climate change directly affects to women. Rising temperatures and unpredictable precipitation patterns are directly related subsistence farming for their livelihoods. In a patriarchal society, women are mostly responsibility for domestic activities such as housekeeping, childrearing, cooking, and fetching water and firewood collection. In this community, the situation is changing as many men and boys are migrating in search of on-farm work and abroad employment. 61% of males are working abroad. In this situation, all women whose male are abroad have extra responsibility in all agricultural and household works, taking care of family and livestock and managing for the food security [19]. This situation makes women more overloaded in the research areas.

4. Conclusion

Food security in the research area is under threat due to impacts of climate change. The linkages of climate change with food production, rising prices, population migration, gender roles, and malnutrition are intricate. Therefore, the issues of food security and livelihoods need to be addressed urgently keeping in view the changing local context, micro climate and

people's capabilities within the broader framework of the entitlements and rights of the local small farmers, women and marginalized groups. Despite these things, Nepal is rich in biodiversity including ecological and ethno-cultural diversity. Nepal has a good prospect in diversifying and increasing agricultural production through integrated sustainable management of wetland for ensuring food/nutritional security. A similar situation is found in the Rupa Lake area.

Food insecurity and climate change are the major challenges of the twenty first century, which directly and indirectly affect the wetland communities. Due to the climate change people are adopting different strategies for their food security and livelihoods. Major adoptions include using early maturing hybrid and improved crop varieties with heat or drought tolerance, integrated pest management, and integrated plant nutrient management.

Climate change is a real challenge for agriculture, forestry and wetland management, and obviously for livelihoods and food security. Its effects cannot be completely controlled but effective micro-level planning is necessary for forestry and wetland management. However, strategies can be developed based on local people's traditional knowledge and practices to reduce the effects of climate change or cope with the changes in various climatic parameters. The governmental and non-governmental actors should come closer to coordinate their meaningful efforts in this direction. Some mitigation oriented or conservation practices such as community forest, private forest conservation, sustainable and eco-friendly crop cultivation, environment friendly development activities and conservation related education are more appropriate. That will positively affect micro climate of small wetlands like Rupa Lake. Similar replication can have positive effect on other micro climates of the country. The community's role in the wetland and lake conservation activities is quite appreciable in the Rupa Lake area. This fact provides ground to suggest that the government at various levels should come up with appropriate skill development, awareness raising activities and marketing assistance plans for the self-reliance oriented capacity building of the local people depending on Rupa Lake for their livelihoods.

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Study Genetic Variation Using DNA Molecular Markers and Identification Physiological Races of Wheat Stripe (yellow) Rust *Puccinia striiformis* f.sp *tritici* during 2010-2014 in Some Regions of Syria

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Abstract: Yellow Rust (stripe) rust (*Puccinia striiformis West.* f. sp. *tritici*) is one of the most epidemic diseases infect wheat in cold and wet regions. In 1988, this disease caused a loss of seasonal production amounted 70% on wheat variety Mexipak in Syria, and recurrent infection in 2010, caused by a virulent race called *Yr27*, caused a considerable loss in the production of bread wheat cultivars (Cham 8, Cham 6 particularly) amounted 90%. Recently, 15 races of yellow rust had been addressed in Syria for seasons 2010-2014; 159E256, 166E254, 166E256, 255 E112, 0 E0, 64 E 6, 230 E150, 0 E 18, 198 E130, 166 E150, 102 E160, 128 E0, 126 E150, 214E150, and 6E16. The race 6E16 was the most frequent during the two seasons, while the race 255E112 was the most virulent, followed by the race 230E222 and the race 0E0 was the weakest one. This study revealed the presence of fourteen newly observed races in Syria. Molecular Variance Analysis of Molecular Variance (AMOVA) of 55 yellow rust *Puccinia striiformis* f.sp *tritici* isolates examined by Amplify Fragment Length Polymorphism (AFLP) revealed high genetic variation within population, and the dimensional scale analysis (MSD) and tree diagram showed that the Syrian yellow rust isolates were clustered in three groups: the first group contained isolates derived from durum wheat, the second one contained bread wheat isolates, but the third was made of isolates derived from both durum and bread wheat species.

Key words: Wheat yellow (stripe) rust, *Puccinia striiformis* West f. sp. *tritici*, DNA molecular markers, AFLP, PCR, races Syria.

1. Introduction

Yellow rust, also known as stripe rust, caused by *Puccinia striiformis f.* sp. *tritici*, is a major disease that affects wheat production worldwide [1-6], since the yield loss can be as high as 70% [7]. In Syria, particularly the irrigated fields and the northern areas, where the rainfall rate is high, the severity of infection was up to 80%, and the yield loss of the susceptible cultivar Mexipak was 29% [8, 9], but the very damaging epidemic, included Iraq and Turkey, took place in 2010, and heavily infected some improved cultivars; Cham 8 and Cham 6 particularly [10]. As that, Yellow rust was considered as a main wheat infection in the country [11].

Moreover, wheat cultivars having excellent stripe rust resistance often become susceptible after being grown for some periods of time because of the frequent development of new virulence by stripe rust races [12-14], and the spores of these emerging races spread by the wind [15, 16] and help overcoming cultivars carrying the resistance genes in other different countries [17]. In addition to virulence studies, molecular markers are being applied widely to characterize the genetic diversity and phylogenetic relationships in the pathogen populations, and to study the disease epidemiology [18].

The Amplified Fragment Length Polymorphism (AFLP) is one of the most important of these

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molecular marker-based techniques, because it also provides a greater understanding of the dynamics of yellow rust fungus community [19, 20].

This study aimed to assess the genetic variance of 55 yellow rust *Puccinia striiformis* f.sp *tritici* isolates collected from many regions in Syria during seasons 2010-2014, using Amplified Fragment Length Polymorphism (AFLP) as a molecular marker to detect the genetic variation related to the geography, within the pathogen population.

2. Materials and Methods

2.1 Yellow Rust Isolates

The infected leaves had been collected from the infected wheat fields in Syria; 171 and 164 fields during the seasons 2010 and 2011-2014, respectively, according to the hierarchical sampling method. The survey included both farmers' fields and that belonging to the agricultural scientific research centers, representing all the Syrian environmental areas. 33 out of 171 samples, of the first season, each of them represented a single spore isolate, but the number of isolates was 22 out of 164 ones for the second season 2011-2014.

2.2 Isolation and Propagation of the Pathogen

This study was carried out in the laboratory of wheat diseases in the International Center for Agricultural Research in the Dry Areas (ICARDA) during season 2010-2014. Because study of genetic variation is so difficult using the DNA from mycelium, due to the penetration process into the tissues of the host [21], the molecular studies depend on the Urediniospore DNA [22]. The Urediniospore of *P. striiformis* were collected by a single pustule, and multiplied on seedlings of the susceptible bread wheat cultivar; Morocco that grown in small plastic pots filled with a sterile mixture of clay soil, sand and pitmose with ratio of 1: 1.3: 2.7, respectively, and after germination, a solution of Maleic Hydrazide (0.25 g/L) was added to the irrigation water. The infected seedlings were incubated at controlled conditions of temperature (10 ± 2 °C), relative humidity (70%-80%) and alternating lighting (16:8 hours of light: dark) for 24 hours, then the temperature was risen to (15 ± 2). The Urediniospore were collected after 17 days of infection and re-multiplied again following the same steps above, till to obtain a quantity of these spores sufficient for downstream applications. Yellow rust assessment was made 17 days after infection using a 0-9 disease-scoring scale [23]. Infection types 0-6 and 7-9 were classified as avirulent and virulent, respectively [24, 13], and the nomenclature of *P. striiformis* was given to each isolate using the standard differential sets according to Johnson et al. [25].

2.3 DNA Extraction

DNA was extracted according to the protocol in the laboratory of biotechnology at (ICARDA), 20-50 mg of Lyophilized Urediniospore isolate was crushed using 50 mg fine sand and metal ball. After that, 1 mL of the extraction solution (1M Tris-HCL, 5M NaCl, 0.5M EDTA, CTAB) was added to the 100 mg of each powdered isolate, and incubated in a water bath at 60-65 °C for 60 minutes. Then 1 mL of the mixture Chloroform/Iso-amyl-alcohol (1:24, v:v) was added, and stirred gently until forming an emulsion, then centrifuged at 10,000 rpm for 10 minutes. The upper layer containing the DNA was transferred to another 1.5 mL tube. DNA was precipitated by adding 1 mL of the cold isopropanol (-20 °C), then centrifuged to discard the supernatant. DNA was washed with 75% cold ethanol (-20 °C) for several times till the pellet became diaphanous to be dried by keeping the tube open at the room temperature. Finally, DNA was dissolved in 100 µL of TE buffer (1 M Tris base, 0.5 M EDTA). The concentration and purity of DNA were estimated using a spectrophotometer at 260 nm.

2.4 AFLP Test

Seven combinations of primers: P16 + M 17, P16 +

M88, P16 + M183, P16 + M269, P20 + M 88, P24 + M 17and P24 + M301 were applied to 10 fungus races. Only primers that gave Polymorphic bands were chosen; P16 + M 17, P16 + M88 (Table 1), and the rest were excluded.

2.5 DNA Digestion

For each isolate, 1.3 µl of genomic DNA (80 ng/µL) was digested with a mixture of tow restriction enzymes; *Ecorl* and *Msel* (0.8µL) for 4h at 37 °C in 1X reaction buffer (2µL of 10 X) at 10µL final volume. The enzymes were deactivated by incubating at 70 °C for 15 min then placing directly in the ice for several minutes.

2.6 Ligation of Adapters

The restricted DNA (10 µL) was ligated with double-stranded adapters by adding 9.6µL of adapter ligation solution, and 0.4 µL of T4-DNA ligase, the whole volume (20 µL) was mixed, centrifuged briefly and incubated at room temperature for 2 h. The restricted-ligated reaction was diluted 1:5 using TE buffer (10 mM Tris-base, 1 mM EDTA, PH 8.0).

2.7 Pre-amplification Reaction

The pre-amplification reaction was prepared with a final volume of 20 µL containing 2 µL of the above diluted ligation- reaction, 16 µL of PCR master mix containing the pre-amplification primers and 0.15 µL Taq DNA polymerase. Or (16 µL of two primers mix and 2 µL of AFLP–PCR buffer and 0.15 µL Taq DNA polymerase). The PCR amplification was performed for 20 cycles as followed: denaturation at 95 °C for 30 s, annealing at 56 °C for 1 minute, and extension 72 °C for 1 min. The pre-amplified DNA was diluted 1:5 using TE buffer.

2.8 Selective AFLP Amplification

The amplification reaction volume contained 8.32 µL of water, 2.5 µL of the diluted pre-amplified reaction, 1 mM AFLP-PCR buffer, 0.09 µL and 2.25 µL of the two combinations P16+ M88 and P16+ M 17 respectively (Table 1) and 0.079 µL Taq DNA polymerase. The PCR conditions included a touchdown program with 13 cycles of the denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s, decreased by 0.7 °C per cycle, and extension at 72 °C for 60 s after each cycle. This was followed by another 23 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min.

2.9 Separation of Amplified DNA Molecules on Acrylamide Gel

The selective amplification products (10 µL) were denatured at 94 °C for 5 min and placed immediately on ice, then separated in 6% denaturing polyacrylamide gel electrophoresis at 70 W and 50 °C for 2 h, and the products were visualized by silver staining.

2.10 Gel Staining

The PCR products were visualized using silver staining as followed: the gel was prefixed by putting in a glacial acetic acid solution, and stained with silver nitrate. The DNA bands was visualized by a developing solution of sodium carbonate and thiosulfate, and then fixed using aglacial acetic acid solution again. The gel photo was transferred to the computer for analyzing the data.

2.11 Data Analysis

After the DNA was amplified according to the method described by Zabeau and Vos [26], and modified

Table 1 Sequence of the Primers used for the amplification of the initial test (P16 + M88), (P16 + M17) AFLP.

Primer	Nucleotide sequence
*Pst*0	5`GACTGCGTCCATGCAG
*Pst*1+ CC	5`GACTGCGTCCATGCAG CC
Mse1 +CG	5` GATGAGTCCTGAGTAA CG
*Mse*0	5` GATGAGTCCTGAGTAA
*Pst*1+ CC	5`GACTGCGTCCATGCAG CC
*Mse*1+ GC	5` GATGAGTCCTGAGTAA CG

by Van et al. and Poowell et al. [27, 28]. PCR products were visualized on acrylamide gel and the data were constructed in an Excel file as a binary matrix for each AFLP fragment ranged between 1,500-100 bp. The data were then analyzed in Power Marker 3.25 [29]. The genetic variance was evaluated based on both of Shannon's diversity index and genetic diversity index, the Principal Co-ordinates Analysis (PCoA) was performed according to the genetic distance, the Correlation Coefficients Matrix and the distance between races [30]. The Analysis of Molecular Variance (MOVA) was done between the fungus races; also the phylogenetic tree of all races was constructed.

3. Results and Discussion

3.1 Nomenclature Races of the Stripe Rust

The species *P. striiformis* f. sp. *tritici*, causing stripe rust on wheat, are further separated into races according to virulence against cultivars or genotypes of wheat. Races are differentiated by infection types on two groups of selected wheat genotypes: the "world differentials" and the "European differentials" [25]. The survey has been conducted in main wheat growing areas of Syria, during two seasons; 2010 and 2011-2014. According to Johnson et al. [25], the nomenclature of 55 single spore isolates, represented 40 different races; 25 ones were recorded only for one season (Fig. 1). Thus, 15 races of yellow rust had been addressed in Syria for season 2010-2014, 159E256, 6E16, 166E254, 166E256, 255 E112, 0 E0, 64 E6, 230 E150, 0 E18, 198 E130, 166 E150, 102 E160, 128 E0, 126 E150, 214E150 (Tables 1 and 2), 7 of them (159E256, 166 E150, 166E254, 166E256, 214E150, 230 E150 and 255 E112) contained the virulence gene *Yr27* (Tables 2 and 3). The 6E16 race was the most frequent during the two seasons, while the race 255E112 was the most virulent, followed by the race 230E222, and the 0E0 race was the weakest one. Moreover, this study revealed presence of fourteen newly observed races in Syria.

The result showed that AFLP amplification test with both primers' combinations *P16+M17* and *P16+M88* had the best efficiency and produced the most

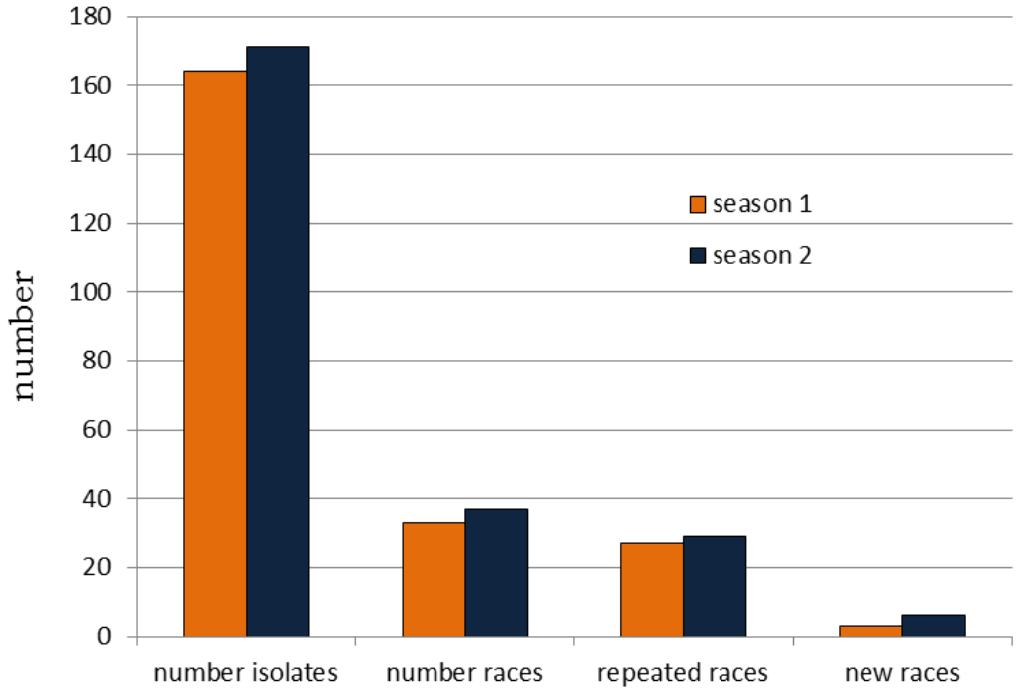

Fig. 1 Number of isolates, identified races , repeated races and new races (2010-2014).

Table 2 Physiological races of the fungus yellow rust on wheat registered in Syria and its pathogenicity genes during the 2010.

Pathogenicity genes	Races	N.
*Yr*6, *Yr*7, *Yr*9+, *Yr*7+, *Yr*6+, *Yr*3N, *Yr*SP, *Yr*2+, *Yr*2A, *Yr*A+, *Yr*9 Gereck 79	230 E 222	1
*Yr*6, *Yr*7, *Yr*1, *Yr*7+, *Yr*SD, *Yr*SU, *Yr*9+, *Yr*6+, *Yr*8, *Yr*2A, *Yr*9 *Gereck 79, Yr27*	230 E 150	2
Yr 7, *Yr* 6, *Yr* SU, *Yr* 2,+, *Yr* 7+, *Yr* 6+, *Yr* 8, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	198 E 130	3
*Yr*6, *Yr*7, *Yr*SU, *Yr*9+, *Yr*6+, *Yr*9, *Gereck 79*	198 E4	4
Yr 6, *Yr* 9+, *Yr* 7+, *Yr* 8, *Yr* SP, *Yr* 2A *Yr* A, *Yr* 9, Gereck	196 E 98	5
*Yr*6, *Yr*7, *Yr*9+, *Yr*7+, *Yr*6+, *Yr*8, *Yr*2+, *Yr*2A, *Yr*A+, *Yr*9 *Gereck 79*	166 E150	6
Yr 7, *Yr* 6, *Yr* SU, *Yr* 6,+, *Yr* 7+, *Yr* 2+, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	164 E 22	7
Yr 6, *Yr* 7, *Yr* 9+, *Yr* 7+, *Yr* 6+, *Yr* 3N, *Yr* 2+, *Yr* 2A, *Yr* A+, *Yr* 9 Gereck 79	142 E 130	8
*Yr*9+, *Yr*7, *Yr*6, *Yr*9+, *Yr* 8, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*	134 E 16	9
*Yr*2+, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*, *Yr*7, *Yr*7+, *Yr* 3N, *Yr*SD	126 E150	10
*Yr*9+, *Yr*2A, *Yr*9, *Gereck 79*	128 E0	11
Yr6+, *Yr* SU, *Yr* SD, *Yr* 10, *Yr* 8, *Yr* 2A, *Yr* A	114 E 16	12
Yr 7, *Yr* 6, *Yr* SU, *Yr* CV, *Yr* 2+, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	102 E 160	13
Yr SU, *Yr* SD, *Yr* 7+, *Yr* 6+, *Yr* 8, *Yr* CV, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	82 E 16	14
Yr 7, *Yr* 3N, *Yr* SU, *Yr* 6+, *Yr* 7+, *Yr* 8, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	78 E 30	15
*Yr*6, *Yr*SU, *Yr*2+, *Yr*7+ *Yr*9, *Gereck* 79	68 E130	16
Yr 6, *Yr* 7, *Yr* SD, *Yr* 6+, *Yr* 7+, *Yr* 8, *Yr* 2+, *Yr* 2A, *Yr* A+, Gereck 79	38 E 150	17
Yr 7+, *Yr* 9, *Yr* 2A, *Yr* 4	36 E 6	18
Yr SD, *Yr* 9, Gereck 79	32 E 0	19
Yr 6, *Yr* r7, *Yr* SD, *Yr* 9, Gereck 79	20 E 0	20
*Yr*6, *Yr*2+, *Yr*6+, *Yr*8, *Yr*9, *Yr*7+, *Yr*10	16 E150	21
Yr 6, *Yr* 6+, *Yr* 8, Gereck 79	6 E 30	22
Yr 6, *Yr* r7, *Yr* 6+, *Yr* 8, *Yr* 2A, *Yr* A+, *Yr* 9, Gereck 79	6E 20	23
Yr 6+, *Yr* 7, *Yr* 6, *Yr* 9, Gereck 79	6 E 16	24
*Yr*6, *Yr*7, *Yr*2A, *Yr*A+, *Yr*9, *Gereck* 79	6 E 0	25
Yr 9, *Yr* 6+, *Yr* 7+, *Yr* CV, *Yr* 6	4 E 40	26
Yr CV, *Yr* 6, *Yr* 2A, *Yr* 9	4 E 32	27
Yr 6, *Yr* 7+, *Yr* 9, Gereck 79	4 E 2	28
*Yr*6, *Yr*2A, *Yr*9	4 E 0	29
Yr 7	2 E0	30
Yr 6, *Yr* 10, *Yr* 3V, *Yr* 9, Gereck 79	0 E 28	31
Yr 3N, *Yr* 6+, *Yr* 7+	0 E 18	32
++++	0 E 0	33

polymorphic markers; 75 and 72 respectively, so they were used for the selective AFLP amplification with addition of two nucleotides (*Pst1+ CC, Mse1 +CC*) and (*Pst1+ CC, Mse1+ GC*), that produced 278 and 302 bands respectively (Figs. 2 and 3), using Adobe Photoshop CS software for reading the gel.

There was no significant difference between the geographic regions, as the PIC was 31.51%. Also, the results of AMOVA pointed out that the variation of the races among the regions was 10% only, and 90% within the region, while the variance components were 2.5% and 97.51%, respectively (Table 4).

In addition, the clustering analysis of the races showed similar results related to genetic variance, using either the PCoA or UPGMA (Fig. 4). The races, representing one area, were separated into different groups, while those related to geographically different areas clustered in same groups, and these results agree with previous studies [31, 32], since the yellow rust spores can travel with air for far distance to anywhere [33], so it is more likely to be one region invaded by many different races simultaneously.

Table 3 Physiological races of the fungus yellow rust on wheat registered in Syria and its pathogenicity genes during the 2011-2014.

N.	Differential cultivars	The resistant gene	The race
1	Chinese 166	----------	0 E0
2	Lee	*Yr*7, *Yr*6, *Yr* 8, *Gereck* 79	6 E 16
3	Heines Kolben	*Yr*6, *Yr*7, *Yr*2A, *Yr*A+, *Yr*9, *Gereck* 79	6 E0
4	Vilmorin 23	*Yr*6, *Yr*7, *Yr*1, *Yr*7+, *Yr*SD, *Yr*SU, *Yr*9+, *Yr*6+, *Yr*8, *Yr*2A, *Yr*9 *Gereck 79*, *Yr27*	230 E150
5	Moro	*Yr*7+ *Yr*9, *Yr*2A, *Yr*4	6E 36
6	Strubes Dickopf	*Yr*6+, *Yr*7+, *Yr* 9, *Gereck* 79	64 E 6
7	Suwon 92 x Omar	*Yr*SU, *Yr*SD, *Yr*7+, *Yr*6+, *Yr*8, *Yr*CV, *Yr*2A, *Yr*A+, *Yr*9, *Gereck* 79	82 E16
8	Clement	*Yr*6, *Yr*2A, *Yr*9	4E0
9	Triticum spelta	*Yr*7	2 E0
10	Hybrid 46	*Yr*9+, *Yr*2A, *Yr*9, *Gereck 79*	128 E0
11	Reichersberg	*Yr*7, *Yr*6, *Yr*SU, *Yr*CV, *Yr*2+, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*	102 E160
12	Heines peko	*Yr6*, *Yr7*, *Yr9*+, *Yr7*+, *Yr6*+, *Yr8*, *Yr2*+, *Yr2A*, *YrA*+, *Yr9 Gereck 79*, *Yr27*	166 E150
13	Nord Desprez	*Yr*7, *Yr*6, *Yr*SU, *Yr*2+, *Yr*7+, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*	198 E130
14	Compare	*Yr*9+, *Yr*2A, *Yr*8, *Yr*7	0 E 18
15	Carstens V	*Yr* 1, *Yr*3V, *Yr*SU, *Yr*6, *Yr*7, *Yr*10, *Yr*9+, *Yr*8, *Yr* SD, *Yr* SP, *Yr* CV, *Yr*2A, *Yr* 9, *Yr27*	255 E112
16	Spaldings prolific	*Yr* 1, *Yr*3V, *Yr*SU, *Yr*6, *Yr*7, *Yr*10, *Yr*9+, *Yr*8, *Yr* SD, *Yr* SP, *Yr*, *Yr27*, CV, *Yr*2A, *Yr* 9	166 E256
17	Heines VII	*Yr*7, *Yr*6, *Yr*SD, *Yr*SU, *Yr*2+, *Yr*3V, *Yr*9+, *Yr*6+, *Yr*8, *Yr*7+, *Yr*2A, *Yr*A, *Yr27*, *Yr* 9	166 E254
18	Anza	*Yr27*, CV, *Yr*2A, *Yr* 9, *Yr*6, *Yr*7, *Yr*9+, *Yr*7+, *Yr*6+, *Yr*8, *Yr*2A, *Yr*A+, *Yr*9 *Gereck 79*	159 E256
19	Sonalika	*Yr*CV, *Yr*7, *Yr*6, *Yr*SU, *Yr*2+, *Yr*7+, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*	166 E120
20	Fed.4/Kavkaz	*Yr*2+, *Yr*2A, *Yr*A+, *Yr*9, *Gereck 79*, *Yr*7, *Yr*7+, *Yr* 3N, *Yr*SD	126 E150
21	Gereck 79	*Yr27*, *Yr*SU, *Yr*SD, *Yr*7+, *Yr*6+, *Yr*8, *Yr*CV, *Yr*2A, *Yr*A+, *Yr*9, *Gereck* 79	214 E150
22	Cham1	*Yr*9, *YrA*+, *Yr2A*, *Yr8*, *Yr7*+, *Yr*6+, *Gereck 79*	134 E16

As that, all races were clustered in four major groups:

Group A: included races belonging to geographic areas near each other; Idlib, Hama and Aleppo, in addition to farther one; Tel Hadya. The genetic relation was only 35.3% according to the values of Bootstrap.

This group contained the races: 230E222, 230E150, 198E130, 196E98, 198E4, 164E22, 142E130, 128E0, 114E16, 102E160, 82E16, 38E150, 36E6, 32E0, 20E0, 14E150, 6E30, 6E20, 6E16, 6E0, 4E40, 4E2, 2E0, 0E28, 0E18, 0E0.

Group B: the genetic relationship was 15.3%, that suggested races of geographically different areas; Dara, Deir AL-Zor and Raqqa. These races are 230E150, 198E130, 134E16, 126E150, 78E30, 38E150, 36E6, 6E16, 4E2 and 4E0.

Group C: contained races from Idlib and Aleppo and Hasaka with a genetic relation was 22.3%, and included the races: 230E222, 6E150, 36E150, 36E6, 6E36, 6E30, 6E16, 4E32, 2E0 and 0E0.

Group D: contained races from Tartous, Homs, Hasaka and Damascus and Latakia The degree of relationship was 42%, and represented the races: 255 E112, 230E150, 214E150, 198E130, 166E256, 166E254, 166E150, 159E256, 128E0, 126 E150, 102 E160, 64 E6, 6E16, 0E18, 230E222, 230E150, 38E150, 6E16, 6E0, 4E0 and 0E0.

The results showed the high similarity among the races representing farther different areas in geography and environment, such as the similar races of Aleppo and Hasakah, and the other races of Aleppo were similar to that of Tartous, Hamah and Tal-hadia (Fig. 5). These can be referred to the transmission of fungus spores by air and spread to far places [34], and the usual pathways of the local wind during the infection season in the spring might play a critical role in the genetic similarity of races among the provinces [35], as the wind generally blows from west to east.

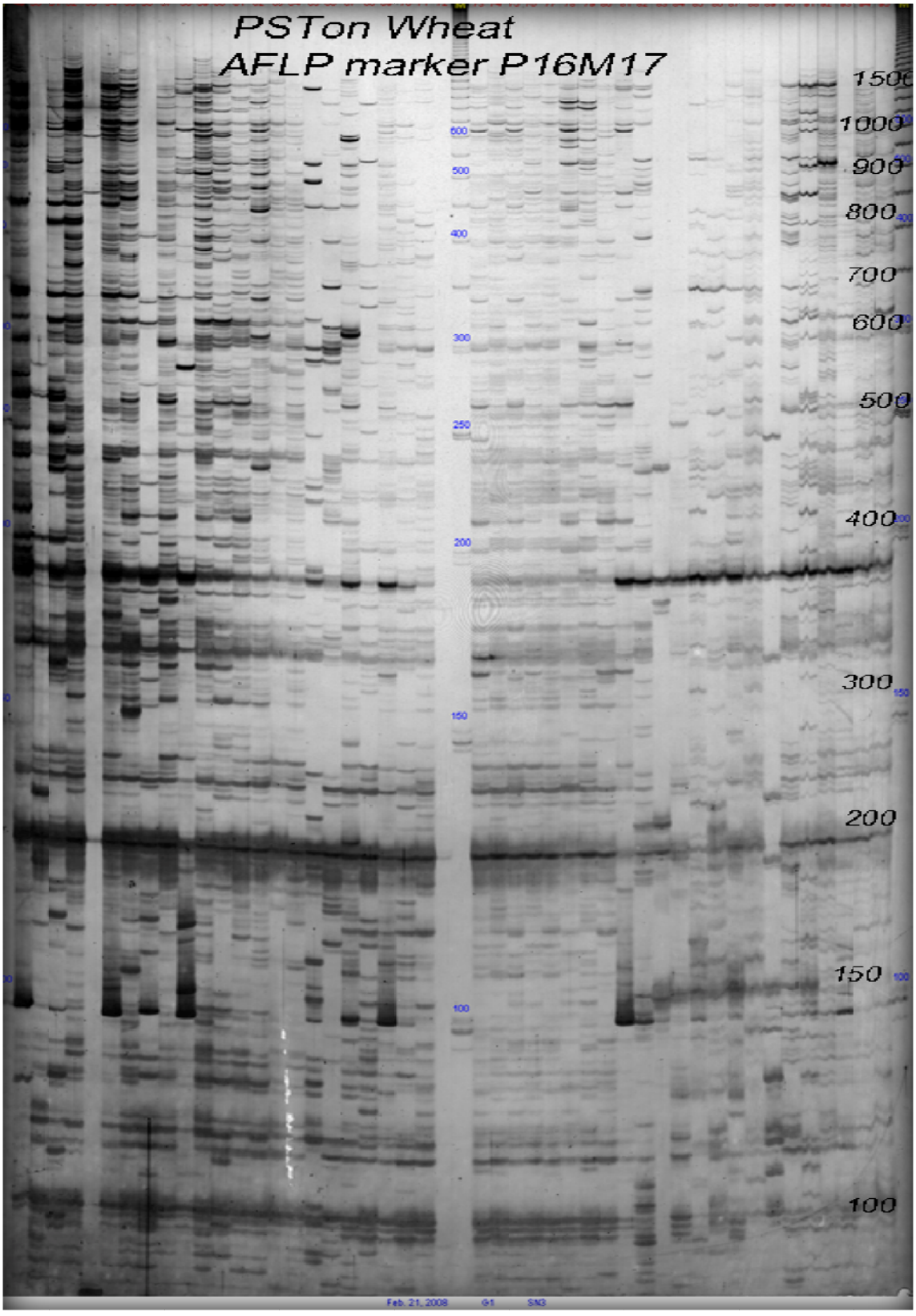

Fig. 2 AFLP product of yellow rust isolates using Primers *P16+ M 17* (*Pst1+ CC, Mse1+CC*).

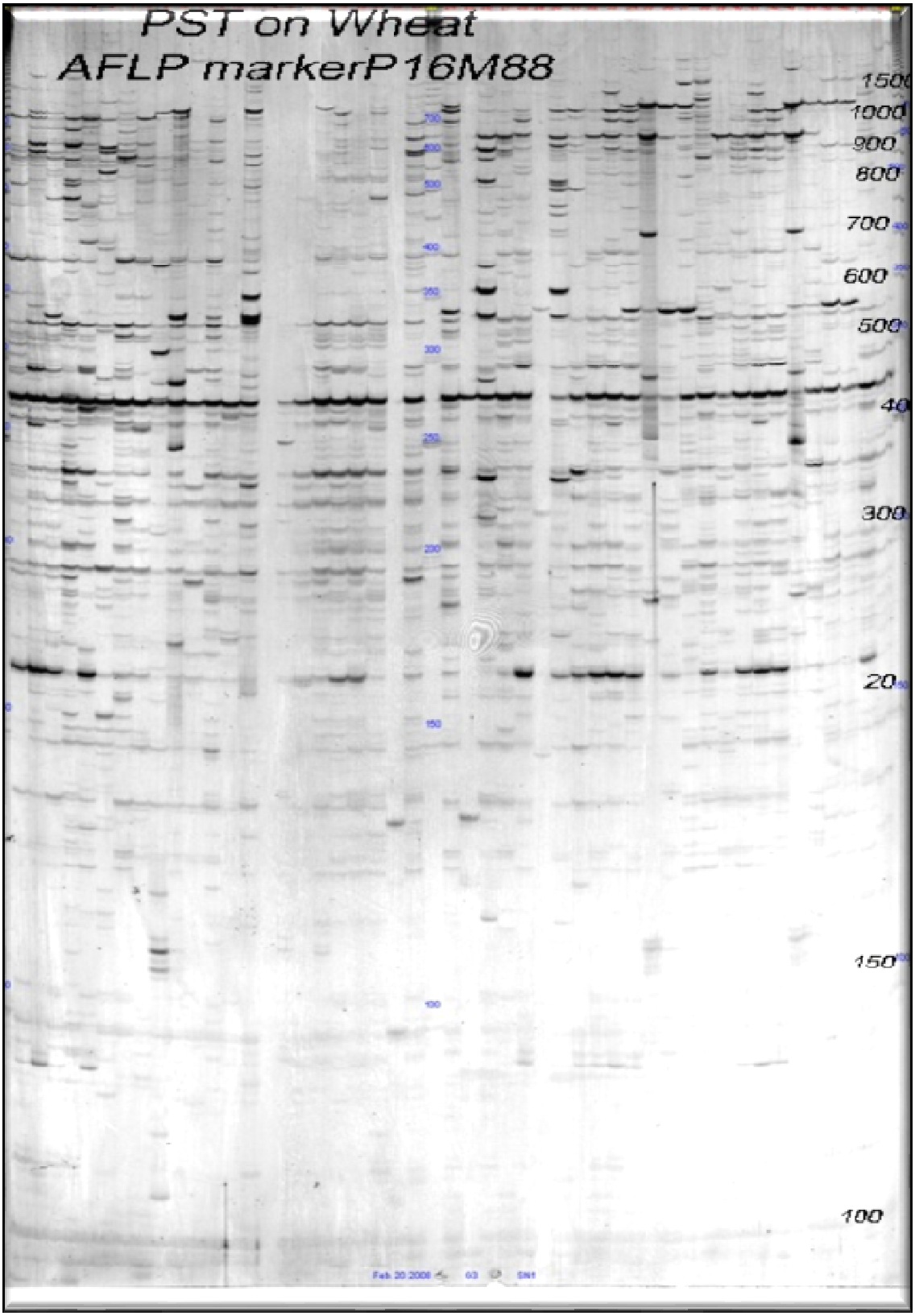

Fig. 3 AFLP product of yellow rust isolates using Primers *P16 + M88* (*Pst1+ CC, Mse1 +GC*).

Table 4 Analysis of partial contrast to the races of the fungus yellow rust between the studied sites in the Syrian society.

Source of variation	Degree of freedom	Sum of squares	Contrast components	Contrast Ratio
Between of cities	10	507.602	50.760	10%
Within the city	85	2,302.377	27.087	90%
Total	95	2,809.979	77.847	

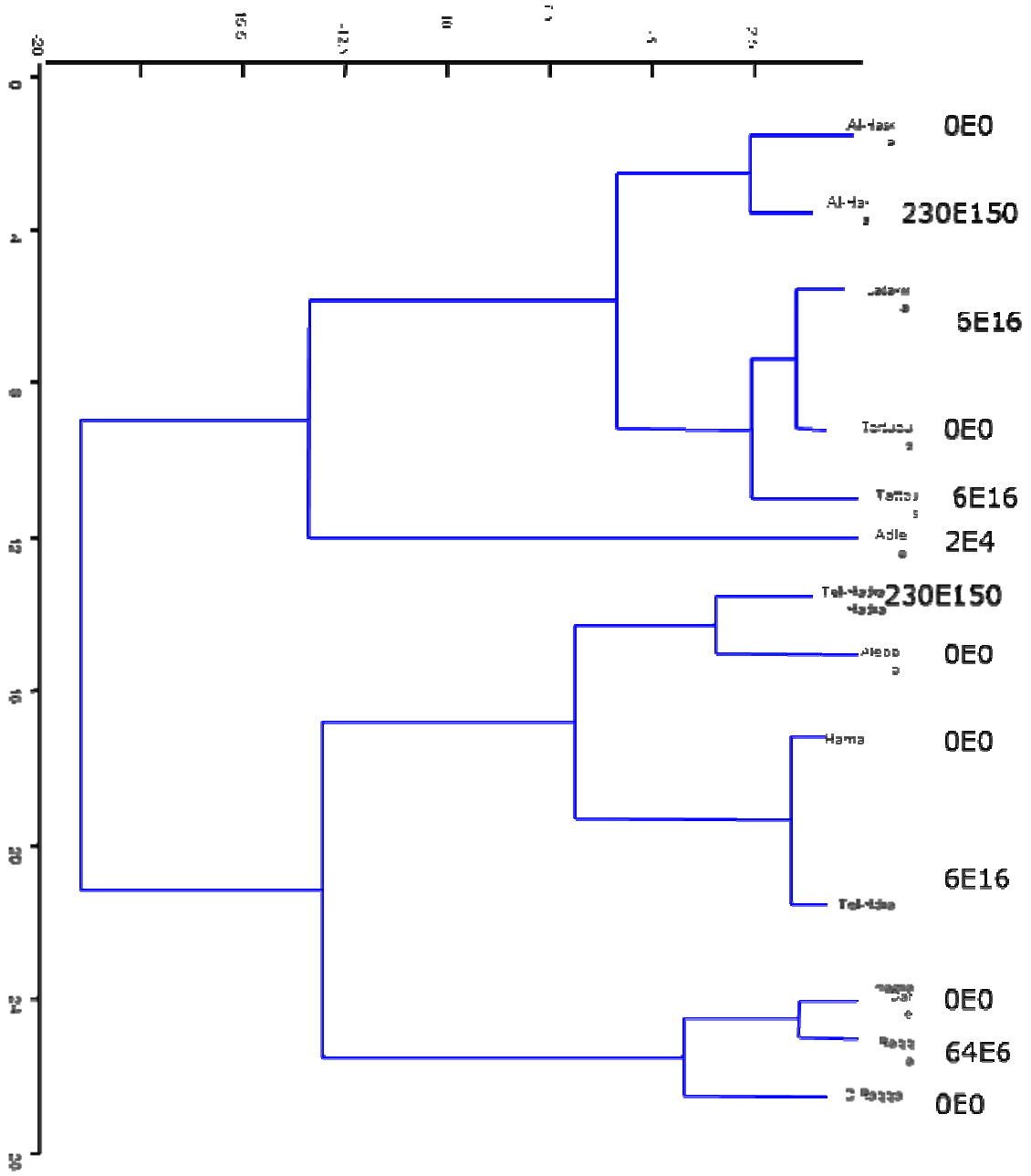

Fig. 4 Dendrogram of yellow rust races on wheat according to Ref. [29].

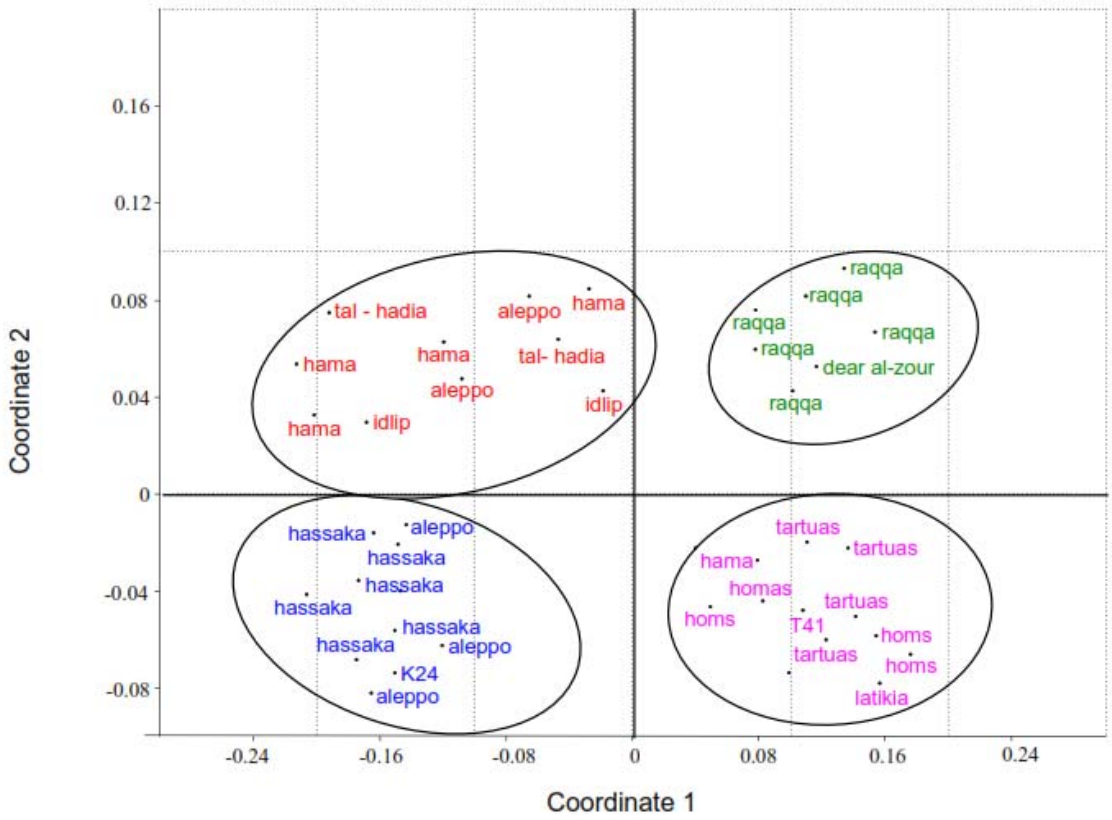

Fig. 5 The results of meta-analysis of genetic tree between fungus yellow rust on wheat races during 2010-2014 [40].

AFLP technique could determine the genetic diversity within the yellow rust community, since it could detect 147 polymorphic sites, i.e. 97.5%. This might declare the epidemic kinetics of this fungus, but couldn't reveal its kinetics within the field [36].

Moreover, the races of Idlib and Aleppo shared many unique AFLP markers, compared with those between Tal-Hadia and Algab that had more polymorphic markers. The result could be explained by the effect of the north area on Tal-Hadia, while the site of Idlib and Aleppo probably involved other cumulative effects.

In specific words, in addition to Tal-Hadia is the most vulnerable area to the fungus infection, it is artificially infected with different races from other areas annually, as it is planted with various genotypes of wheat that have varying capability to infect with this pathogen [37] (have varying degrees of susceptibility against this pathogen), all of that factors increases the probability for emerging of new races from thus complex fungal community.

In the north region, UPGMA analysis determined 10 groups with 69.73% similarity, one of them had 11 different types including type 0, i.e. 90% of races. That might suggest more study to have more specific results about the genetic structure of the pathological zone [38]. North Syria is affected with the fungal Turkish community by wind which carries the Uredospore's faraway up to 800 Km [39], so it might suppose that the whole genetic variance in this area is derived from differentially genetic material belonging either to a major source; type 0, to other ones come from immigrated spores, or the survival ones along the summer.

In this work, there were two virulent genotypes: 230 E 222 and 230 E 150, separating into different groups with 88.7% similarity, which proposed that AFLP markers were unrelated to the virulence [40]. Also, the dendrogram confirmed that, as the bootstrap value was 11.33% and the genetic distance between

two genotypes was 4.1% only, while the number of alleles was 68.23%.

4. Conclusion

P. striiformis f. sp. *tritici* communities could be similar over all the fields in north Syria, as that must be considered in developing a new strategy of wheat breeding to resist this pathogen.

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