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# IDENTIFICATION OF *E.COLI NISSLE 1917* PROTEINS BY USING 2-D GEL ELECTROPHORESIS UNDER THE INFLUENCE OF *COCOS NUCIFERA SAP* AND WINE

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*E. coli* Nissle, protein, 2-D electrophoresis, up and down regulation.

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**ABSTRACT:** Proteomics is the large scale study of proteins**.** Before going to analyse the differentially expressed proteins, through 2-D gel electrophoresis the proteins were extracted in a sample under stress. In the present study, we were isolated the protein samples of *E. coli* Nissle 1917 treated with cocoti sap and wine stress. The isolated proteins washed with 2-D clean up kit because it was very sensitive to salts and detergents. Quantify the protein concentration for loading the samples in 2-D electrophoresis. Proteins were separated by based on IEF and Molecular weight. By using Image master 2-D platinum 6.0 software programmes analyze the protein spots in the gel. We noticed over all 800 proteins in our gel, 370 protein spots were visualized clearly, in that considers 15 spots were isolated based on the regulation. Ten spots shows up regulation and remaining shows down regulation. One newly expressed protein was isolated from wine treated gel when compared to the control and also sap treated gels. Independent t-test was performed to analyze the significant difference between up and down regulation values. Further, this study helps to undergo the proteins identification and its functions through MALDI-TOF analysis.

**INTRODUCTION:** *Cocos nucifera* sap is commonly called as cocoti sap. Sap is a juice collected from the cocoti palm plants. After fermentation, cocoti sap is converted into cocoti wine. It is a sweetish, milky white, effervescent alcoholic beverage with evolved $CO_2$ bubbles as mildly alcoholic beverage similar to beer. This alcohol gives sour smell and sulphur like odor may also be present. It acts as toxicants. Many scientists have analyzed the chemical and microbial properties of cocoti sap [1, 2, 3]. *Escherichia coli* Nissle 1917 is one of the probiotic present in gut flora of our body and served as a model organism for countless biochemical, biological, and biotechnological studies, since the completion of the *E. coli* genome-sequencing project.

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*E. coli* Nissle 1917 has demonstrated to reduce intestinal inflammatory bowel disease (IBD) [4, 5].

Proteomics is regarded as a powerful approach as far as biochemical research is concerned because it directly studies the key functional components of biochemical systems, namely proteins [6].

Proteomics provides a powerful tool for analysing the various molecular mechanisms employed by plants, animals, insects, and microorganisms.

In most cases protein samples are compared in healthy versus infected (or) treated[7].Proteomics that aims at the determination of proteins constituents and their isoforms in a given sample[8].

2-D clean-up kit facilitates the preparation of low conductivity samples suitable for isoelectric focusing (IEF) and 2-D gel electrophoresis. Additionally, the kit concentrates proteins from samples that are too dilute, allowing for higher protein loads that can improve spot detection.

2-D gel electrophoresis is derived from 1-D SDS-PAGE, and expands the number of proteins resolved on an electrophoresis gel by separating the proteins based on their native charge and molecular mass [9, 10]. 2-D electrophoresis technique captured detailed information about protein expression, complex formation, isoforms, and post translational modifications (PMF) [11]. 2-D electrophoresis is regarded as a powerful technique, because it can be used to separate and resolved complex protein mixtures into thousands of individual compounds [12]. This technique has been widely used and successfully applied in a variety of biological systems.

Although a comparison of protein expression profiles from regular 2-D gel electrophoresis can be carried out with the assistance of various software programs [13]. It typically requires some computerized justification of 2-D gel images so that two images can be superimposed and composed [14]. After separation, proteins in 2-D gels were visualized by staining, with colloidal Coomassie blue stain. In this manner, proteins can be quantified and spot patterns in multiple gels can be matched and compared. Statistical analysis can be performed on group of spots in gel, and variations, differences, and similarities can be evaluated [15].

The proteins are finally visualized by radiolabeling or detected with a variety of staining methods such as silver, coomassie blue or fluorescent stains. Adapted image capture devices are used to generate digital images that can be analyzed with 2-D gel softwaresuch as ImageMaster 2-D platinum.

Down regulation is the process by which a cell decreases the quantity of a cellular component, such as RNA or protein, in response to an external variable. An increase of a cellular component is called up-regulation. Cells can increase and decrease their sensitivity to cells by regulating the number of their receptors. Receptors are proteins and are manufactured by the cell itself, so a cell can increase and decrease the amount of receptors within its plasma membrane. If a cell increases the number of receptors then we call it up-regulation and if the cell decreases the number of receptors we call it down regulation.

Down regulation is when a cell decreases its sensitivity to a hormone by decreasing the amount of available receptors.

Up regulation is used by cells to increase their sensitivity to a specific hormone. Up regulation occurs when a cell produces more receptors, the cell decreases its degradation of receptors or by activating already present receptors. Cells typically up regulate when the concentration of a hormone is very little.

If there is a lower concentration of a hormone in the blood stream and the cell increases the number of receptors, it increases the chances of interacting with that hormone. Hormones themselves can also cause cells to up regulate. Genomic techniques, including microarrays allow genomic profiling of cells under stress [16].

T-test is used to test differences in means between two groups. The t- test can be used even if sample sizes are very small, as long as the variables within each group are normally distributed and the variation of scores within the two groups is equal. With the t-test, the test statistic used to generate p-values has a student's distribution with n-1 degrees of freedom [17].

## MATERIALS AND METHODS:

**Sample collection:** Fresh cocoti palm sap samples were collected from coconut palm trees in a sterilized reagent bottles in Tirupati rural, A.P, INDIA. Samples were transported immediately to the laboratory for analysis, some of the sap was separated, allowed for fermentation to make palm wine (fermented palm sap is known as palm wine) at room temperature 25-28$^{o}$C. These samples were filtered by using vacuum pump nitrocellulose membrane filters to separate the microorganisms present in the sample (Filter pore size is 0.02μ).

**Culture collection:** *Eschericia coli* Nissle 1917 was obtained from culture collection center of Ardeypharm GmbH, Herdecke, Germany and the culture was maintained on Luria-Bertani (LB) Medium. LB medium is the most commonly used for *E. coli* culture.

**Protein extraction and Quantification:** The MIC of the two samples, i.e., cocoti sap and wine was determined by micro dilution method. The proteins of three samples (control, sap and wine treated) of *E. coli* Nissle 1917 were extracted by using Trizol protein extraction kit method through sonication and centrifugation process. Quantify the protein concentration by using BCA kit method and all samples were adjusted to 400 mg.

**2-D gel electrophoresis:** Collected protein sample is purified because 2-D gel electrophoresis is very sensitive to salts and detergents. Sample contains some amount of salts and detergents. In this process protein samples were separated by two independent properties i.e. isoelectric point and based on molecular weight. 4 – 7 pH gradient strips were used; the strip length is 24cms.

2-DE protein sample was loaded into the strip holder, placed the strip and covered the strip by using covering gel, allowed for IEF with the help of electrodes, according to the optimal conditions. Remove the strip from the strip holder after the IEF step was completed. Then the sample treated for rehydration with the help of equilibrium buffer Iodoacetamide (IAA). Put the strip into the casting plate, set the instrument and loaded with running buffer allowed for second dimension.

After completion of the second dimension, the gel was separated from the plate and allowed for staining. Colloidal coomassie brilliant blue stain was used.

**Image scanning and analysis:** All destained gels were digitized using gel scanner (Typhon Variable Mode imager), and allowed for gel analysis by using Image master 2-D platinum 6.0 software programme. It quantifies the protein spots, and showed the variation between the control and treated gel samples, the spot size indicates, up-regulation and down-regulation of the protein.

The expressed protein spots were separated by using spot cutter and these spots can be analyzed by MS- for protein identification.

## RESULTS AND DISCUSSION:

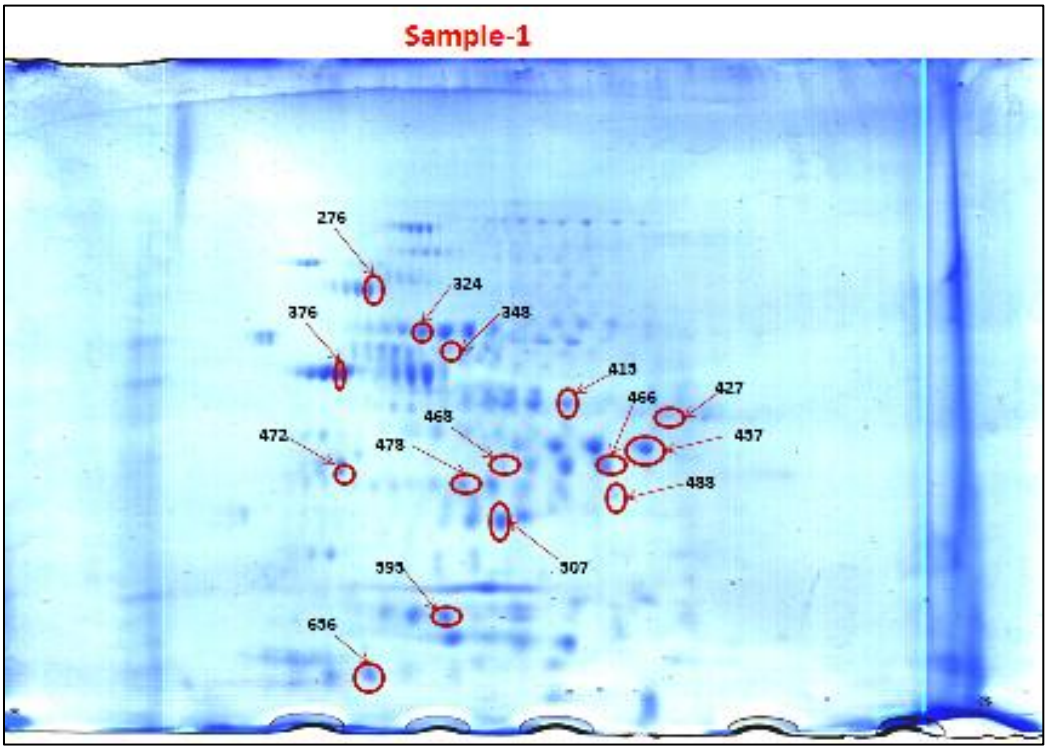



**FIG. 1: REPRESENTATIVE 2-D GEL ELECTROPHORESIS IMAGE OF CONTROL *E.COLI NISSLE 1917*, COVERING PIRANGE OF 4 TO 7. THE LOCATIONS OF THE SPOTS ARE MARKED ON THE GEL**

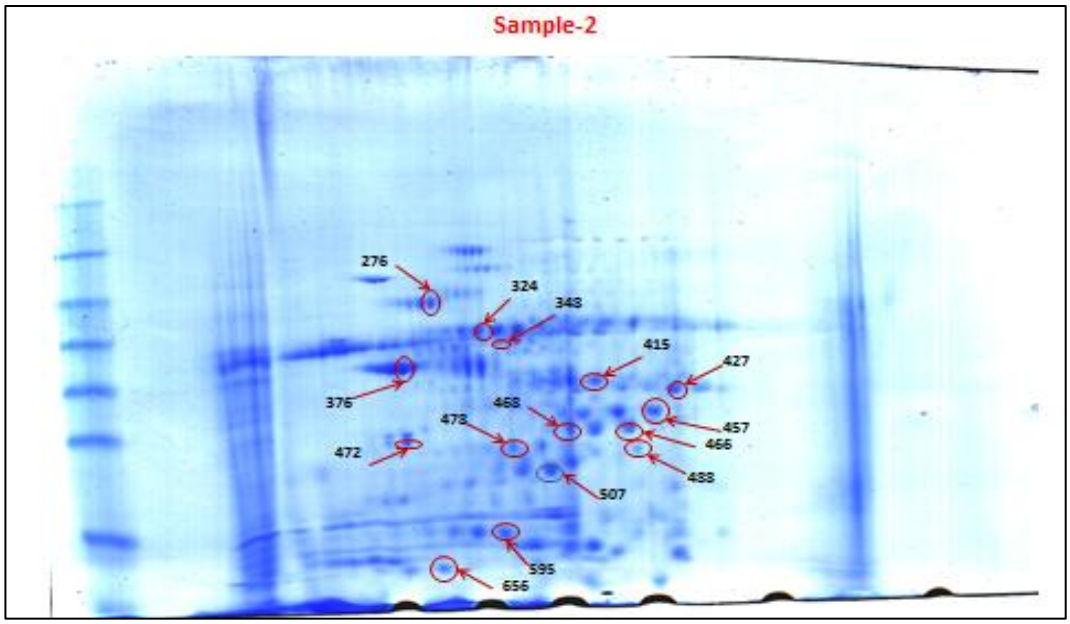



**FIG- 2: REPRESENTATIVE 2-D GEL ELECTROPHORESIS IMAGE OF COCOTI SAP TREATED *E.COLI NISSLE 1917*, COVERINGPI RANGE OF 4 TO 7. THE LOCATIONS OF THE SPOTS ARE MARKED ON THE GEL.**

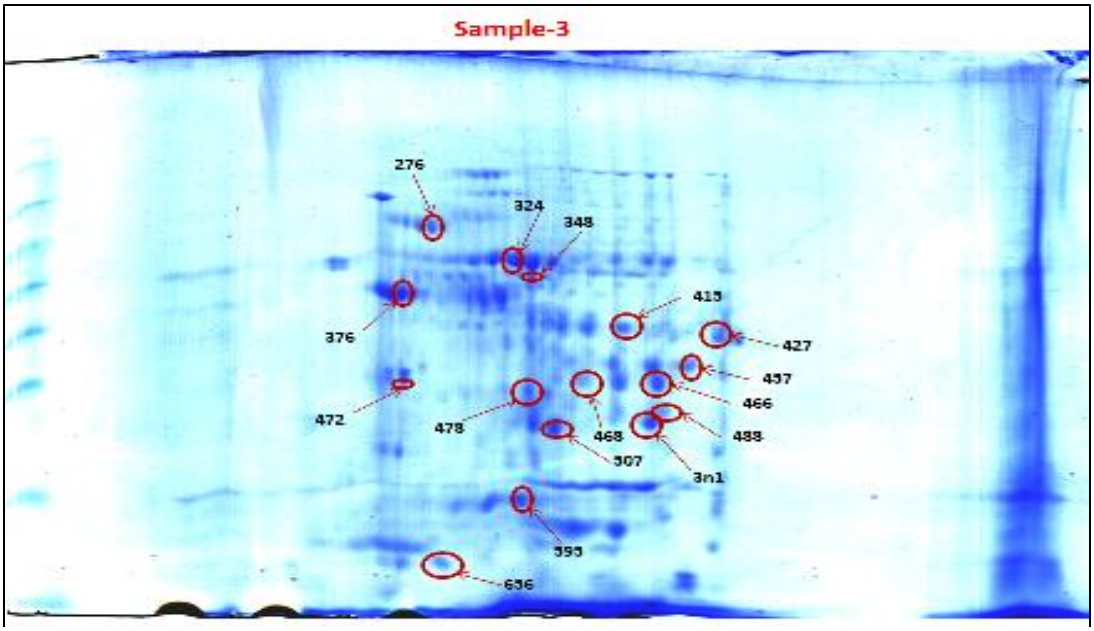
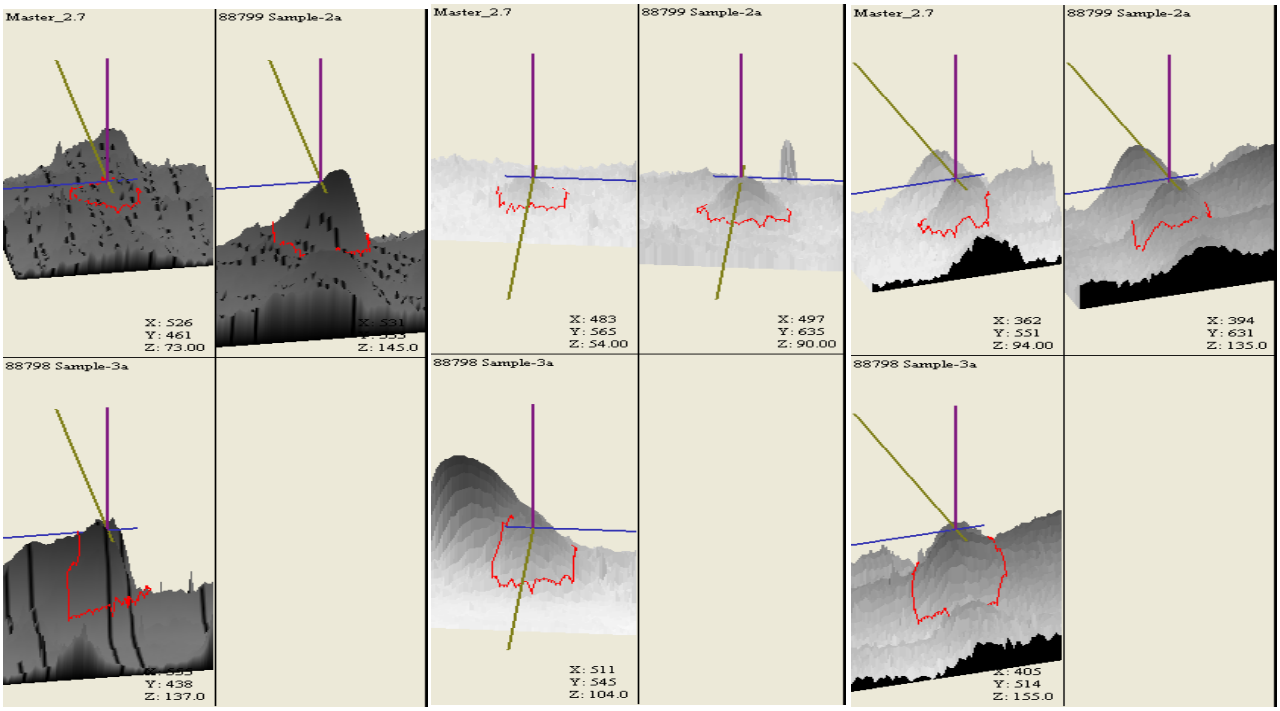
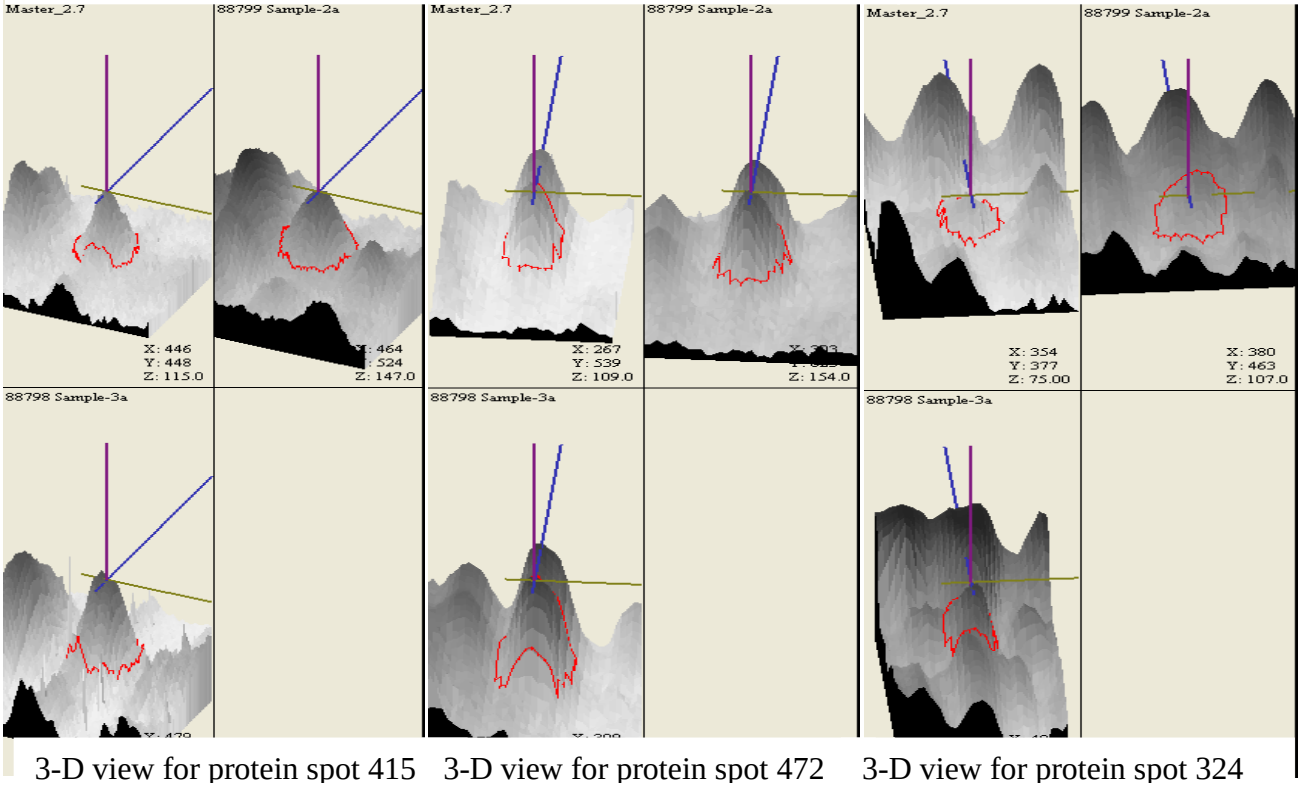



**FIG- 3: REPRESENTATIVE 2-D GEL ELECTROPHORESIS IMAGE OF COCOTI WINE TREATED *E.COLI NISSLE1917* COVERINGPIRANGE OF 4 TO 7. THE LOCATIONS OF THE SPOTS ARE MARKED ON THE GEL.**

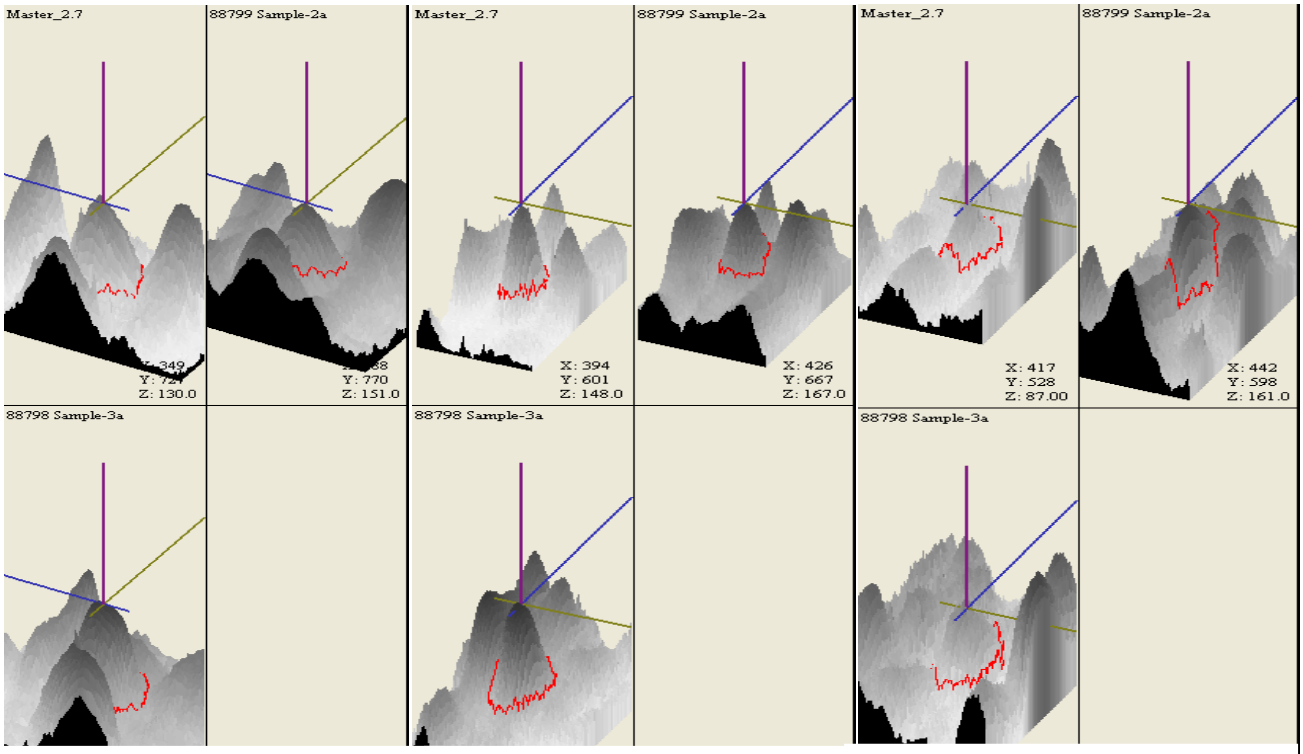
In the present study, we analyzed control, cocoti sap and wine treated protein samples by using 2-D gel electrophoresis. In gel images, it shows the variations in all the three samples i.e. control, cocoti sap and cocoti wine treated samples. Based on the protein regulation, we were noticed 15 differentially expressed spots in cocoti sap treated sample when compared to control sample **(Fig. 1 & 2)**. In the case of wine treated sample, totally 16 differently expressed spots were noticed. Specifically, we were identified one newly expressed protein spot i.e. **$3n_1$** (**Fig. 3**) spot which was not present in both control and cocoti sap treated samples. The protein spots sizes were increased in up-regulation and spots size were decreased in down regulation, when compared to the control. Based on this, the protein spots were separated from the gel. Over these ten samples shows ten up-regulation and five protein spots shows down-regulation. The 3 - D gel images of all the differentially expressed proteins were generated and presented in the following images **(Fig. 4 & 5).**



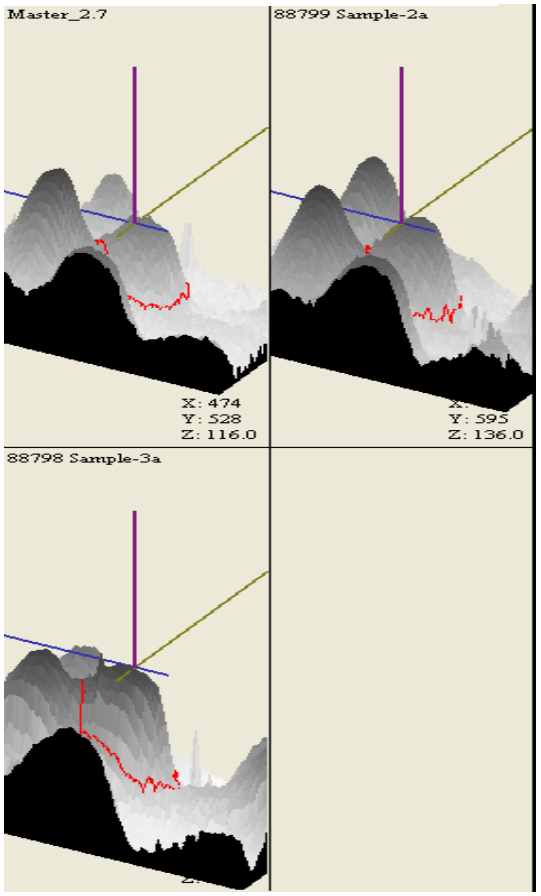

3-D view for protein spot 427 3-D view for protein spot 488 3-D view for protein spot 478




3-D view for protein spot 415 3-D view for protein spot 472 3-D view for protein spot 324

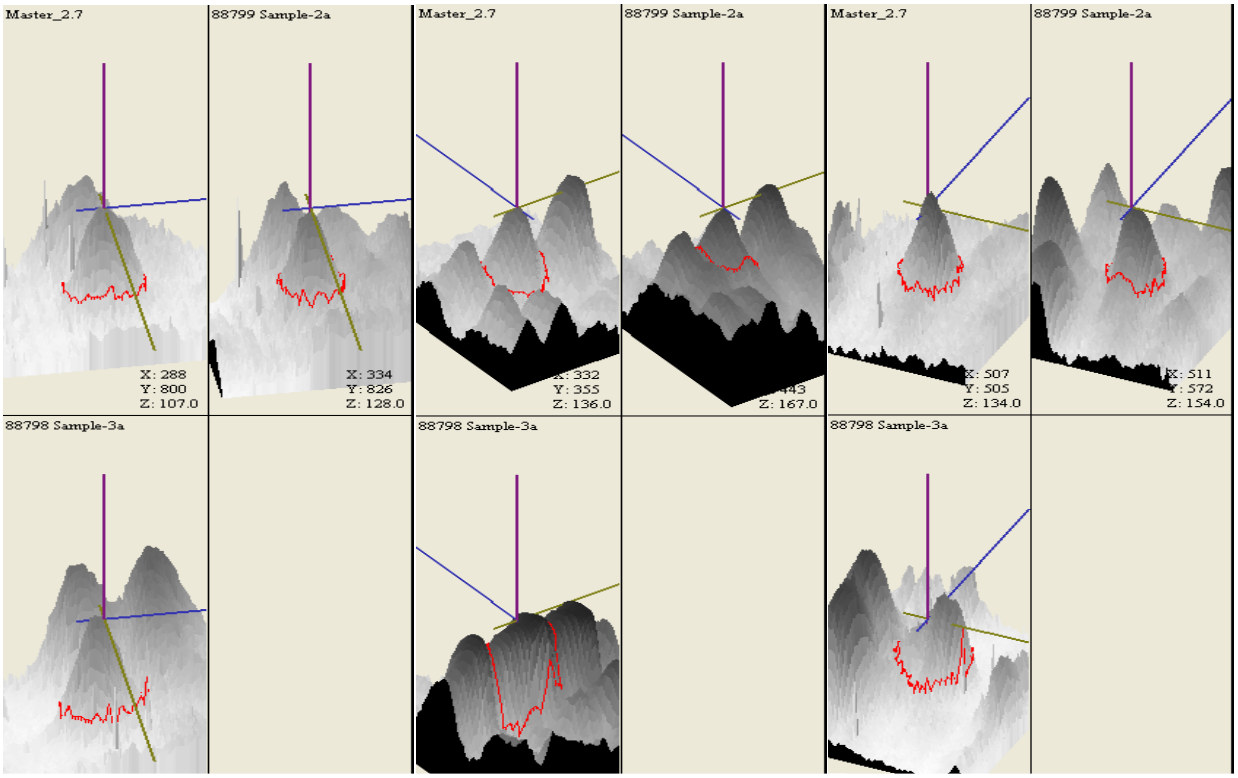



3-D view for protein spot 348 3-D view for protein spot 507 3-D view for protein spot 468

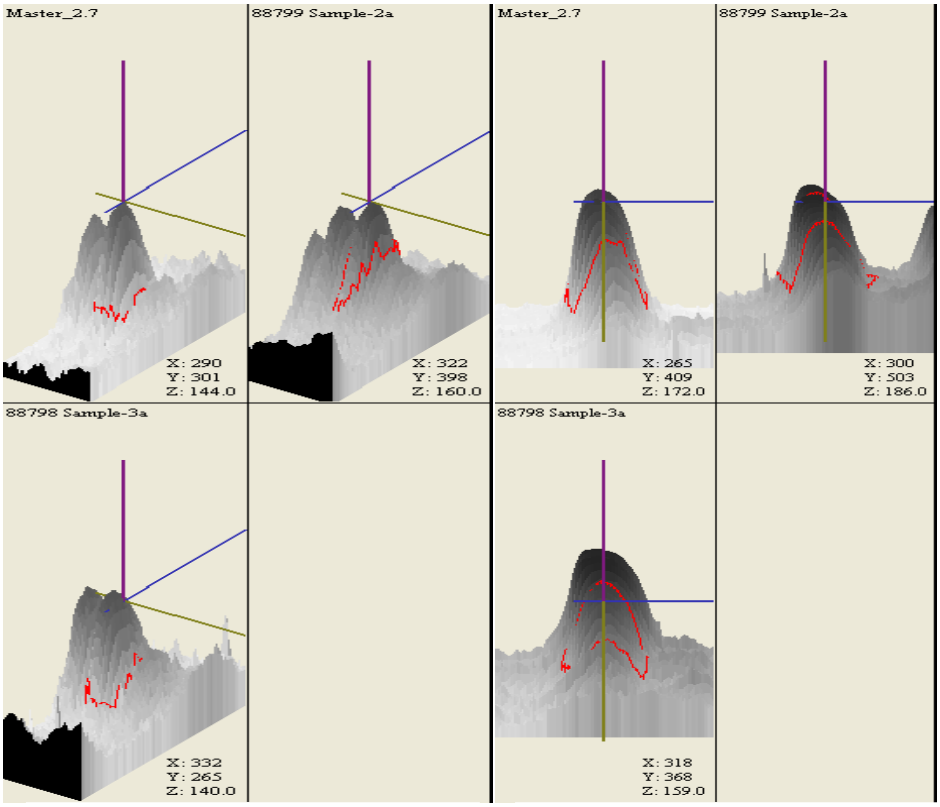



3-D view for protein spot 466

**FIG. 4: UP- REGULATION OF PROTEIN EXPRESSION 3-D IMAGES**




3-D view for protein spot 595 3-D view for protein spot 656 3-D view for protein spot 457




3-D view for protein spot 276 3-D view for protein spot 376

**FIG. 5: DOWN REGULATION OF PROTEIN 3-D IMAGES**

**TABLE 1: UP REGULATION VALUES OF PROTEINS UNDER EXPOSER OF SAP AND WINE TREATMENT**

| Spot no. | Sap up regulation | Wine up regulation | Average up regulation | p-value | Square root(p) | Variable |
|---|---|---|---|---|---|---|
| 427 | 1.4392 | 1.0146 | 1.2269 | 0.1021 | 0.3195 | 18.6327 |
| 488 | 1.3880 | 1.6292 | 1.5086 | 0.1255 | 0.3542 | 20.7444 |
| 478 | 1.0343 | 1.4729 | 1.2536 | 0.1043 | 0.3229 | 18.8384 |
| 415 | 1.3391 | 1.4478 | 1.3934 | 0.1160 | 0.3405 | 19.9073 |
| 472 | 1.0400 | 1.3275 | 1.1841 | 0.0985 | 0.3138 | 18.2884 |
| 324 | 0.9836 | 1.1779 | 1.0807 | 0.0899 | 0.2998 | 17.4456 |
| 348 | 1.0285 | 1.1774 | 1.1029 | 0.0918 | 0.3029 | 17.6319 |
| 507 | 0.6155 | 1.0965 | 0.8560 | 0.0712 | 0.2668 | 15.4739 |
| 468 | 1.1728 | 1.0039 | 1.0883 | 0.0906 | 0.3009 | 17.5117 |
| 345 | 1.6316 | 1.0024 | 1.3170 | 0.1096 | 0.3310 | 19.3295 |
| | | | **Total = 12.0115** | | | |

**Up regulation** Total numbers-10 Mean- 18.38038
Variance- 2.19323 Standard deviation- 1.48096

**TABLE 2: DOWN REGULATION VALUES OF PROTEINS UNDER EXPOSER OF SAP AND WINE TREATMENT**

| Spot no. | Sap down regulation | Wine down regulation | Average Down regulation | p-value | Square root(p) | Variable |
|---|---|---|---|---|---|---|
| 595 | 2.1437 | 1.1589 | 1.6513 | 0.20607 | 0.45395 | 26.9974 |
| 656 | 1.9544 | 1.4716 | 1.7130 | 0.21377 | 0.46236 | 27.5395 |
| 457 | 1.7074 | 1.4133 | 1.5603 | 0.19472 | 0.44127 | 26.1849 |
| 276 | 1.3755 | 0.9965 | 1.1860 | 0.14801 | 0.38472 | 22.6264 |
| 376 | 1.4453 | 2.3600 | 1.9026 | 0.23743 | 0.48727 | 29.1613 |
| | | | **Total =8.0132** | | | |

**Down regulation** Total numbers- 5 Mean-26.5019
Variance-5.8786 S.D- 2.4246
t- Calculated value: 6.8687 t- Critical value: 2.4469
P- value: 0.00047

**RESULT:** There is no significant difference between up and down regulation in the samples. Sonull hypothesis is rejected.

**DISCUSSION:** Transferred the data square root to $Sin^{-1}\sqrt{p}$. After performing $Sin^{-1}\sqrt{p}$ transformation independent samples t- test was conducted. There was no significant difference in up-regulation (Mean-18.38038, Standard deviation- 1.48096) (**Table 1**) and down regulation (Mean- 26.5019, Standard deviation- 2.4246) (**Table 2**). t- Calculated value is 6.8687, and t- critical value is 2.4469. p – Value is 0.00047.

Where t- calculated value is higher than t- critical value, so null-hypothesis is rejected.

**CONCLUSION:** In the present study, the proteins were separated from cocoti sap and wine treated gel samples. Both cocoti sap and wine treated samples shows up and down regulations and also a new protein was evaluated under wine stress.

The results indicating that the sap and wine can cause stress on probiotic *E.coli Nissle 1917*. Further, the spots are undergoing for MALDI-TOF-MS analysis and the differentially expressed proteins are to be noticed. This will be useful for drug designing in pharmaceutical industries for the treatment against sap and wine caused by the intestinal disorders.

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